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## Forecasting Cattle Population: A Case Study of Türkiye


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### ABSTRACT

Cattle breeding is of critical importance in meeting the animal protein needs of the increasing population due to its significant contribution to meat and milk production, which are the main animal protein sources. In addition, cattle breeding has important potential for both the agricultural economy and the general economy in terms of the production and export of value-added agricultural goods and processed products, especially for countries with a large number of cattle. In order to maximize these and similar benefits, to evaluate the structural problems in the livestock sector and to implement effective policies to increase the cattle population to optimum levels, it is of great importance to make data-based decisions and therefore produce sufficient and necessary data. Achieving this will be possible not only with existing data but also by making forward projections with solid scientific methods and estimating the necessary data to plan for the future now. The purpose of this research is to estimate the number of cattle for the next ten years by comparing the results of the artificial neural networks (ANN) and autoregressive integrated moving average (ARIMA) models, using Türkiye's cattle number at the beginning of the year for the years 1930-2024. According to the research results, ARIMA had a greater ability to forecast than ANN. Box-Jenkins method was used in the ARIMA estimations. The ARIMA (1,1,0) model was determined to be the most appropriate model for the data, and it was estimated that the number of cattle at the beginning of the year will increase in the next ten years, reaching 17313762 head in 2025 and 17317161 head in 2033, representing a 5.5% increase in the ten-year period.

**Key words:** Cattle population; forecasting; time series; ARIMA; Türkiye

### Sığır Popülasyonunun Tahmini: Türkiye'den Bir Örnek Çalışma

### ÖZ

Sığır yetiştiriciliği, temel hayvansal protein kaynakları olan et ve süt üretimine yaptığı önemli katkı nedeniyle artan nüfusun hayvansal protein ihtiyacının karşılanmasında kritik öneme sahiptir. Ayrıca sığır yetiştiriciliği, özellikle sığır sayısı fazla olan ülkeler için katma değerli tarımsal mal ve işlenmiş ürün üretimi ve ihracatı açısından hem tarım ekonomisi hem de genel ekonomi için önemli bir potansiyele sahiptir. Bu ve benzeri faydaların en üst düzeye çıkarılması, hayvancılık sektöründeki yapısal sorunların değerlendirilmesi ve sığır varlığının optimum seviyelere çıkarılmasına yönelik etkin politikaların uygulanabilmesi için veriye dayalı kararlar alınması ve dolayısıyla yeterli ve gerekli verinin üretilmesi büyük önem taşımaktadır. Bunu başarmak sadece mevcut verilerle değil, güçlü bilimsel yöntemlerle ileriye dönük projeksiyonlar yapmak ve geleceği şimdiden planlamak için gerekli verileri tahmin etmekle mümkün olacaktır. Bu araştırmanın amacı, 1930-2024 yılları için Türkiye'nin yılbaşındaki sığır sayısını kullanarak yapay sinir ağları (YSA) ve otoregresif bütünleşik hareketli ortalama (ARIMA) modellerinin sonuçlarını karşılaştırarak gelecek 10 yıl için sığır sayısını tahmin etmektir.

Araştırma sonuçlarına göre ARIMA, YSA'ya göre daha yüksek tahmin yeteneğine sahip bulunmuştur. ARIMA tahminlerinde Box-Jenkins yöntemi kullanılmıştır. ARIMA (1,1,0) modelinin veriler için en uygun model olduğu belirlenmiş ve yılbaşındaki sığır sayısının önümüzdeki on yıl içinde artarak 2025 yılında 17313762 başa, 2033 yılında ise 17317161 başa ulaşacağı ve on yıllık periyotta %5,5'lik bir artış göstereceği tahmin edilmiştir.

**Anahtar kelimeler:** Sığır popülasyonu; tahmin; zaman serisi; ARIMA; Türkiye

## INTRODUCTION

Cattle breeding is crucial to satisfying the expanding population's animal protein needs because of its considerable contribution to meat and milk production, which are the main animal protein sources. Furthermore, because Türkiye ranks 19th in the world and first in Europe in terms of cattle population (Faostat, 2023), cattle breeding has a significant potential for both the agricultural and general economies in terms of producing and exporting value-added agricultural goods and processed products. To optimize these and related benefits, to evaluate structural difficulties in the livestock sector, and to execute successful policies to raise the cow population to optimal levels, it is critical to make data-driven decisions and thus generate adequate and high-quality cattle. This will be achievable not only with existing data, but also by using solid scientific methodologies to make forward projections and estimate the essential data to plan for the future now.

From 1930 to 1982, the number of cattle in Türkiye fluctuated and increased by 241%, reaching 15.981 million from 4.685 million. It fell by 39% after this year until 2004. The number of cattle then increased till it reached 16.421 million at the end of 2023. The increase over the last ten years was 15%, whereas the change over the last twenty years was 63% (TurkStat, 2023; TurkStat, 2024a). This high fluctuation in the time series of cattle population requires the use of sophisticated forecasting models and techniques, such as time series decomposition, autoregressive integrated moving average (ARIMA) models, and machine learning approaches for decision making, risk management, resource and budgeting planning, formulating policies and strategies, and supply chain management.

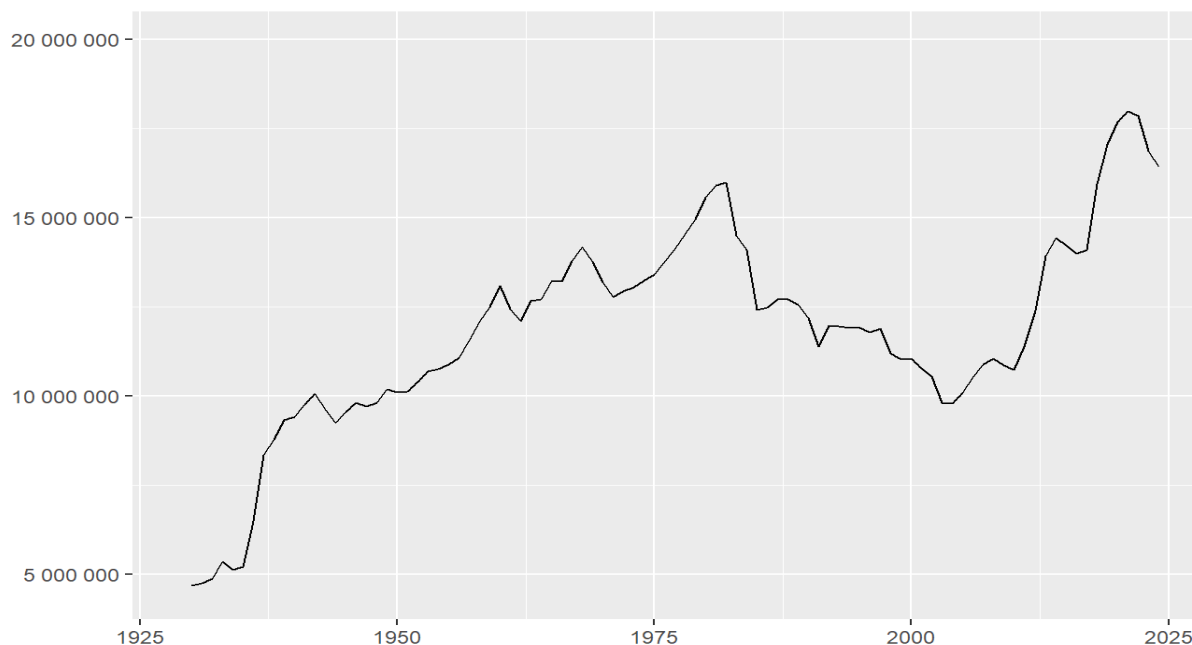
We used artificial neural networks (ANN) and ARIMA models, which are the most commonly used models to produce forecasts with considerable accuracy (Ma, 2020), in order to forecast the cattle population in Türkiye. The ARIMA, also known as the Box-Jenkins time series method, is commonly used in forecasting agricultural commodities or prices (e.g. Novanda et al. 2018; Alhas Eroğlu et al. 2019; Putri et al. 2019; Yıldız & Atış 2019). Artificial neural networks predict approaches based on basic mathematical models of the brain. They allow intricate nonlinear interactions between the response variable and its predictors (Hyndman & Athanasopoulos 2021). The ANN models, which also have applications in agricultural commodity or price predictions (e.g. Özbek, 2017; Çelik & Köleoğlu 2022), are capable of predicting new observations from other observations after executing a process known as learning from existing data (Wang & Meng 2012).

Although there has been some research in Türkiye on future projections of animal populations or animal products (e.g. Cenan & Gürçan 2011; Alhas Eroğlu et al. 2019; Dalgıç et al. 2023), it appears that these studies have recently focused on red meat production estimations. Cenan and Gürçan (2011) forecasted the cattle population in Türkiye. However, their estimates were rather outdated, as they do not include data from 2006 to 2023, and the fact that just one model was employed without comparison with alternative models has necessitated making comparative estimates for the cattle population with multiple models.

The goal of this study is to estimate the number of cattle for the next ten years by comparing the results of the ANN and ARIMA models, using Türkiye's cattle number at the beginning of the year for the years 1930-2024.

## MATERIALS AND METHODS

Türkiye's cattle number at the beginning of the year for 1930–2024, whose source is the Ministry of Agriculture and Forestry, was used in this study (TurkStat 2023; TurkStat 2024a) (Figure 1). The historical data for the number of cattle was obtained from the Turkish Statistical Institute (TurkStat) Publication of 'Indicators of 100 Years'.



**Figure 1.** The number of cattle by year (heads)

### The ARIMA Model

ARIMA can be described as a hybrid of two models: the autoregressive (AR) model combined with the Moving Average (MA) model. ARIMA notation (p, d, q) is commonly used to represent the ARIMA model. P is the AR process degree, d is the differentiation order, and q is the MA process degree (Putri et al., 2019; Box et al., 2015; Akdi, 2010). ARIMA (p, d, q) can be expressed as follows (Equation 1):

$$\hat{y}_t = \mu + \phi_1 y_{t-1} + \dots + \phi_p y_{t-p} - \theta_1 e_{t-1} - \dots - \theta_q e_{t-q} \quad (1)$$

Here,  $\mu$  is the constant term,  $\phi$ s and  $\theta$ s are the parameters of autoregressive and moving average models.  $e_{t-1} \dots e_{t-q}$  are random shocks that are supposed to have been chosen at random from a normal distribution (Alhas Eroğlu et al. 2019; Duke University, 2023).

We used Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) values in order to decide which ARIMA model is the most appropriate.

### The Artificial Neural Networks Model

The feed-forward neural network was used in this study with a single hidden layer, which was used for models used for time series modeling and forecasting and lagged inputs for forecasting univariate time series. The neural network autoregression (NNAR) model, a tool for fitting a neural network with lagged time series values as inputs, was used. It is a feed-forward network with one hidden layer to signify that the hidden layer has p lagged inputs and k nodes (Hyndman, 2023).

This network is made up of layers, weights and activation functions. The layers are an input layer, a single hidden layer, and an output layer. The input layer stores the values of the dataset's N features. The hidden layer is made up of M neurons, each of which holds the value created by an activation function. The projected outputs are kept in the output layer. Each node in the input layer is connected to each neuron in the hidden layer by a weight value, which is used to calculate the weighted sum of each neuron in the hidden layer. Other weights connect each neuron in the hidden layer to each node in the output layer. The activation function is in charge of creating the hidden neuron values (Qaddoura et al., 2021).

The training procedure begins by calculating the weighted sum  $WS_j$  (Equation 2) of each neuron j of the hidden layer J by summing the product of the value  $x_i$  of node i of the input layer and each weight  $w_{ij}$  connecting the input nodes i and the neuron j. The result is then added to the bias  $b_j$ . The activation function uses the weighted sum  $WS_j$  for each neuron j in M neurons to generate an input value for the following layer representing the output layer (Hyndman & Athanasopoulos 2021; Qaddoura et al., 2021).

$$WS_j = \sum_{i=1}^N w_{ij} x_i + b_j \quad (2)$$

### Comparison of model performance

To assess the forecasting capability, following assessment statistics are applied to each model: root mean square error (RMSE), mean absolute error (MAE), mean absolute percentage forecast error (MAPE), and mean absolute scaled error (MASE). They are expressed as follows (Equation 3-6):

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (Y_t - YF_t)^2}{n}} \quad (3)$$

$$MAE = \frac{\sum_{i=1}^n |Y_t - YF_t|}{n} \quad (4)$$

$$MAPE = \frac{\sum_{i=1}^n |(Y_t - YF_t) / YF_t|}{n} \times 100\% \quad (5)$$

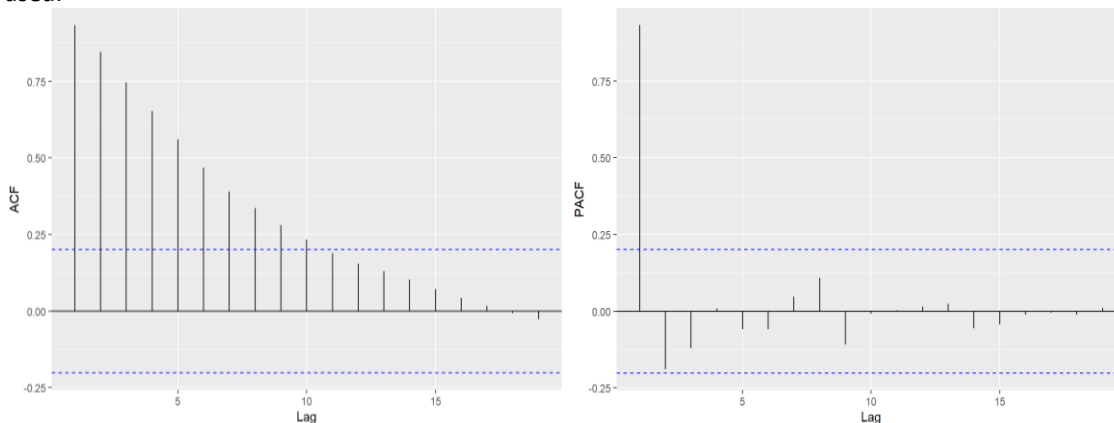
$$MASE = \frac{MAE}{Q} \quad (6)$$

Where  $Y_t$  and  $YF_t$  represent the  $i$ -th actual and forecasting values. The total number of forecasts is  $n$ , and  $Q$  is a scaling constant.

## RESULTS AND DISCUSSION

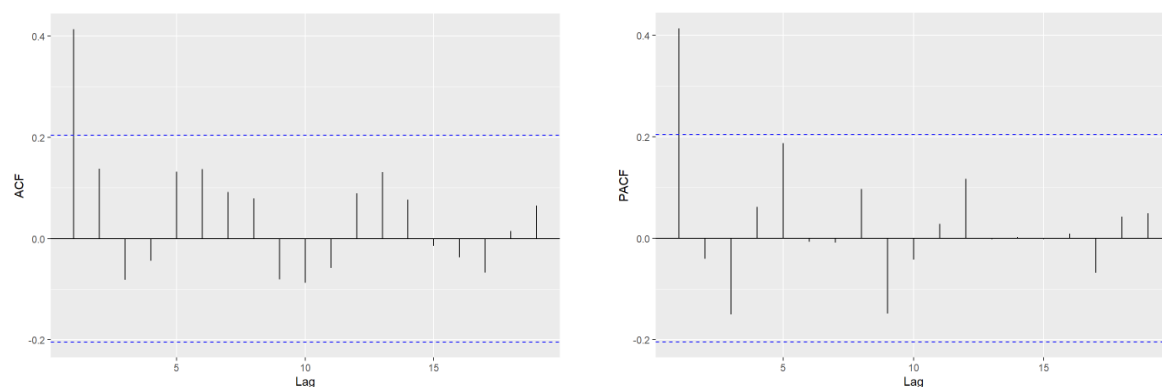
### The ARIMA Model

When looking at the autocorrelation function (ACF) graph of the series, it is observed that it decreases and approaches zero, and in the partial autocorrelation function (PACF) graph, except for the first delay, almost all other delays are within the confidence interval (Figure 2). So, we concluded that the ARIMA model can be used.



**Figure 2.** ACF and PACF graphs of the original series

It is seen that the cattle population in Türkiye has a fluctuating series over the years (Figure 1). According to the KPSS Unit Root Test results, it was decided that the series was not stationary because the test-statistic value was above the critical value. In this case, the series must be detrended, that is, made stationary, by applying first-order differencing. After this process, according to the KPSS Unit Root Test results, the first order difference series (Figure 3) was stationary.



**Figure 3.** ACF and PACF graphs of the first difference of the series

The AIC and BIC values were used to compare alternative ARIMA models, with lower values indicating a better match (Table 1). We selected ARIMA (1,1,0), which had the lowest AIC and BIC values in the ARIMA models, as the forecast model. The parameter of this model is AR(1) (0.449), and it is significant because the p-value (<0.001) of this coefficient is smaller than 0.05. Therefore, the null hypothesis that the parameter is zero can be rejected.

**Table 1.** The AIC and BIC results for model selection

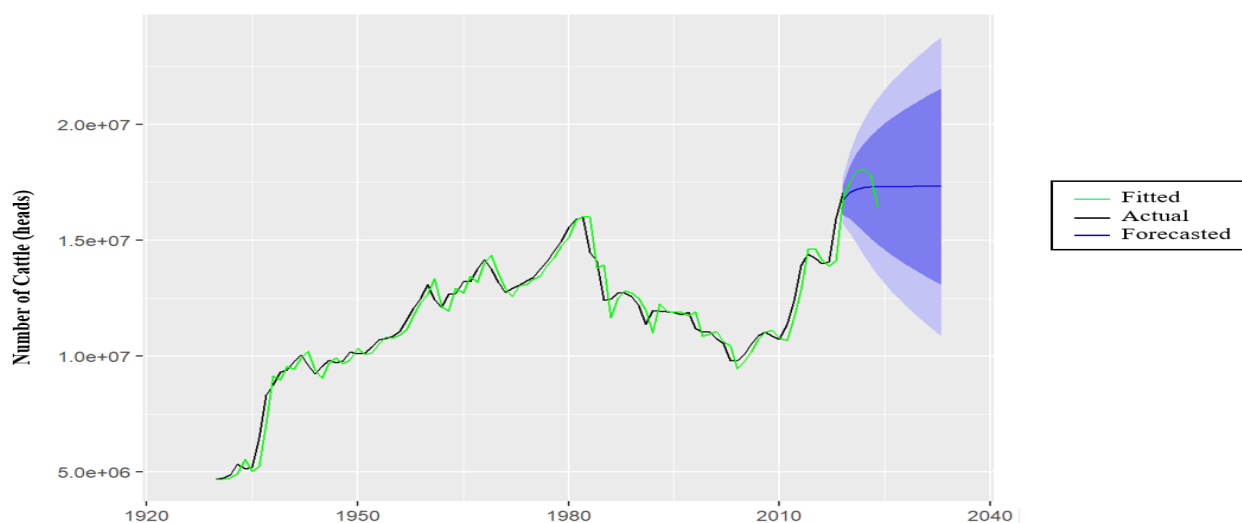
AIC	BIC	Loglikelihood	Model type
2759.227	2761.727	-1378.592	model_0_1_0
2739.748	2744.703	-1367.808	model_1_1_0
2741.811	2749.174	-1367.772	model_2_1_0
2741.839	2749.202	-1367.786	model_1_1_1
2743.842	2748.797	-1369.855	model_0_1_1

It was estimated in the ARIMA model that the number of cattle at the beginning of the year would increase in the next ten years, reaching 17313762 head in 2025 and 17317161 head in 2033 (Table 2). The fitted, actual and forecasted data are given in Figure 4.

**Table 2.** ARIMA Model forecast results of the number of cattle in Türkiye (2025-2033)

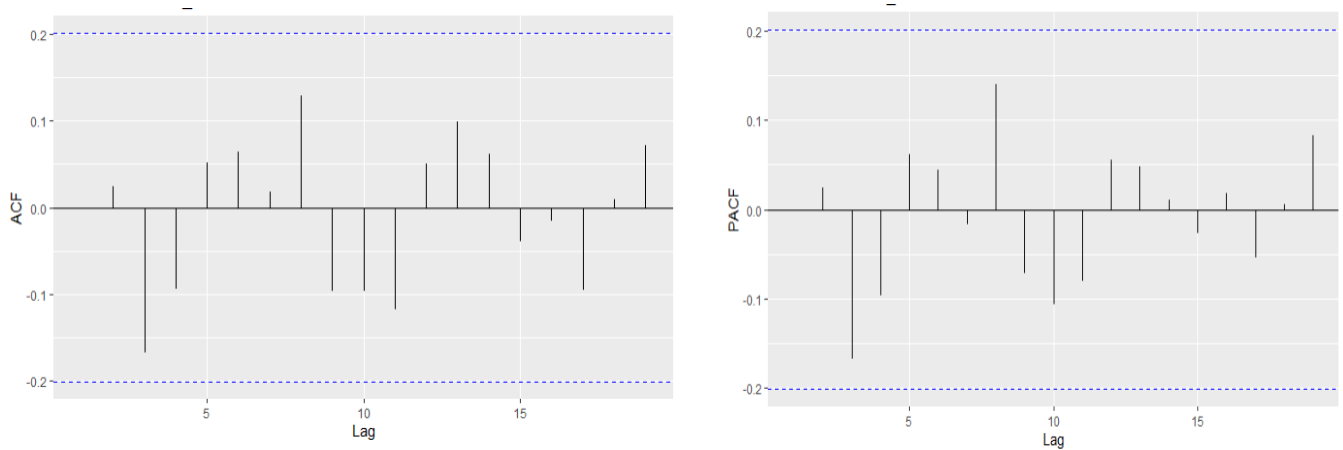
	Point Forecast	Lo* 80	Hi* 80	Lo 95	Hi 95
2019	16734306	16077441	17391172	15729717	17738896
2020	17069837	15926677	18212997	15321525	18818150
2021	17212215	15657395	18767034	14834324	19590106
2022	17272631	15365692	19179570	14356219	20189042
2023	17298267	15084030	19512505	13911883	20684652
2024	17309146	14821235	19797057	13504214	21114078
2025	17313762	14577840	20049684	13129530	21497994
2026	17315721	14351848	20279594	12782868	21848574
2027	17316552	14140801	20492303	12459660	22173444
2028	17316905	13942455	20691355	12156129	22477681
2029	17317054	13754932	20879177	11869259	22764850
2030	17317118	13576712	21057524	11596661	23037575
2031	17317145	13406568	21227722	11336434	23297856
2032	17317156	13243509	21390804	11087050	23547263
2033	17317161	13086723	21547600	10847264	23787059

\*Lo: Lower prediction at related confidence limit; Hi: Upper prediction at related confidence limit



**Figure 4.** Forecast, actual and fitted data by year for the ARIMA model

The ACF and PACF plots of the error terms (Figure 5) suggest that the series appears stationary and resembles white noise. All autocorrelations fall within the confidence interval (represented by the blue lines), and the Ljung-Box test statistic (0.4217) exceeds the significance threshold of 0.05. Thus, it can be concluded that the series exhibits white noise characteristics.



**Figure 5.** The ACF and PACF plots of the error terms

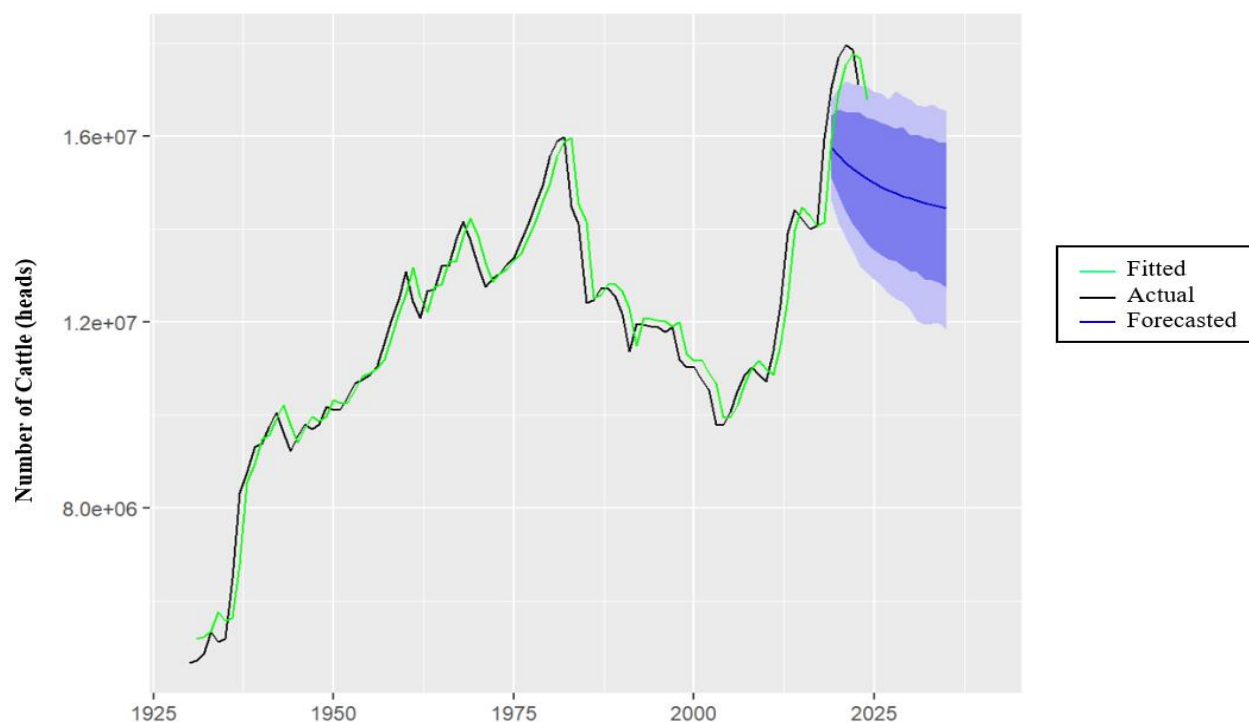
### The Artificial Neural Networks Model

We used a single hidden layer feedforward neural network in the ANN model using the R function NNETAR to forecast the number of cattle. The series was non-seasonal because it was a yearly period. In the model, the number of non-seasonal lags is utilized as inputs. The number of lags was automatically determined according to the AIC. The number of nodes in the hidden layer was determined as half of the number of input nodes plus 1. The Box-Cox transformation parameter lambda is set to "auto," thus a transformation is chosen automatically and sigmoid function was used.

According to the ANN model results, the number of cattle at the beginning of the year decreases in the next ten years, reaching 14988275 head in 2025 and 14526228 head in 2033 (Table 3). The fitted, actual, and forecasted data are given in Figure 4.

**Table 3.** ANN model forecast results of the number of cattle in Türkiye (2024-2033)

	Point Forecast	Lo 80	Hi 80	Lo 95	Hi 95
2019	15746894	15024950	16448224	14620871	16867463
2020	15576417	14616206	16502506	14061292	16988834
2021	15427607	14395710	16508135	13851243	17033219
2022	15296927	14092228	16485840	13508346	17041606
2023	15181575	13885807	16399067	13334686	17021366
2024	15079302	13724934	16362398	13026879	17077446
2025	14988275	13666117	16311889	12842197	16922725
2026	14906987	13478877	16235367	12525304	16937139
2027	14834181	13254505	16115830	12536839	16945040
2028	14768803	13171202	16159178	12453062	16827174
2029	14709960	13052157	16153587	12226108	16831251
2030	14656892	12904160	16110563	12075669	16825599
2031	14608944	12848886	16052622	11967487	16745110
2032	14565553	12907993	16035489	11905885	16794195
2033	14526228	12836285	16014918	11822787	16743936



**Figure 4.** Forecast, actual and fitted data by year for the ANN model

#### Comparison of Model Performance

Table 4 illustrates the RMSE, MAE, MAPE, and MASE from the ANN and ARIMA models in order to evaluate the forecasting capability of the models. Despite the fact that the R-squared values of both models are high (>95%), which means a good fit of both models to the data, the error levels in the ARIMA model are lower than in the ANN, leading us to the conclusion that the ARIMA has greater ability and hence delivers better outcomes (Table 4).

**Table 4.** Forecasting performances of the models

	R <sup>2</sup>		RMSE	MAE	MAPE	MASE
ARIMA	0.969	Training set	506763.3	357580.8	3.249045	0.9145099
		Test set	627988.9	598647.1	3.464956	1.5310348
ANN	0.964	Training set	528614.4	381511.3	3.484319	0.9757119
		Test set	1987073.2	1918526.7	11.009492	4.9066159

According to the results for the ARIMA and Neural Network approaches, overlearning was detected in the Neural model results because of an excessive increase in MAPE, but not in the ARIMA model. The results in the train set were close for both approaches, whereas the results in the test set were good for ARIMA but not for Neural Networks.

Cenan and Gürçan (2011) concluded in their research that the ARIMA (1,1,0) model was the most suitable model in the ARIMA models for forecasting the cattle population in Türkiye, in accordance with our study. Our result is also coherent with Alhas Eroğlu et al.'s (2019) prediction that beef production would increase steadily until 2028. However, the increase in their study (%7.4) is higher than that in ours (%2.8). The main reasons for this are the decrease in the number of cattle after 2021, which was forecasted to increase by Alhas Eroğlu et al.'s (2019), and the regular increase in carcass weight, causing a greater rise in beef production.

The forecasted results closely align with the average values of the “Towards Sustainability” and “Business-As-Usual” scenarios from the FAO cattle projections for 2025 and 2030. The differences between the projected values and the FAO scenarios are 0.4% for 2025 and -4.0% for 2030, respectively (FAO, 2024).

When we examined the trend of the number of cattle per capita over the years, which can be used in evaluating the animal protein consumption trend (Doğan & Kan, 2021), we observed that the number of cattle per capita in 2033, calculated using the population projections estimated by TurkStat (TurkStat, 2024b) and the



number of cattle estimated in this study, decreased by 6,7% in the last ten years. However, this value had increased by 4,5% in the last ten years in 2023. This shows that the change in the number of cattle per capita in the coming years will be downward, unlike in recent years. Thus, it is critical to assess the domestic cattle supply security in terms of sustainability using the appropriate policy and strategies, and to take the necessary steps within the context of food security.

In order to secure the domestic cattle supply, it is important to take the following measures and establish policies aimed at these measures: guaranteeing the income level of cattle breeders with state aids, encouraging biotechnological methods for animal breeding in order to increase efficiency and quality in animal production, optimizing business scales, taking measures to ensure regular raw material supply to the livestock-based industry, supporting livestock cooperatives and maximizing their functionality in order to achieve these goals in the livestock sector, taking measures to protect dairy cattle enterprises that are important in the supply of animal materials, increasing the proportion of small cattle in the animal product supply by following policies that direct consumer preferences towards animal products obtained from small cattle, expanding veterinary services and increasing controls and inspections in order to combat animal diseases effectively, by increasing the production of forage crops, eliminating the deficit of quality forage, encouraging the cultivation of forage crops, increasing capacity utilization in the feed industry, and protecting and rehabilitating meadow and pasture areas (Cenan & Gürcan 2011, Akgül & Yıldız 2016; Çiçek & Doğan 2018; Aral et al. 2020).

High inflation and economic problems, especially with the sharp increase in foreign exchange since the end of 2021, have brought about decreases in the demand for animal products in recent years, but the economic improvements expected from 2024 may cause the supply-demand balance to deteriorate further in favor of demand. This situation makes the measures to be taken to increase the supply of cattle even more important.

Turning to imports of fattening and slaughtering cattle in order to meet the rising demand caused by the increase in population in the coming years will weaken the economic competition of domestic cattle breeders, as in the past and today, and will cause the cattle breeding sector in Türkiye to shrink. In order to prevent this, it is important to plan policies that will allow the domestic cattle supply to increase at a certain level and to start implementing the necessary measures instead of solving the problem of decreasing domestic cattle supply with cattle imports.

If the cattle supply that will adequately meet the increase in demand for meat and dairy products in the coming years is not secured, the supply-demand imbalance caused by this situation will cause red meat and milk prices to increase sharply. This increase will also cause inflation to increase. Government interventions to reduce the market prices of meat and dairy products in order to reduce this effect on inflation will cause cattle and animal product breeders to be unable to cover their production costs and will subsequently abandon cattle breeding or at least not make new investments for the necessary growth that the sector needs (Özbek, 2023). In order to deal with this problem, instead of interventions to reduce market prices in order to prevent the increase in meat and milk prices due to the reasons mentioned above or fluctuations in foreign currency or a sharp increase in imported raw material prices, it is necessary to determine the problems that cause this increase in advance and to make constructive interventions for these problems.

## CONCLUSIONS

The Box-Jenkins ARIMA and ANN models were used in this study to forecast the number of cattle at the beginning of the year for 2025-2033. A comparative study was conducted in order to assess the forecasting capability of these models. According to the research results, ARIMA has a greater ability to forecast than ANN. The ARIMA (1,1,0) model was determined to be the most appropriate model for the data, and it was estimated that the number of cattle at the beginning of the year will increase in the next ten years, reaching 17313762 head in 2025 and 17317161 head in 2033. However, this increase is substantially below the increase over the past decade. This demonstrates that the necessary precautions should be taken to raise the number of cattle over the next ten years in order to meet the increasing population's animal protein needs due to its substantial contribution to meat and milk production. Another study finding is that the ARIMA model can be used to forecast the number of cattle in other countries and can be applied to forecast the number of other animal types.

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### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Evaluation of Fruit Characteristics of Bitter Almond (*Prunus dulcis* var. *amara*) Genotypes Selected from Hilvan District of Şanlıurfa

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### ABSTRACT

The aim of this study was to evaluate the fruit characteristics of selected bitter almond genotypes in Hilvan district of Şanlıurfa, Türkiye. The study was conducted in 2015-2016 and examined 18 bitter almond genotypes grown from seed. Fruit characteristics were determined, including the weight of shelled and kernel almonds, size and various physical properties. The weight of the shelled almonds ranged from 2.20 g to 6.21 g, while the kernel weight ranged from 0.50 g to 1.04 g. The width, length and height of the shell fruits ranged from 15.92 to 24.32 mm, 24.43 to 40.68 mm and 11.29 to 14.88 mm, respectively, while the width, length and height of the kernel fruits ranged from 8.90 to 13.57 mm, 17.45 to 28.85 mm and 4.51 to 6.68 mm, respectively. The shape of the kernels was predominantly classified as medium-wide and flat. A weighted scoring method was used to assess the overall quality of the fruit, with scores ranging from 536 to 624 points. In this study, further research is recommended to evaluate the properties of the rootstock of the genotypes and explore their potential in various industries such as cosmetics, pharmaceuticals and food. It is expected that this study will contribute to a better understanding of bitter almond diversity and thus provide important support for future breeding efforts.

**Key words:** Bitter almond, performance, fruit characteristics, Şanlıurfa.

## Şanlıurfa'nın Hilvan İlçesinden Seçilen Acı Badem (*Prunus dulcis* var. *amara*) Genotiplerinin Meyve Özelliklerinin Değerlendirmesi

### ÖZ

Bu çalışmanın amacı, Şanlıurfa'nın Hilvan ilçesinde seçilen acı badem genotiplerinin meyve özelliklerini değerlendirmektir. Çalışma 2015-2016 yıllarında yürütülmüş ve tohumdan yetiştirilen 18 acı badem genotipi incelenmiştir. Kabuklu ve iç badem ağırlığı, irilik ve çeşitli fiziksel özellikler de dahil olmak üzere meyve özellikleri belirlenmiştir. Kabuklu bademlerin ağırlığı 2.20 g ile 6.21 g arasında değişirken, çekirdek ağırlığı 0.50 g ile 1.04 g arasında değişmiştir. Kabuklu meyvelerin eni, boyu ve yüksekliği sırasıyla 15.92 ile 24.32 mm, 24.43 ile 40.68 mm ve 11.29 ile 14.88 mm arasında değişirken, çekirdekli meyvelerin eni, boyu ve yüksekliği sırasıyla 8.90 ile 13.57 mm, 17.45 ile 28.85 mm ve 4.51 ile 6.68 mm arasında değişmiştir. Çekirdeklerin şekli ağırlıklı olarak orta genişlikte ve yassı olarak sınıflandırılmıştır. Meyvelerin genel kalitesini değerlendirmek için ağırlıklı puanlama yöntemi kullanılmış ve puanlar 536 ile 624 puan arasında değişmiştir. Bu çalışmada, genotiplerin anaçlarının özelliklerini değerlendirmek ve kozmetik, ilaç ve gıda gibi çeşitli endüstrilerdeki potansiyellerini keşfetmek için daha fazla araştırma yapılması önerilmektedir. Bu çalışmanın acı badem çeşitliliğinin daha iyi anlaşılmasına katkıda bulunması ve böylece gelecekteki ıslah çalışmaları için önemli bir destek sağlaması beklenmektedir.

**Anahtar kelimeler** Acı badem, performans, meyve özellikleri, Şanlıurfa

## INTRODUCTION

Almond (*Prunus amygdalus* Batsch) is a fruit species belonging to the Rosaceae family known for its high economic value. Thanks to its geographical location and ecological diversity, Turkey has very favorable conditions for almond cultivation (Şimşek & Gülsoy, 2017). Turkey has great potential for almond cultivation. The GAP region in particular, with its favorable climatic conditions, has developed into an important region for almond cultivation. The high summer temperatures are a great advantage for almond cultivation in this region (Kaşka et al., 2005; Şimşek & Osmanoğlu, 2010; Emre & Tapkı, 2022). Almonds are classified pomologically as bitter almonds (*Prunus amygdalus* var. *amara*) and sweet almonds (*Prunus amygdalus* Dulcis) (Karatay et al., 2014).

Bitter almonds are used in oil production and the cosmetic industry because they contain cyanogenic acid and can have a toxic effect when consumed in excess (Thodberg et al., 2018). In contrast, sweet almonds contain very little or no cyanogenic acid at all (Topçuoğlu & Ersan, 2020). Bitter almond kernels contain amygdalin (C<sub>20</sub>H<sub>27</sub>NO<sub>11</sub>), a colorless and crystalline glycoside (Keser et al., 2014; Del Cueto et al., 2017). These kernels also contain a special enzyme. When the kernel is chewed, crushed or otherwise damaged, this enzyme breaks down the soluble amygdalin in the presence of water and converts it into glucose, hydrocyanic acid and benzaldehyde (Atapour & Kariminia, 2013; Bolarinwa et al., 2014). The genotypes of bitter almonds are used differently due to their higher amygdalin content compared to sweet almonds. Bitter almonds, which are used as raw materials in the cosmetics and pharmaceutical industries, are also used in traditional medicine (Bouزيد et al., 2021; Guici et al., 2023).

The use of bitter almonds in almond breeding programs plays an important role in increasing genetic diversity and improving resistance to environmental stresses (Dicenta et al., 2007; Sánchez-Pérez et al., 2010). In addition, when used as rootstock, bitter almonds have been reported to be more resistant to rodents and certain diseases (Kodad & Socias I Company, 2008; Wani et al., 2012). Therefore, the evaluation of the agronomic performance of bitter almond genotypes is both economically and scientifically important. In addition, bitter almond genotypes are of great importance as rootstocks in modern fruit production. Although there are numerous studies in the literature investigating the fruit characteristics of sweet almond genotypes (Ağlar, 2005; Şimşek, 2011; Gülsoy & Balta, 2014; Bozkurt, 2017; Büyükfırat et al., 2022). No study was found investigating the fruit characteristics of bitter almond genotypes. In this respect, this study is a first both in our country and worldwide. The aim of this study was to determine the agronomic fruit characteristics of selected bitter almond genotypes from Hilvan district (Şanlıurfa).

## MATERIALS AND METHODS

This study was conducted in 2015-2016 in Hilvan district of Şanlıurfa. The plant material used for the study consisted only of almond trees grown from seed. A total of 140 almond trees were selected in the 2015 harvest season. As the study focused on bitter almond types, sweet almonds were eliminated and the performance of the remaining 18 bitter almond types was examined.

During the harvest season (August – September), 30 fruit samples from each type were separated from their shells and dried in the shade. The analyses were carried out in the laboratory of the Department of Horticulture of the Faculty of Agriculture of Dicle University. In the study, which was conducted over two years, the average shelled and kernel fruit weight were weighed on a digital scale with an accuracy of 0.01 g. The width, length, height and shell thickness of shelled and kernel almonds were measured with a digital caliper with an accuracy of 0.01 mm. Some other characteristics of shelled and kernel almonds were determined by observation.

The performance of bitter almond types was determined using the weighted rating method. For this purpose, the weighted rating method defined by Şimşek (1996) and Balta (2002) was appropriately modified and applied. This method aimed to evaluate the almond types based on specific criteria, allowing for a more objective assessment of their performance. In the weighted rating method, total scores were calculated by multiplying the value score of each characteristic by the relative scores and summing the scores separately (Table 1).

**Table 1.** Criteria taken into account in the weighted evaluation, the value points of these criteria and the relative scores awarded based on the quality status of the fruit.

Criteria and value points	Contribution Shares (%)
Inshell fruit size (3-5-7-9)	10
Shell suture aperture (0-5-9)	8
Shell hardness (1-3-5-7-9)	12
Kernel almond color (1-3-5-7-9)	7
Yield (3-5-7)	20
Kernel almond shell smoothness (1-5-7)	8
Kernel almond hairiness (3-5-7-9)	10
Kernel almond taste (3-5-7)	20
Double kernel ratio (1-5-7)	5
Total	100

## RESULTS AND DISCUSSION

Based on the weighted rating of fruit quality characteristics, the 18 examined bitter almond genotypes scored between 536 (HLV136) and 624 (HLV15, HLV114, HLV130) (Table 2).

**Table 2.** Weighted rating scores according to quality status of bitter almond genotypes

Genotype No	According to quality	Genotype No	According to quality
HLV7	564	HLV98	564
HLV15	624	HLV103	618
HLV23	576	HLV114	624
HLV38	586	HLV118	578
HLV52	598	HLV120	558
HLV58	618	HLV125	584
HLV64	538	HLV130	624
HLV77	606	HLV136	536
HLV90	596	HLV139	596

Although bitter almonds are not consumed directly, almond oil and cyanide derivatives are widely used in the cosmetics and pharmaceutical industries (Čolić et al., 2019; Guici et al., 2023). Therefore, the size and weight of bitter almonds are important factors that increase the raw material yield by providing more oil for these industries

In the 18 bitter almond genotypes examined, the weight of shelled almonds ranged from 2.20 g (HLV120) to 6.21 g (HLV118); the width of shelled almonds ranged from 15.92 mm (HLV139) to 24.32 mm (HLV58 and HLV118); the length of shelled almonds ranged from 24.43 mm (HLV120) to 40.68 mm (HLV52); height of shelled almonds ranged from 11.29 mm (HLV139) to 14.88 mm (HLV118) (Table 3).

In almonds, there is generally an inverse relationship between the thickness of the shell and the proportion of kernel; as the thickness of the shell increases, the proportion of kernel decreases and vice versa. Therefore, in the industries where bitter almonds are used as raw materials, thin shells and high kernel content are preferred. In the study, the shell thickness of the bitter almond genotypes ranged from 2.47 mm (HLV23) to 4.47 mm (HLV118).

In previous studies, Şimşek and Osmanoğlu (2010) reported that in the promising genotypes selected from Derik district of Mardin, the weight, width, length and thickness of shelled fruit ranged from 1.75 to 4.77 g, 17.11 to 24.90 mm, 26.13 to 35.71 mm and 11.84 to 16.77 mm, respectively. Akçalı (2015) reported that in the almond genotypes growing on the foothills of Mount Erciyes, the length of the fruit shelled was between 19.90 and 40.74 mm, the height between 10.29 and 17.37 mm and the width between 11.79 and 27.62 mm. Büyükfırat et al. (2022), found that in the almond genotypes from the Yeşilyurt (Malatya) region, the average weight of the fruit shelled was between 3.50 g and 12.07 g (44-YE-69), the width between 19.15 and 30.68 mm, the length between 29.79 and 45.38 mm and the thickness between 2.43 mm and 5.26 mm.

**Table 3.** Shelled fruit characteristics of bitter almond genotypes

Genotype No	Shelled almond weight (g)	Shelled almond width (mm)	Shelled almond length (mm)	Shelled almond height (mm)	Shell thickness (mm)
HLV7	3.06±0.26	18.54±0.45	28.79±1.24	12.83±0.64	2.97±0.29
HLV15	3.33±0.21	18.31±0.77	30.61±0.69	12.22±0.40	2.71±0.15
HLV23	3.00±0.46	19.68±0.36	28.89±1.17	11.59±0.37	2.47±0.11
HLV38	3.74±0.09	20.03±0.62	32.39±0.56	12.52±0.14	3.48±0.07
HLV52	6.01±0.27	23.17±0.76	40.68±1.48	14.38±0.76	4.25±0.41
HLV58	6.16±0.49	24.32±0.91	34.55±1.54	14.33±0.32	3.44±0.20
HLV64	2.29±0.10	16.42±0.41	25.29±0.91	12.77±0.32	2.85±0.52
HLV77	3.48±0.29	19.18±0.86	32.06±1.55	12.07±0.52	3.25±0.62
HLV90	2.75±0.40	17.84±1.10	29.65±0.34	12.24±0.74	2.95±0.58
HLV98	2.93±0.29	18.52±0.57	27.92±0.72	12.86±0.58	2.93±0.09
HLV103	5.49±0.20	22.91±1.09	33.90±1.22	14.36±0.26	3.66±0.29
HLV114	3.06±0.20	17.62±0.44	29.44±0.46	11.89±0.27	2.67±0.33
HLV118	6.21±0.21	24.32±1.35	39.92±1.55	14.88±0.40	4.47±0.15
HLV120	2.20±0.17	15.99±0.55	24.43±0.79	12.41±0.24	2.92±0.06
HLV125	2.64±0.10	17.34±0.58	28.36±0.34	12.21±0.53	2.79±0.23
HLV130	2.53±0.12	17.70±0.78	27.35±0.08	12.21±0.40	3.05±0.19
HLV136	2.29±0.06	16.93±0.93	28.51±1.05	11.46±0.10	2.64±0.34
HLV139	2.41±0.33	15.92±0.92	27.81±1.82	11.29±0.29	2.79±0.28

When evaluating the kernel characteristics of the 18 bitter almond genotypes examined, it was found that the kernel weight was between 0.50 g (HLV130 and HLV139) and 1.04 g (HLV58 and HLV103). The width of the kernel ranged from 8.90 mm (HLV139) to 13.57 mm (HLV58), the length of the kernel between 17.45 mm (HLV120) and 28.85 mm (HLV52), the height of the kernel between 4.51 mm (HLV58) and 6.68 mm (HLV64) and the ratio of the kernel between 16.24 % (HLV118) and 25.54 % (HLV120). No double kernel was observed in any of the genotypes examined (Table 4).

**Table 4.** Kernel fruit characteristics of bitter almond genotypes

Genotype No	Kernel almond weight (g)	Kernel almond width (mm)	Kernel almond length (mm)	Kernel almond height (mm)	Kernel ratio (%)	Double ratio (%)
HLV7	0.60±0.02	11.59±0.62	20.73±1.27	5.30±0.40	19.28±0.60	0
HLV15	0.60±0.02	10.58±0.25	20.25±0.76	4.94±0.15	17.94±0.23	0
HLV23	0.58±0.09	9.85±0.71	19.85±0.86	4.65±0.27	19.45±0.21	0
HLV38	0.76±0.03	11.91±0.45	23.68±0.96	5.47±0.09	20.23±0.39	0
HLV52	1.01±0.05	11.66±0.49	28.85±0.97	5.57±0.17	16.86±0.74	0
HLV58	1.04±0.03	13.57±0.70	24.43±0.29	4.51±0.24	16.93±0.97	0
HLV64	0.55±0.03	9.69±0.26	17.60±0.92	6.68±0.60	23.87±0.43	0
HLV77	0.78±0.07	11.45±0.63	23.76±0.61	5.62±0.20	22.41±0.25	0
HLV90	0.58±0.08	11.40±0.43	19.10±1.00	5.51±0.58	21.08±0.19	0
HLV98	0.64±0.03	12.12±0.35	20.19±0.56	5.40±0.26	21.83±1.20	0
HLV103	1.04±0.02	13.32±0.64	23.76±0.43	4.67±0.19	18.91±0.66	0
HLV114	0.61±0.03	10.29±0.33	18.91±0.79	5.02±0.26	20.08±0.48	0
HLV118	1.02±0.06	11.57±0.91	28.43±1.93	5.67±0.14	16.24±0.52	0
HLV120	0.56±0.03	9.40±0.27	17.45±0.82	6.45±0.02	25.54±0.84	0
HLV125	0.59±0.04	10.19±0.30	19.77±1.04	5.77±0.20	22.18±0.54	0
HLV130	0.50±0.02	10.07±0.82	18.44±0.57	5.54±0.42	19.61±0.13	0
HLV136	0.54±0.01	10.47±0.53	19.15±0.69	5.80±0.21	23.74±0.51	0
HLV139	0.50±0.08	8.90±0.47	18.81±1.00	4.78±0.26	20.54±0.55	0

**Table 5.** Some physical properties genotypes of bitter almond

Genotype No	SH	SAS	TSSA	WAS	PA	SSO	SAC	FSSA
HLV7	VH	H	P	N	S	C	ML	SM
HLV15	VH	LO	B	N	S	C	ML	SM
HLV23	VH	H	P	N	S	C	ML	SM
HLV38	VH	LO	B	N	S	C	ML	SM
HLV52	VH	LO	B	SW	S	C	ML	L
HLV58	VH	LO	B	N	S	C	ML	L
HLV64	VH	LO	SP	N	S	C	ML	SM
HLV77	VH	LO	B	N	S	C	ML	SM
HLV90	VH	H	P	N	N	C	ML	SM
HLV98	VH	H	P	N	S	C	ML	SM
HLV103	VH	LO	B	N	S	C	ML	ML
HLV114	VH	LO	B	N	S	C	ML	SM
HLV118	VH	LO	B	SW	S	C	ML	L
HLV120	VH	LO	SP	N	S	C	ML	SM
HLV125	VH	LO	P	SW	N	C	ML	SM
HLV130	VH	LO	P	SW	N	C	ML	SM
HLV136	VH	H	P	N	N	C	ML	SM
HLV139	VH	H	P	N	S	C	ML	SM

SH: Shell hardness (VH: Very Hard); SAS: Shelled almond shape (H: Hearth, LO: Long Oval); TSSA: Tip shape of shelled almonds (P: Pointed, B:Blunt,SP:Slightly Pointed); WAS: Wingedness of almonds shelled (N: None, SW: Slightly winged); PA: Protrusion of the abdomen (S: Slightly, N:None); SSO: Shell suture opening (C: Closed); SAC: Shelled almond color (ML: Medium Light); FSSA: Fruit size of shelled almonds (SM: Small, L:Large, ML: Medium Large)

**Table 6.** Some physical properties of the kernel fruit of bitter almond genotypes

Genotype No	AHS	KAH	KAC	KAT	NAPO (Piece)	KAS	KAWI (%)	KASAWI	KATI (%)	KASATI
HLV7	S	SH	N	B	47.17	SM	55.91	MW	25.57	F
HLV15	S	SH	N	B	47.17	SM	52.25	MW	24.40	F
HLV23	SW	SH	N	B	48.79	SM	49.62	N	23.43	F
HLV38	S	MH	N	B	37.24	SM	50.30	MW	23.10	F
HLV52	SW	MH	N	B	28.02	MS	40.42	N	19.31	F
HLV58	SW	MH	N	B	27.21	MS	55.55	MW	18.46	F
HLV64	SW	MH	N	B	51.45	SM	55.06	MW	37.95	MT
HLV77	S	MH	N	B	36.28	SM	48.19	N	23.65	F
HLV90	SW	SH	N	B	48.79	SM	59.69	MW	28.85	F
HLV98	S	SH	N	B	44.22	SM	60.03	MW	26.75	F
HLV103	SW	MH	N	B	27.21	MS	56.06	MW	19.65	F
HLV114	S	SH	N	B	46.39	SM	54.42	MW	26.55	F
HLV118	SW	MH	N	B	27.75	MS	40.70	N	19.94	F
HLV120	SW	MH	N	B	50.54	SM	53.87	MW	36.96	MT
HLV125	S	SH	N	B	47.97	SM	51.54	MW	29.19	F
HLV130	S	SH	N	B	56.60	SM	54.61	MW	30.04	MT
HLV136	SW	SH	N	B	52.41	SM	54.67	MW	30.29	MT
HLV139	SW	SH	N	B	56.60	SM	47.32	N	25.41	F

AHS: Almond hull smoothness (S: Smooth,SB: Slightly Wrinkled); KAH: Kernel Almond Hairiness (SH: Slightly Hairy, MH: Moderately Hairy); KAC: Kernel Almond Color (D: Dark); KAF: Kernel Almond Taste ( B:Bitter); NAPO : Number of Almonds Per Ounce; KAS: Kernel Almond Size (SM: Small, MS: Medium Small); KAWI: Kernel Almond Width Index ; KASAWI: Kernel Almond Shape According to Width Index (MW: Medium-Width, N:Narrow); KATI: Kernel Almond Thickness Index ; KASATI: Kernel Almond Shape According to Thickness Index (F: Flat, MT: Medium Thick)

In all of the 18 bitter almond genotypes examined, the shell hardness was classified as 'very hard '(VH) As for the shape of the almonds shelled, 12 genotypes were classified as 'long oval'(LO) and 6 genotypes as 'heart-shaped' (H) The tip shape of the almonds shelled was observed to be 'pointed' (P) in 8 genotypes, 'blunt' (B) in 8 genotypes and 'slightly pointed'(SP) in 2 genotypes. When examining the wing structure, it was found that 14 genotypes had no wings, (N) while 4 genotypes (HLV52, HLTV118, HLTV125 and HLTV130) had a 'slightly winged' (SW) structure. The degree of abdominal protrusion was classified as 'slight' (S) in 14 genotypes, while 'none' (N) was observed in 4 genotypes (HLV90, HLTV125, HLTV130 and HLTV136) The opening of the shell suture was observed

as 'closed' (C) in all genotypes, and the color of the almond of shelled was classified as 'medium light' (ML) in all genotypes. With regard to fruit size, 14 genotypes were classified as 'small', (SM) 3 genotypes (HLV52, HLV58 and HLV118) as 'large' (L) and 1 genotype (HLV103) as 'medium-large' (ML) (Table 5).

In the 18 bitter almond genotypes examined, the smoothness of the kernel almond shell was determined to be "smooth" (S) in 9 genotypes and "slightly wrinkled" (SW) in 9 genotypes. The hairiness of the kernel almond was observed as "slightly hairy" (SH) in 10 genotypes and "moderately hairy" (MH) in 8 genotypes. The kernel almond color was "dark" (D) in all genotypes and the taste was "bitter" (B). The number of almonds per ounce (28.35 grammes) ranged from 27.21 (HLV58 and HLV103) to 56.60 (HLV130 and HLV139). Almond size was classified as "small" (SM) for 14 genotypes and "medium-size" (MS) for 4 genotypes. The kernel width index ranged from 40.42 % (HLV52) to 60.03 % (HLV98). Based on the width index, the kernel shape was categorized as 'medium-width' (MW) for 14 genotypes and 'narrow' (N) for 4 genotypes. The kernel thickness index ranged from 18.46 % (HLV58) to 37.95 % (HLV64). Based on the thickness index, the kernel shape was identified as 'flat' (F) in 14 genotypes and as 'medium thick' (MT) in 4 genotypes (Table 6). These data show that the bitter almond genotypes studied exhibit considerable diversity in terms of their kernel characteristics. In particular, differences between genotypes were found in aspects such as smoothness, hairiness, number of kernels, size and shape indices. This diversity enables selection for different uses and breeding studies. In addition, the bitter taste of all genotypes is an important factor in determining the potential uses and processing requirements of these bitter almond genotypes.

## CONCLUSION

While there are many studies on sweet almond genotypes in the literature, there are no studies examining the fruit characteristics of bitter almond genotypes. In this respect this study is considered a first in this field, both in our country and worldwide. This study is the first to investigate the fruit characteristics of bitter almond genotypes in the Hilvan district of Şanlıurfa. 18 bitter almond genotypes examined showed considerable diversity in terms of fruit weight, size and physical characteristics. When evaluated using the weighted scoring method, the genotypes received scores between 536 and 624. Almond cultivation continues to spread in the southeast Anatolia region. As in other fruit-growing areas, rodents are also observed in almond-growing areas. When bitter almond genotypes are used as rootstock in modern almond cultivation, they are less susceptible to rodent damage. Therefore, it is important to study the adaptation of bitter almonds alongside other almond rootstocks in the same environment. Future adaptation studies will provide valuable insights in this regard.

As a result, bitter almond genotypes can improve almond breeding programs by increasing genetic diversity and contributing traits such as disease resistance and drought tolerance. These genotypes are often more resistant to environmental stress factors and can be used as rootstock for sweet almond varieties. The unique flavor and aroma components of bitter almonds can enhance the flavor profile of sweet almonds, while their bioactive compounds have potential applications in the development of functional foods. They are also valuable for almond cultivation in arid regions due to their drought tolerance. It is expected that this study will contribute to a better understanding of bitter almond diversity and thus provide important support for future breeding efforts.

## Declaration of Interests

The authors declare that they have no conflict of interest.

## Author Contributions

The authors declare that they have contributed equally to the article.

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## Identification of the Parasitoid Wasp *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) in Pistachio Orchards Using DNA Barcoding

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### ABSTRACT

Identification of *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae), a parasitoid of various species within the Coccinellidae family, is essential for the development of effective biological control strategies in pistachio orchards. This study aimed to accurately identify *D. coccinellae* utilizing DNA barcoding techniques. Research was conducted throughout 2023 on coccinellid specimens collected regularly from pistachio orchards in Siirt. Among the collected Coccinellidae species, *D. coccinellae* DNA was successfully identified in the *Hippodamia variegata* specimen among a total of 11 individuals. The mitochondrial COI gene region of *D. coccinellae* was analyzed by DNA barcoding analysis using Polymerase Chain Reaction (PCR) and sequencing methods. PCR results and DNA sequencing confirmed the accurate identification of *D. coccinellae*, demonstrating high similarity with reference sequences in the NCBI database. The sequencing results exhibited high accuracy and reliability, with a similarity rate of 97.13% and an E-value of 0.0. Phylogenetic relationships were assessed using the Maximum Likelihood (ML) method. Phylogenetic analyses clearly illustrated the relationships of *D. coccinellae* with other Hymenoptera species. The phylogenetic tree indicated that *D. coccinellae* is most closely related to *Meteorus obfuscatus*, with a genetic distance of 0.10, providing insights into interspecies relationships.

**Key words:** *Dinocampus coccinellae*, Coccinellidae, DNA barcoding, parasitoid, COI gene.

### Siirt Fıstığı Bahçelerinde Coccinellidae Türlerinin Parazitoidi *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae)'nın DNA Barkodlama ile Tanımlanması

### ÖZ

Siirt fıstığı bahçelerinde, Coccinellidae familyasından çeşitli türlerin parazitoidi olan *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae)'nın tanımlanması, etkili biyolojik kontrol stratejilerinin geliştirilmesi açısından kritik öneme sahiptir. Bu çalışma, *D. coccinellae*'nin DNA barkodlama yöntemi kullanılarak kesin tanımlanmasını amaçlamıştır. Araştırma, 2023 yılı boyunca Siirt fıstığı bahçelerinden düzenli olarak toplanan coccinellid örnekler üzerinde gerçekleştirilmiştir. Çalışma sonucunda, toplanan Coccinellidae türleri arasında, toplam 11 birey içinde *Hippodamia variegata* örneği üzerinde *D. coccinellae* DNA'sı doğru bir şekilde tespit edilmiştir. *Dinocampus coccinellae*'nin mitokondrial COI gen bölgesi, Polimeraz Zincir Reaksiyonu (PCR) ve dizileme yöntemleri kullanılarak DNA barkodlama analizi ile incelenmiştir. PCR sonuçları ve DNA dizileme analizi, *D. coccinellae*'nin doğru bir şekilde tanımlandığını ve yüksek bir benzerlik oranıyla NCBI veritabanındaki referans dizilerle uyumlu olduğunu ortaya koymuştur. Dizileme sonuçları, %97.13'lük bir benzerlik oranı ve 0.0 E-değeri ile yüksek doğruluk ve güvenilirlik sağlamıştır. Filogenetik ilişkilerin belirlenmesinde Maximum Likelihood (ML) yöntemi kullanılmıştır. Filogenetik analizler, *D. coccinellae*'nin diğer Hymenoptera türleriyle olan akrabalık ilişkilerini net bir şekilde göstermiştir. Filogenetik ağaç, *D. coccinellae*'nin en yakın akrabası olarak *Meteorus obfuscatus* ile 0.08 genetik uzaklığa sahip olduğunu ortaya koymuş, bu da türler arasındaki akrabalık ilişkileri hakkında bilgiler sunmuştur.

**Anahtar kelimeler:** *Dinocampus coccinellae*, Coccinellidae, DNA barkodlama, parazitoid, COI geni

## INTRODUCTION

Pistachios are of significant importance to Türkiye's agricultural diversity and substantially contribute to the regional economy. Effective management of harmful organisms is essential for achieving high productivity in pistachio cultivation. While traditional chemical control methods are commonly employed for pest management, they have raised growing concerns due to their negative environmental and health impacts (Dhankhar and Kumar, 2023). As a result, there is an increasing interest in exploring more environmentally friendly and sustainable alternatives. Biological control, one such alternative, focuses on regulating pest populations through the utilization of their natural enemies (Nazir et al., 2019).

The Coccinellidae family, which includes predatory insects, plays a crucial role in biological control applications. These beetles are particularly effective in managing populations of pest insects, such as aphids (Riddick, 2017). However, members of the Coccinellidae family can also be susceptible to their own natural enemies. In this context, the parasitoid wasp *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) is recognized for parasitizing ladybugs within the Coccinellidae family (Koyama and Majerus, 2008). This cosmopolitan wasp has been documented parasitizing more than 50 species of ladybugs in both natural and infested environments (Ricupero et al., 2023). In Europe, *Coccinella septempunctata* L. serves as its most prevalent host (Berkvens et al., 2010). The taxonomic classification of *D. coccinellae* is as follows:

- Phylum Arthropoda (Arthropods)
  - Subphylum Hexapoda (Hexapods)
    - Class Insecta (Insects)
      - Order Hymenoptera (Sawflies, wasps, ants, bees)
        - Family Braconidae (Braconid wasps)
          - Genus (*Dinocampus*)
            - Species (*Dinocampus coccinellae*)

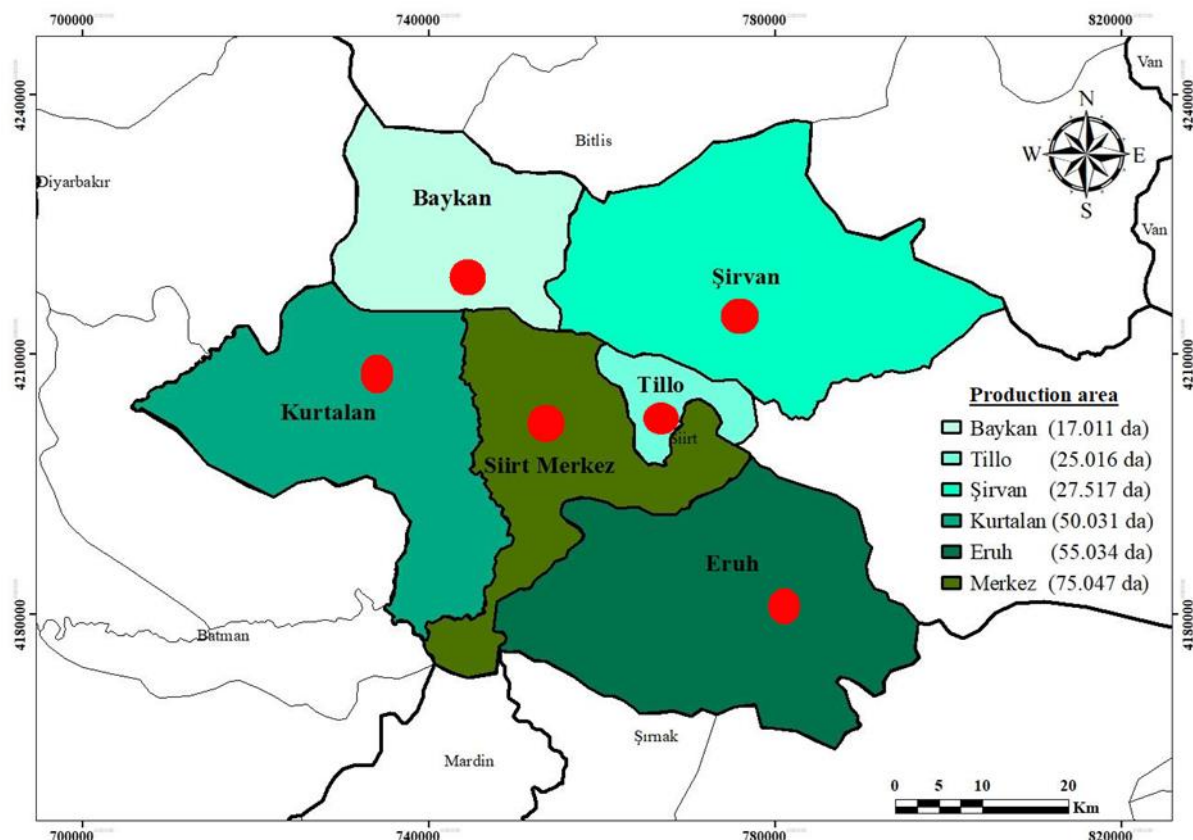
Coccinellidae, commonly known as ladybugs, play a crucial role in maintaining ecological balance and providing biological control against various pest species within agricultural ecosystems (Ranjbar et al., 2024). Numerous species of Coccinellidae have been in pistachio orchards globally, including in Türkiye (Souliotis et al., 2002; Bolu, 2004; Özgen and Karsavuran, 2005; Salehi et al., 2013; Dilmen and Özgökçe, 2020). Research has identified approximately 22 species of Coccinellidae in Turkey, 17 species in Iran, and 8 species in Greece. Notably, *Coccinella septempunctata*, *Hippodamia variegata*, *Adalia bipunctata*, and *Oenopia conglobata* are particularly prevalent. Therefore, the identification and monitoring of *D. coccinellae* can be considered a critical element in the biological control of pests. However, distinguishing between closely related species with similar morphological features requires the expertise of taxonomic specialists for precise identification. Accurate taxonomic classification and identification based on external morphology and genital organs demand significant expertise, time, and resources (Wang et al., 2024). Consequently, in recent years, DNA barcoding has increasingly been employed for the accurate and rapid identification of species. DNA barcoding is a molecular technique that allows for species-level identification using genetic material (Antil et al., 2023). This method is typically performed by sequencing the mitochondrial COI (cytochrome c oxidase I) gene region, which is sufficiently variable to distinguish between species in many groups (Hebert et al., 2003). DNA barcoding offers a significant advantage in differentiating morphologically similar species (Smith et al., 2006) and has thus been widely used in taxonomic studies and biodiversity monitoring (Poolprasert et al., 2019; Huang et al., 2020; Abdalla et al., 2022; Baena-Bejarano et al., 2023).

The aim of this study is to identify the parasitoid *D. coccinellae* associated with Coccinellidae species in pistachio orchards in Siirt DNA barcoding. This identification will facilitate the assessment of the impact of this parasitoid on beneficial ladybugs and contribute to the evaluation of biological control strategies, ultimately leading to the development of more effective pest management approaches.

## MATERIALS AND METHODS

### Sample Collection and Field Study

The study was conducted in 2023, during which coccinellid specimens were collected from pistachio orchards across various districts of Siirt, including Merkez, Tillo, Baykan, Kurtalan, and Şirvan (Figure 1).



**Figure 1.** Sampling locations (H. Dilmen, Original, Esri ArcGIS 10.2).

Sampling occurred every 15 days from April to October. During this process, a minimum of 10 ladybug specimens from the Coccinellid species were collected from each orchard (Table 1). Specimens were gathered using Japanese umbrellas and sterile collection vials. Each specimen was separated and placed in individual labeled tubes. The collected specimens were separated and labeled with only one individual per tube. To prevent damage to the specimens, the tubes were transported to the laboratory in portable coolers containing refrigerants.

**Table 1.** Coccinellidae species collected in Siirt pistachio orchards

Orchard location	Coccinellidae Species
Merkez, Tillo, Baykan, Kurtalan, Şirvan, Erüh	<i>Hippodamia variegata</i> (Goeze), <i>Oenopia conglobata</i> (Linnaeus, 1758), <i>Coccinella septempunctata</i> (Linnaeus, 1758), <i>Adalia decempunctata</i> (Linnaeus, 1758).

#### Examination of Ladybug Specimens for Parasitoid

In the laboratory, ladybug specimens were meticulously examined using an Olympus SC61 stereo microscope, which was equipped with an Olympus SC50 camera and CellSens Entry software. The Coccinellidae individuals collected during the study exhibited noticeable changes in external appearance, such as paleness or darkening, and displayed signs of weakness and lethargy. Furthermore, some specimens presented swelling or deformities in specific body regions. These individuals were set aside for molecular analysis due to suspicions of parasitism. Suspected parasitized specimens were preserved by freezing at -20°C for subsequent DNA isolation. All individuals were utilized in the analysis.

#### DNA Isolation and PCR Amplification

DNA isolation from coccinellid specimens was conducted utilizing the Invitrogen PureLink Genomic DNA Mini Kit, which is specifically designed to isolate high-purity DNA from a range of biological samples. The isolation procedure was carried out in accordance with the manufacturer's guidelines. The isolated DNA was subsequently stored in 1.5 mL Eppendorf tubes at -20°C. DNA purity and quantity were measured using a NanoDrop spectrophotometer, confirming the acquisition of DNA at appropriate concentrations.

The isolated DNA was amplified for the mitochondrial COI (cytochrome c oxidase I) gene region using Polymerase Chain Reaction (PCR). The primers targeted a region of approximately 658 base pairs: LCO1490-F (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198-R (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). The PCR reaction mixture was prepared in a total volume of 50 µL, which included 5 µL of 10X PCR buffer, 2.5 mM MgCl<sub>2</sub>, 200 µM dNTP mix, 0.2 µM of each primer, 1.25 U of Taq DNA polymerase, and 100 ng of DNA template. The PCR program on the MiniAmp Plus Cyclor was optimized as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute, concluding with a final extension at 72°C for 5 minutes.

#### Electrophoresis and DNA Purification

PCR products were analyzed using 1.5% agarose gel electrophoresis, which was prepared by dissolving agarose in 1x TAE buffer. The electrophoresis was performed at 90 volts and 100 mA for approximately 45 minutes. A 100 bp DNA ladder (used as a molecular weight marker) was run alongside the samples to determine the size of the PCR products. After electrophoresis, the gel was stained with ethidium bromide for 20 minutes and visualized under UV light using a transilluminator to confirm the presence and size of the amplified DNA fragments. Once the correct size of the amplification products was confirmed, gel extraction and purification processes were carried out. The purified DNA was prepared for sequencing.

#### DNA Sequencing and Bioinformatics Analysis

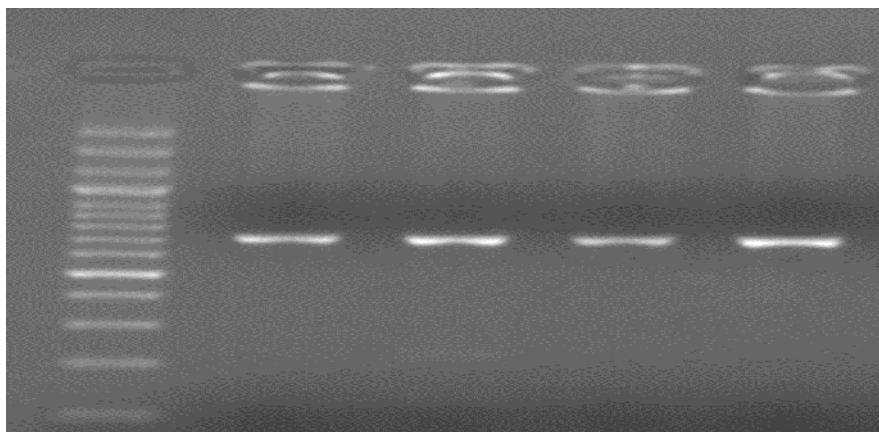
PCR products were sent to MedSantek (Istanbul) for sequencing, which was performed using the Sanger sequencing method. The resulting sequences were compared with reference sequences in the NCBI GenBank database using the BLAST tool, enabling the accurate identification of *Dinocampus coccinellae*. Sequence alignment was carried out using Clustal W within the MEGA X software, an approach known for its reliability in aligning homologous sequences (Kumar et al., 2018). For phylogenetic analysis, the Maximum Likelihood (ML) method, as described by Felsenstein (1981), was employed. This method is well-suited for estimating evolutionary relationships due to its robustness in modeling nucleotide substitutions and its ability to handle varying rates of evolution across sites, making it a reliable choice for constructing accurate phylogenetic trees. This method was chosen as it provides high accuracy in generating phylogenetic trees, especially when dealing with large datasets and complex models of evolution, making it a widely accepted approach in molecular phylogenetics.

### RESULTS AND DISCUSSION

This study identified the parasitoid *Dinocampus coccinellae* from Coccinellidae species in pistachio orchards in Siirt using DNA barcoding techniques.

PCR amplification targeting the mitochondrial COI gene region was successfully achieved, yielding the expected 658 base pair products in all samples. The PCR results for *D. coccinellae* are presented in the agarose gel electrophoresis image (Figure 2). The presence of a clear and single band in all samples indicates that the DNA fragments were of uniform size and that the target gene region was successfully amplified. The results of the study show that the amplification was successful and that *D. coccinellae* DNA was accurately detected in the samples (Figure 2). Therefore, this gel electrophoresis result supports the accurate identification of *D. coccinellae* and the reliability of the DNA barcoding method. As a result of the study, among the 11 different individuals collected from four distinct Coccinellid species, *D. coccinellae* DNA was accurately identified in *Hippodamia variegata* specimens collected from the central district of Siirt. This finding confirms that *H. variegata* in this region can host the parasitoid, highlighting the need for further research on this relationship.





**Figure 2.** Polymerase chain reaction results of the mitochondrial COI gene region of *Dinocampus coccinellae*. M Marker (DNA leader; 100bp).

Additionally, the sequencing results were compared with the NCBI database. In the "Matched Sequences" column, the reference sequence for the analyzed sample, *D. coccinellae*, is shown. The "Score" column indicates a high similarity score of 819, suggesting a strong match between the sequences. The "E-value" is given as 0.0, indicating that the comparison is statistically significant and not due to random chance. The "Percentage Identity" column shows a value of 97.13%, meaning that the analyzed sequence is 97.13% identical to the reference sequence (Table 2). Furthermore, the comparison of the obtained sequences with the NCBI database confirms the accurate identification of *D. coccinellae* and shows a high level of agreement with other records in the NCBI database. These results confirm that the *D. coccinellae* specimen was correctly identified using DNA barcoding.

**Table 2.** BLAST analysis results from NCBI database.

Matched Sequences	Score	E-value	Percentage Identity	Accession length	Accession
<i>Dinocampus coccinellae</i>	819	0.0	%97.13	658	OR039339.1
<i>Dinocampus coccinellae</i>	819	0.0	%97.13	658	OR039335.1
<i>Dinocampus coccinellae</i>	819	0.0	%97.13	658	OR039333.1
<i>Dinocampus coccinellae</i>	819	0.0	%97.13	658	OR039332.1

The phylogenetic tree generated from the sequencing data in this study illustrates the genetic relationships among various Hymenoptera species, with a specific focus on *D. coccinellae*, a parasitoid of Coccinellidae species. Constructed using the Maximum Likelihood (ML) method and DNA barcoding, the tree provides insights into the genetic affinities and evolutionary relationships among these species (Figure 3).

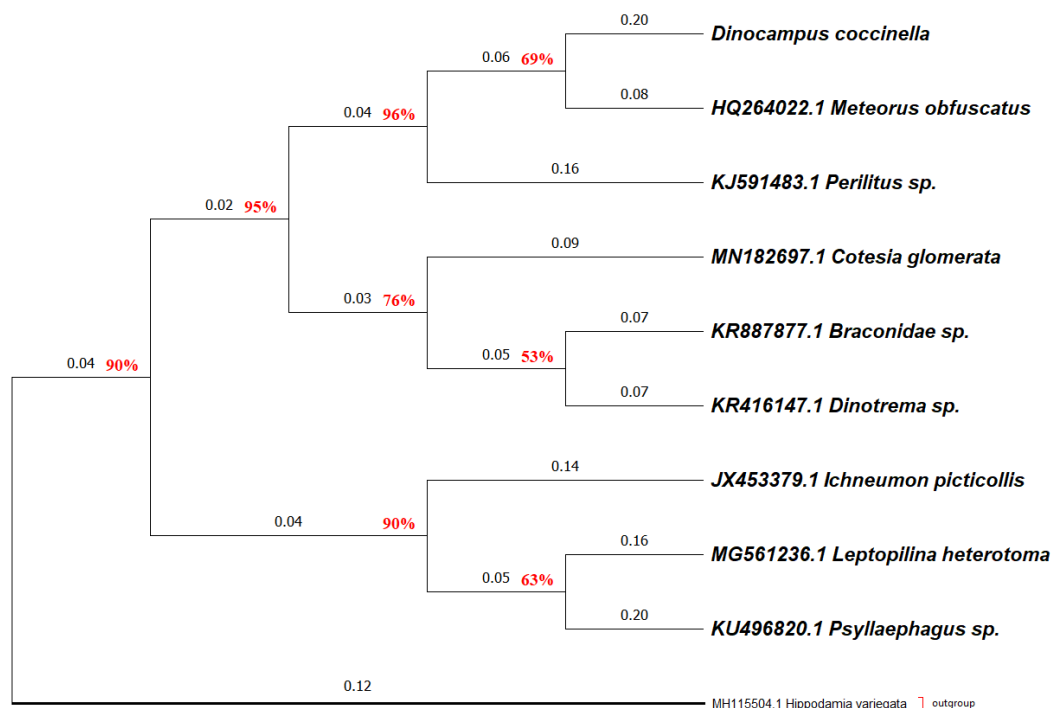
This phylogenetic tree was created to show the evolutionary relationships of *D. coccinella* with other braconid and closely related species. *Hippodamia variegata*, used as an outgroup in the phylogenetic analysis, is evolutionarily distinct from the other species in the analysis and has been used as a reference point. Located at the bottom of the tree, *H. variegata* was selected as the outgroup in this study. The outgroup is distinctly separated from the other species. The distance value of 0.12 in the tree indicates how different the outgroup is from the ingroup. This shows that *H. variegata* is a species that diverged much earlier than all the other species in this tree. *Dinocampus coccinella* is at the top of the tree, and when examining its evolutionary relationships with other species, its closest relatives are *Meteorus obfuscatus* and *Perilitus* sp. The close evolutionary relationship of these three species is represented by short branches that indicate their genetic distances. For example, there is only a 0.08 short branch length between *D. coccinella* and *M. obfuscatus*, which indicates that these two species are genetically quite close to each other. It can be said that they diverged from a common ancestor a short time ago.

Additionally, the presence of some short branches (e.g., 0.02, 0.04) in the phylogenetic tree indicates that these species are genetically closer to each other. On the other hand, longer branches (e.g., 0.16) represent greater genetic differences and more ancient evolutionary separations. Notably, *D. coccinella* has a longer branch length compared to other braconid species, indicating a relatively early separation in the evolutionary process within this group.

The red bootstrap support values (e.g., 96%, 69%, 63%) presented on the tree represent the statistical reliability of the branches. High bootstrap values (90% and above) indicate that the corresponding branching is strong and reliable. For example, the 96% support value between *D. coccinella* and *M. obfuscatus* provides high

reliability for their relationship as relatives. However, some branches have low support values, such as the 53% support value between *Braconidae* sp. and *Cotesia glomerata*. This indicates that the reliability of this branching is weaker and that more data or analysis is needed regarding the relationships between these species.

In conclusion, this phylogenetic analysis has revealed the genetic relationships and evolutionary distinctions of *D. coccinella* with other species. The branch lengths in the tree indicate the evolutionary distances between species, while the bootstrap support values reflect the confidence in these relationships. *Dinocampus coccinella* has a close evolutionary relationship with its nearest relatives, *M. obfuscatus* and *Perilitus* sp., but this trio represents an earlier separation compared to other species. The low support values on the branches indicate uncertainties in some familial relationships and suggest that further investigation of these relationships is needed.



**Figure 3.** Phylogenetic relationships among *Dinocampus coccinellae* and its closely related species (Maximum Likelihood analysis).

The findings of this study indicate that *D. coccinellae* is present in Siirt pistachio orchards and can be effectively identified using DNA barcoding. The presence of *D. coccinellae* is a significant finding for pest management in pistachio orchards, highlighting the importance of understanding this species' role in biological control strategies. The DNA barcoding method has provided high accuracy and reliability in identifying *D. coccinellae*. Specifically, the use of the COI gene region has facilitated the differentiation of *D. coccinellae* from other similar parasitoid species. This confirms that DNA barcoding is an effective tool in the taxonomic studies of parasitoids and other insect species.

In recent years, mitochondrial gene regions, particularly the COI gene, have been widely used for species identification and diagnostics. Mitochondrial DNA, with its high evolutionary rate and nucleotide diversity, is recognized as an effective biomarker for distinguishing closely related species and populations. This method offers significant reliability for assessing genetic diversity and accurately identifying species (Hebert et al., 2003; Boehme et al., 2010).

The use of the mitochondrial COI gene region for diagnostic purposes has been successfully validated in many studies. For instance, Hebert et al. (2003) demonstrated the effectiveness of the COI gene region in species identification. It has been proven effective in distinguishing known species and discovering previously unknown ones (Cheng et al., 2023). In recent years, many parasitoid species have been identified using the COI gene region (Ceccarelli et al., 2012; Franjević et al., 2015; Chen et al., 2021; Al-Jalely et al., 2022; Tiring et al., 2023). Ricupero et al. (2023) sequenced and characterized the mtDNA of *D. coccinellae* using COI and 16S rRNA gene markers in ladybug species collected from various geographic regions, including China, the USA, Canada, Chile, and Italy. In this study, the identification of *D. coccinellae* using DNA barcoding has once again validated the effectiveness of mitochondrial gene regions in species diagnostics, and the results are consistent with other studies. The

97.13% similarity rate obtained indicates that the DNA barcoding method is reliable for the accurate identification of *D. coccinellae*. This result shows that mitochondrial gene regions provide valuable information for biological control strategies and pest management, offering an important tool for ecosystem management. The findings emphasize the reliability and effectiveness of mitochondrial gene regions in species identification and suggest that future studies using this method could be highly beneficial for identifying insect species. Such analyses represent a crucial step in developing biological control strategies for pests and other insects.

## CONCLUSION

This study has successfully identified *Dinocampus coccinellae* using DNA barcoding and has confirmed that this species acts as a parasitoid of coccinellid beetles in Siirt pistachio orchards. The 97.13% similarity rate obtained validates the accurate identification of *D. coccinellae* and confirms its parasitism of coccinellids. Furthermore, the phylogenetic analysis conducted in this study highlights the close genetic relationship of *D. coccinellae* with *Meteorus obfuscatus* and *Perilitus* sp., while also revealing the genetic distances between *D. coccinellae* and other Hymenoptera species, thus demonstrating the effectiveness of DNA barcoding in elucidating genetic similarities and their phylogenetic relationships.

Identifying *D. coccinellae* provides significant advantages for plant protection and integrated pest management (IPM) programs. Coccinellid species, which are beneficial insects providing biological control against pests, may have their effectiveness impacted by parasitism by *D. coccinellae*. Thus, determining *D. coccinellae* as a parasitoid is a critical step in understanding the populations of coccinellids and their impact on pest management. Additionally, identifying *D. coccinellae* in this manner allows for monitoring of the parasitoid's population dynamics and effectiveness, helping assess whether coccinellids are affected by its parasitism and the implications for pest control strategies. This identification also enables more targeted and effective implementation of biological control strategies. Determining the extent to which coccinellids are affected by *D. coccinellae* can assist in developing strategies to enhance the effectiveness of these species and reduce pest impacts. In conclusion, this study highlights the importance of DNA barcoding technology in biological control strategies and its contribution to accurately assessing the effects of parasitoids. Accurate identification using this method will provide valuable insights for future research on *D. coccinellae*.

## Declaration of Interests

The Author declare that there is no conflict of interest.

## Author Contributions

**1<sup>st</sup> Halil DİLMEN:** Conceptualization; supervision; data curation; molecular analysis; investigation; methodology; software; writing— original draft; writing—review and editing.

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## Türkiye'deki Robotik Sağım Sistemli Çiftliklerin Yönetim Uygulamalarının İncelenmesi<sup>§</sup>

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### Öz

Bu araştırmada, robotik sağım sistemli (RMS) çiftliklerin Türkiye’de nasıl tasarlandığı ve yönetildiğini belirlemek amaçlanmıştır. Araştırmanın yapıldığı dönemde tümü ithal ürün olan üç farklı marka RMS tespit edilmiş olup, bu sistemlerin kurulduğu süt çiftlikleriyle 65 adet sorudan oluşan bir çevrimiçi anket yapılmıştır. Anket soruları sırasıyla çiftlik ve robotik sağım sistemi bilgileri, besleme, meme sağlığı, çiftlik ve robot hijyeni, robot/inek yönetimi ve süt üretimi gibi ana başlıklar altında sorulmuştur. Anket için 73 adet çiftlik ile görüşülmüş ve 2024 yılı Ağustos ayı sonuna kadar 39 adet çiftlikten dönüş sağlanmıştır. Araştırma sonuçlarına göre, anketi cevaplandıran çiftliklerde toplam 109 adet robotik sağım sistemi bildirilmiştir. Çiftliklerin %46’sının tek robotlu, %26’sının ise iki robotlu planlandığı, kalan %28’inin ise 3 ve üzeri robota sahip olduğu görülmüştür. Araştırmada çiftliklerin %74’ü serbest inek trafiğini, %26’sı ise zorunlu trafik sistemini tercih etmişlerdir. Ayrıca çiftliklerin %67’si ineklerini gruplandırmadığı, %54’ü de doğrudan düve ile faaliyete başladığını belirtmiştir. Robotlu sağıma geçtikten sonra hayvanlarını elden çıkaran çiftliklerin oranı %62 olarak tespit edilmiştir. Robot sistemin satın alınma sebeplerine en fazla verilen cevaplar sırasıyla, süt üretimini artırması, minimum çalışma saatleri, meme sağlığını iyileştirmesi ve ilave işgücü olmadan sürüyü büyütme olanağı olmuştur. Çiftliklerin %92’si robot sistemin beklentilerini büyük oranda karşıladığını ve çok memnun olduklarını belirtmişlerdir. Ankete katılan tüm RMS kullanıcıları, robot sisteme geçişlerinin kendileri ve inekleri için stres seviyelerini azalttığını, RMS sayesinde çiftlik içindeki diğer idari görevlere ve en önemlisi de kendileri ve aileleri için daha fazla zaman ayırdıklarını belirtmişlerdir.

**Anahtar kelimeler:** Otomatik sağım sistemi, çiftlik yönetimi, yemleme stratejisi, robot hijyeni, meme sağlığı, süt üretimi.

## Investigation of Management Practices of Farms with Robotic Milking Systems in Türkiye

### ABSTRACT

This study aimed to determine how farms with robotic milking systems (RMS) are designed and managed in Türkiye. At the time of the research, three different brands of RMS, all imported products, were identified and an online survey consisting of 65 questions was conducted with dairy farms where these systems were installed. The survey questions were asked under main headings such as farm and robotic milking system information, feeding, udder health, farm and robot hygiene, robot/cow management and milk production. For the survey, 73 farms were interviewed and 39 farms returned by the end of August 2024. According to the results of the research, a total of 109 robotic milking systems were reported in the farms that answered the questionnaire. It was seen that 46% of the farms were planned with one robot, 26% with two robots, and the remaining 28% had 3 or more robots. In the study, 74% of the farms preferred free cow traffic and 26% preferred forced traffic system. In addition, 67% of the farms stated that they did not group their cows and 54% stated that they started their activities directly with heifers. The rate of farms that disposed of their animals after switching to robotic milking was 62%. The most common answers to the reasons for purchasing the robot system were, respectively,

increasing milk production, minimum working hours, improving udder health, and the opportunity to expand the herd without additional labor. 92% of the farms stated that the robot system met their expectations to a great extent and they were very satisfied. All RMS users surveyed stated that their transition to the robotic system has reduced stress levels for themselves and their cows, and thanks to RMS, they have more time for other administrative tasks on the farm and most importantly, more time for themselves and their families.

**Key words:** Automatic milking system, farm management, feeding strategy, robot hygiene, udder health, milk production.

## GİRİŞ

Tarımda uygulanan teknolojik ilerlemeyi gösteren pek çok örnek vermek mümkündür. Dünyada gönüllü sağım sistemi (VMS), otomatik sağım sistemi (AMS) ve robotik sağım sistemi (RMS) adıyla bilinen teknolojik sağım, modern süt çiftliklerinde ilerlemeyi ifade eden en önemli çözümlerden biridir. Hassas hayvancılık gelişmelerinden biri olan robotik sağım sistemleri (RMS), süt hayvancılığında dünya çapında devrim yaratmıştır.

Süt sağım robotları, bilgisayarlar ve özel sürü yönetim yazılım sistemi kullanılarak sütün elde edildiği otomatik makinelerdir. İnekler, sistemin temizlenmesi için gereken 1-2 saat de dahil olmak üzere gün boyunca sağılmaktadır. Robotik süt sağım teknolojisi Avrupa'da süt çiftliklerindeki işgücü sorunlarını çözmek için geliştirilmiştir. Geleneksel sağım tesislerine göre robotik sistemler sağım sürecini kontrol ederken, diğer yandan tüm çiftlik sisteminin nasıl yönetildiği konusunda da çok sayıda değişikliğe sebep olmaktadır. Robotik süt sağımı gıda zincirinin önemli bir halkasıdır ve süt sağım robotlarının kullanıldığı bir çiftliğin idaresi geleneksel süt sağımından farklı bir yaklaşım gerektirir. Süt sağım robotu, geleneksel bir durumda elde edilemeyen inekle ilgili bilgileri depolar ve mevcut senaryoda hayvanları bireysel olarak yönetmeyi mümkün kılar. RMS süt kalitesi, miktarı ve inek sağlığı hakkında bilgi toplayarak çiftçilerin sürülerini daha iyi yönetebilmelerini sağlar. Avrupa'da RMS kullanımına genellikle aileler tarafından yönetilen ve işletilen çiftliklerde sağılan inek sayısında orta düzeyde bir artış eşlik etmektedir. Çiftliklerde RMS kullanımı ile ilgili sorunları geliştirmek ve tartışmak için araştırma çalışmalarında çeşitli yönler dikkate alınmaktadır.

Robotik sağım sürecine ilişkin ilk fikirler 1970'li yılların ortalarında ortaya çıkmış ve sağım sisteminin teknik olarak iyileştirilmesine odaklanılmıştır. RMS çiftliklerdeki mevcut sorunların sosyo-ekonomik yönlerini, çiftlik ve sağım sistemi hijyenini, hayvan sağlığını, süt kalitesini, refah, otlatma, çiftlik ve sürü yönetimi gibi tüm işlemleri kapsamaktadır (Markey, 2013; Gaworski et al., 2016). İlk süt sağım robotları 1992 yılında Hollanda'daki ticari süt çiftliklerinde kurulmuştur. Otomatik süt sağımında gerçek atılım 90'lı yılların sonunda gerçekleş ve bugün dünya çapında ineklerini otomatik olarak sağan yaklaşık 1500 süt çiftliği bulunmaktadır. Otomatik süt sağım sistemlerine sahip tüm süt çiftliklerinin %95'inden fazlası kuzeybatı Avrupa'da yer almakta olup, en önemli sayı robotik süt sağımının doğum yeri olan Hollanda'dadır (Anonim, 2023).

Türkiye'de ilk robotik sağım teknolojisi 2012 yılında bir markaya ait 4 adet robot ile Tekirdağ ilinde bir çiftlikte kurulmuştur. 2024 yılı ortasına gelindiğinde Türkiye'deki robotik sağım sistemli çiftlik sayısının yaklaşık 120, robot sayısının da yaklaşık 400 civarında olduğu tahmin edilmektedir. Robotik süt sağımı hakkında bilgi geliştirmek için araştırma çalışmaları ve değerlendirmeler yurtdışında yoğun olarak yapılmaktadır. Türkiye'de de robot sağım ile ilgili spesifik çalışmalara son yıllarda başlanmış, ancak yapılan çalışmalar oldukça sınırlı kalmıştır (Gonulol, 2016; Akar Çıkrıkçı, 2019).

Yemleme yöntemi, inek akışı, robotun performansı ve sürü büyüklüğü gibi birçok faktör sağım sıklığını etkilemektedir. Serbest inek trafiğinde inekler günde ortalama 2.4 ila 2.8 kez sağılırken, zorunlu inek trafiğinde bu sayılar 2.5 ila 2.9 arasındadır (Ipema, 1997; Unal ve ark., 2017). Bu sonucu sadece inek trafiği doğrudan etkilememektedir. İneklerin getirilme ve istemsiz sağılma yüzdeleri de buna eklenebilir (Rodriguez, 2012). Bir başka araştırmada, 64 sağlıklı ineğin günlük ortalama sağım sıklığı 2.4 ila 2.6 arasında olduğu bildirilmiştir (Castro et al., 2012). Bu tür araştırmalar robotların yaygın olarak kullanıldığı ülkelerde birçok kez yapılmıştır. Ancak farklı ülkelerdeki süt çiftliklerinin kendine has özelliklerinden kaynaklanan farklılıklar sonuçları etkileyebilmektedir (Unal ve Kuraloglu, 2015). Robotik süt sağım ünitesinin satın alma maliyetinin yüksek olması, robotun mümkün olan en yüksek kapasitede kullanılmasını gerektirmektedir. İneğin sağım sıklığı, robotun tam kapasitede yüklenmesini etkileyen ana faktörlerden biridir (Laurs et al., 2009; Unal ve Kuraloglu, 2015).

Bu araştırmada, Türkiye'deki robotik sağım sistemli çiftliklerin nasıl yönetildiğini belirlemek için bu sistemleri kullanan çiftliklerle çevrimiçi anket yapılmıştır. Ankette çiftçilere sırasıyla genel çiftlik-robotik sağım sistemi bilgileri, besleme, meme sağlığı, çiftlik-robot hijyeni, robot/inek yönetimi ve süt üretimi şeklinde sekiz ana başlıktan oluşan toplam 65 soru sorularak, çiftliklerin sorunları ve memnuniyet ölçümlerinin belirlenmesi amaçlanmıştır.

## MATERYAL VE METOT

Bu araştırmada, Türkiye’de faaliyet gösteren robotik sağım sistemli çiftliklerin çiftlik içi yönetim yapılarının ele alınması planlanmıştır. Çalışma için, Türkiye’de tümü ithal ürün olan üç farklı marka sağım robotu teknolojisini kullanan 73 çiftlikle temas kurulmuş ve bu çiftliklerle çevrimiçi ortamda anket soruları paylaşılmıştır. Ankete, 2024 Ağustos ayı sonuna kadar 39 çiftlikten dönüş olmuştur. Robotik sağım sistemi markasına göre anket yapılan çiftliklerin ve sağım robotu sayılarının dağılımı Çizelge 1’de verilmiştir.

**Çizelge 1.** Robotik sağım sistemi markalarına göre anket yapılan çiftliklerin ve sağım sistemlerinin dağılımı

Marka	Görüşme yapılan çiftlik sayısı	Anketi cevaplandıran çiftlik sayısı	Robotik sağım sistemi sayısı
Lely	46	24	72
Delaval	22	11	31
Gea	5	4	6
Toplam	73	39	109

Araştırmadaki anket soruları 8 ana başlıktan oluşmuştur (De Jong et al., 2003; Gonulol, 2016; Unal ve ark., 2017; Akar Çıkrıkçı, 2019):

A-Genel Çiftlik Özellikleri: Çiftlikteki sağmal inek sayısı, çiftlikte kapasite fazlası durumu, çiftlikteki inek trafiği tipi, ineklerin süt verimlerine göre gruplanıp gruplanmadığı ve ilk robot sağıma düveyle mi inekle mi başlandığına ilişkin bilgileri kapsamaktadır.

B-Robotik Sağım Sistemi Özellikleri: Kullanılan sağım robotu markası, sağım robotunun mevcut çiftliğe mi yoksa yeni çiftliğe mi kurulduğu, çiftlikte kaç adet sağım robotu olduğu, robotlu sağıma hangi yılda başlanıldığı, robotlu sağımda günlük ortalama sağım sıklığı, robot başına günlük sağılan inek sayısı ve çiftliğin otomasyon seviyesini belirlemek için çiftlikte bulunan makine, cihaz ve ekipmanlara ait soruları içermektedir.

C-Yemleme Stratejileri: Günlük hazırlanan rasyon yem miktarının hayvan başına kaç kg olduğu ve robotta verilen maksimum konsantre (kesif) yem miktarının ne kadar olduğu bilgilerini kapsamaktadır.

D-Meme Sağlığı: Meme sağlığı ve süt kalitesinin ne sıklıkla kontrol edildiği gibi soruları içermektedir.

E-Çiftlik Hijyeni: Gübre temizleme (sıyırma) işleminin nasıl yapıldığı, ahır tabanında gübre sıyırma işleminin günde kaç kez yapıldığı, serbest durakların (yataklıkların) günde kaç kez temizlendiği ve sağım robotu etrafının ne kadar sıklıkla temizlendiğine ilişkin bilgileri kapsamaktadır.

F-Robot Hijyeni: Memeliklerin durulama işleminin hangi durumlarda yapıldığı (kısa durulama) ve süt filtresinin ne sıklıkla değiştirildiği bilgilerini içermektedir.

G-Robot/İnek Yönetimi: Hayvanların robota güdülmesinin (tut getir) hangi sıklıkla kontrol edildiği, her seferinde kaç tane hayvanın gidip getirildiği, süt veriminin hangi sıklıkla kontrol edildiği, sağım aralığının hangi sıklıkla kontrol edildiği, sağım robotuna geçildiğinde elden çıkarılan hayvan sayısı, hayvanları elden çıkarmanın sebepleri, sağımın en yoğun olduğu zamanlar, robot sağıma geçtiğinde karşılaşılan en büyük sorunlar, robotu satın alma sebepleri ve robotu satın almanın beklentileri karşılayıp karşılamadığı gibi soruları içermektedir.

H-Süt Üretimi: Ortalama hayvan başına günlük süt veriminin (kg) ne kadar olduğu, ortalama robot başına günlük toplam süt üretimi (kg veya ton) ne kadar olduğu, ön süt depolama tankının (buffer tank) olup olmadığı ve ana süt soğutma tank kapasitesi ve çiftliklerin sağım robotu kullanacak diğer çiftlikler için önerilerinin olup olmadığına ilişkin soruları kapsamaktadır.

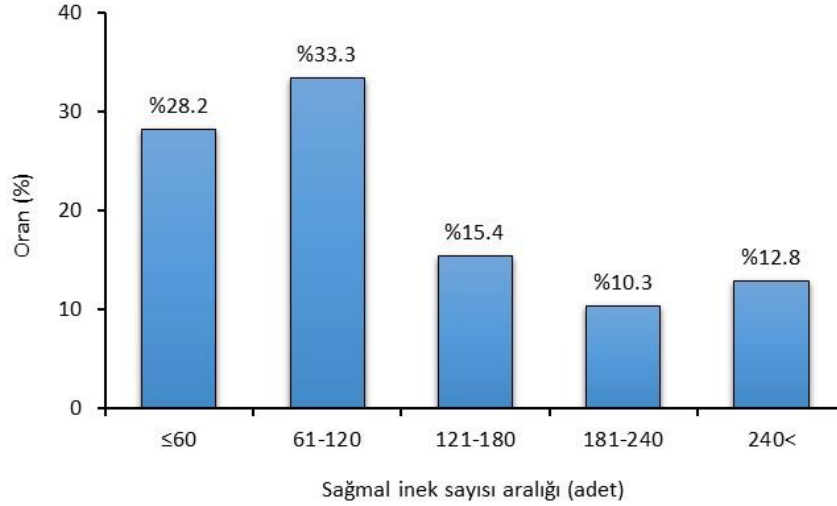
Anket soruları genellikle çoktan seçmeli olup, bu soruların büyük çoğunluğu tek cevaplı, bir kısmı da birden fazla cevaplı sorular şeklindedir. Az sayıda soru da açık uçlu sorulmuştur. Birden fazla cevabın işaretlendiği çoktan seçmeli seçeneği olan (“Diğer” seçeneği dahil) sorularda, her katılımcının en çok dikkate aldığı etkeni işaretlemesi ve böylece daha nitelikli bir sonuca ulaşılması amaçlanmıştır. Bu tür sorularda, katılımcıların sorulara verdiği cevaplar hesaplanırken cevaplar toplam cevap sayısına değil, katılımcı sayısına bölünmüştür. Bu nedenle araştırma sonuçları gösterilirken her bir cevaba ait yüzdesel oran, toplam katılımcı içinde o cevabı seçen katılımcıların oranını göstermektedir (KKB, 2022).

## BULGULAR VE TARTIŞMA

Araştırmaya katılan 39 çiftliğin 18 şehire dağıldığı görülmüştür. Buna göre, Afyonkarahisar, Ankara, Bursa, Çorum, Denizli, Edirne, Karaman, Konya ve Muğla illerinde 1’er adet; Çanakkale, Isparta ve İstanbul illerinde 2’şer adet; Aydın, Kırklareli, Manisa ve Tekirdağ illerinde 3’er adet, Balıkesir ilinde 5 adet ve İzmir ilinde 7 adet robotik sağım sistemli çiftlikler bulunmaktadır. Çiftliklerle yapılan anket sonuçları aşağıda maddeler halinde verilmiştir.

### A-Çiftlik Özellikleri

Ankete katılan çiftliklerin sağmal inek sayıları çoğunlukla 61-120 arasında belirlenmiştir (Şekil 1). Bu çiftliklerdeki oran %33.3'tür. Çiftliklerin %28.2'inde 1-60 arası, geriye kalan %38.5 oranındaki çiftliklerde ise 120'nin üzerinde sağmal inekler mevcuttur. Yapılan incelemede en az sağmal inek sayısı 1 adet robota sahip bir çiftlikte 25 adet olarak belirlenmiştir. En fazla sağmal inek ise, 14 adet robota sahip bir çiftlikte olup bu çiftlikte 782 adet inek vardır.

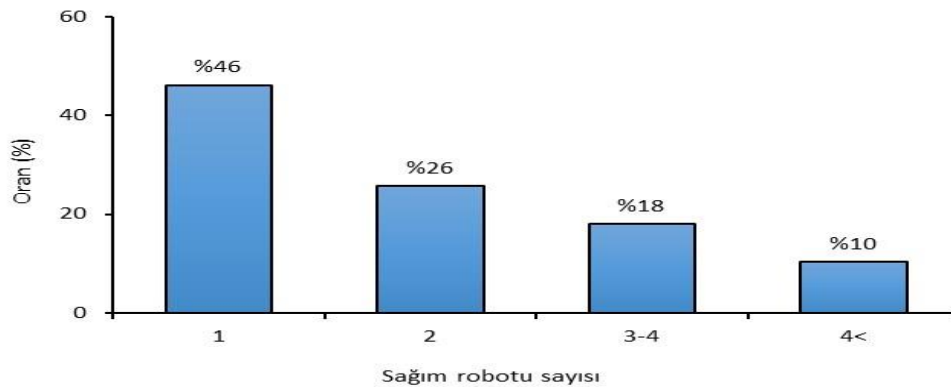


Şekil 1. Çiftliklerin sağmal inek sayıları

Çiftçilere kapasite fazlasının olup olmadığı sorulduğunda, çiftliklerin %62'si kapasite fazlasının olmadığını iletmişlerdir. Robotik süt sağım sistemine sahip çiftliklerin %72'sinin serbest inek trafiğine, %28'inin ise kılavuzlu (zorlamalı) inek trafiğine sahip oldukları bildirilmiştir. Çiftçilerin %67'si hayvanlarını süt verimlerine göre gruplandırmadığını, kalan çiftçiler gruplandırmayı tercih ettiklerini belirtmişlerdir. Diğer yandan çiftliklerin %54'ü robotlu sağıma ilk olarak düve ile, kalan %46'nın ise inek ile başladıkları bildirilmiştir.

### B-Robotik Sağım Sistemi Özellikleri

Araştırmada görüşülen toplam 73 çiftlikten 39 çiftlik ile anket çalışması yapılmıştır. Anket yapılan çiftliklerdeki robotların %66'sı Lely, %28'i Delaval ve %6'sı ise Gea markasına ait olduğu bulunmuştur. Bu üç markadaki çiftliklere sağım robotu mevcut çiftliğe mi yoksa yeni çiftliğe mi kuruldu sorusuna; çiftçilerin %54'ü (21 çiftlik) yeni çiftliğe kurduğunu bildirmiştir. Çiftliklerdeki robot sayıları incelendiğinde, işletmelerin %46'sı (18 çiftlik) 1 adet robota, %26'sı (10 çiftlik) 2 adet robota sahip olduğu görülmüştür (Şekil 2). Geri kalan çiftliklerdeki robot sayıları incelendiğinde; 7 çiftlikte 3 veya 4 adet sağım robotu, 4 çiftlikte ise 5 ve üzeri sağım robotu olduğu bildirilmiştir. Bu dört çiftlikteki birer çiftlik 8 ve 10 adet robota, iki çiftlik ise 14'er adet robotik sağım sistemine sahip olduğu belirlenmiştir.

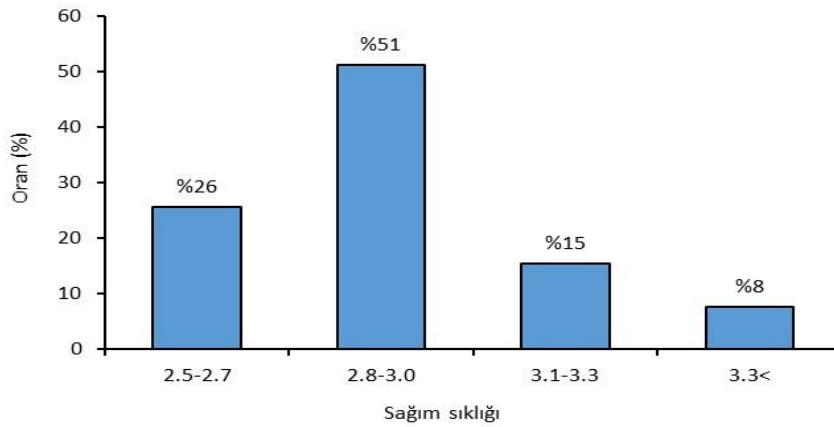


Şekil 2. Çiftliklerdeki robot sağım sistemi sayıları

Çiftliklere robotlu sağıma hangi yıl başlandığı sorulduğunda, 2013-2023 yılları arası cevaplar verilmiştir. Ülkemizde ilk robotik sağım sistemli çiftliğin 2012 yılında faaliyete başlandığı belirtilmiştir. Ancak, bu çiftlik daha sonraki yıllarda işletmesini kapatmış ve robotları ikinci el olarak satmıştır. Ankete katılan çiftliklerin %56'sı 2021-

2023 yılları robotlu sisteme geçtiklerini, %26'sı 2017-2020 yılları arasında başladıklarını belirtmişlerdir. Bir robotik sağıım sisteminin kurulum maliyeti marka ve modele göre değişmekle birlikte 120-150 bin Euro aralığında olduğu tahmin edilmektedir. Türkiye'de yükselen enflasyonla birlikte Türk Lirasının döviz kurlarındaki değer kaybı, robotik sağıım sistemlere olan talebi azaltması beklenirken, aksine son üç senede (2021-2023) çok fazla talep gördüğü belirlenmiştir. Buna, Avrupa ülkelerinde robotun başlamasına temel sebeplerden biri olarak gösterilen, ülkemizde de son üç senedir aşırı yükselen işçilik maliyetleri ve kalifiye eleman eksikliklerinin ana nedeni olduğu söylenebilir.

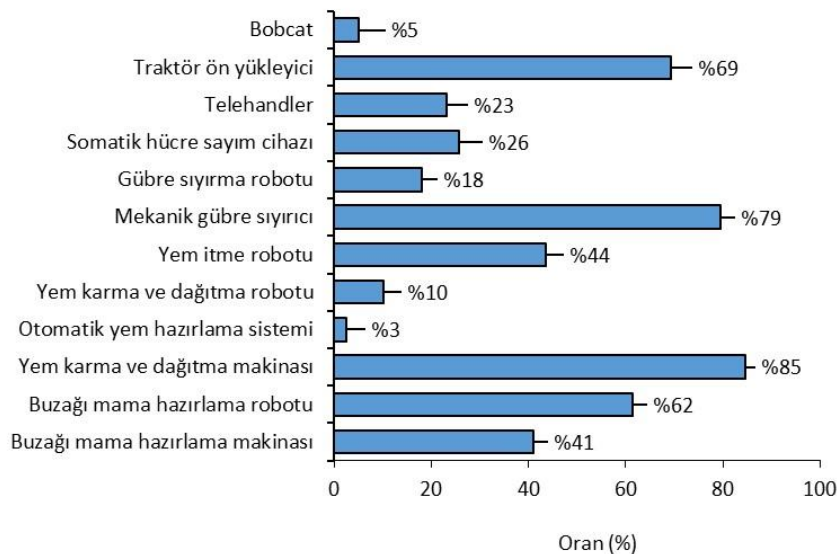
Ankete katılan RMS çiftliklerinin %26'sı 2.5-2.7 sağıım/inek/gün arasında, %51'i 2.8 ile 3.0 sağıım/inek/gün arasında ve %15'i 3.1-3.3 sağıım/inek/gün arasında sağıım sayısına sahiptir. Üç işletme ise (%8'i) sağıım sıklığı ortalamasının 3.3'ün üstünde olduğunu iletmiştir (Şekil 3). Birçok çiftçi, hayvanlarının günde daha fazla sayıda beslendiğinde daha fazla sağıım/inek/gün elde ettiğini bildirmiştir. Günlük sağıım sayısındaki bu artış, yemleme periyotlarının inekleri robot ünitesini daha sık ziyarete teşvik etmesine bağlanabilir.



**Şekil 3.** Çiftliklerde ineklerin günlük sağıım sayıları dağılımı

Ankete katılan çiftliklerin %85'inde robot başına günlük sağıım sayısının 150'den fazla olduğu bildirilmiştir. Yukarıda verilen günlük sağıım sıklıklarının yüksek değerlerde olması, inek ziyaret sayısını artırdığını göstermektedir.

Çiftliklerin robotik sağıım sistemi dışında kullandıkları otomasyon araçlarına en fazla cevap, %62 ile buzağı mama hazırlama robotunda verilmiştir. Bunu %44 ile yem itme robotu, %26 ile somatik hücre sayım cihazı, %18 ile gübre sıyırma robotu ve %10 ile de yem karma ve dağıtma robotu izlemiştir. Sadece bir çiftlikte otomatik yem hazırlama sistemi vardır (Şekil 4). Öte yandan, robotik sağıımlı çiftliklerin çoğunda konvansiyonel tarım makineleri kullanımının ön planda olduğu görülmüştür. Buna göre çiftliklerde %85 ile yem karma ve dağıtma makinası, %79 ile mekanik gübre sıyrıcı ve %69 ile traktör ön yükleyicinin en fazla kullanım oranlarına sahip araçlar olduğu bulunmuştur (Şekil 4).



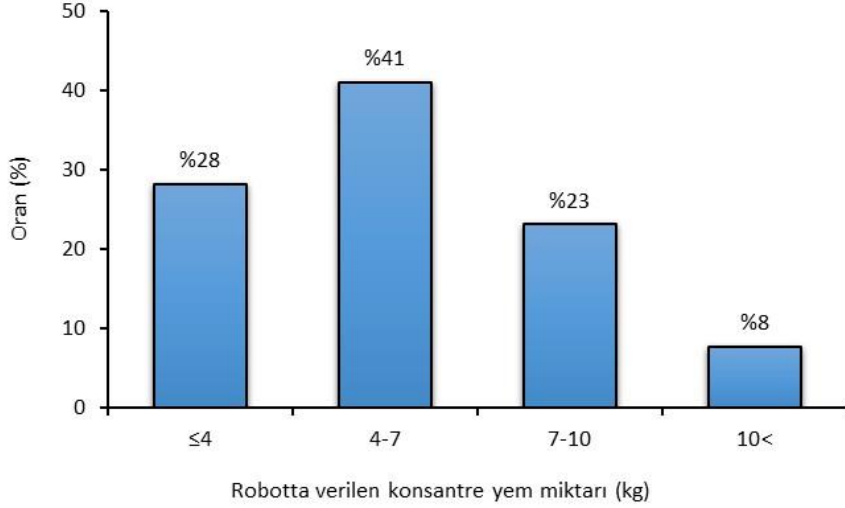
**Şekil 4.** Robotik sağıım sistemi hariç çiftliklerin sahip olduğu önemli makine ekipmanların dağılımı



### C-Yemleme Stratejileri

Çiftliklerde hayvanlara verilen rasyon yem miktarı ortalama 37.2 kg/gün bildirilmiştir. Süt verimi yüksek olan bazı çiftliklerde bu miktar 45 kg'ın üzerine çıkmış olup, 9 işletmede ortalamada 47.5 kg rasyon yem verildiği saptanmıştır.

Ankete katılan çiftliklerin %41'i robotta 4-7 kg arasında konsantre yem verildiğini bildirmiştir. Çiftliklerin sadece %8'i 10 kg'ın üzerinde konsantre yem kullanmıştır (Şekil 5). Laurs ve ark. (2009) yapmış oldukları çalışmada, robotta verilen konsantre yem miktarının büyük oranda 4-7 kg arasında verildiğini bildirmişlerdir. Bu çalışmada verilen sonuç ile araştırmacıların sonuçları benzerlik göstermiştir.



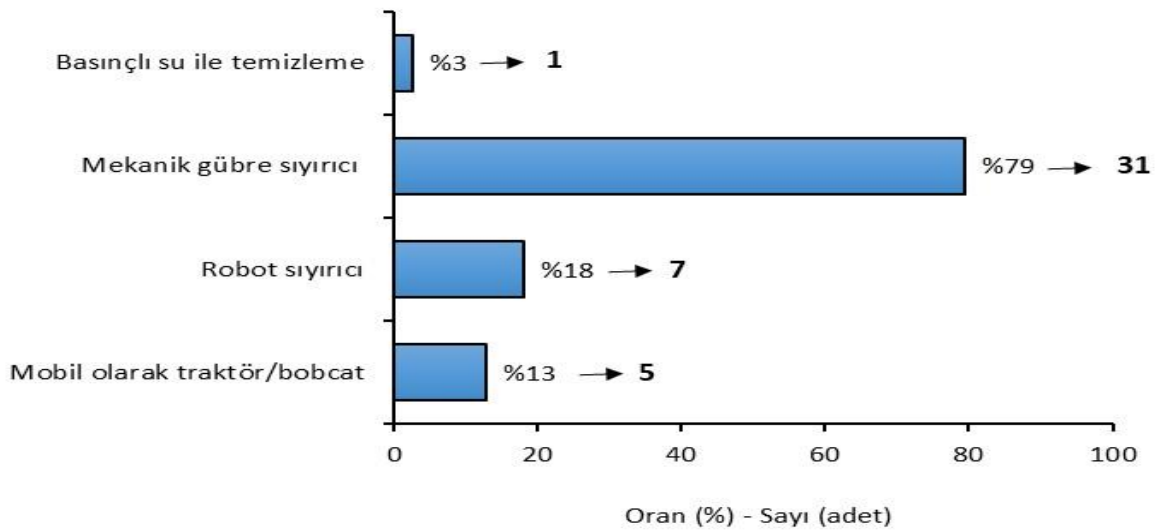
Şekil 5. Robotta verilen maksimum konsantre (kesif) yem miktarı (KYM)

### D-Meme Sağlığı

RMS kullanıcılarının %79'u (30 çiftlik) meme sağlığı ve süt kalitesini korumak için kontrollerini her gün yaptıklarını belirtmişlerdir. Çiftliklerin beşi haftada bir, biri ayda bir ve ikisi gerektiğinde meme sağlığını ve süt kalitesini kontrol etmekte olduğunu bildirmişlerdir. Çiftliklerin büyük çoğunluğunun inek meme sağlığı ve süt kalitesine önem verdiği anlaşılmaktadır.

### E-Çiftlik Hijyeni

Ankete katılan çiftlikler, işletmelerinde gübre temizleme (sıyırma) işlemini %79 oranında mekanik gübre sıyrıcı ile yaptıklarını belirtmişlerdir. Bu işlemi %18 robot sıyrıcı, %13 mobil olarak traktör/bobcat ve %3 oranında basınçlı su ile temizleme işlemi takip etmiştir (Şekil 6). Elde edilen araştırma sonuçlarına göre, gübre temizleme (sıyırma) işlemi için en çok tercih edilen ve kullanılan makinenin mekanik gübre sıyrıcıların olduğu görülmektedir.



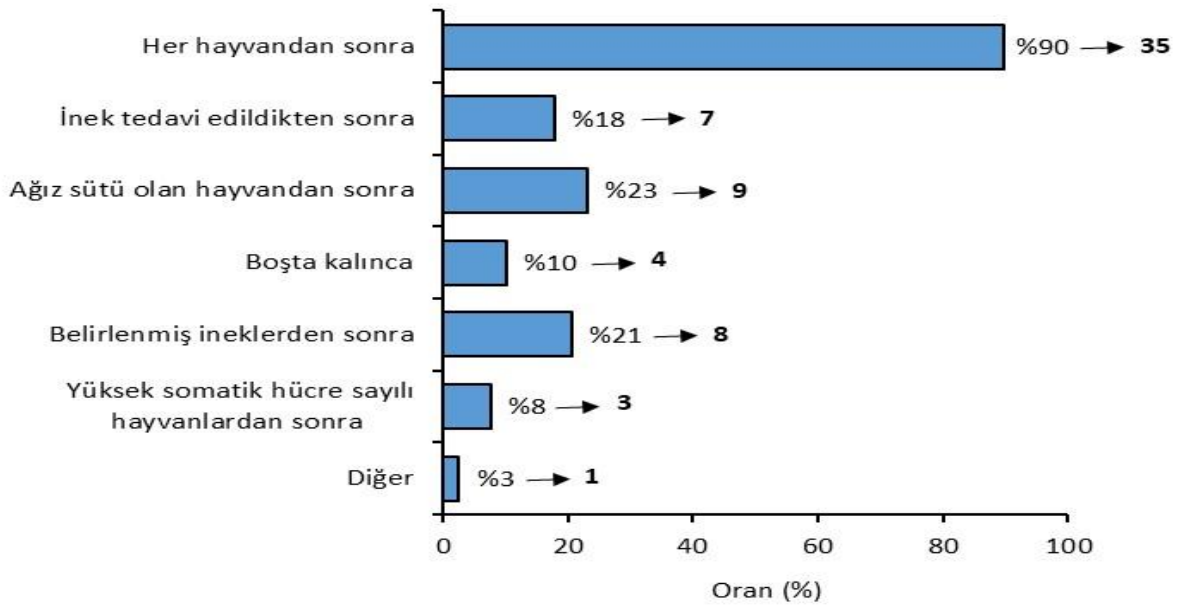
Şekil 6. Gübre temizleme işleminin yapılma şekli



Çiftliklerin %62'si gübre kanallarında çoğunlukla günde dört kez ve üzerinde temizlik yapıldığını bildirmişlerdir. Günde iki kez temizleyenlerin oranı %16, günde bir ve üç kez temizleyenler de %11 oranındadır. Diğer taraftan çiftliklerin çoğu (%56'sı) serbest durakları (yataklıkları) günde bir kez temizlediklerini belirtmiştir. Günde iki kez temizlediklerini ileten çiftçilerin oranı ise %31'dir. Üç işletme yataklıkların temizliğini muhtemelen en azından günde bir kez yapmadıklarından, bu soruya cevap vermemişlerdir. Sağım robotu etrafının ne sıklıkla temizlendiği sorusuna ise işletmelerin %36'sı günde bir kez temizlendiği bildirmişlerdir. Bu oranlar dikkate alındığında işletmelerin çiftlik hijyenine büyük oranda önem verdikleri görülmektedir. Bu da aynı oranda hem hayvan sağlığını hem de süt kalitesini etkilemektedir.

#### F-Robot Hijyeni

Robot hijyeni için meme lastiklerinin temizlenmesi amacıyla yapılan yıkama işlemlerinin uygulanmasına ilişkin cevaplar oransal olarak Şekil 7'de verilmiştir. Şekil 7'den de görüldüğü üzere çiftliklerin %90'ı her hayvandan sonra meme lastiklerinin durulama işlemini yaptıklarını belirtmişlerdir. İşletmelerin %23'ü ağız sütü olan hayvandan sonra, %21'i belirlenmiş ineklerden sonra, %18'i inekler tedavi edildikten sonra da durulama işlemlerini yaptıklarını bildirmişlerdir.



Şekil 7. Meme lastiklerinin kısa durulama işleminin yapılma sıklığı

Çiftliklerin %42'si (16 çiftlik) süt filtresini günde bir kez, %37'si (14 çiftlik) günde iki, %18'i (7 çiftlik) günde üç ve %3'ü de (1 çiftlik) günde 4 ve üzeri değişim yaptıklarını belirtmişlerdir. Süt filtresi değiştirme sıklığının fazla olması bakteri oluşumunu da büyük oranda engellediği görülmüştür.

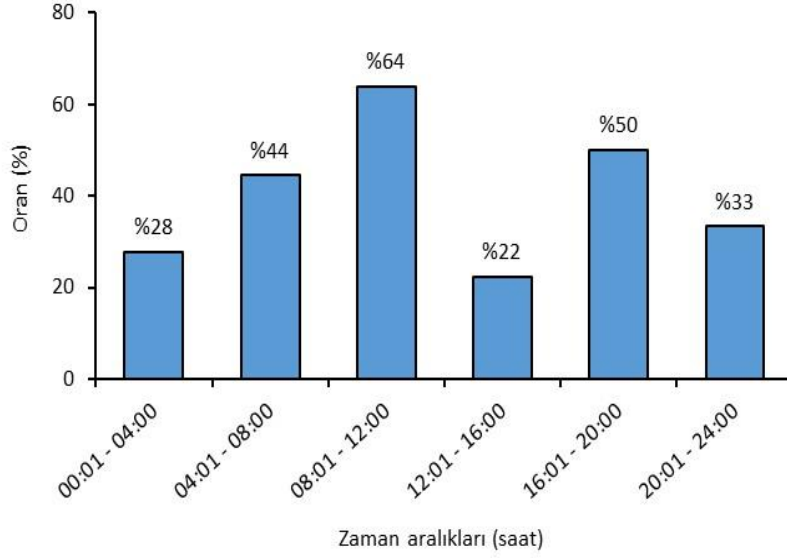
#### G-Robot/İnek Yönetimi

Yapılan değerlendirmede robotlara yönlendirilen hayvanların günde bir, günde iki ve günde üç kez ve üzeri şeklindeki cevaplarının çiftlikler tarafından eşit oranlarda (%33'er) tercih edildiği belirlenmiştir. Yapılan incelemede, robot markalarının ineklerin robotlara yönlendirilme oranlarına bir etkisinin olmadığı görülmüştür. Her üç marka robotlu çiftliklerde farklı sayılarda hayvanın toplanıp getirildiği belirlenmiştir. Robot/inek yönetimi için çiftçilerin %46'sı her seferinde 2-3, %35'i ise 4-6 arasındaki ineği yönlendirdiği belirlenmiştir.

Çiftlikler süt verimlerini günde en az bir kez kontrol etmektedirler. Buna göre çiftliklerin %36'sı günde bir kez, %26'sı iki kez ve %38'i üç kez ve üzeri kontrol ettiklerini iletmışlerdir. İneklerin sağım aralığının kontrolü ise, 16'şar çiftlik tarafından günde bir kez ile üç ve üzeri sayıda, 7 çiftlik tarafından da iki kez kontrol edildiği belirlenmiştir.

Ankete katılan çiftliklerin %62'si sağım robotuna geçtikten sonra bazı ineklerini elden çıkardıklarını bildirmişlerdir. Çiftliklere göre değişmekle birlikte elden çıkarılan inek sayısı en az 1, en fazla 15 adet olmuştur. Hayvanların elden çıkarılmasına en önemli neden olarak %85 oranında şekilsiz (uygunsuz) meme yapısı olduğu bildirilmiştir. Bunu, hayvanın robotta rahat durmaması, robota rahat gelmemesi ve süt verimi düşüklüğü gibi diğer sebepler izlemiştir.

İşletmelerde robotların sağım yoğunluğunun en fazla olduğu saat aralıkları 08:00-12:00 (%64) ve 16:00-20:00 (%50) olarak bildirilmiştir. Buna karşın, 12:00-16:00 ve 00:00-04:00 zaman aralıkları daha az sağımın yapıldığı süreler olarak iletilmiştir (Şekil 8).



**Şekil 8.** Sağımın en yoğun olduğu zaman aralıkları

Robot sağıma geçildiğinde karşılaşılan en büyük soruna ilişkin çiftçilerin büyük çoğunluğu, robota alıştırma sürecinde büyük zorluklar yaşadıklarını bildirmiştir. Kış koşullarındaki don olayı, robot arızası, meme takma başarısızlıkları, kalifiye eleman sıkıntısı, serbest inek trafiğini yönetme ve sağımı gecikmiş hayvanların takip zorluğu işletmelerin yaşadığı diğer sorunlar içerisinde yer almıştır.

Çiftliklerin robotu satın almalarına sebep olarak, %69'u süt üretimini arttırmak, %64'ü meme sağlığını iyileştirmek, %62'si ilave iş gücü olmadan sürüyü büyütme olanağı ve %46'sı minimum çalışma saatleri gerekçelerini sunmuşlardır (Şekil 9). Sadece bir çiftlik yetkilisi başka bir çiftliğin tavsiyesiyle robota geçtiğini bildirmiştir. Stresli hayvanların sakinleşmesi ve kiralık işgücünün azaltılması gibi cevaplar robotun satın alınmasında etkisi az olan cevaplar olmuştur.



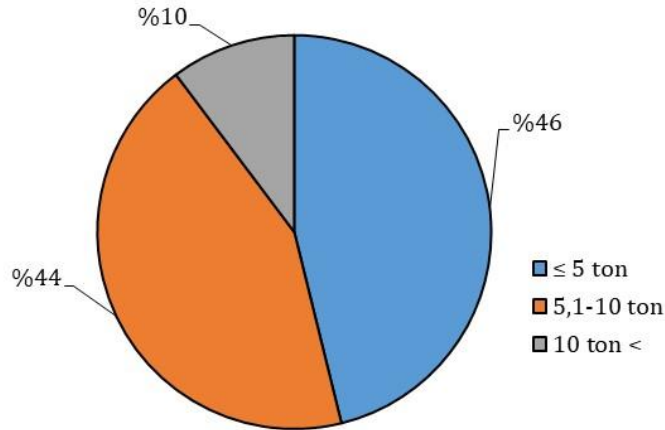
**Şekil 9.** Robotik sistemi satın alma sebepleri

### H-Süt Üretimi

Ankete katılan 39 çiftlik, hayvan başına günlük ortalama süt verimlerinin 31.5 kg/gün olduğunu bildirmişlerdir. Çiftliklerin 15 adedi süt verimlerinin 30 kg/gün'ün altında, 16 çiftlik 30-35 kg/gün arasında ve 8 çiftlik te 35 kg/gün üzerinde süt verimlerine sahip olduğunu bildirmiştir.

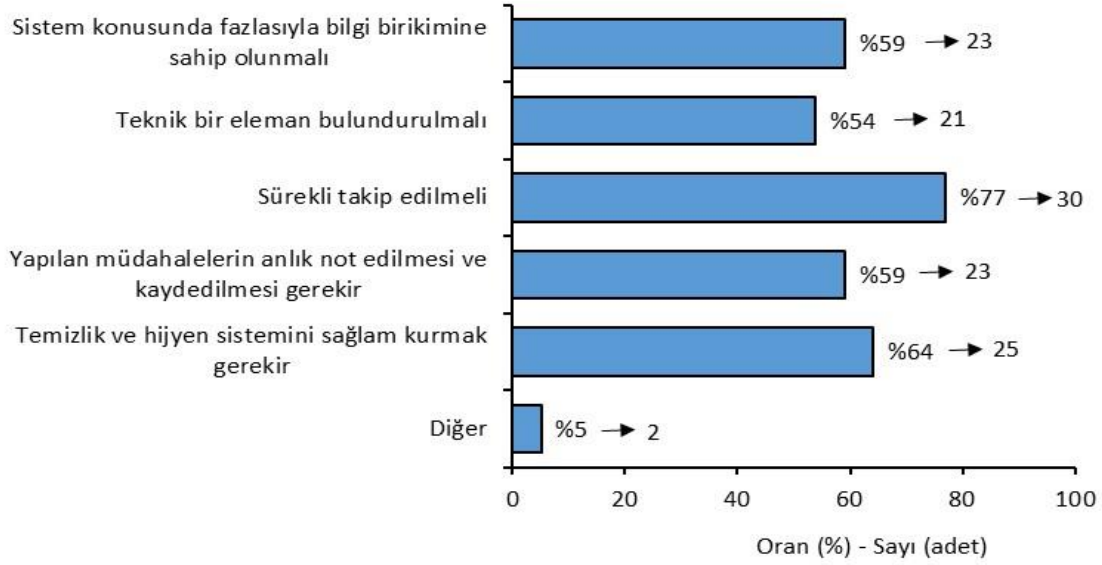
Ortalama robot başına günlük toplam süt üretimi 1712 kg olduğu belirtilmiştir. Ankete cevap veren 38 çiftlikten 4 tanesi (%10) 1000 kg altında, 7 tanesi (%18) 1000 ila 1500 kg arasında, 19 tanesi (%49) 1500 ila 2000 arasında, 8 tanesi (%21) ise 2000 kg üzerinde günlük toplam süt üretimini sağlamaktadır. Anket sonucu elde edilen sonuçlara göre robot başına günlük toplam süt üretiminin en fazla 1500-2000 kg arasında olduğu 19 adet çiftlik olduğu görülmektedir.

Robotik sağım sistemli çiftliklerde bulunan süt soğutma tankları konvansiyonel sağım sistemli çiftliklerden farklı yapıya sahiptir. Robotik çiftliklerdeki ana süt soğutma tankları, akış kontrollü soğutma özelliğine sahip tanklar olduğundan, soğutma işlemini süt akışına göre otomatik olarak ayarlamaktadır. Böylece değişen hacimlerdeki süt karıştırılmadan ve donma riski olmadan hızlı ve tutarlı bir şekilde sütü soğutmaktadır. Anket yapılan tüm çiftliklerdeki süt soğutma tankları bu özelliğe sahip tanklar olduğu bildirilmiştir. Konvansiyonel sağım sisteminden robotik sisteme dönüşüm yapan çiftliklerin büyük çoğunluğu, eski tanklarını elden çıkardıklarını, bazı çiftlikler ise ya yedekte duran çalışır durumdaki konvansiyonel sağım sisteminde sağılan süt için ya da ek depolama tankı olarak kullandıklarını bildirmişlerdir. Ankete katılan çiftliklerin %67'si (26 çiftlik) işletmelerinde ana süt soğutma tankı dışında ön süt depolama tankı da (buffer tank) bulundurduğunu bildirmişlerdir. Geriye kalan çiftliklerde sadece ana süt soğutma tankları mevcuttur. Çiftliklerdeki ana süt soğutma tankı kapasitesi sağılır hayvan sayısı, ineklerin laktasyon verim ortalaması ve robotik sağım ünitesi sayısı ile uyumlu olmaktadır. İşletmelerin %46'sının 1 ila 2 adet robota sahip olması nedeniyle, her bir çiftlikte bulunan ana süt soğutma tank kapasitesi 5 ton'a kadar olduğu görülmüştür (Şekil 10). Diğer yandan 3 veya 4 robota sahip işletmelerin %44'ünün de 5-10 ton arası tank kapasitesine sahip olduğu bildirilmiştir. Çiftliklerin 4'ünde sağılır hayvan sayısı fazla olduğundan 10 ton'un üzerinde birden fazla tanklara sahiptir. Bu çiftliklerdeki ana tankların toplam kapasiteleri 22, 24, 42 ve 72 ton kapasitelerdedir.



**Şekil 10.** Çiftliklerin ana süt soğutma tankı kapasite aralıkları

Elde edilen sonuçlara göre, sağım robotu kullanan çiftliklerin %77'si robota yeni başlamış veya başlayacak diğer çiftliklerin robotik sağım sistemini sürekli olarak takip etmesi gerektiğini önermişlerdir. Çiftliklerin diğer önemli önerileri içerisinde; %64 ile temizlik ve hijyen sisteminin sağlam kurulması, %59 ile sistem konusunda fazlasıyla bilgi birikimine sahip olunması ve yapılan müdahalelerin anlık not edilmesi ve kaydedilmesi yer almıştır (Şekil 11). Ayrıca, çiftliklerin %54'ü robotta meydana gelecek anlık sorunların çözümü için bir teknik eleman bulundurulmasını da önermişlerdir. Diğer seçenek içinde yer alan cevapta ise, birer çiftlik tarafından robotta verilen konsantre yemlemenin çok iyi yönetilmesi ve zorunlu inek trafiğinin çiftlik için daha uygun olacağı önerisi yapılmıştır. Bu araştırmada elde edilen sonuçlar bazı araştırmacıların çalışmalarıyla da desteklenmiştir (De Jong et al. 2003; Castro et al. 2015).



**Şekil 11.** Sağım robotu kullanan çiftliklerin diğer çiftliklere önerileri

Araştırmada çiftliklere son olarak, başta robotik sağım sistemi yönetimi olmak üzere çiftliğin genel yönetimi üzerinde başka görüşlerinin olup olmadığı sorulmuştur. Ankete yorum yapan çiftçilerin birçoğu RMS teknolojisini benimsemenin sadece sağım yönetimini değil, aynı zamanda tüm çiftlik yönetim stratejisini değiştirdiğini de vurgulamışlardır. Birçok çiftçi yeni sisteme adapte olurken sabırlı olunması gerektiğini söylemiştir. İlk iki haftanın genellikle çok stresli geçtiğini, ancak birçok çiftçinin ortak fikri, bu başlangıç döneminden sonra işlerin sakinleştiği ve çalışmanın kolaylaştığı iletilmiştir.

De Jong ve ark (2003) ve Castro ve ark. (2015)'nin çalışmalarında da, bu çalışmaya benzer görüşler elde edildiği bildirilmiştir. Araştırmacıların bilgi edindiği çiftlik sorumluların cevapları arasında; sistemin çalışması için RMS kullanıcılarının gerçek bakıcılar olması gerektiği; çiftlik yönetimi becerileri RMS'ye geçip geçmemeye karar verirken göz önünde bulundurulması gereken en önemli faktör olduğu; RMS'ye karar verirken sistemin nasıl planlanacağı ve yönetileceği konusunda fikir edinmek için diğer çiftliklerin ziyaret edilmesi ve farklı sistemlere bakılmasının önemli olduğu; mevcut ahır yerine, yeni bir ahıra robotu yerleştirilmekle inek trafiğinin daha kolay yönetilebilir olduğu yer almıştır. Castro ve ark. (2015) robotik sağım sistemine sahip 38 çiftlikteki çalışmalarında, mevcut çiftliklerin yüksek oranda tam zamanlı çalışanların olduğu aile yapısına dayandığı bildirilmiştir. Yazarlar, bu çiftliklerin RMS'nin benimsenmesiyle süt üretimini artırmayı, işgücünden tasarruf etmeyi ve daha fazla esnekliğe sahip olmayı amaçladıklarını iletmışlerdir. Ayrıca serbest inek trafiğine sahip çiftliklerin %87'sinde çiftçinin en çok zaman alıcı faaliyetin inekleri sağıma getirmek olduğu; yine çiftçilerin yaklaşık %58'inin RMS'lerinden çok memnun olmakla birlikte, bu değer sürü büyüklüğü ortalamasının altında olan ve bir RMS kullanımına daha iyi adapte olan çiftliklerde %91'e ulaştığı belirtilmiştir.

## SONUÇ

Ankete katılan çiftliklerdeki robotik süt sağım kullanıcılarının büyük çoğunluğu çiftliklerinde kapasite fazlasının olmadığını, hayvanları süt verimlerine göre gruplandırmadığını ve robotlu sağıma ilk olarak düve ile başladıklarını bildirmişlerdir. Araştırmadaki çiftliklerin %72'si serbest inek trafiğini uygulamaktadır. Çiftliklerin %54'ü robotik sistemi yeni çiftliğe kurduğunu bildirmiştir. Ayrıca çiftliklerin %72'si 1 ve 2 adet robota sahiptir.

Ankete katılan çiftliklerdeki ineklerin günlük ortalama sağım sıklığı 3.1 sağım/inek/gün belirlenmiştir. Günlük sağım sıklığı beraberinde robotun etkin kullanımını artırmış olup çiftliklerin %85'i bir robot ünitenin günde 150 den fazla sağım yaptığı belirtmiştir.

Çiftliklerin robotik sağım sistemi dışında kullandığı otomasyon araçları içerisinde buzağı mama hazırlama robotu %62 ile en fazla kullanılan araç olduğu belirlenmiştir. Bu aracı sırasıyla yem itme robotu, somatik hücre sayım cihazı, gübre sıyırma robotu ile yem karma ve dağıtma robotu takip etmiştir. Otomasyon araçları içerisinde yer alan otomatik yem hazırlama sistemini ise sadece bir çiftliğin kullandığı görülmüştür. Anket yapılan çiftliklerin çoğunda konvansiyonel tarım makineleri kullanılmaktadır. Bu makineler içinde yem karma ve dağıtma makinası, mekanik gübre sıyırıcı ve traktör ön yükleyici gibi makineler en yüksek kullanım oranlarına sahiptir.

Çiftlikler robotu satın alma sebeplerine büyük oranda süt üretimini arttırması, meme sağlığını iyileştirmesi, ilave iş gücü olmadan sürüyü büyütme imkânının olması ve minimum çalışma saatleri gerekçelerini sunmuşlardır.

Ankete katılan tüm çiftlikler genel olarak robotik süt sağım sistemlerinden memnun olduklarını belirtmiştir. Çiftçiler, robot sağıma geçildiğinde hayvanın robota alıştırma sürecinde büyük zorluklar yaşadıklarını bildirmiştir. Bunun dışında kış koşullarındaki don olayı, robot arızası, meme takma başarısızlığı, kalifiye eleman sıkıntısı, serbest inek trafiğini yönetme ve sağımı gecikmiş hayvanların takip zorluğu işletmelerin yaşadığı diğer sorunlardır.

Sonuç olarak, çiftçilerin robotla yaşadıkları genel deneyim olumlu görünmüştür. Çoğu çiftçi, robotun yönetsel görevler için daha fazla zaman ve daha da önemlisi kendileri için daha fazla zaman sağladığını belirtmiştir. Çiftçiler ayrıca robot sağımın inekler ve kendileri için stresi azalttığını bildirmiştir.

Y: Bu çalışma, Bursa Uludağ Üniversitesi, Biyosistem Mühendisliği Anabilim Dalı, Fen Bilimleri Enstitüsünde devam eden Yüksek Lisans çalışmasının bir bölümünü içermektedir.

#### Çıkar Çatışması Beyanı

Makale yazarları, aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

#### Yazar Katkıları

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan ederler.

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**Zencefil (*Zingiber officinale* rosc.)’in Hızlı ve Etkili In Vitro Mikroüretimi**Müge Arkadaş<sup>1</sup> , Sebahattin Özcan<sup>2</sup> <sup>1</sup>Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Tarla Bitkileri Anabilim Dalı, Ankara, Türkiye<sup>2</sup>Ankara Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Ankara, Türkiye<sup>1</sup> <https://orcid.org/0000-0002-7375-6058>, <sup>2</sup> <https://orcid.org/0000-0001-5127-1482>✉: [ozcans@ankara.edu.tr](mailto:ozcans@ankara.edu.tr)**Öz**

Zingiberaceae familyasından olan Zencefil (*Zingiber officinale* Rosc.) tıp, gıda ve kozmetik gibi birçok kullanım alanına sahip olması nedeniyle ticari açıdan oldukça önemli bir tıbbi ve aromatik bitkidir. Çiçeklenme ile tohum tutumunun zayıf olması nedeniyle tohumdan çoğaltılması oldukça zor olan zencefilin rizom yoluyla uzun süreli vejetatif çoğaltımı da rizomların zayıflamasına neden olmaktadır. Bundan dolayı zencefil tarımının geliştirilmesi için kısa sürede ve çok sayıda hastaliksız bitki üretiminin yapılması gerekmektedir. Bu çalışmada da Murashige and Skoog (MS) besin ortamına ilave edilen 6-benzil amino pürin (BAP) ve  $\alpha$ -naftalen asetik asit (NAA)’in 20 farklı kombinasyonunun zencefilin in vitro mikroüretimine etkisi araştırılmıştır. En yüksek oranda sürgün (9.25 adet/eksplant) ve kök oluşumu (11.41 adet/eksplant) besin ortamına 0.5 mg/L BAP ve 0.25 mg/L NAA ilave edildiğinde elde edilmiştir. Öte yandan, besin ortamlarına ilave edilen BAP ve NAA oranları bu miktarların üzerinde kullanıldığında sürgün ve kök oluşumunda çok önemli düşüşler olmuştur. Aynı şekilde BAP ile birlikte 0.1 mg/L NAA kullanımı da sürgün ve kök oluşumunu düşürmüştür. Bu sonuçlar, zencefil bitkisinde iyi bir oksin-sitokinin dengesinin kurulmasının in vitro mikroüretim için son derece önemli olduğunu göstermiştir. Elde edilen in vitro bitkicikler de %100 oranında dış şartlara adaptasyon sağlamıştır.

**Anahtar kelimeler:** BAP, doku kültürü, NAA, rejenerasyon, zencefil.**Rapid and Efficient In Vitro Micropropagation of Ginger (*Zingiber officinale* rosc.)****ABSTRACT**

Ginger (*Zingiber officinale* Rosc.), a member of the *Zingiberaceae* family, is a commercially important medicinal and aromatic plant with many uses in medicine, food and cosmetics. Ginger is very difficult to propagate from seed due to poor flowering and seed set. Long-term vegetative propagation through rhizomes also causes weakening of the rhizomes. Therefore, in order to develop ginger cultivation, it is necessary to produce a large number of disease-free plants in a short time. In this study, the effect of 20 different combinations of 6-benzyl amino purine (BAP) and  $\alpha$ -naphthalene acetic acid (NAA) added to Murashige and Skoog (MS) medium on in vitro micropropagation of ginger was investigated. The highest rate of shoot (9.25 shoots/explant) and root formation (11.41 roots/explant) was obtained when 0.5 mg/L BAP and 0.25 mg/L NAA were added to the medium. On the other hand, there were significant decreases in shoot and root formation when BAP and NAA were added to the nutrient media above these amounts. Similarly, the use of 0.1 mg/L NAA with BAP also decreased shoot and root formation. These results showed that the establishment of a good auxin-cytokinin balance in ginger plants is extremely important for in vitro micropropagation. The in vitro plantlets regenerated were 100% adapted to external conditions.

**Key words:** BAP, ginger, NAA, tissue culture, regeneration.



## GİRİŞ

Zencefil (*Zingiber officinale* Rosc.) *Zingiberaceae* ailesine ait çok yıllık, yaprakları rizom dallarından çıkan, adventif saçak köklere ve çoğunlukla tek yıllık toprak üstü aksamaya sahip yaklaşık 100 cm boylanan önemli bir tıbbi ve aromatik bitkidir (Aleem ve ark., 2020; Kumari ve ark., 2020; Udounang ve ark., 2022). Güney Doğu Asya'ya özgü tropik ve subtropik bölgelerde yetiştirilen Zencefil bitkisi ilaç, gıda ve kozmetik gibi birçok kullanım alanına sahip olması sebebiyle ticari açıdan oldukça önemlidir (Chakraborty ve ark., 2023). Zencefile keskin bir aroma veren uçucu yağ gingerol ve diğer keskin bileşikler, kan akışını ve iltihabı etkileyen kimyasallardan olan prostaglandin ve lökotrien üretimini engelledikleri için tıbbi açıdan oldukça önemlidir. Ayrıca, zencefil yüzyıllardır geleneksel tedavide soğuk algınlığı, boğaz ağrısı, astım, iştahsızlık kabızlık, hazımsızlık, mide bulantısı, çarpıntı, eklem iltihabı, yüksek tansiyon ve migren gibi hastalıklarının tedavisinde kullanılmaktadır (Aleem ve ark., 2020).

Tıbbi ve aromatik bitkilerin gelişi güzel hasat edilmesi, biyolojik çeşitliliğinin azalması ve bitki kalitesindeki potansiyelin düşmesine yol açmaktadır. Endüstriyel faaliyetler için oldukça önemli olan bu bitkilerin sürdürülebilir bir şekilde hasat edilmeleri onların ticari tarım potansiyellerini de arttırmaktadır (Tasheva ve Kusturkova, 2013; Kunene ve ark., 2018). Düşük kök sap çoğalma oranına sahip olan zencefilin ekonomik değere sahip rizomlarının toprak kaynaklı patojenlere maruz kalması verim kayıplarını önemli oranda arttırmaktadır. Çoğunlukla rizom yoluyla üretilen zencefilin uzun süreli vejetatif çoğaltımı rizomların zayıflamasına da neden olmaktadır (Kambaska ve Santilata, 2009). Çiçeklenme ile tohum tutumunun zayıf olması nedeniyle tohumdan çoğaltılması da oldukça zor olan bitkilerin başında gelmektedir (Das ve ark., 2010; Mosie, 2019). Bu nedenlerden dolayı ticari olarak zencefil tarımı için kısa sürede yüksek miktarda hastaliksız bitki üretiminin yapılması gerekmektedir. Diğer taraftan in vitro teknikler kullanılarak kısa süre içerisinde aynı genotipe sahip hastaliksız milyonlarca bitki üretmek mümkün olmaktadır. Bu çalışmada da farklı bitki büyüme düzenleyicileri kullanılarak Zencefil bitkisinin in vitro hızlı ve yüksek oranda çoğaltımı için bir protokol geliştirilmesi amaçlanmıştır.

## MATERYAL VE METOT

### Bitki Materyali ve Yüzey Sterilizasyonu

Piyasada satışa sunulan zencefil rizomlarından sağlıklı olanları seçilerek musluk suyuyla sabunla yıkandıktan sonra 21 gün karanlıkta bekletilerek rizomların filizlenmesi sağlanmıştır. Yüzey sterilizasyonu için rizomlardan izole edilen filizler öncelikle %70'lik etanolde 2 dakika bekletildikten sonra üç defa steril saf su ile durulanmıştır. Etanol uygulamasını takiben filizler %30'luk ticari çamaşır suyuyla 20 dakika tutulduktan sonra steril saf su ile üç kez yıkanmıştır. Yüzey sterilizasyonuna tabi tutulan bu sürgünler mikroçoğaltım başlangıç materyali elde etmek amacıyla aseptik şartlarda in vitro temel besin ortamında kültüre alınmıştır. Ayrıca Güney Agripark Tarımsal Araştırmalar Ltd. (Antalya) Şirketinden de in vitro steril fideler sağlanarak araştırmalarda kullanılmıştır.

### Besin Ortamı ve Kültür Koşulları

Temel besin ortamı olarak 30 g/L sükröz ve 7 g/L agar içeren Murashige and Skoog (MS) ortamı (Murashige ve Skoog, 1962) kullanılmıştır. Bu besin ortamına zencefil bitkisinin hızlı çoğaltımı için 0.5, 1, 2 ve 4.0 mg/L 6-benzilaminopürin (BAP) ile 0.1, 0.25, 0.5, ve 1.0 mg/L  $\alpha$ -naftelen asetik asit (NAA) farklı kombinasyonlarda ilave edilmiştir. Hazırlanan besin ortamlarının pH değeri 5.6-5.8'e ayarlandıktan sonra 121°C de 20 dakika süreyle otoklavda steril edilmiştir. Tüm kültürler 24±1°C sıcaklık ve 16 saat aydınlık fotoperiyodun uygulandığı iklim dolaplarında inkübe edilmiştir.

### Mikroüretim

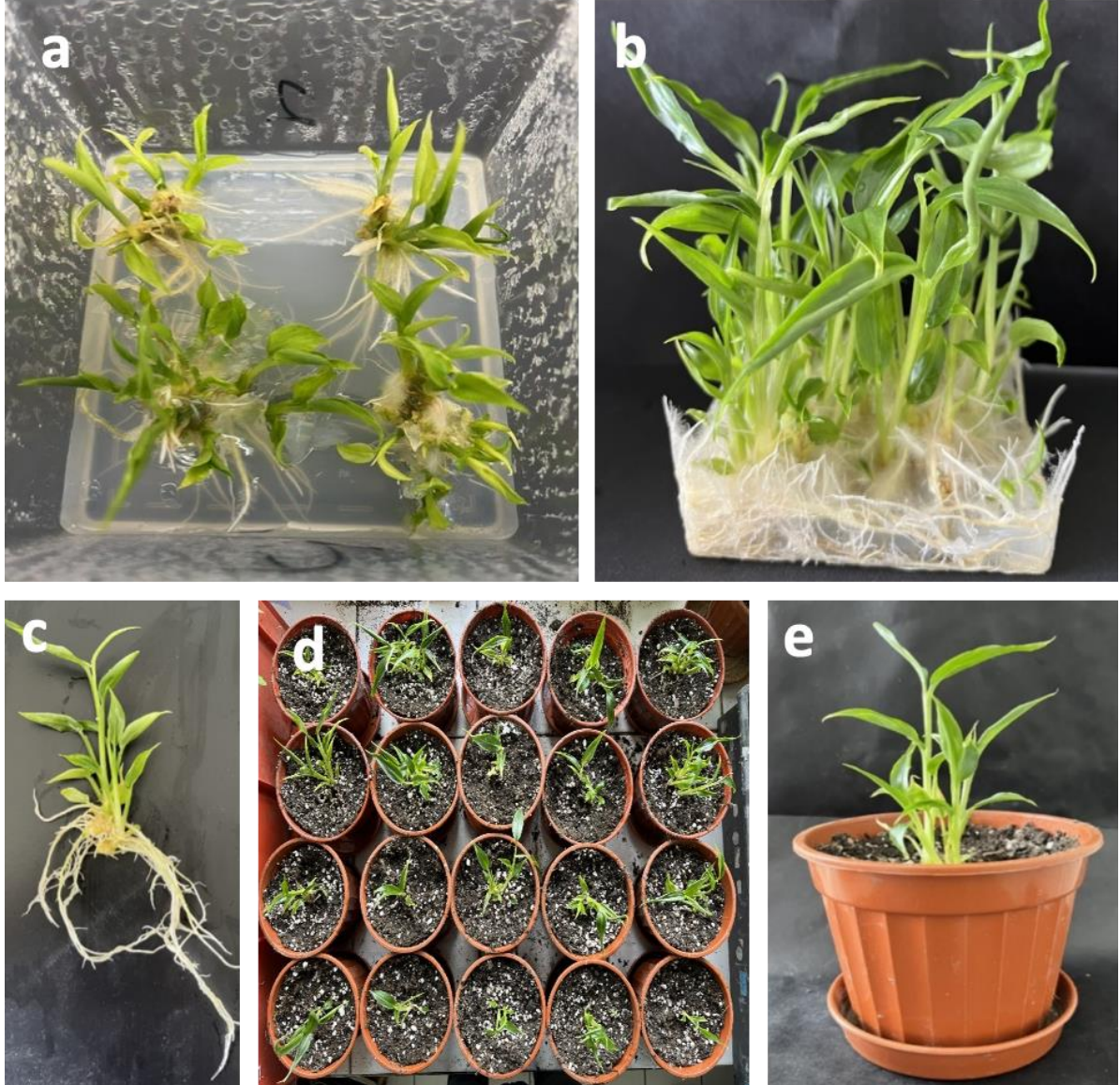
Yüzey sterilizasyonuna tabi tutulan rizom filizlerinden 6 hafta sonra gelişen sürgünler ile Güney Agripark Tarımsal Araştırmalar Ltd. (Antalya) Şirketinden elde edilen in vitro bitkiler hızlı çoğaltma için başlangıç materyali olarak kullanılmıştır. Bu sürgünlerden izole edilen koltuk altı ve tepe meristem bölgeleri farklı oranlarda BAP ve NAA içeren MS besin ortamında iklim dolaplarında kültüre alınmıştır. Kültür başlangıcından 7 hafta sonra eksplant başına sürgün sayısı ve sürgün uzunluğu ile kök sayısı ve kök uzunluğu belirlenmiştir. In vitro şartlarda gelişen ve köklenen bitkicikler 7 hafta sonra köklerindeki agar tamamen yıkanarak dış koşullara adaptasyon için 2:1 oranında torf ve perlit karışımı içeren saksılara aktarılmıştır. Nem kaybını önlemek için saksıların üzerine şeffaf naylon poşet geçirilmiş ve bir hafta süreyle poşetler açılarak su sprey edilmiştir. Bir hafta sonra poşetlerde delikler açılarak havalandırma sağlanmış ve 15 gün sonra da poşetler tamamen kaldırılmıştır.

### İstatistik Analizler

Tüm denemeler 3 tekerrürlü olarak kurulmuş ve her bir tekerrürde 4 adet eksplant kültüre alınmıştır. Elde edilen veriler varyans analizi için JMP istatistik programına tabi tutulmuş ve ortalamalar arasındaki fark LSD testi ile belirlenmiştir.

### BULGULAR VE TARTIŞMA

Rizomlardan gelişen filizler besin ortamında kültüre alındıktan bir hafta sonra sürgünler gelişmeye başlamıştır. Gelişen bu sürgünlerden 6-7 hafta sonra elde edilen tepe ve koltuk altı meristemleri farklı oranlarda BAP ve NAA içeren MS besin ortamlarında kültüre alınmıştır. Kültüre alınan bu eksplantlardan da sürgün gelişimi bir hafta sonra başlamış ve 7 hafta sonra da iyi bir kök yapısına sahip tam gelişmiş sürgün ve bitkicikler elde edilmiştir (Şekil 1a-c).



**Şekil 1.** Zencefilin in vitro mikroüretimi. Kültür başlangıcından 4 hafta (a) ve 7 hafta (b-c) sonra 0.5 mg/L BAP ve 0.25 mg/L NAA içeren MS besin ortamında sürgün gelişim. d-e) Dış koşullara alıştırılmış in vitro bitkiler.

Çizelge 1’de MS besin ortamına ilave edilen 20 farklı BAP ve NAA konsantrasyonunun zencefilde in vitro sürgün oluşumu ve köklenmesi üzerine etkisi verilmiştir. Çizelge incelendiğinde farklı BAP ve NAA konsantrasyonlarının zencefilin mikroçoğaltımı üzerine önemli etkilerinin olduğu gözlenmiştir. Eksplant başına en yüksek sürgün sayısı 9.25 adet ile 0.5 mg/L BAP ve 0.25 mg/L NAA içeren besin ortamından elde edilmiş olup, diğer uygulamalarla arasındaki fark da istatistiki olarak önemli bulunmuştur. Eksplant başına en düşük sürgün sayısı ise 0.5 adet ile 4 mg/L BAP ve 0.5 mg/L NAA içeren MS besin ortamından elde edilmiştir (Çizelge 1). Sürgün

sayısında olduğu gibi en yüksek sürgün uzunluğu da 5.58 cm ile yine 0.5 mg/L BAP ve 0.25 mg/L NAA içeren MS besin ortamında gözlenmiştir. En düşük sürgün uzunluğu da aynı şekilde 4 mg/L BAP ve 0.5 mg/L NAA içeren MS besin ortamından elde edilmiştir.

**Çizelge 1.** Kültür başlangıcından 7 hafta sonra farklı oranlarda BAP ve NAA içeren MS besin ortamının zencefilin in vitro mikroüretimine etkisi.

Büyüme Düzenleyicileri (mg/L)		Eksplant Başına Sürgün Sayısı (Adet)	Ortalama Sürgün Uzunluğu (cm)	Eksplant Başına Kök sayısı (Adet)	Ortalama Kök Uzunluğu (cm)
BAP	NAA				
0	0	4.91 cd*	3.09 b	7.00 bc	2.99 defg
0.5	0	2.75 efgh	2.81 bc	7.08 bc	5.30 bc
1	0	2.91 efgh	2.40 bcd	3.75 efg	2.70 efgh
2	0	7.75 b	1.73 de	3.91 efg	2.37 fgh
4	0	3.83 de	2.16 cd	3.75 efg	2.81 efgh
0.5	0.1	2.33 ghı	1.05 efg	2.41 gh	1.34 hı
1	0.1	2.16 ghı	0.99 efg	3.75 efg	3.34 def
2	0.1	2.08 hı	1.36 ef	3.16 fg	1.49 ghı
4	0.1	3.16 efgh	0.95 efg	5.00 def	2.23 fgh
0.5	0.25	9.25 a	5.58 a	11.41 a	8.91 a
1	0.25	2.08 hı	1.00 efg	3.75 efg	1.75 ghı
2	0.25	5.75 c	2.66 bc	8.83 b	6.08 b
4	0.25	3.41 efg	0.99 efg	3.50 efg	2.00 fgh
0.5	0.5	3.75 def	2.20 cd	3.16 fg	2.34 fgh
1	0.5	2.91 efgh	2.23 cd	5.41 cde	2.27 fgh
2	0.5	1.33 ij	0.73 fg	0.66 h	0.35 ı
4	0.5	0.50 j	0.51 g	0.83 h	0.33 ı
0.5	1	3.25 efgh	2.30 bcd	6.75 cd	3.91 cde
1	1	2.50 fghı	1.28 efg	4.91 def	4.45 cd
2	1	2.75 efgh	1.03 efg	2.00 gh	1.98 fgh
4	1	3.08 efgh	1.21 efg	2.00 gh	1.75 hı

\*Aynı sütunda farklı harflere gösterilen ortalamalar arasındaki farklılık LSD testi sonuçlarına göre 0.01 düzeyinde önemlidir.

Sürgün oluşumunda olduğu gibi kök gelişiminde de 0.5 mg/L BAP ve 0.25 mg/L NAA içeren besin ortamı en yüksek sonuçları vermiştir (Çizelge 1; Şekil 1b-c). Bu ortamda eksplant başına kök sayısı 11.41 adet olurken, ortalama kök uzunluğu ise 8.91 cm olmuştur. Eksplant başına en düşük kök sayısı 2 mg/L BAP ve 0.5 mg/L NAA içeren besin ortamından elde edilirken, en düşük kök uzunluğu ise 4 mg/L BAP ve 0.5 mg/L NAA içeren ortamdan elde edilmiştir (Çizelge 1). İyi bir kök sistemine sahip olan in vitro bitkicikler torf ve perlit karışımı içeren saksılara aktarıldıklarında %100 oranında dış şartlara adaptasyon sağlamıştır (Şekil 1 d-e).

Besin ortamları ve özellikle de bitki büyüme düzenleyicileri kültüre alınan hücre, doku ve organlardan yeni bitkilerin elde edilmesinde son derece önemli bir yer tutmaktadır (Çakmak ve ark., 2016; Trolinder, 2020). Farklı araştırmacılar tarafından çok sayıda yapay besin ortamı geliştirilmesine rağmen MS besin ortamı halen çok sayıda bitki türü için en yaygın kullanılan besin ortamı olma özelliğini sürdürmektedir (Özcan ve ark., 2023; Özcan ve ark., 2025). Dolaylı olarak bitki büyüme ve gelişme ile birlikte in vitro rejenerasyonu sağlayan bitki büyüme düzenleyicileri in vitro bitki gelişimi için mutlak gerekli olan bileşiklerdir (Trolinder, 2020; Ibrahim, 2022). Çoğu bitki türünde bu bileşikler olmadan in vitro şartlarda yeni bitkilerin elde edilmesi mümkün olmamaktadır. Genel olarak in vitro şartlarda sitokinler sürgün oluşumu ve gelişimde etkili olurken, oksinler ise kallus ve kök oluşumunda etkili olmaktadır. Ancak en iyi sürgün ve bitki rejenerasyonu iyi bir oksin-sitokin dengesi kurulduğunda elde edilebilmektedir (Nazir ve ark., 2022). Genellikle yüksek oranda sitokin ve düşük oranda oksin kullanıldığında iyi bir bitki rejenerasyonu sağlanabilmektedir (de Oliveira ve ark., 2022; Asghar ve ark., 2023). Düşük oranlarda kullanılan oksinler hücrelerin hızlı bir şekilde bölünmesini ve kallus oluşumunu sağlarken, sitokinler ise hızlı bölünen bu hücrelerin farklılaşarak morfogenez yoluyla sürgünlere dönüşümünü ve

gelişmelerini sağlamaktadır. Doku kültürlerinde sitokinin olarak en yağın kullanılan bileşik BAP iken, oksin olarak da NAA yaygın olarak kullanılmaktadır. Bu büyüme düzenleyicileri zencefilinde içinde bulunduğu çok sayıda bitki türünde yüksek oranlarda in vitro rejenerasyon sağlamıştır.

Bu çalışmada BAP ve NAA'nın 20 farklı kombinasyonu zencefil bitkisinin in vitro hızlı ve etkili mikroüretimi için kullanılmıştır. En yüksek oranda sürgün ve kök oluşumu besin ortamına 0.5 mg/L BAP ve 0.25 mg/L NAA ilave edildiğinde elde edilmiştir. Öte yandan, besin ortamlarına ilave edilen BAP ve NAA oranları bu miktarların üzerinde kullanıldığında sürgün ve kök oluşumunda çok önemli düşüşler olmuştur. Aynı şekilde BAP ile birlikte 0.1 mg/L NAA kullanımı da sürgün ve kök oluşumunu düşürmüştür. Büyüme düzenleyici içermeyen besin ortamından da önemli oranda sürgün ve kök gelişimi elde edilebilmiştir. Ancak, 0.5 mg/L BAP ve 0.25 mg/L NAA içeren besin ortamıyla karşılaştırıldığında oldukça düşük kalmıştır. Elde edilen bu sonuçlar zencefil bitkisinde iyi bir oksin-sitokinin dengesinin oluşturulmasının in vitro mikroüretim için son derece önemli olduğunu göstermiştir.

Farklı araştırmacılar tarafından zencefilin in vitro mikroüretimi üzerine araştırmalar yürütülmüştür. Shaaban ve ark. (2023) sadece 1 ve 2 mg/L BAP ile 0.1 mg/L NAA kullandıkları çalışmalarında kontrole göre yüksek sonuçlar elde etmişlerdir. Ancak, elde edilen sonuçlar bizim çalışmamıza göre düşük kalmıştır. Benzer şekilde sınırlı sayıda BAP ve NAA kombinasyonunun kullanıldığı diğer bir çalışmada ise en yüksek sürgün rejenerasyonu 7.8 adet ile tek başına 2.5 mg/L BAP kullanıldığında elde edilmiştir (Shaik ve Rajani Kanth, 2018). Ancak bu uygulamada kök gelişimi zayıf olduğundan dolayı sürgünler ayrıca NAA veya IBA içeren başka bir ortamda köklendirilmiştir. Bu çalışmaya göre bizim çalışmamızdan elde edilen sonuçlar (eksplant başına 9.25 adet sürgün) daha yüksek olduğu gibi, köklendirme için sürgünlerin ayrı bir ortama aktarılmasına da gerek duyulmamıştır. Kullanılan rejenerasyon ortamında gelişen sürgünler aynı zamanda çok iyi bir kök sistemi de oluşturmuştur (Şekil 1b). Bu durum ise zencefilin hızlı üretiminde zaman ve maliyetten önemli oranda tasarrufa gidilmesini sağlayabilecektir. Ayrıca besin ortamlarında yüksek oranlarda sitokinin kullanımı anormal gelişmeler ile vitrifikasyona da neden olabilmektedir.

Zuraida ve ark. (2016) yaptıkları çalışmada öncelikle rizom filizlerinden farklı büyüme düzenleyicisi içeren besin ortamlarında mikro sürgünler elde etmişler ve daha sonra bu sürgünleri farklı oranlarda BAP ve NAA içeren besin ortamında kültüre almışlardır. En yüksek sürgün sayısını 19.5 adet ile 3 mg/L BAP ve 0.5 mg/L NAA içeren ortamdan elde etmişlerdir. Ancak bu çalışmada elde edilen sürgünlerin eksplant başına mı yoksa kültür kabı başına mı olduğu ile ilgili bilgi vermemişlerdir. Ayrıca mikro sürgünlerin ve daha sonra da gelişmiş sürgünlerin elde edilmesi için 90 günlük bir zamana ihtiyaç duyulmuştur. Halbuki bizim çalışmamızda 7 hafta sonra iyi bir kök sistemine sahip bitkicikler elde edilebilmiştir. Zencefilin in vitro mikroüretimine yönelik çalışmalar diğer araştırmacılar tarafından da yürütülmüştür (Bhattacharya ve Sen, 2006; Miri, 2020; Zahid ve ark., 2021). Ancak halihazırdaki çalışmadan elde edilen sonuçlar düşük oranlarda bitki büyüme düzenleyicilerinin kullanımı, iyi bir kök sistemine sahip yüksek oranda bitkicik eldesi ve süre yönüyle önemli avantajlar sağlamıştır.

## SONUÇ

Bu çalışmada zencefil bitkisinin hızlı ve etkili in vitro mikroüretimi için BAP ve NAA'nın çok sayıda farklı kombinasyonu kullanılarak 7 hafta gibi kısa bir süre içerisinde iyi bir kök yapısına sahip bitkicikler elde edilebilmiştir. Üstelik düşük oranlarda büyüme düzenleyicilerinin kullanımı sayesinde elde edilen bitkiciklerinde son derece sağlıklı olduğu gözlenmiştir. Bu bitkiciklerin köklendirilmesi için ikinci bir alt kültür ve köklendirme ortamına da ihtiyaç duyulmamıştır. Elde edilen in vitro bitkicikler saksılara aktarılarak %100 oranında dış şartlara adapte olmuştur. Geliştirilen bu yöntem zencefil tarımında kullanılabilecek niteliktedir.

## Teşekkür

Başlangıç materyali için in vitro bitki sağlayan Güney Agripark Tarımsal Araştırmalar Ltd. (Antalya) Şirketine ve istatistik analizlerde yardımları için Prof. Dr. Cengiz SANCAK'a teşekkür ederiz.

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## Author Contributions

**Müge ARKADAŞ:** Bu çalışma, SÖ danışmanlığında yürütülen ve MA tarafından yapılan Doktora tezinin sonuçlarını içermektedir. MA laboratuvar denemelerin planlanması ve yapılması ile makalenin yazılması konularında görev almıştır.

**Sebahattin ÖZCAN:** Tez konusunun belirlenmesi, sonuçların değerlendirilmesi ve yönlendirilmesi ile makalenin düzeltilmesinde katkıda bulunmuştur.



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## Kırsal Alanda Uygulanan Kalkınma Projelerinde Yer Alan Kişilerin Kırsal Turizm Eğitimlerine Katılma Durumlarının İncelenmesi

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### Öz

Sürdürülebilir Kalkınma Amaçları, Birleşmiş Milletler tarafından daha yaşanabilir bir dünya için ekonomik, sosyal ve ekolojik olarak gelişime yönelik ilan edilen bir dizi müdahale yol ve yöntemlerini içeren hedefler silsilesidir. Üye tüm ülkelerin ortak kabulü ve deklarasyonu ile uygulanmaya başlanan hedefler kapsamında tüm dünyada çalışmalar gerçekleştirilmekte ve 2030 yılına hedeflenen amaçlara ulaşmak üzere destekleme programları uygulanmaktadır. Birçok alt sektörle etkileşim halinde olan ve tüm coğrafyalarda ekonomik gelişime katkı sağlayan turizm sektörü de bu programlar içerisinde yer almaktadır. 60'lı yıllarda kırsal alanlarda kalkınmanın sağlanması, kentsel alanlara göçün önlenmesi ve ters yönde ivmelendirilmesi amacıyla özellikle Avrupa'da uygulanan politikalarla daha fazla duyulmaya başlanan Kırsal Turizm de ülkeler bağlamında en yaygın desteklenen turizm branşları içerisinde yer almaktadır. Çalışma kapsamında, Erzurum ilinde Sürdürülebilir Kalkınma Amaçları çerçevesinde uygulanan projelerde yer alan kişilerin kırsal turizm eğitimlerine katılma durumları incelenmiştir. Projelere katılan kişilerin 230'u ile yapılan anket çalışması sonucunda elde edilen veriler, katılma durumu esas alınarak, kişilerin ve işletmelerin özellikleri ortaya konulmasında SPSS programı yardımıyla yapılan crosstab analizinde ve kırsal turizm eğitimlerine katılma kararı üzerinde etkili olan faktörlerin belirlenmesi için ise LIMDEP paket programı yardımıyla probit analizinde kullanılmıştır. Analiz sonuçlarına göre, eğitimlere katılan kişilerin genellikle 20-30 yaş aralığında, erkek, lisans mezunu, kırsal alanda doğup, şehir merkezinde ikamet eden, SGK güvencesine sahip, sabit bir işi olmayan, 20 000 TL üstünde aylık geliri olan ve en fazla girişimcilik eğitimlerine katılma özelliklerine sahip oldukları belirlenmiştir. Ayrıca probit analiz sonucunda ise, erkek, yaşı fazla, geliri yüksek, sosyal güvenceye sahip, daha önce farklı konularda eğitim almış, girişimde bulunmuş, kırsal alana yönelik desteklerden devamlı haberdar olan kişiler daha fazla kırsal turizm eğitimlerine katılırken, ankete katılanların eğitim düzeyleri arttıkça kırsal turizm eğitimlerine katılmadıkları belirlenmiştir.

**Anahtar Kelimeler:** Kırsal kalkınma projeleri, kırsal turizm eğitimi, Erzurum

## Examining the Participation Status of People Participating in Rural Tourism Trainings in Development Projects Implemented in Rural Areas

### ABSTRACT

Sustainable Development Goals are a series of goals that include a series of intervention methods and methods declared by the United Nations for economic, social, and ecological development for a more livable world. Within the scope of the targets, which have been implemented with the common acceptance and declaration of all member countries, studies are carried out all over the world, and a series of support programs are implemented to achieve the targets targeted for 2030. The tourism sector, which interacts with many sub-sectors and contributes to economic development in all geographies, is also included in these programs. Rural Tourism, which became more popular in the 1960s with the policies implemented especially in Europe with the aim of ensuring development in rural areas, preventing migration to urban areas, and accelerating it in the opposite direction, is among the most widely supported tourism branches in the context of countries. Within the



scope of the study, the participation status of people participating in rural tourism training in projects carried out within the framework of sustainable development goals in Erzurum was examined. The data obtained as a result of the survey conducted with 230 of the people participating in the project were used in crosstab analysis with the help of the SPSS program to reveal the characteristics of people and businesses based on participation status, and probit analysis with the help of the LIMDEP package program to determine the factors affecting the decision to participate in rural tourism training. was used in the analysis. According to the analysis results, the people who participated in the trainings were generally young, male, undergraduate, born in rural areas, residing in the city center, having SSI security, not having a fixed job, having a monthly income above 20 000 TL, and having the characteristics of participating in entrepreneurship training the most. has been determined. In addition, as a result of the probit analysis, people who are male, older, have high income, have social security, have previously received training in different subjects, have taken initiative, and are constantly aware of supports for rural areas, participate in more rural tourism trainings, while as the education levels of the survey participants increase, rural tourism increases. It was determined that they did not participate in their training.

**Key words:** Rural development projects, rural tourism education, Erzurum

## GİRİŞ

Kırsal kalkınma, tarım ve hayvancılıkla geçinen kırsal alanlarda ekonomik, sosyal ve çevresel koşulların iyileştirilmesini hedefleyen bir süreçtir (Kaypak, 2011). Bu yaklaşım, kırsal nüfusun yaşam kalitesini artırmanın yanı sıra, doğal kaynakların korunması ve sürdürülebilir kullanımını da içerir (Pretty, 2008). Sürdürülebilirlik, özellikle kırsal kalkınma bağlamında, doğal kaynakların tükenmesini önleyen ve gelecek nesillere yaşanabilir bir çevre bırakmayı amaçlayan bir paradigmadır (WCED, 1987).

Kırsal kalkınma süreçlerinde sürdürülebilirliğin önemini artıran en önemli etkenlerden biri, kırsal alanlarda yoğun tarımsal faaliyetlerin ekosistem üzerindeki baskısıdır. Tarımsal üretimin sürdürülebilir yöntemlerle yapılması, hem ekonomik büyümeyi destekler hem de çevresel bozulmayı engeller (Altieri, 1995). Örneğin, toprak koruma tekniklerinin ve organik tarım uygulamalarının yaygınlaştırılması, hem verimliliği artırmakta hem de toprak erozyonunu önlemektedir (Tilman et al., 2002). Bunun yanı sıra, kırsal bölgelerde yenilenebilir enerji kaynaklarının kullanılması, enerji bağımlılığını azaltarak ekonomik faydalar sağlamaktadır (IEA, 2021).

Kırsal kalkınmanın başarılı bir şekilde sürdürülebilirlik ilkelerine entegre edilmesi için toplumsal katılım büyük önem taşır. Yerel halkın karar alma süreçlerine dâhil edilmesi, uygulamaların benimsenmesini ve sürdürülebilirliğin sağlanmasını kolaylaştırır (Chambers, 1997). Ayrıca, eğitim ve teknik destek programları ile çiftçilerin bilinçlendirilmesi, sürdürülebilir tarım uygulamalarının yaygınlaşmasını hızlandırabilir (FAO, 2014). Kırsal kalkınma ve sürdürülebilirlik arasındaki ilişki, yalnızca çevresel değil, aynı zamanda ekonomik ve sosyal boyutlarıyla da ele alınmalıdır. Bu doğrultuda, kırsal kalkınma stratejilerinin, sürdürülebilir kalkınma hedefleriyle uyumlu bir şekilde planlanması gereklidir (UN, 2015). Bu şekilde hem kırsal bölgelerin ekonomik olarak güçlenmesi sağlanabilir hem de çevresel ve sosyal sürdürülebilirlik için önemli adımlar atılabilir. “Kalkınma ve Sürdürülebilirlik” kavramları özellikle sanayileşme ve etkileriyle birlikte dünya gündeminde yerini almaya başlamış, Sanayi Devrimi ve sonrasında ise uluslararası arenada en çok işlenen konulardan biri olmuştur (Arı, 2019). Sürdürülebilir kalkınma sosyal, ekonomik ve ekolojik adalet temelinde büyümenin, bugünün gereksinimlerini gelecek nesillerin ihtiyaçlarını karşılamalarından ödün vermeden devam etmesi hem kapsayıcı hem de çevreci olması gerektiği ana fikrindedir. Bu doğrultuda Sürdürülebilir Kalkınma ekonomik büyüme, sosyal dahil etme ve etkin ve etkili bir çevre yönetimi konularından oluşan 3 temel sac ayağı üzerine inşa edilmektedir. Ocak 2016 itibariyle 193 ülke tarafından ortak kabulde uygulanmaya başlayan Sürdürülebilir Kalkınma Amaçları, “Kimseyi geride bırakma” / “Leave no one behind” sloganı ile, tüm dünyada mevcut sorunların çözümü için ulaşılmaması gereken hedefler olarak ele alınmıştır. Bu doğrultuda 17 amaç, 169 alt amaçtan oluşan hedefler belirlenmiş ve bu hedeflere de 2030 yılına kadar ulaşılması kararlaştırılmıştır (Arı 2019).

Sürdürülebilir Kalkınma Amaçları tüm yönleriyle değerlendirildiğinde görülmektedir ki, kalkınma çalışmalarında ekonomik, sosyal ve çevresel denge doğrultusunda odaklanılmalıdır. Yoksulluğun bitirilmesi; Açlığa Son; Sağlıklı Bireyler; Nitelikli Eğitim, Kadınların Güçlendirilmesi, Cinsiyet Eşitliği, Temiz Su ve Sanitasyon, Enerji, İstihdam ve İstihdam edilebilirlik, Endüstri, İnovasyon, Eşitsizlikle Mücadele, Sürdürülebilir Yaşam Alanları, Üretim ve Tüketimde Sürdürülebilirlik, İklim Değişikliği ve etkilerine yönelik mücadele, Su ve Karasal Yaşam, Barış ve Adalet ve Uygun ve Amaçlar için Ortaklıklar başlıklarıyla amaçlar, tüm sektörleri kapsayan oldukça geniş ve odaklı bir yapıya sahiptir (Anonim 2020a).

Sürdürülebilir Kalkınma Amaçları (SKA) ve bunların alt amacı olan 169 hedef incelendiğinde de birçoğunun tarım ve çevre konularıyla doğrudan ya da dolaylı olarak ilgisi ortaya çıkmaktadır. Çevresel etki, doğa ve doğal kaynakların kullanımı boyutlarıyla tarımsal faaliyetlerin, dolayısıyla da kırsal kalkınmanın tüm amaçlarla

doğrudan ya da dolaylı ilişkili olduğu görülmektedir. Sosyal, ekonomik ve ekolojik boyutuyla kırsal faaliyetler, sahip olduğu çeşitlilik anlamında kırsal kalkınmanın merkezinde yer almaktadır. Bu çeşitlilik içerisinde ise son yıllarda özellikle doğaya yönelme ardından kırsal turizm faaliyetlerinin dikkat çekici bir gelişim ve hareketlilik oluşturmaya başladığı görülmektedir (Arı, 2019).

Kırsal turizm, yerel toplulukların sosyoekonomik kalkınmasında önemli bir potansiyele sahiptir. Bu potansiyelin etkin bir şekilde kullanılmasında eğitim programları kilit bir rol oynamaktadır. Kırsal turizm eğitimleri, hem bireysel hem de toplumsal boyutta ekonomik, sosyal ve kültürel faydalar sağlayarak kırsal alanlarda sürekli kalkınmaya katkıda bulunur (Gökmen ve Sert, 2018)

Kırsal turizme yönelim, yapılan yatırımlar ve elde edilen gelişim ile sürdürülebilir kırsal kalkınmada turizmin önemini artırmaya başlamıştır. Kırsal Turizm uygulama alanları ve getirileri değerlendirildiğinde, birebir yöre halkına dönen gelirleri ve sağladığı ekonomik iyileşme; doğal değerler, yerel kültür, sosyal entegrasyon ve gelişim; sosyal, kültürel ve çevresel değerlerin korunması ve yaşatılması; yaşam koşullarının iyileşmesine ve kalitesinin artmasına katkısı; istihdam ve doğal olarak kırsal alandan kente göçün önlenmesi ve dolayısıyla kentsel alanlara yönelik oluşan göçün baskı etkisinin azaltılması bağlamında değerlendirildiğinde, kırsal turizmin önemi ortaya çıkmaktadır. Yine kırsal turizm faaliyetleri destinasyon özellikleri ve cazibesi doğrultusunda sürdürülebilirliği, güvenli yapısı, çevresel duyarlılığı ile gelecek nesillerin gereksinimlerinin karşılanmasından ödün verilmeden bugünün ihtiyaçlarının karşılanmasına uygun içeriklendirilebilirliği ile kırsal kalkınma ve sürdürülebilirlik noktasında önem arz etmektedir (Doğan ve Özasan, 2017). Bu kapsamda kırsal turizmin; yerel kültür ve doğal değerleri kapsayıcı bir şekilde ele alan; tüm alanlarla birebir ilişki içerisinde olan, ekonomik-sosyal ve ekolojik anlamda koruma ve sürdürülebilirlik esaslı çalışan bir yapıda olduğu görülmektedir. Uygulandığı yerelin birebir halkını içine alan; sosyal, kültürel ve ekolojik değerlerin ve çeşitliliğin sürdürülebilir kullanım şekilleriyle ele alınıp yapılandırıldığı ve uygulandığı bölgelerin taşıma kapasiteleri detaylarıyla incelenerek kullanıldığı sürece sürdürülebilir kırsal kalkınmanın da lokomotifi rolünü üstleneceği düşünülmektedir.

Bu kapsamda kalkınma planları içerisinde yer alan kırsal turizmin geliştirilmesine yönelik çalışmalar birçok ulusal programla ele alınmaya başlanmış, uluslararası ve ulusal fonlar aracılığıyla destekleme çalışmaları başlatılmıştır. Tarımsal - Kırsal Kalkınmayı Destekleme Kurumu (TKDK) aracılığıyla uygulanan Avrupa Birliği (AB) tarafından birliğe aday statüsündeki ve potansiyel aday durumundaki ülkeleri desteklemek amacıyla oluşturulmuş Katılım Öncesi Yardım Aracı (Instrument for Pre-Accession Assistance-IPA) olarak adlandırılan programın Kırsal Kalkınma bileşeni (IPARD), sorumluluk alanında yer alan kentsel ve kırsal alanların öncelikli ihtiyaçlarına göre kalkınma plan ve programları yürütmekte olan kalkınma ajansları ve girişimciliğin geliştirilmesi ve desteklenmesi misyonuyla çalışan Küçük ve Orta Ölçekli İşletmeleri Geliştirme ve Destekleme İdaresi Başkanlığı (KOSGEB) tarafından birçok proje ve destek faaliyeti uygulanmaktadır. Destekler kapsamında çeşitli mali destek programları ve projeler gerçekleştirilmekte ve bu projelerle çeşitli eğitim faaliyetleri uygulanarak yeni girişimlerin oluşturulması teşvik edilmektedir. Erzurum'un içerisinde yer aldığı Kuzeydoğu Anadolu Bölgesi'nde de gerek IPARD ve gerek Kuzeydoğu Anadolu Kalkınma Ajansı (KUDAKA) ve KOSGEB tarafından mali ve teknik destek programları, girişimcilik eğitim programları uygulanmakta, özellikle kırsal alan ve girişimciler desteklenmeye çalışılmaktadır. IPARD kapsamında ve kalkınma ajansı mali destek programları hali hazırda kurulu işletmelerin yatırımları desteklenirken, kalkınma ajansı teknik destek programları kapsamında daha çok eğitim ve danışmanlık destekleri ve yine KOSGEB aracılığıyla da yeni işletme kuracaklara girişimcilik alanında eğitim ve iş kurma destekleri uygulanmıştır (Kuşat, 2014)

Birleşmiş Milletler tarafından belirlenen ve dünyadaki sorunların çözümüne yönelik 2030 yılına kadar uygulanması ve nihai olarak ulaşılması planlanan amaç ve hedefleri içeren Sürdürülebilir Kalkınma Amaçları belirlenmiştir. Bu amaçlar içerisinde kırsal alanların geliştirilmesi için birçok proje hayata geçirilmiştir. Bu projelerden birisi de Kırsal Turizm Projeleridir. Bu kapsamda projenin amaçlarına ulaşması, potansiyelin kullanılması ve halkın bilinçlendirilmesi için birçok eğitim çalışması düzenlenmektedir ve yöre halkının bu eğitimlere katılması büyük önem arz etmektedir. Bu amaçla, Erzurum ilinde kırsal kalkınma projeleri yoğunlukta uygulanmış olup bu projeler sonucunda çeşitli eğitimler düzenlenmiş ancak bu eğitimlere katılım isteğinde bazen sorunlar yaşanmıştır. Bu kapsamda çalışmada uygulanan projelerin önemli bir sac ayağını oluşturan eğitim dallarından biri olan kırsal turizm eğitimlerine katılma durumları esas alınarak kişilerin katılma kararı üzerinde etkili olan faktörlerin ortaya konularak katılımlardaki sorunlara çözüm yolu bulunabilmesi amaçlanmıştır. Ayrıca bu çalışmanın verilen kırsal kalkınma kapsamında verilen eğitimlerin irdelendiği ilk çalışma olması açısından da literatüre katkı sağlayacağı düşünülmektedir.

## MATERYAL VE METHOT

Araştırmada Erzurum'da bulunan ve Erzurum-Erzincan-Bayburt illerine yönelik faaliyet gösteren KUDAKA, TKDK ve KOSGEB'den alınan veriler doğrultusunda sayısal Tesadüfi Örneklem Yöntemi kullanılarak belirlenen anketlerle hareket edilmiş, 230 kişiye uygulanan anket verileri birincil veri kaynağı olarak

değerlendirilmiştir. Çalışmaya ait ikincil veri kaynakları ise; Türkiye İstatistik Kurumu (TÜİK) gibi kurum ve Birleşmiş Milletler kaynaklarından elde edilen bilgilerden oluşmaktadır.

Anket yapılan işletme sayısı daha önce kırsal kalkınma projelerine katılan kişiler esas alınarak tesadüfi örnekleme yöntemine göre 220 ancak anket çalışması ile elde edilen verilerin hatalı olması problemine karşı ise örnek hacmi %5 genişletilerek toplam anket sayısı 230 olarak belirlenmiştir. Anket çalışmalarının tamamı kırsal turizm alanında 29 proje ile mevcut potansiyelin en yüksek olduğu ve hali hazırda kırsal turizm faaliyetlerinin devam ettiği, destekleme projelerinin uygulanmış olduğu Erzurum ili kuzey ilçeleri arasında bulunan İspir, Tortum ve Uzundere ilçelerinde yürütülmüştür. Uygulanan anketlerin ilçe bazında dağılımı Çizelge 1’de gösterilmiştir.

**Çizelge 1.** Yapılan anketlerin ilçelere göre dağılımı

İlçeler	Yapılan Anket		Eğitimlere Katılanlar		Eğitime Katılmayan	
	N	%	N	%	N	%
İspir	85	37,0	68	29,6	17	7,4
Tortum	70	30,4	47	20,4	23	10,0
Uzundere	75	32,6	63	27,4	12	5,2
<b>Toplam</b>	<b>230</b>	<b>100,0</b>	<b>178</b>	<b>77,4</b>	<b>52</b>	<b>22,6</b>

Çalışmada anket yolu ile elde edilen veriler, kişilere ait özelliklerin belirlenmesi için SPSS ‘te crosstab analizlerinin yapılmasında ve kişilerin eğitime katılma isteğinde belirleyici faktörleri tespit etmek için, LİMDEP istatistik programı ile Probit Modeli analizinde kullanılmıştır. Araştırmada Bağımlı Değişken; 0 =Kırsal Turizm Eğitime Katılmamış, 1 = Kırsal Turizm Eğitime Katılmış şeklinde kodlanarak iki kategoriye ayrılmıştır. Bağımlı değişkenin sınırlı oluşu probit modelinin kullanımını cazip kılmıştır. Böylece basit bir regresyon modeli (OLS), bağımlı değişkenin iki kategorisi arasında eşit farklılıklar varsayacağından objektif sonuçlar vermemektedir. Bağımlı değişkenin doğal bir şekilde olması, multinominal logit modelinin kullanılması akabinde taraflı sonuçlar vermektedir. Bundan dolayı bu şekilde olan kategorilerde doğal sınırlamanın olması ve bağımlı değişkenin kesikli kategoriler şeklinde olması probit modelini gerektirmektedir (Abdel-Aty and Radwan, 2000).

Model şu şekilde ifade edilebilir.

$$y^* = bx + \varepsilon \quad \varepsilon \sim N [0,1]$$

$$y = 0 \text{ eğer } y^* \leq 0,$$

$$y = 1 \text{ eğer } 0 < y^* \leq \mu_1$$

## RESULTS AND DISCUSSION

Kırsal ve kentsel alanda yerleşik hedef gruplara yönelik anket çalışmaları toplam 230 kişiye uygulanmıştır. Elde edilen veriler kişilerin kırsal turizm eğitimlerine katılma durumları ve kişisel özelliklerin ortaya konulduğu çapraz tablolarını gösteren crosstab analizinde kullanılmıştır. Crosstab analiz sonuçları Çizelge 2’de gösterilmiştir. Ankete katılım konusunda demografik yapılar incelendiğinde de en fazla katılımın genç ve orta yaş grubundan geldiği görülmektedir. Katılımcıların yaş grupları içerisinde en yüksek oranın 20-30 yaş aralığındaki grup olduğu ve toplam katılımcılar içerisinde %43,5’lük bir orana sahip oldukları gözlemlenmektedir. Dijital ortamda uygulanan çalışmalara hemen her yaş gurubundan katılımın olduğu, ağırlıkta genç nüfus olarak nitelendirilen 20-30 yaş grubundaki katılımcılar ağırlıkta olmak üzere 20-40 yaş aralığındaki grubun anket çalışmalarına katılımında ön sırada yer aldıkları görülmüştür (Anonim, 2020a).

Türkiye İstatistik Kurumu Nüfus Kayıt Sistemi’nin 2021 yılı verilerine göre Türkiye’de kadınların nüfusu 42 milyon 252 bin 172 kişi, erkeklerin nüfusu ise 42 milyon 428 bin 101 kişi olarak belirlenmiştir. Bu sonuçlara göre toplam nüfusun %49,9’unu kadınlar, %50,1’ini ise erkekler oluşturmaktadır. Erzurum il nüfusuna bakıldığında 2021 yılı verileri doğrultusunda il nüfusun 377.261 erkek ve 379.632 kadından oluştuğu, yüzde olarak ise %49,84 erkek, %50,16 kadınlardan oluştuğu görülmektedir (Anonim 2020b) TÜİK 2021 verileri doğrultusunda nüfus Erzurum özelinde incelendiğinde erkeklerin toplam nüfus içerisindeki payı %49,84, kadınların oranı %50,16’dır. Cinsiyete göre yapılan değerlendirmelerde ankete katılanların % 32,2 ‘lik kısmının kadın katılımcılar, % 67,8’inin erkek katılımcılardan oluştuğu görülmektedir.

Çizelge 2. Crosstab analiz sonuçları

Değişkenler	Gruplar	1		0		Toplam	
		N	%	N	%	N	%
Yaş	20-30	76	33	24	10,4	100	43,5
	31-40	73	31,7	21	9,1	94	40,9
	41-50	22	9,6	7	3	29	12,6
	51-60	5	2,2	0	0	5	2,2
	61 <	2	0,9	0	0	2	0,9
Cinsiyet	Kadın	66	28,7	8	3,5	74	32,2
	Erkek	112	48,7	44	19,1	156	67,8
Eğitim	İlköğretim	12	5,2	1	0,4	13	5,7
	Orta Öğretim	53	23	17	7,4	70	30,4
	Ön Lisans	38	16,5	8	3,5	46	20
	Lisans	57	24,8	20	8,7	77	33,5
	Lisansüstü	18	7,8	6	2,6	24	10,4
Doğum yeri	Kırsal Alan	103	44,8	22	9,6	125	54,3
	Şehir Merkezi	75	32,6	30	13	105	45,7
İkamet yeri	Kırsal Alan	72	44,8	21	9,6	93	40,4
	Şehir Merkezi	106	32,6	31	13	137	59,6
Sosyal güvence	Güvencesi yok	18	7,8	13	5,7	31	13,5
	Yeşil Kart	10	4,3	3	1,3	13	5,7
	SGK	128	55,7	27	11,7	155	67,4
	Özel Sigorta	21	9,1	5	2,2	26	11,3
	Diğer	1	0,4	4	1,7	5	2,2
Meslek	İşsiz	69	30	17	7,4	86	37,4
	Çiftçi	12	5,2	5	2,2	17	7,4
	İnşaat İşçisi	7	3	0	0	7	3
	Tüccar/Esnaf	32	13,9	6	2,6	38	16,5
	Memur	31	13,5	12	5,2	43	18,7
	Diğer	27	11,7	12	5,2	39	17
Gelir (TL)	1.000-5.000	20	8,7	9	3,9	29	12,6
	5.001-10.000	31	13,5	4	1,7	35	15,2
	10.001-15.000	9	3,9	12	5,2	21	9,1
	15.001-20.000	21	9,1	4	1,7	25	10,9
	20.001 - <	97	42,2	23	10	120	52,2
Eğitime katılma durumu ve türü	Bitkisel Üretim	7	3	0	0	7	3
	Hayvansal Üretim	8	3,5	0	0	8	3,5
	Girişimcilik	88	38,3	0	0	88	38,3
	Proje Hazırlama	16	7	0	0	16	7
	Katılmadım	2	0,9	52	22,6	54	23,5
	Diğer	6	2,6	0	0	6	2,6
	Birden çok	51	6,1	0	0	51	22,2
TOPLAM		178	77,3	52	22,6	230	100,0

Türkiye’de yaşı 25 ve üzerinde olan ve en az bir eğitim düzeyini tamamlamış bireylerin toplam nüfus içerisindeki oranının yıllar itibarıyla değiştiği gözlemlenmiştir. Bu oran 2008 yılında %81,1 iken 2018 yılına gelindiğinde %90,1 olarak belirlenmiştir. Oranlar cinsiyete göre incelendiğinde aynı artışın gerçekleştiği görülmektedir. 2008 yılında kadınlarda %72,6 olan oranlar, 2018 yılında %84,5’e yükselmiştir. Erkeklerde ise %89,8’den %95,9’a çıkmıştır (Anonim 2019a). Türkiye’de Millî Eğitim Bakanlığı yaygın eğitim kapsamında çeşitli kursların uygulanmasına devam etmiştir. Bu kurslara katılım oranları incelendiğinde özellikle kadın katılımcıların genel katılım içindeki oranının %58 olduğu tespit edilmiştir. Toplam 7.034.152 kursiyerin dahil olduğu eğitim çalışmalarına erkek katılımcıların dahil olma oranı ise genel katılım içerisinde %41 olarak gerçekleşmiştir. Kadınların düzenlenen çalışmalara katılım oranı genel nüfus ortalamasına göre ise %10,14’e tekâmül etmektedir (Anonim, 2019b).

Ankete katılanların %54,3 'ü kırsalda doğarken, aynı grubun %59,6 oranında şehir merkezinde ikamet ettikleri gözlenmiştir. Bölgenin en büyük ili konumundaki Erzurum kentli nüfus bakımından da oransal olarak diğer illerden öndedir. Yürürlükte olan Büyükşehir Yasası doğrultusunda 2013 yılı itibarıyla Kır-kent nüfusu takibi yapılamadığından, büyükşehir statüsünde iller arasında yer alan Erzurum ilinde kırsal nüfusu kentsel nüfusa dönüşmüş durumdadır (Anonim, 2015).

Katılımcıların %67,4'lük kısmı sosyal güvenceye sahibi olduğu, 13,5'lik kısmının ise sosyal güvencesi bulunmadığı görülmektedir. %11,3 'lük kısmının özel sağlık sigortası kapsamında sosyal güvence sahibi olduğu tespit edilmiştir.

Eğitim çalışmalarına katılım durumunda ise en fazla katılımın sırasıyla hali hazırda işsiz olanlar, tüccar/esnaf olarak faal olanlar ve memurlardan geldiği gözlemlenmiştir. Anket uygulanan bölgede eğitim çalışmalarına hali hazırda çiftçilik yapanların katılım durumları ise %5,2 olarak tespit edilmiştir.

TÜİK'in hazırladığı 2020 yılı raporlarına göre Türkiye'de yıllık ortalama hane halkı kullanılabilir fert geliri 33.428 TL. olarak belirlenmiştir (Anonim, 2020c). Bu kapsamda bulgularımızda bu rakama yakın olarak 20 000 TL üzerindeki payın fazla olmasından dolayı ortalama fert geliri de Türkiye ortalamasına yakındır.

İl ve özellikle kırsal alanında proje bazlı eğitim çalışmaları yansırı il ve ilçe tarım müdürlüklerinde farklı dönemlerde eğitim çalışmaları da uygulanmaktadır. Bu kapsamda düzenlenen girişimcilik eğitimine katılanların oranı en fazla olup %38,3 olarak tespit edilmiştir.

Ekonometrik analiz sonuçları esas alındığında, Erzurum ilinde kırsal turizm eğitimlerine katılıp katılmama durumu üzerinde etkili olan değişkenlerin tamamı ekonomik teoriye uygun çıkmıştır. "Yaş" ve "Kırsal faaliyetlerle ilgili desteklerden haberdar olma durumu" değişkenleri %1 seviyesinde, "Daha önce herhangi bir eğitim çalışmasına katılma durumu" değişkeni %5 ve "Girişimde bulunma durumu" değişkeni ise %10 seviyesinde önemli bulunmuştur. Yaş, daha önce başka eğitimlere katılma durumu, girişimde bulunma ve kırsal faaliyetlerle ilgili desteklerden haberdar olanlar ile kırsal turizm eğitimine katılma durumu arasında pozitif bir ilişki vardır. Yani kişilerin yaşı arttıkça kırsal turizm eğitimlerine daha çok katıldıkları, daha önce farklı eğitim çalışmalarına katılanların kırsal turizm eğitimine katılımda daha çok yer aldıkları, aldıkları eğitimler ardından girişimde bulunanların kırsal turizm eğitimlerine katılımda daha aktif oldukları, kırsal faaliyetlere yönelik desteklerden haberdar olanların kırsal turizm eğitimlerine daha çok katıldıkları gözlemlenmiştir. Yapılan bir çalışmada eğitim programlarının içeriği, yerel halkın mevcut bilgi düzeyi ve bölgesel özellikler dikkate alınarak şekillendirilmesi ve gerekli desteklerinde buna göre verilmesinin önemi vurgulanmıştır. Çalışmada verilen örnekte, bir bölgede ekolojik turizm potansiyeli var ise, doğa koruma ve sürdürülebilirlik konularına özel vurgu yapılması gerekli olduğu belirtilmiştir (Eren, 2022). Yapılan diğer bir çalışmada da, . Yapılan bir çalışmada, eğitim yoluyla geliştirilen turizm faaliyetleri, yerel ekonomiye önemli bir katkı sağladığı, kırsal turizmin işletme maliyetlerinin düşük olması ve yerel kaynakları kullanma özelliği, bu alanlarda ekonomik hareketliliği artırarak girişimde bulunmayı desteklediği vurgulanmıştır (Altın, 2023). Bunun yanı sıra, eğitimlerle kazanılan bilgi ve beceriler, yerel halkın turizm faaliyetlerine daha bilinçli katılımını sağlar ve toplumsal dayanışmayı güçlendirir.

Analiz sonucunda çizelge 3'de yer alan marjinal etki değerleri bağımsız değişkenin bir birim artırılması durumunda bağımlı değişkende oluşacak etkileri göstermektedir. Çizelge 3'e göre, katılımcıların yaşının 1 birim artması kırsal turizm eğitimlerine katılım durumunu %10 oranında artırdığı, daha önce eğitim çalışmalarına katılma durumunun %12, daha önce aldığı eğitimlerden sonra girişimde bulunanların kırsal turizm eğitimlerine katılma durumunu %10 ve kırsal faaliyetlerle ilgili desteklerden haberdar olma durumunun ise %50 oranında artırdığı belirlenmiştir.

**Çizelge 3.** Regresyon Analizi Sonuçları

Değişkenler	Katsayı	Standart Hata	Marjinal Etki
Sabit Terim	2,15403	0,65335	-
Yaş	0,41247***	0,13831	0,10164
Cinsiyet	0,44410	0,24042	0,01094
Eğitim Durumu	-0,08508	0,09800	-0,02116
Yıllık Gelir	0,01864	0,05966	0,00459
Sosyal Güvence	0,07819	0,18299	0,01927
Daha önce eğitime katılma durumu	0,47322**	0,22314	0,12299
Girişimde Bulunma Durumu	0,42408*	0,23594	0,10911
Kırsalda uygulanan desteklerden haberdar olma durumu	1,58884***	0,217781	0,50089

\*\*\* %1 önem seviyesi, \*\* %5 önem seviyesi, \* %10 önem seviyesi

## SONUÇLAR

Kırsal ve Turizm başlıkları bir arada incelendiğinde, bugün birçok ülkede 60'lı yıllardan itibaren değerlendirmeye alınmış olduğu ve kalkınmada önemli gelişmelerin elde edilmesine destek sağladığı görülmektedir. Kırsal alanlar ve yerel değerlerin sadece ekonomik değil, sosyal, kültürel ve sağlıklı yaşam boyutuyla da değerlendirildiği görülmektedir. Bu bağlamda konunun kalkınma boyutu içerisinde sürdürülebilirlik yönüyle de ele alındığı ve çevresel etkinin de uygulama çalışmalarında dikkat edilen boyutlar içerisine dahil edildiği görülmektedir.

Turizm hizmet sektörü içerisinde birçok alt sektörle etkileşim halindedir ve birçok sektörü beslemektedir. Etki alanı geniş olan turizm sektörü, özellikle kırsal alan düşünüldüğünde, özel müşteri gruplarına hitap eden ve odağında müşteri memnuniyetinin oldukça fazla olduğu bir sektör olarak ön plana çıkmaktadır. Dolayısıyla yapılacak yatırımların çok detaylı düşünülmesi, ürün kalitesinden çeşitliliğine, sunum şekillerinden içeriklerine kadar çok yönlü hareket edilmesi gerektiği açıktır. Bu noktada elbette gerek kamu gerek özel sektör ve gerekse de sivil toplum alanında iş birliklerinin ve desteklerin sağlanması gerekmektedir. Bu anlamda kalkınma planlarından teşvik politikalarının planlamasına kadar birçok yerde ele alınmış olan kırsal turizm için destek mekanizmalarına özel yerler ayrılmış ve Erzurum ili özelinde de devlet eliyle desteklemeler gerçekleştirilmiştir. Destekleme çalışmaları proje uygulamaları şeklinde gerçekleştirilmiştir. 2010 yılı itibarıyla bölgesel kalkınma strateji raporları doğrultusunda yoğunlaştığı görülen programların gerek kamu gerekse sivil toplum kuruluşları aracılığıyla uygulanmıştır. Projeler kapsamında teknik destekler uygulamalı mesleki eğitimler ve girişimcilik eğitimleri şeklinde, mali destekler ise üretim ve tesisleşme yatırım destekleri şeklinde gerçekleştirilmiştir.

Bu çalışmada, Düzey 2 bölgesinde yer alan Erzurum ilinde kırsal turizme yönelik aktif kamu, sivil toplum ve özel sektör kurum kuruluşlarınca uygulanan proje çalışmaları, hibe/fon kaynakları ve bunlarla uygulanan eğitim çalışmaları ele alınmış, Sürdürülebilir Kalkınma Amaçları doğrultusunda incelenmeye, değerlendirilmeye çalışılmıştır. Bölgede özellikle ifade edilen 3 kurumun destekleriyle uygulanan çalışmalarda ağırlıkta Erzurum ili kuzey ilçelerinde proje çalışmalarının yoğunlaştığı, destek programlarıyla girişimlerin gerçekleştirildiği belirlenmiştir. Bu doğrultuda kırsal turizm açısından daha çok ön planda olan ve uygulama çalışmaları gerçekleştirilen kuzey ilçeler İspir, Tortum, Uzundere ilçeleri özelinde uygulanan çalışmalar incelenmiştir.

Elde edilen verilerle yapılan analiz sonuçlarına göre, eğitimlere katılan kişilerin genellikle 20-30 yaş arasında, erkek, lisans mezunu, kırsal alanda doğup, şehir merkezinde ikamet eden, SGK güvencesine sahip, sabit bir işi olmayan, 20.000 TL üstünde aylık geliri olan ve en fazla girişimcilik eğitimlerine katılma özelliklerine sahip oldukları belirlenmiştir.

Ayrıca probit analiz sonucunda ise, erkek, yaşı fazla, geliri yüksek, sosyal güvenceye sahip, daha önce farklı konularda eğitim almış, girişimde bulunmuş, kırsal alana yönelik desteklerden devamlı haberdar olan kişiler daha fazla kırsal turizm eğitimlerine katılırken, ankete katılanların eğitim düzeyleri arttıkça kırsal turizm eğitimlerine katılmadıkları belirlenmiştir. Analiz sonuçları itibarıyla genç nüfusun ve kadınların eğitim almada geride kaldıkları bu kapsamda bu kitlelerin ilgisini çekecek nitelikte eğitim çalışmalarının yeniden düzenlenmesinin gerekli olduğu düşünülmektedir. Bunun yanı sıra, bu çalışma sadece Erzurum bölgesinde tek bir eğitim çalışması esas alınarak yürütülmüş olup, sonuçlar bu eğitimi kapsamaktadır. Bu çalışma pilot bir çalışma niteliğinde olup, kendinden sonra yapılacak farklı eğitimlerinin esas alındığı ve daha geniş araştırma evreninde uygulanacak çalışmalara klavuz olacağı ayrıca sonuçları itibarıyla de proje uygulayıcılarına etkin eğitim düzenlenmesi ve hedef kitlenin seçilmesi vb konularda yol gösterici olacağı ve konu ile ilgili politikalara da yön vereceği düşünülmektedir.

## Çıkar Çatışması Beyanı:

Makale yazarları arasında herhangi bir çıkar çatışması yoktur.

## Yazarların Katkısı

Yazarlar makaleye eşit oranda katkı sağlamışlardır.

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## Effects of Wheat Bran and Molasses Additives on Quinoa Silage Quality

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### ABSTRACT

This study was carried out to determine the effects of different additives (molasses and wheat bran) and their rates (0%, 5%, 10% and 15%) on the silage quality of quinoa (*Chenopodium quinoa* Willd.). Molasses and wheat bran had significant effects on quality characteristics of quinoa silage. Molasses and wheat bran increased the dry matter rate, crude protein, lactic acid and propionic rates of quinoa silage, while pH, ammonia, crude ash and acetic acid contents decreased. While molasses had no effect on the ADF (acid detergent fibre) rate, wheat bran caused an increase in the ADF rate. While wheat bran caused a significant increase in the NDF (neutral detergent fibre) rate compared to the control, molasses caused a decrease in the NDF rate. While molasses significantly increased the RFV value, wheat bran caused a decrease in the RFV (relative feed value). While the butyric acid rate was high only in the 5% wheat bran application, no significant difference was observed in other applications compared to the control.

**Key words:** Organic acid, lactic acid, dry matter, pH, crude protein, neutral detergent fibre

### Kinoa Silaj Kalitesine Buğday Kepeği ve Melas Katkı Maddelerinin Etkileri

#### Öz

Bu çalışma, farklı katkı maddelerinin (melas ve buğday kepeği) ve bunların oranlarının (% 0, % 5, % 10 ve % 15) kinoa (*Chenopodium quinoa* Willd.) silaj kalitesi üzerine etkilerini belirlemek amacıyla yürütülmüştür. Melas ve buğday kepeği kinoa silajının kalite özellikleri üzerine önemli etkilere sahiptir. Melas ve buğday kepeği kinoa silajının kuru madde oranını, ham proteini, laktik asit ve propiyonik oranlarını artırırken, pH, amonyak, ham kül ve asetik asit içeriklerini azaltmıştır. Melas ADF (asit çözücülerde çözünmeyen lif) oranı üzerine etki etmezken, buğday kepeği ADF oranında artışa neden olmuştur. Buğday kepeği NDF (nötr çözücülerde çözünmeyen lif) oranında kontrole göre önemli artışa neden olurken, melas NDF oranında azalmaya neden olmuştur. Melas NYD (nispi yem değeri) değerini önemli ölçüde artırırken, buğday kepeği NYD'de azalmaya neden oldu. Büterik asit oranı sadece %5 buğday kepeği uygulamasında yüksek iken, diğer uygulamalarda kontrole kıyasla önemli bir fark gözlenmedi.

**Anahtar kelimeler:** Organik asit, laktik asit, kuru madde, pH, ham protein, nötr çözücülerde çözünemeyen lif

### INTRODUCTION

Silage is an important feed source in animal nutrition. Silage is the feed made to meet the roughage needs of animals during the winter months when roughage and green feed are scarce and grazing is not possible. Silage has an important place in meeting the green feed needs of animals in winter. Silage is becoming increasingly widespread due to reasons such as its high digestion rate, its consumption with pleasure by animals, its long shelf life and low loss of nutrients, its high water content and most importantly, its economical nature. Corn, alfalfa, meadow grass, sainfoin, barley grain, sorghum and sunflower plants are generally used in silage making. The number of plants that are resistant to extreme climate and soil conditions such as salty and arid and which can

be preferred for silage making is limited. Quinoa plant, which is resistant to salty, cold and dry conditions, has the potential as an alternative plant for silage production in extreme climate and soil conditions (Razzaghi, 2011; Jacobsen, 2003; Keskin et al., 2023). Quinoa is an annual and dicotyledonous plant belonging to the *Chenopodiaceae* family.

Depending on the ecological and soil conditions of the region, plant height varies between 50-350 cm (Kadereit et al., 2005; Temel and Keskin, 2022). Quinoa is now widely grown in China, India, America and Canada. The fact that the United Nations declared 2013 the "Year of Quinoa" and that the National Aeronautics and Space Administration included quinoa seeds in the astronauts' food list made a significant contribution to the spread of quinoa (Kır and Temel, 2016; Geren and Güre, 2017). Quinoa has the potential as an alternative feed source for animals due to its high seed and dry matter yield per unit area (Keskin and Önkür, 2019, Temel and Keskin 2019). The straw, harvest residues, green parts and silage of the quinoa plant are used in animal nutrition (Van Schooten and Pinxterhuis, 2003; Kakabouki et al., 2014; Podkowska et al., 2018; Yacout et al., 2021; Salama et al., 2021).

Since quinoa has low carbohydrate content and high water content, making silage without using additives will result in poor silage quality. Therefore, using additives that will increase the dry matter content and fermentation of quinoa silage will increase the quality of quinoa silage. Barley grain, urea, molasses, salt, cracked wheat, wheat bran, cracked barley and whey are generally used as additives to silages (Gülümser et al., 2019). Molasses and cracked wheat additives contribute significantly to increasing silage quality (Kordi and Naserian, 2012; Qin and Shen, 2013).

This study was conducted to determine the effects of molasses and wheat bran used as additives on the silage quality of quinoa.

## MATERIALS AND METHODS

Silage material was taken at Iğdır University Agricultural Application and Research Center in the field of Red Head variety of quinoa grown under irrigated conditions in 2022 year. The quinoa plant in this area was sowed with a row spacing of 35 x 10 cm and row spacing, and 80 kg ha<sup>-1</sup> of phosphorus and 7.5 kg of nitrogen were given with the sowing. When the plants were 30-40 cm tall, an additional 50 kg ha<sup>-1</sup> of nitrogen was given. Plant chopped theoretically 2-4 cm length and ensiled by using plastic vacuum packed, and approximately 400 g of fresh forage material was placed into the plastic silage bags. Molasses and wheat bran were added to the silage samples at the rates of 5%, 10% and 15%. No additives were added to the control silages. Silage samples were kept in a dark place for fermentation for 60 days. Each application was prepared in 3 replicates. Silage samples were opened after 60 days and subjected to the following silage quality analyses.

**pH:** 20 grams of mature silage samples were put into the blender together with the wet silage sample and 180 ml of pure water and mixed well. It was then passed through a cloth filter to remove solids and the pH of the filter was determined with a pH meter (AOAC 1990).

**Dry matter rate:** 20 grams of mature silage samples were taken and placed in aluminum containers and dried in drying ovens set at 65 °C until their weight stabilized. The dry matter rate was determined by dividing the initial wet weight (AOAC 1990).

**Ammonia:** The amount of ammonia was determined by applying the titration and distillation stages of the Kjeldahl method, which is used to determine the amount of nitrogen in the silage filter used in pH measurement (AOAC 1990).

**Crude protein rate:** The amount of nitrogen was determined using the micro Kjeldahl method, and the amount of protein was determined by multiplying the amount of nitrogen by 6.25 (AOAC 2003).

**Crude ash rate:** After the samples were kept in the muffle furnace set at 550 °C for 8 hours, the amount of ash was determined by proportioning the remaining amount to the initial amount (AOAC 1990).

**NDF and ADF rate:** It was determined according to the method specified by Van Soest et al. (1991).

**Relative feed value:** %KMS (Dry Matter Digestibility = 88.9 - (0.779 \* % ADF) was determined by using the ADF rate and %KMT (Dry Matter Consumption = 120 / % NDF) was determined by using the NDF rate. By multiplying the KMT and KMS values and dividing by 1.29 NYD value was also determined (Sheaffer et al. 1995).

**Organic Acids:** The amounts of lactic acid, propionic acid, acetic acid and butyric acid, among the silage organic acids were determined using the method specified by De Baere et al. (2013) on the HPLC-DAD device.

Research data were analyzed for variance in the SPSS Statistics 17.0 statistical program, and important factor averages were grouped according to the Duncan test (SPSS, 2008).

## RESULTS AND DISCUSSION

The effects of wheat bran and molasses additives on the pH, dry matter, ammonia, crude protein, crude ash, NDF, ADF, RFV, lactic acid, acetic acid, propionic acid and butyric acid amounts of quinoa silage were determined.

### pH

When the control, molasses additive, wheat bran additive and molasses + wheat bran additive were examined in quinoa silage, pH values were found between 3.90-5.23. The highest pH value (5.23) was detected in quinoa silages without additives. In control silage, Podkowska et al. (2018), Yacout et al. (2021), Güner and Temel (2022) and Salama et al. (2021) determined pH values of 4.13, 4.36, 4.23 and 4.36, respectively, and while these values were found to be higher than the pH values obtained in our current study, the pH value (5.65) determined by Fang et al. (2022) was low. When molasses and wheat bran were added, silage pH values varied between 3.0 and 4.80. Additives caused the pH value of quinoa silage to decrease. The addition of 15% wheat bran and 10% molasses contributed more to the decrease in silage pH. It has been reported that the use of additives such as molasses, wheat bran, and crushed wheat causes significant decreases in the pH value of silages (Qin and Shen 2013, Silva et al. 2014, Bolakar and Yüksel 2021, Fang et al. 2022, Gül 2023). When wheat bran was used at 15% and molasses additive was used at 5%, 10% and 15% rates, silage pH values were found to be between 3.90-4.00 and close to the optimum silage pH values of 3.80-4.20 (Leterme et al. 1992). McDonald et al. (1991) and Limin Kung et al. (2003) stated that molasses, which contains high amounts of water-soluble carbohydrates, causes the pH value of silages to decrease because it accelerates the activity of lactic acid bacteria and prevents the conversion of proteins in the silage to ammonia. The food source of lactic acid bacteria is soluble sugars. Silage materials containing sufficient amounts of sugar cause lactic acid bacteria to multiply and ultimately decrease the pH value of the silage. In silages that do not contain enough sugar, rotting, putrefaction and mold occur in silages due to slow fermentation and pH value not decreasing (Çiftçi et al., 2005; Şakalar and Kamalak, 2016).

### Dry Matter Rate (%)

The dry matter rate in the control silage was determined as 15.43%. In control silages of quinoa, Podkowska et al. (2018), Salama et al. (2021), Pulido Suarez et al. (2019) and Güner and Temel (2022) determined the dry matter rates as 20.93%, 26.89%, 16.9% and 24.39%, respectively, and found them lower than the values in our current study. On the other hand, Fang et al. (2022), the dry matter rate of the control quinoa silage (10.7%) was higher than our current finding. Using increasing amounts of wheat bran, molasses and molasses + wheat bran additives increased the dry matter rate of quinoa silage. If additives were added, dry matter rates varied between 16.93% and 32.20%. The highest dry matter rate (32.2%) was obtained in the application where the highest amount of additives were used (15% M and 15% WB). As a matter of fact, in studies conducted on different plant silages, it was reported that additives such as molasses, wheat bran, broken wheat and cracked barley caused significant increases in the silage dry matter rate (Kordi and Naserian, 2012; Silva et al., 2014; Dumlu Gül et al., 2015; Bolakar and Yüksel, 2021; Fang et al., 2022; Gül 2023).

### Ammonia (%)

The ammonia content of quinoa silage without additives was 11.10%. Podkowska et al. (2018), Salama et al. (2021), Güner and Temel (2022) and Yacout et al. (2021) determined the ammonia rates in quinoa silage as 8.02%, 1.27%, 5.51% and 1.27%, respectively, and these values were found to be higher than the values obtained in our current study. On the other hand, it was found to be lower than the values (22.90%) determined by Fang et al. (2022). When wheat bran additive was used in quinoa silage, ammonia levels were found to be between 4.56-15.06%. While using 5% wheat bran increased the ammonia content compared to the control, adding higher amounts of wheat bran (10% and 15%) to quinoa silage significantly reduced the ammonia content. As a matter of fact, in studies conducted, different researchers determined that when wheat bran additive was used in silages, the ammonia rate decreased compared to the control group (Silva et al., 2014; Qin and Shen, 2013). When molasses additive was used in quinoa silage, ammonia levels were found to be between 5.26-9.20%. Due to the increase in the molasses additive rate, decreases in the ammonia rate were also observed. As a matter of fact, in studies conducted on different plants, it was determined that the addition of molasses caused a significant decrease in the ammonia content of silage (Fang et al., 2022; Gül 2023). When molasses + wheat bran additives were used together, the ammonia levels of quinoa silage were found to be between 2.73-7.86%. The application that reduced the ammonia rate the most in quinoa silage was 10% molasses + 15% wheat bran additives.

### Crude Protein Rate (%)

Crude protein content in quinoa silage without additives was determined as 16.10%. Crude protein values of quinoa silage were determined as 17.60% and 16.67%, respectively, by Güner and Temel (2022) and Fang et al. (2022). These detected values were similar to our current study. On the other hand, crude protein values were determined by Podkowska et al. (2018), Pulido Suarez et al. (2019) and Yacout et al. (2021) as 10.31%, 15.10% and 14.59%, respectively. These values were found to be higher than our current study. When wheat bran additive was used, crude protein rates were found to be between 15.93%-17.60%. The use of increasing amounts of wheat bran has caused a decrease in the crude protein content of silage. As a matter of fact, in studies conducted on different plant silages, many researchers found that using additives such as wheat bran, wheat cracked, barley cracked or barley paste caused a decrease in the crude protein rate (Dumlu Gül et al., 2015; Acar and Bostan, 2016, Gülümser et al., 2019, Gül, 2023). When molasses additive was used, crude protein rates were found to be between 15.80-20.23%. The addition of molasses caused an increase in the crude protein content of quinoa silage. However, the high use of molasses (15%) caused a decrease in the crude protein rate. It has been reported that adding molasses to silages made with different plant species causes decreases in the crude protein rate (Canbolat et al., 2019; Gülümser et al., 2019). This may be related to the crude protein content of the plant. As a matter of fact, it has been determined that adding molasses to the silage of the alfalfa plant, which has a high protein content, causes decreases in the crude protein rate (Acar and Bostan, 2016). On the other hand, some studies have determined that the addition of molasses increases the crude protein rate of silage (Bolakar and Yüksel, 2021; Fang et al., 2022; Gül, 2023). It has been observed that when molasses + wheat bran are used together, crude protein rates vary between 15.96% and 18.76%. While the crude protein content was high in applications with 5% and 10% molasses content, it was observed that there were decreases in the crude protein content due to the increase in the wheat bran content.

**Table 1.** Effects of molasses (M) and wheat bran (WB) additives on quinoa silage quality

Application	pH	Dry matter (%)	Ammonia (%)	Crude protein (%)	Crude ash (%)
Control	5.23 a	15.43 i	11.10 b	16.10 d	27.76 a
5% WB	4.80 b	17.43 i	15.06 a	15.93 d	22.70 c
10% WB	4.10 c	22.06 f	5.43 e-g	17.16 cd	16.90 e
15% WB	4.00 d	25.53 d	4.56 f-i	17.60 b-d	15.46 f
5% M	4.00 d	16.93 i	9.20 c	19.46 ab	24.00 b
10% M	3.90 e	18.76 h	7.90 cd	20.23 a	23.10 bc
15% M	3.90 e	21.93 f	5.26 e-h	15.80 d	22.10 c
5% M x 5% WB	4.00 d	21.13 g	7.86 cd	18.30 bc	19.96 d
5% M x 10% WB	3.90 e	24.13 e	4.00 f-i	18.16 bc	17.13 e
5% M x 15% WB	3.93 e	27.63 c	3.50 hi	15.96 d	14.83 f
10% M x 5% WB	3.90 e	22.40 f	5.76 ef	18.76 a-c	19.36 d
10% M x 10% WB	3.90 e	25.46 d	3.16 i	17.50 cd	17.06 e
10% M x 15% WB	3.90 e	29.63 b	2.73 i	17.53 cd	14.53 f
15% M x 5% WB	3.90 e	25.23 d	6.90 de	16.86 cd	18.93 d
15% M x 10% WB	3.90 e	28.20 c	4.46 f-i	15.96 d	16.73 e
15% M x 15% WB	3.90 e	32.20 a	3.73 g-i	16.23 d	14.46 f
F value and significance	1012.9**	446.2**	61.5**	5.4**	93.1**

\*\*P<0.01<sup>a,b,c</sup> Means within a row with different letters differ by Duncan test.

### Crude Ash Rate (%)

The raw ash rate of silage without additives was determined as 27.76%. This value was determined by Podkowska et al. in studies conducted to determine the crude ash rate in quinoa silage. (2018), Pulido Suarez et al. (2019), Yacout et al. (2021) and Güner and Temel (2022) were found to be higher than the values reported (14.76%, 16.70%, 9.42% and 26.37%). When wheat bran additive was used, crude ash rates were found to be between 15.46-22.70%. The addition of wheat bran caused a decrease in the crude ash content. Studies conducted by some researchers have found that adding wheat bran or crushed wheat to silages reduces the crude ash rate (Kordi and Naserian, 2012; Gül, 2023). When molasses additive was used, crude ash rates were found to be between 22.10-24.00%. The highest value of raw ash rate was obtained with the addition of 5% molasses. The addition of molasses at higher rates (10% and 15%) caused a significant decrease in the raw ash

rate. As a matter of fact, in studies, many researchers have found that when they add molasses to plant silage, it reduces the raw ash rate compared to the control (Şahin, 2019; Gül, 2023). When molasses + wheat bran were used together, crude ash rates were found to be between 14.46-19.96%. All of these values had lower raw ash contents compared to the control quinoa silage without additives.

#### NDF Rate (%)

The NDF rate in quinoa silage without additives was determined as 26.20%. In the studies carried out by Podkowska et al. (2018), Fang et al. (2022) and Güner and Temel (2022) found silage NDF rates of 45.31%, 29.10% and 37.02%, respectively. However, the NDF values determined in the current study were lower than previous studies. When wheat bran additive was used, NDF rates were found to be between 33.13%-34.53%. Wheat bran additive significantly increased the NDF rate of quinoa silage. As a matter of fact, in studies conducted on different plant silages, many researchers have found that the NDF rate is higher than the control group when they use wheat bran, broken wheat and cracked barley as additives (Kordi and Naserian 2012, Gülümser et al. 2019). When molasses additive was used, NDF rates were found to be between 22.86-28.23%. While the addition of 5% molasses did not cause a significant change in the NDF rate, it was determined that the addition of molasses at high rates (10% and 15%) caused a significant decrease in the NDF rate compared to the control silage. As a matter of fact, in studies conducted on silages of some plants, many researchers have found that when they use molasses as an additive, the NDF rate decreases compared to the control group (Bolakar and Yüksel, 2021; Gül, 2023). On the other hand, some studies reported that the addition of molasses caused an increase in the NDF rate (Gülümser et al., 2016; Fang et al., 2022). When molasses + wheat bran were used together, the NDF contents of quinoa silage varied between 26.03% and 30.40%. The lowest NDF rate was obtained when 15% molasses and 5% wheat bran additives were used together.

**Table 2.** Effects of molasses (M) and wheat bran (WB) additives on nutrients and organic acids of quinoa silage

Application	NDF (%)	ADF (%)	RFV	Lactic acid (%)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)
Control	26.20 d-f	17.46 de	268.20 bc	2.94 h	15.33 a	0.14 i	0.013 b
5% WB	33.13 ab	19.83 ab	206.63 ef	5.64 g	12.84 b	0.27 h	0.724 a
10% WB	33.83 a	19.50 a-c	202.93 ef	9.90 f	3.44 c	0.28 h	0.026 b
15% WB	34.53 a	20.56 a	196.43 f	9.68 f	3.34 cd	0.43 g	0.034 b
5% M	28.23 c-e	17.26 de	250.50 b-d	14.22 c	2.78 c-e	0.52 f	0.015 b
10% M	24.90 fg	18.16 b-e	280.46 b	14.37 c	2.61 de	0.82 d	0.033 b
15% M	22.86 g	17.26 de	307.86 a	18.00 a	2.68 c-e	1.52 a	0.018 b
5% M x 5% WB	28.23 c-e	17.86 c-e	247.56 cd	11.16 ef	2.50 e	0.56 f	0.027 b
5% M x 10% WB	30.40 bc	18.56 b-d	227.80 de	10.16 f	2.30 e	0.54 f	0.026 b
5% M x 15% WB	27.96 c-f	17.70 de	252.76 b-d	11.05 ef	2.40 e	0.71 e	0.034 b
10% M x 5% WB	29.46 cd	16.70 ef	239.73 cd	14.23 c	2.69 c-e	0.81 d	0.029 b
10% M x 10% WB	28.73 c-e	14.96 g	249.86 cd	13.74 cd	2.69 c-e	1.04 c	0.025 b
10% M x 15% WB	29.30 c-e	15.46 fg	244.43 cd	12.13 de	2.06 e	1.16 b	0.019 b
15% M x 5% WB	26.03 ef	17.76 c-e	268.43 bc	16.14 b	2.41 e	1.19 b	0.030 b
15% M x 10% WB	28.30 c-e	18.16 b-e	245.53 cd	13.34 cd	2.19 e	1.12 bc	0.038 b
15% M x 15% WB	28.73 c-e	17.26 de	244.36 cd	13.48 cd	2.18 e	1.17 b	0.034 b
F value and significance	10.1**	7.2**	9.4**	38.9**	282.9**	212.0**	352.3**

\*\*P<0.01<sup>a,b,c</sup> Means within a row with different letters differ by Duncan test.

#### ADF Rate (%)

In the control application of quinoa silage, ADF rates were determined as 17.46%. Silage ADF value was determined by Podkowska et al. (2018), Fang et al. (2022), Güner and Temel (2022) found it to be 34.24%, 20.50% and 24.40% lower, respectively, while Güner and Temel (2022) found it to be 17.60%, close to the values in the current study. When wheat bran additive was used, ADF rates were found to be between 19.50%-20.56%. Wheat bran caused an increase in the ADF rate of quinoa silage. As a matter of fact, some studies have stated that ADF rates increased compared to the control when wheat bran, wheat cracked and barley crushed additives were used in plant silages (Çiftçi et al. , 2005; Kordi and Naserian, 2012; Gülümser et al., 2019). When molasses additive was used, ADF rates were found to be between 17.26-18.16%. The lowest value of the ADF rate was detected in the M 5% x WB 0% and M 15% x WB 0% groups (17.26%). Molasses addition did not cause a significant change in the ADF content of quinoa silage. Some studies have found that when molasses additives are used in silage plants, ADF rates decrease compared to the control group (Şahin, 2019; Bolakar and Yüksel, 2021). Some



studies have found that when molasses additive is used in silages, it increases ADF rates compared to the control (Gülümser et al., 2019; Fang et al., 2022). When molasses + wheat bran additives were used together, ADF rates were found between 14.96-18.56%. The lowest ADF rate (14.96%) was obtained when 10% molasses and 10% wheat bran were applied together as additives.

#### Relative Feed Value

The relative feed value of quinoa control silage was determined as 268.20. Dumlu Gül et al. (2015), Acar and Bostan (2016), Canbolat et al. (2019), Bolakar and Yüksel (2021) and Gül (2023) determined the relative feed value of quinoa silage as 132.10, 143.19, 155.52, 61.53 and 92.78, respectively. When wheat bran additive was used in quinoa silage, relative feed values were found between 196.43-206.63. The highest relative feed value was determined in the 5% wheat bran application, and the lowest was determined in the 15% wheat bran application. The addition of wheat bran reduced the RFV value of silage. As a matter of fact, in studies conducted on different plant silages, many researchers found that when they used wheat bran, cracked barley and barley paste as additives, RFV was lower than the control group (Dumlu Gül et al., 2015; Acar and Bostan, 2016; Gül, 2023). In case of using molasses additive, relative feed values were found between 250.50-307.86. While 5% molasses application did not cause a significant change in the RFV value compared to the control silage, increased molasses application caused the RFV value to increase. It has been reported that molasses addition increases the RFV value of silage (Dumlu Gül et al., 2015; Acar and Bostan, 2016; Canbolat et al., 2019, Bolakar and Yüksel, 2021; Gül, 2023). When molasses + wheat bran were used together, the RFV values of silage were found to be between 227.80-268.43. In all combinations of molasses + wheat bran application, RFV value was lower than the control. This may be due to the fact that molasses increases the RFV value and wheat bran decreases the RFV value.

#### Lactic Acid Rate (%)

Lactic acid rates in quinoa silage were determined as 2.94 in the control group. While the lactic acid values of quinoa silage were found to be high in some studies based on the values obtained in the current study (Podkowska et al., 2018; Güner and Temel, 2022), in some other studies the lactic acid values were found to be low (Salama et al., 2021; Yacout et al., 2021; Fang et al., 2022). When wheat bran additive was used in quinoa silage, lactic acid rates were found to be between 5.64-9.90%. The addition of wheat bran caused a significant increase in lactic acid content. Application of wheat bran more than 10% did not provide an additional increase in lactic acid content. For this reason, it has been determined that the application of more than 10% of wheat bran is unnecessary. In studies conducted on different plant silages, many researchers have found that when they use wheat bran and barley paste as additives, there are increases in the lactic acid rate compared to the control group (Qin and Shen, 2013; Acar and Bostan, 2016; Gül, 2023). When molasses additive was used, lactic acid rates were found between 14.22-18.00%. The addition of molasses provided a significant increase in the lactic acid content. This increase was greater than the addition of wheat bran. In studies, many researchers have found that when they add molasses to plant silage, the lactic acid rate increases compared to the control (Acar and Bostan, 2016; Canbolat et al., 2019; Fang et al., 2022). It is estimated that the use of easily soluble carbohydrate content of molasses by silage microorganisms causes the increase in lactic acid content. When mixtures of molasses + wheat bran in different proportions were added to quinoa silage, lactic acid rates were found to be between 10.16-16.14%. Lactic acid rates were found to be higher in all molasses + wheat bran mixture rates compared to the control silage. The highest lactic acid rate was obtained in the application of 15% molasses + 5% wheat bran.

#### Acetic Acid Rate (%)

Acetic acid rates in quinoa silage were determined as 15.33% in the control group. Podkowska et al. (2018), Salama et al. (2021), Yacout et al. (2021), Fang et al. (2022) and Güner and Temel (2022) found acetic acid values of 0.37%, 3.06%, 3.06%, 7.08%, 8.54%, respectively, and they were found to be higher than the values obtained in the current study. When wheat bran additive was used in quinoa silage, acetic acid levels were found to be between 3.34-12.84%. It was determined that 5% wheat bran was not sufficient to reduce the acetic acid rate in quinoa silage, and the addition of 10% wheat bran was appropriate to reduce the acetic acid rate. Application of wheat bran more than 10% did not provide an additional reduction in acetic acid. In studies conducted by some researchers, they found that adding wheat bran to silages reduced the acetic acid rate compared to the control group (Qin and Shen, 2013; Silva et al., 2014). When molasses additive was used, acetic acid rates were found to be between 2.61-2.78%. The addition of molasses to quinoa silage caused a significant decrease in the acetic acid content. The addition of high amounts of molasses did not cause an additional increase in the acetic acid content. For this reason, it was determined that the addition of 5% molasses was sufficient to reduce the acetic acid

content. Some studies have reported that using molasses additives in silages causes a decrease in the acetic acid rate (Canbolat et al., 2019; Fang et al., 2022). When molasses + wheat bran additives were used together at different rates, acetic acid rates were found to be between 2.06-2.69%. Compared to the control silage, the acetic acid rates obtained in all molasses + wheat bran applications mixed in different proportions were lower.

#### Propionic Acid Rate (%)

Propionic acid rates in quinoa silage were determined as 0.14% in the control group. In some studies, the propionic acid rates of quinoa silage were found to be lower (Fang et al., 2022; Güner and Temel, 2022). Compared to the control silage, the use of wheat bran and molasses additives increased the propionic acid rates. The largest increase rate was detected in the 15% molasses application. A study reported that adding molasses to forage pea silage increased the propionic acid content (Canbolat et al., 2019).

#### Butyric Acid Rate (%)

Butyric acid content in the control silage of quinoa was determined as 0.013%. In some studies, butyric acid rates of quinoa silage were found to be lower (Salama et al., 2021; Yacout et al., 2021; Fang et al., 2022; Güner and Temel, 2022). The addition of molasses, wheat bran and mixtures of both at different rates (except 5% wheat bran) to quinoa silage did not cause a significant change in the butyric acid rate compared to the control silage.

#### CONCLUSION

Molasses was used as an additive to increase the carbohydrate content in quinoa plant silage, and wheat bran additive was used to increase the dry matter content. Molasses used as an additive increased the dry matter, ADF, RFV, lactic acid, butyric acid and propionic acid values compared to the control group; It has also been found to reduce pH, ammonia, NDF, crude ash, crude protein and acetic acid values. It was determined that wheat bran as an additive increased the dry matter, crude protein, ADF, NDF, lactic acid and propionic acid values in quinoa silage, and decreased the pH, ammonia, RFV, crude ash, acetic acid and butyric acid values. It has been determined that molasses + wheat bran as an additive increases the dry matter, lactic acid, propionic acid, NDF and butyric acid values in quinoa silage, while it decreases the pH, crude protein, ammonia, ADF, crude ash, acetic acid and RFV values. When the research data were examined, molasses used as an additive in quinoa plant silage contributed to the increase in the carbohydrate content of the silage, resulting in the improvement of silage quality. On the other hand, it has been determined that the addition of wheat bran increases the dry matter level. It was concluded that adding 5% molasses and 15% wheat bran into the quinoa silage would be sufficient to obtain good quality quinoa silage.


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## New Record of the *Melanopsis buccinoidea* (Olivier, 1801) from two streams of Mersin (Türkiye) with Evaluation Some Morphometric Parameters

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### ABSTRACT

In this study, some biometric characteristics of the freshwater snail *Melanopsis buccinoidea* (Olivier, 1801) were determined in Koca Stream and Bayat Stream (Mersin, Türkiye). Weight (W) (g), shell length (SL) (mm), shell width (SW) (mm), aperture length (AL) and width (AW) (mm) measurements were made on the samples taken from different stations. Morphological relationships were determined by multivariate statistical evaluation. The highest values in terms of shell length and weight were determined to be  $16.53 \pm 3.67$  mm and  $0.47 \pm 0.23$  g, living in Koca Stream. The largest shell length measured in the samples was 21.93 mm and the aperture width was 10.62 mm. The results were analyzed using Principal Component Analysis (PCA), a versatile statistical technique employed to explore the relationships between variables and account for a significant portion of the dataset. Samples taken from different stations showed a strong relationship between aperture width and spir height.

**Key words:** *Melanopsis buccinoidea*, biometric, freshwater snail.

## Mersin (Türkiye) İki Çayından *Melanopsis buccinoidea* (Olivier, 1801)'nin Bazı Morfometrik Parametrelerin Değerlendirilmesiyle Yeni Kaydı

### ÖZ

Bu çalışmada, Koca Deresi and Bayat Dere'sinde (Mersin, Türkiye) yaşayan tatlı su salyangozu *Melanopsis buccinoidea*'nın (Olivier, 1804) bazı biyometrik özelliklerini incelenmiştir. Farklı istasyonlardan alınan örneklerde ağırlık (A) (g), kabuk genişliği (KG) (mm), kabuk yüksekliği (KY) (mm), apertür uzunluğu (AU) ve genişlik (AG) (mm) ölçümleri yapılmıştır. Çok değişkenli istatistiksel değerlendirme ile morfolojik ilişkilerin belirlenmiştir. Kabuk uzunluğu ve apertür ağırlığı açısından en yüksek değerler  $16.53 \pm 3.67$  mm ve  $0.47 \pm 0.23$  g olarak Koca Deresi'nde yaşayanlar olduğu belirlenmiştir. Örneklerde ölçülen en büyük kabuk uzunluğu 21.93 mm ve apertür genişliği 10.62 mm olarak ölçülmüştür. Sonuçlar, değişkenler arasındaki ilişkileri araştırmak ve veri kümesinin önemli bir bölümünü açıklamak için kullanılan çok yönlü bir istatistiksel teknik olan Temel Bileşen Analizi (TBA) kullanılarak analiz edilmiştir. Farklı istasyonlardan alınan örneklerde apertür genişliği ve spir yüksekliği arasında güçlü bir ilişki olduğunu göstermiştir.

**Anahtar kelimeler:** *Melanopsis buccinoidea*, biometrik, tatlısu salyangozu.

### INTRODUCTION

Snails in the Prosobranchia subclass are distributed in various fresh and brackish waters, mostly in the sea. They live depending on aquatic systems and do not tolerate different ecological systems (Yıldırım, 1999). Unlike Pulmonates, they do not prefer stagnant water and are indicators of clean water. Despite this, they have

been observed to live in various habitats such as canals, pools, streams and lakes (Farahnak et al., 2006; Gürlek et al., 2012).

Germain (1921) performed one of the first studies on the *Melanopsis* genus and examined the terrestrial and freshwater molluscs of Syria (Bilgin, 1980). The *Melanopsis* genus is found in large quantities in aquatic ecosystems in the Middle East and is distributed in northern Africa and Europe (Por, 1963; Schütt, 1983). The majority of this genus contains many species and lives in the Mediterranean (Tchernov, 1971; Brown and Wright, 1980). In addition, it has been observed that they have a wide distribution and subspecies because they easily adapt to different ecological regions. In Türkiye, it is distributed in the Aegean, Mediterranean and Southeastern Anatolia regions (Yıldırım, 1999; Gürlek et al., 2019). With their strong radula teeth, they scrape algae and diatoms from all hard surfaces and use them as food. Apart from these, they also feed on animal carcasses, macrophytes and various aquatic plants (Glaubrecht, 1996; Mouahid et al., 1996). They are also intermediate hosts of several trematode species, as well as some other gastropod species (Bilgin, 1967). Farahnak et al. (2006) examined *Melanopsis* species (*M. doriae*, *M. costata*, *M. preamorsa* and *M. nodosa*) parasitically and observed that 72 of a total of 2266 samples (3.1%) were infected with parasites belonging to Cercaria.

Nägele (1901) reported the existence of *Melanopsis buccinoidea* species from Seyhan River (Adana, Türkiye). Sturany (1905) identified the *M. buccinoidea* species belonging to the freshwater Molluscs in Kayseri Erciyes Mountain and its surroundings. Kinzelbach (1989) reported the presence of *Melanopsis p. buccinoidea*, *Melanopsis p. costata* in the Middle East (Türkiye-Syria). Heller et al. (1999) studied the systematics and distribution of *Melanopsis* in the central and northern regions of the Jordan Valley, recognizing *M. buccinoidea* based on shell morphology and biometric measurements. Gürlek et al. (2012) determined morphometric characteristics of *M. buccinoidea buccinoidea* in the Kumaşır lake (Kahramanmaraş, Türkiye) and forms the dominant populations in the lake. Gürlek et al. (2016) reported presence of *M. buccinoidea* from Limonlu (Lamos) Stream (Mersin, Türkiye). Bilgin and Şeşen (1991) studied distribution of some freshwater molluscs in Mersin, Adana and Antakya regions. This study is the first scientific report on the population of *M. buccinoidea* (Olivier, 1801) living in Koca Stream and Bayat Stream (Mersin, Turkey) and some of its biometrics characteristics.

## MATERIALS AND METHODS

Samples were collected from Koca Stream (36,726386-34,399936 North/East) and Bayat Stream (36,116333-32 N, 977681 E) between August and September 2024 (Figure 1).



Figure 1. Sampling area.

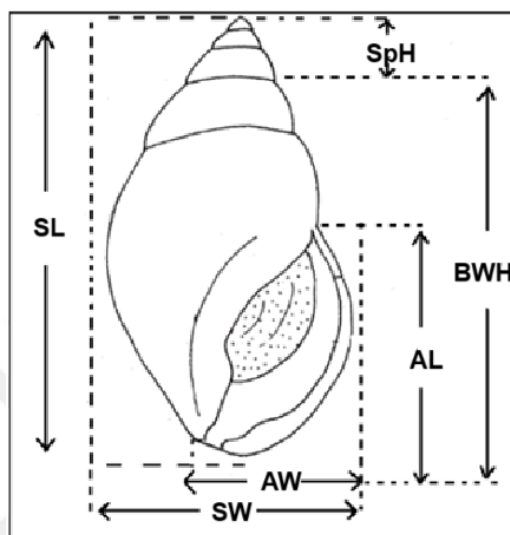
To collect the samples, metal-framed scoops and shovels, a rake for bottom dredging were used, individuals were collected by hand from sand and areas up to 1 m water depth, and the samples were stored in styrofoam boxes at +4°C. Identification of the collected snails were carried out by comparing them with

descriptions and drawings of different *Melanopsis* species available in the literature (Gürlek et al., 2016) (Figure 2).



**Figure 2.** *Melanopsis buccinoidea* collected from Koca Stream and Bayat Stream

Shell measurements (SL, SW, AL, AW, BWH, SpH) were made with a digital caliper ( $\pm 0.01$  mm), and weight measurements were made with a precision scale ( $\pm 0.001$  g) (Figure 3).



**Figure 3.** Morphometric measurement of *Melanopsis buccinoidea* (Gürlek et al., 2012). shell length (SW) (mm), shell width (SW) (mm), aperture length (AL) and width (AW) (mm), weight (W) (g), spir height (SpH), body whorl height (BWH)

Correlation analysis was used to determine the relationships between biometric parameters, and Microsoft Excel® was used to analyze and process the data. Principal component analysis (PCA) was applied using the Past 4.03 program to determine the relationships between variables.

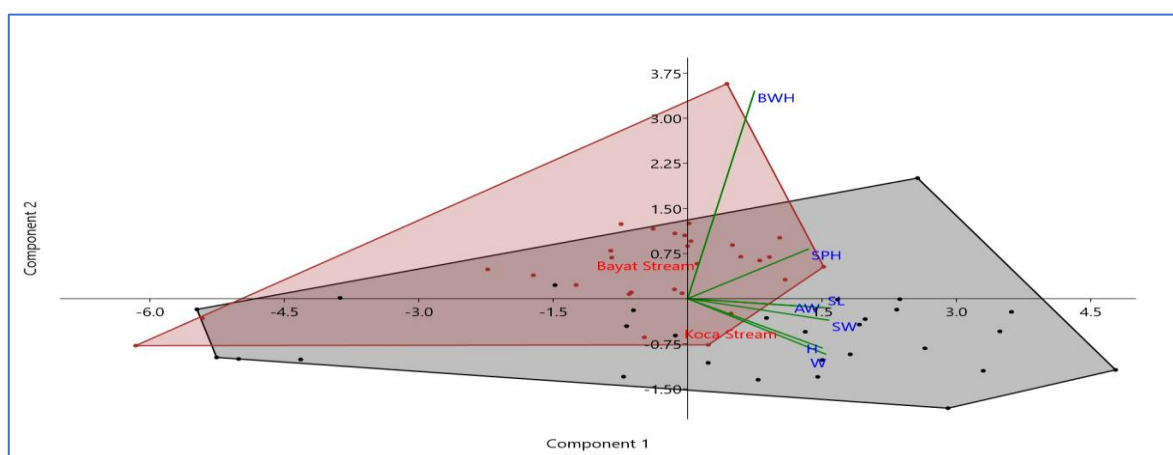
## RESULTS AND DISCUSSION

In this study, the presence of *M. buccinoidea* in Koca Stream and Bayat Stream was reported for the first time. Phenotypic variations in shell morphology make species identification highly challenging in many taxa, with

similarities in shell characteristics often leading to the oversight of new species in research. Understanding the malacofauna of Koca Stream and Bayat Stream is crucial for gaining insights into Türkiye's freshwater snail fauna and zoogeographic distribution.

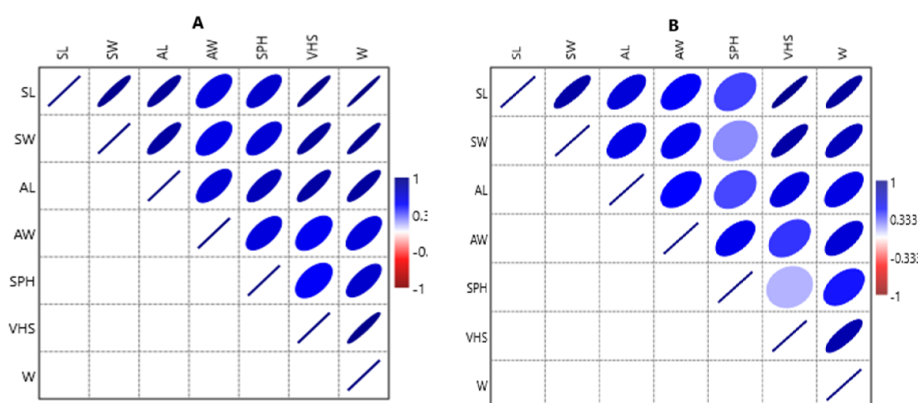
Heller et al. (2005) reported the maximum SL and AL of *M. buccinoidea* as 35.1 mm and 17.8 mm in the inland waters of the countries of east coast of the Mediterranean. Gürlek et al. (2012) determined morphometric measurements of *M. buccinoidea buccinoidea* individuals collected from Kumaşır Lake and reported the means of SL, SW, AL, AW, BWH, SPH and W; 25.95 mm, 12.18 mm, 14.13 mm, 8.02 mm, 18.47 mm, 8.01 mm and 2.05 g, respectively. They also reported the maximum shell length and aperture height of *M. buccinoidea* as 36.94 mm and 20.73 mm, respectively. In the current study, the mean of SL, SW, AL, AW, BWH, SPH and W for Koca Stream and Bayat Stream were assessed as  $16.53 \pm 3.67$  and  $15.54 \pm 2.29$  mm;  $7.41 \pm 1.47$  and  $6.81 \pm 0.87$  mm;  $8.10 \pm 2.11$  and  $7.36 \pm 1.19$  mm;  $4.86 \pm 1.06$  and  $4.60 \pm 0.76$  mm;  $1.32 \pm 0.54$  and  $1.88 \pm 0.57$  mm;  $15.22 \pm 3.35$  and  $13.66 \pm 2.02$  mm;  $0.47 \pm 0.23$  and  $0.33 \pm 0.10$  g, respectively. The maximum shell length is 21.93 mm and aperture height is 10.62 mm.

PCA indicated a strong relationship between SL, SW, BWH and W (Figure 4). In gastropods, these morphometric traits may change with potentially highlighting the role of underlying biological and ecological influences on both shell structure and body size (Akça Atıl et al., 2024).



**Figure 4.** Biplot of Principal Component Analysis (PCA) analysis of the shell characteristics (SL, SW, AL, AW, SPH, BWH) and weight (W) of *M. buccinoidea*.

Gürlek et al. (2012) demonstrated a strong correlation between SL-SW and SW-W of *M. buccinoidea buccinoidea* individuals collected from Kumaşır Lake. In this study, there was a strong correlation between SL-SW, SL-AL, SL-BWH, SL-W, SW-AL, SW-BWH, SW-W, AL-BWH and BWH-W in Koca Stream and Bayat Stream (Figure 5).



**Figure 5.** Correlation matrix of the shell characteristics (SL, SW, AL, AW, SPH, BWH) and weight (W) of *M. buccinoidea* in A) Koca Stream, B) Bayat Stream.



## CONCLUSIONS

Consequently, Koca Stream and Bayat Stream are new locality records for *M. buccinoidea* in Türkiye. The morphological differences observed across various stations are likely due to factors such as nutrition, water chemistry in the habitats, and water temperature with a high potential for sub speciation or even speciation. Anatolia exhibits high diversity and endemism, especially among mollusks. Therefore, further molecular taxonomy studies on the collected materials will reinforce our findings and help clarify the status of the genus in Türkiye, while also exploring the possible causes of morphological variations.

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## Declaration of interests


The authors declare that they have no conflict of interest.

## Author Contributions

**Mehmet KOCABAŞ:** Conceptualization; investigation; methodology; writing—review and editing.

**Filiz KUTLUYER KOCABAŞ:** Conceptualization; data curation; formal analysis; investigation; methodology; software; writing— original draft; writing—review and editing.

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## Legal Framework Through Meta-Analysis For Environmental Sustainability

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### ABSTRACT

Despite of being only species, which know the fact that their existence without natural environment is close to impossible, human still places as detrimental species that causes environment the most. The industrial revolution, together with advances in science and technology, urbanisation and population growth, have led to irreversible damage to the habitable environment in the process of meeting various needs, especially for energy and food. The growing impact of environmental problems have brought the “right to the environment” issue to the light. This right, which aims to ensure the sustainable protection of the environment, is recognised as a fundamental human right within the scope of “third generation rights”. The absence of spatial and temporal limitations on the right to the environment distinguishes it from other human rights. Its beyond-time features limits means that it belongs to both present and future generations. Similarly, the absence of spatial limitations means that the rights and obligations arising from environmental degradation or the threat thereof cannot be confined within national administrative boundaries. This study examines the meaning, function, types and scope of legal personality and emphasises the need to create a new type of legal personality specifically designed for natural persons. This new legal framework is proposed as a more effective tool for the protection of natural assets.

**Key words:** Naturel assets, conservation, legal personality, right of nature, rights of nature

### Çevresel Sürdürülebilirlik İçin Meta-Analiz Yoluyla Yasal Çerçeve

#### ÖZ

Doğal çevreden bağımsız olarak varlığını sürdürmesinin mümkün olmadığını “bilen” yegâne varlık olmasına rağmen ona en fazla zarar veren varlık da yine insanoğlunun kendisidir. Sanayi Devrimi ile bilim ve teknolojiye ilerlemeler, şehirleşme ve artan nüfus gibi nedenlerden doğan, başta enerji ve gıda olmak üzere farklı ihtiyaçların karşılanması süreci, yaşanılabilir çevreye geri döndürülemez zararlar vermektedir. Çevresel sorunların etkilerinin artması, “çevre hakkını” ön plana çıkarmıştır. Bu hak, çevrenin sürdürülebilir şekilde korunmasını amaçlayan bir üçüncü kuşak temel insan hakkıdır. Çevre hakkının yersel ve zamansal bir kısıtlamaya bağımlı olmaması, onu, diğer insan haklarına nazaran farklı bir noktaya taşımaktadır. Zamansal kısıtlamaya tabi tutulmaması, onun şimdiki ve gelecekteki insanlara ait olması anlamına gelmektedir. Yersel sınırlandırmalara sığmamasının bir sonucu olarak da çevrenin tahribi veya tahrip tehlikesi durumunda ortaya çıkan hak ve yükümlülükler yalnızca ulusal idari sınırlarla tayin edilememektedir. Bu çalışmada, hukuki kişiliğin anlamı, işlevi, türleri ve kapsamı incelenerek doğal varlıkların korunmasında çok daha elverişli bir araç olarak görülen “hukuki kişiliğin” bu varlıklara özel yeni bir türünün oluşturularak kullanılması gerektiği üzerinde durulmuştur.

**Anahtar kelimeler:** Doğal varlıklar, koruma, yasal kişilik, doğa hakkı, doğanın hakları

## INTRODUCTION

An anthropocentric approach posits that “the environment can be sacrificed for the benefit of humans” or that “the environment should be preserved solely for the purposes of human interests.” (Kayaer, 2013). However, in recent years, with the recognition that no natural entity is superfluous or infinite, there has been a shift toward the conservation of natural resources on the grounds of sustainability. At this point, the necessity arises of overcoming the detrimental effects of the anthropocentric perspective on natural resources and establishing an effective legal framework to ensure their protection. Within this context, the concept of legal personhood provides a framework for safeguarding the rights of nature. The combination of these two concepts offers an innovative pathway toward sustainable environmental protection. From a practical standpoint, this approach aims to sustain the services that natural resources provide to human existence. Such resources are of vital importance and non-transferable in terms of ensuring human survival. However, it is impossible to predict the future importance of a non-human species currently deemed insignificant or even viewed as unworthy of protection due to its looming extinction for human life. This unpredictability accentuates the need for a holistic attitude toward natural entities, one that can be summarized as follows: “The preservation of a holistic environment of which humans are merely one integral part is ultimately to humanity’s benefit, as humans themselves are a complementary component of this whole.” In this regard, it is suggested that attitudes towards natural entities be framed by a more integrative approach. The emphasis is on protecting the environment as a whole, rather than focusing on merely human-centered benefits. Such a shift not only aligns with ecological sustainability but also highlights the intrinsic value of all natural entities beyond their immediate utility to humans.

Regardless of which approach is adopted, there is no dispute regarding what constitutes the entities encompassed by the term “environment” or the necessity of their protection. The central issue revolves around determining the most effective method to achieve this protection. In this context, rather than perpetuating the punishment-oriented conservation methods that dominate today’s practices, the granting of legal personhood to natural entities is proposed as an alternative. Under the current legal system, the law centers exclusively on human interests. A natural entity is only protected when harm to it results in a loss or injury to humans. Therefore, the entity being protected by the law is not the natural entity itself, but rather the human entity that benefits from it. In other words, the preservation of such an entity is inherently dependent on the existence of a human beneficiary who derives utility from it. Secondly, in cases where harm is inflicted upon a natural entity, the scope of the penalty imposed on the perpetrator is determined not by the damage inflicted upon the entity itself, but by the harm incurred by the human beneficiaries. Ultimately, the beneficiary of the sanction applied to the perpetrator is not the natural entity itself but the humans who utilize and benefit from it. In contrast, the proposal to grant legal personhood to natural entities rests on the principle that every entity, by virtue of its inherent significance within a holistic framework, warrants legal protection even if there is no direct relationship between the entity and any human beneficiary. This approach shifts the focus from anthropocentric utility to the intrinsic value of natural entities, recognizing their importance within the broader ecological system and emphasizing their right to legal safeguarding independent of their service to humanity.

In legal terms, entities are classified as either persons (subjects) or objects (things). In cases where personhood is not granted, entities are regarded as objects. Legal personhood, however, is divided into two categories: “natural persons” and “legal persons” (Ustahaliloğlu, 2022). Natural persons refer to individuals, while legal persons encompass legally recognized organizations such as corporations, associations, and foundations.

Legal personhood states to the recognition of an entity by the legal system as a holder of rights and obligations. For instance, the granting of legal personhood to the Whanganui River in New Zealand obliges as a prominent example supporting this approach (Boyd, 2020). Legal personhood, nevertheless, is no longer solely exclusive to humans and juridical entities; it is progressively being consumed as a mechanism for the protection of natural entities. Addressing nature’s rights under the framework of legal personhood permits natural entities to claim rights on their own behalf and facilitates the establishment of effective protection mechanisms. This approach redefines the legal status of natural entities, shifting them from being mere objects that serve human interests to autonomous entities with rights of their own.

Within this context:

**Natural Personhood:** Discusses to the legal personhood of individuals. It permits individuals to hold rights and participate in legal transactions.

**Juridical Personhood:** Indicates to the legal personhood of non-human entities such as corporations, associations, and foundations. Juridical personhood permits these entities to own property, initiate legal proceedings, and enter into contracts.

The development of new forms of legal personhood necessitates diverse approaches. For instance, Boyd (2020) establishes a connection between granting legal personhood to natural entities and advancing environmental sustainability. Kaufmann and Sheehan (2019), instead, discuss that recognizing legal personhood for natural entities has the potential to transform environmental protection policies primarily.

In the literature, research on granting legal personhood to natural entities remnants limited, necessitating broader conceptual discussions. Particularly after 2020, studies on the effects of legal personhood for natural entities on environmental sustainability have started to fill a critical gap. For instance, Stucki (2020) focuses the practical challenges faced in granting legal personhood to the Ganges and Yamuna Rivers, whereas Kaufmann and Sheehan (2019) debate how legal personhood can bring about a paradigm shift in environmental protection policies. Meta-analysis can contextualize the findings from such studies within a broader framework (Boyd, 2020). Juridical personhood grants objects the rights to own property, enter into contracts, and initiate legal proceedings. Modern legal theory interprets juridical personhood as an extension of individual rights (Kurki, 2019). Accordingly, while a legal system harmonies personhood to an entity under its jurisdiction, it transfers that entity from the “realm of objects” to the “realm of persons.” This recognition not only guarantees the protection of natural entities’ rights but also promotes a hidden understanding of their ecological roles (Boyd, 2020). Such a transition can also be characterized as a form of promotion. Being granted personhood equates to possessing “unrestricted authority over objects” and is a requirement for assigning “rights” or “obligations” to an entity. According to legal frameworks, only Tekinay and Ayan (Tekinay, 1992; Ayan, 2016) can oblige as bearers of rights and obligations under the law. As a result, persons can pursue lawsuits and take legal action to safeguard their interests, with the protection of these interests enforced by public authority. Conversely, objects that lack legal personhood can only be protected if their preservation concerns a legally recognized person who demands such protection. In summary, if no person is directly affected or no stakeholder wishes to benefit from the protection, legal safeguards for objects cannot be enforced. This limitation underscores the importance of granting legal personhood to natural entities to ensure their rights are preserved irrespective of their utility to humans or human intervention. Through such recognition, the focus shifts from anthropocentric benefit to the intrinsic value of nature itself.

Before the recognition of "juridical" legal personhood, only certain individuals among all being whether human or non-human, tangible or intangible were endowed with this status. Over time, this status was extended to include individuals who had previously been excluded, such as family members outside of the patria potestas in Roman law, women, the elderly, children, and individuals with congenital disabilities. As a result, all living humans, with the exception of fetuses, eventually became entitled to the benefits of legal personhood. In addition to humans, entities that are neither human nor possess a physical form have also been granted legal personhood. Consequently, new entities with legal personhood have emerged under classifications such as "state," "corporation," "association," and "foundation." This development reflects the evolving understanding of personhood and its application beyond a strictly human-centered framework, establishing non-human entities as subjects of legal rights and obligations.

In addition to widely accepted entities with legal personhood, certain minority practices have extended this status to non-human entities with physical forms, such as ships and Hindu idols (Smith, 1914), as well as, on occasion, to rivers (Suykens, 2019), animals (Bevilaqua, 2019), and even nature as a whole (Kauffman, 2018). The most significant finding from these practices is that protecting the inherent interests of an entity itself is far more effective than safeguarding the interests of individuals who own or benefit from that entity. This point is illustrated by a fundamental dilemma: what happens when an entity that is not owned by any individual, yet is recognized as requiring protection, suffers harm? To address this dilemma, the "rights of nature" theory was proposed. According to this theory, the protection of natural entities is not solely tied to ownership; instead, the interests of current or future human populations may justify and necessitate the safeguarding of these natural entities. This turns the focus from ownership-based protections to recognizing the intrinsic value and rights of the natural world.

## MATERIALS AND METHODS

Meta-analysis is a technique that combines the results of independent studies on a specific topic toward provide a broad evaluation (Tyler & Last, 1992). This method suggestions the advantage of synthesizing a broad range of literature to present general trends and insights (Smith & Taylor, 2022). It is predominantly useful for examining complex and interdisciplinary topics, such as environmental sustainability and legal frameworks (Brown & Miller, 2023). According to Smith and Taylor (2022), meta-analysis not only participates the findings of

individual studies but also uncovers contextual differences between them. The reason for employing this method in the study was to deliver a comparative evaluation of the existing literature on legal personhood for natural entities. Conversely, the reliability of the results found through this method is directly tied to the diversity of the data used and observance to methodological standards.

The primary objective of selecting the meta-analysis method was to categorize the general trends and shared outcomes in existing studies on the legal personhood of natural entities. This approach targets to compare the findings of different studies, present a broad perspective, and highlight areas in the literature that require further investigation (Smith & Taylor, 2022).

The data comprised in the study were initially sourced from international academic databases such as Web of Science (WoS) and Scopus. Key terms such as “legal personhood,” “natural entities,” “environmental law,” and “meta-analysis” were used during the search process. Data selection criteria included studies published after 2020, those appearing in peer-reviewed journals, and those presenting concrete findings related to environmental law.

The collected data were examined using fixed-effects and random-effects models. Although the fixed-effects model emphasizes on homogeneous effects within the studies, the random-effects model allows the evaluation of heterogeneity. These methods were used together to improve the reliability of the meta-analysis and confirm the generalizability of the results (Brown & Miller, 2023).

Additionally, statistical methods like Funnel Plots and Egger's tests were used to identify potential issues with publication bias. These tests helped detect systematic errors in the dataset and increased the accuracy of the findings. The core issue addressed in this study pertains to whether nature itself should be authentically recognized as possessing rights and whether respect should be granted to “the rights of nature” or “the rights belonging to nature.” The hypothesis of the study is that granting legal personhood to natural individuals would support environmental protection mechanisms and encourage a more sustainable approach within environmental legal systems (Stone, 2019).

### Purpose of the Research

This study objects to appraise the general trends in research directed on themes like environmental law and the rights of nature (Stone, 2019; Boyd, 2020). The central hypothesis of the study theorizes that granting legal personhood to natural entities will reinforce environmental protection mechanisms and contribute to the development of a more sustainable environmental legal system. Within this context, the study pursues to address the subsequent questions:

- To what extent can granting legal personhood to natural entities enhance existing environmental protection policies?
- What new mechanisms can legal personhood activate in the preservation of natural entities?
- What are the long-term implications of this approach for environmental sustainability?

Practical examples show both the successes and limitations of granting legal personhood to natural entities. For instance, the recognition of legal personhood for Colombia's Atrato River has raised greater inclusion of local communities in decision-making processes and produced positive outcomes for the preservation of the region's biodiversity (Vélez, 2018). Still, it is highlighted that for such practices to be stretched effectively, it is necessary to strengthen the associated legal infrastructure.

By exploring these examples and addressing the critical questions, the study objects to provide insights into how legal personhood can reshape the framework of environmental protection and contribute to achieving broader sustainability goals.

## RESULTS AND DISCUSSION

### General Effect Sizes

The total effect sizes designed in the meta-analysis are abridged below according to the fixed-effects and random-effects models: The average effect size was designed as 19.45 using the fixed-effects model and 2.73 using the random-effects model. These findings demonstrate how the impact of environmental regulations can vary across different regions. The relatively lower effect size in the fixed-effects model may be credited to the evaluation of environmental regulations within specific contexts. For example, the energy sector reforms under the European Green Deal have fashioned consistent outcomes in certain countries (European Commission, 2021). Conversely, the higher effect sizes observed under the random-effects model reflect the varied impacts of such regulations across different social, economic, and legal contexts. Notably, renewable energy investments in countries like India and China are cited as primary drivers of these differences (IEA, 2021). In addition to these findings, it is critical to present the temporal variations in the meta-analysis data through visual aids such as graphs, which can highlight annual distributions and trends. Understanding the reasons behind these temporal

changes necessitates a focus on both national and international developments. For example, the recognition of legal personhood for New Zealand's Whanganui River established an innovative legal mechanism for its preservation (Boyd, 2020). Similarly, granting legal personhood to the Ganges and Yamuna Rivers in India represented a significant step toward protecting environmental rights, though challenges related to representation and implementation were encountered (Stucki, 2020). The reasons underlying these yearly variations can also be linked to political, social, and economic factors influencing the development of environmental law. For instance, Kaufmann and Sheehan (2019) argue that to better comprehend the impacts of changes in environmental protection policies, both fixed-effects and random-effects models should be considered together. Moreover, the notable increase observed after 2020 could be associated with the renewed prominence of the Paris Climate Agreement, the European Green Deal, and heightened societal discussions on the environmental impacts of the COVID-19 pandemic (Yalçın et al., 2021). The results of the study reveal that the effects captured through meta-analysis vary across years. Visualization of yearly distributions and trends through graphs provides a clearer understanding of the findings (Kaufmann & Sheehan, 2019). This analysis underscores the need to interpret the interplay between evolving environmental regulations and broader socio-political dynamics over time.

The findings from the meta-analysis subsidize to environmental sustainability policies in various ways: Strengthening Renewable Energy Policies:

- The meta-analysis outcomes highlight the impact of granting legal personhood to the Ganges and Yamuna Rivers in India on renewable energy investments.
- The case of New Zealand's Whanganui River validates the significance of involving local communities in decision-making processes for the sustainable management of water resources.

Influence on International Environmental Policies:

- When paralleled with the examples analyzed in the meta-analysis, the environmental regulations under the European Green Deal offer insights into how granting legal personhood could shape international environmental protection policies. For instance, recognizing the legal personhood of the Whanganui River has stimulated similar regulatory frameworks in other countries.

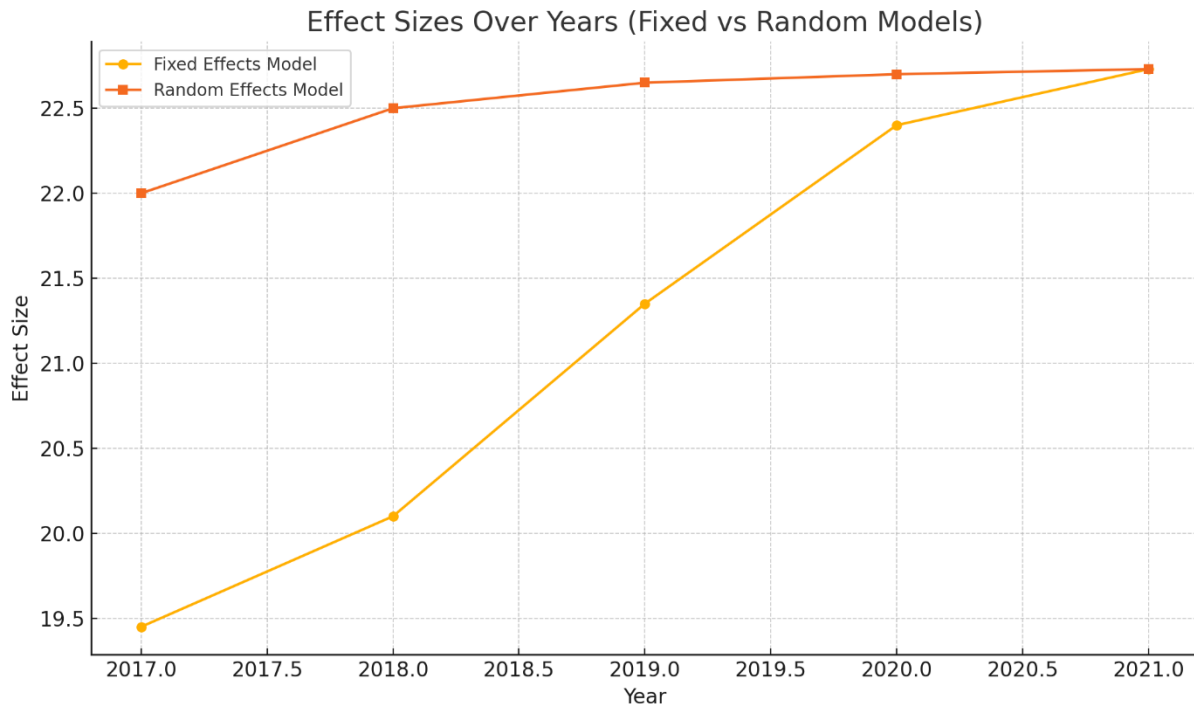
These examples emphasize the broader implications of legal personhood for addressing global environmental challenges and highlight its potential role in fostering innovative and sustainable approaches to environmental governance.

Annual trends are closely related to the implementation processes of environmental regulations in different countries. For instance, the implementation of environmental policies under the European Green Deal objectives has created an increasing awareness trend in Europe-centered publications, as reflected in graphical data (European Commission, 2021). Similarly, the emphasis on renewable energy investments in Asian countries is observed to have had a positive impact on post-2020 trends (IEA, 2021). The annual variations depicted in graphs can be linked to specific policy events and levels of social awareness. For example, the signing of the Paris Climate Agreement in 2015 heightened interest in environmental regulation, with this interest becoming more pronounced in legal mechanisms after 2020. Stucki (2020) focuses that the integration of environmental rights into legal regulations cannot be examined self-sufficiently of social and political contexts. Hence, the increase detected in meta-analysis results reflects not only legal reforms but also the influence of environmental awareness campaigns within society. The fluctuating annual effects may stem from the rise in environmental awareness or changes in legal frameworks. Graphical visualizations of these contexts permit readers to better understand the results (Kaufmann & Sheehan, 2019; Stucki, 2020). For instance, the decision to grant legal personhood to the Ganges and Yamuna Rivers in India clearly illustrates how heightened environmental awareness impacts legal regulations. Alike, the recognition of legal personhood for New Zealand's Whanganui River proves how locally effective policies can contribute significantly to international legal discourse (Boyd, 2020).

- The random-effects model, given its high heterogeneity rate (99.99%), has delivered more reliable results. This high heterogeneity stems from the diversity of economic and social factors across the countries wherever environmental regulations are applied. For instance, in developing countries, the applicability of environmental regulations may profit different results compared to developed countries due to development priorities and financial limitations (OECD, 2020). This explains wherefore the random-effects model is more operative for analyzing broader contexts within environmental regulations. Whilst the random-effects model yields more dependable results in analyzing heterogeneity, there are cases wherever the exact reasons behind this heterogeneity persist undefined, which can limit the generalizability of findings. Precisely, the methodological diversity observed in datasets collected from different regions may affect the applicability of results to diverse social and political contexts (Stucki, 2020). The fixed-effects model assumes homogeneous effects across the studies being analyzed, whereas the random-effects model

accounts for heterogeneity and provides a broader context. Researchers such as Boyd (2020) and Kaufmann & Sheehan (2019) discuss that the random-effects model is healthier at capturing the impact of increasing environmental awareness on annual changes. Remarkably, higher effect sizes have been experimental during periods of heightened environmental awareness under the random-effects model. In this regard, the post-2020 rises can be deduced as being shaped by the growing impact of environmental regulations (Stucki, 2020). This further underlines the value of the random-effects model in detecting variations and highlighting the influence of diverse factors within dynamic environmental frameworks.

**Graph 1:** Overall Effect Sizes According to Fixed and Random Effects Models



Graph 1, likens the average effect sizes of the fixed-effects and random-effects models over the years. The evolution of environmental law across time aligns closely with significant political and social events. For instance, the post-2020 rise in environmental responsiveness has been formed by the renewed prominence of the Paris Climate Agreement and international policies such as the European Green Deal (European Commission, 2021). Furthermore, discussions on environmental protection and sustainability during the COVID-19 pandemic contributed to heightened public awareness, which is reflected in the meta-analysis results. Kaufmann and Sheehan (2019) highlight that environmental protection policies are effective not only through legal processes but also via societal awareness campaigns. The stronger results detected in the random-effects model for the years following 2020 reflect the heterogeneous impacts of international environmental policies during this period. In this context, Boyd (2020) reviews the effects of environmental law reforms on individuals and societies, highlighting how international collaborations support environmental protection policies. The fixed-effects model captures the years in which environmental regulations were based on stable legal frameworks, whereas the random-effects model reliably analyzes the effects of studies with greater heterogeneity. The pronounced growth observed post-2020 can be accredited to the rise in environmental awareness during this period (Boyd, 2020; Kaufmann & Sheehan, 2019). This accentuates the influence of global environmental policies and societal shifts in shaping the trajectory of environmental law.

### Subgroup Analyses

Findings by Year: The analysis of annual effects revealed notable differences:

#### 2009 and 2012:

Tau-squared ( $\tau^2$ ) values were calculated as 512.07 and 3568.96, respectively. These high values specify significant differences amongst the studies conducted in these years, classifying heterogeneity as a key source of variation.



**2011:**

The results from the fixed-effects and random-effects models were initiate to be remarkably consistent (4.40 vs. 4.33). Also, the low variance value (18.40) proposes a high degree of homogeneity among the studies conducted during this year.

**Table 1.** Subgroup Analysis Results by Year

Year	Fixed Effects Effect Size	Random Effects Effect Size	Tau-Squared	Number of Studies
2007	9.10	6.40	64.69	15
2009	46.63	42.29	512.07	20
2011	4.40	4.33	18.40	18
2012	21.01	39.80	3568.96	22
2014	24.06	14.85	258.26	25

The detailed analysis of the data presented in Table 1 can elucidate the impacts of environmental awareness and legal regulations more obviously. Particularly, the differences observed in specific years may be linked to political changes or societal awareness campaigns. In this context, interpreting the visuals while considering their social and political contexts will aid readers in better understanding the results of subgroup analyses (Kaufmann & Sheehan, 2019).

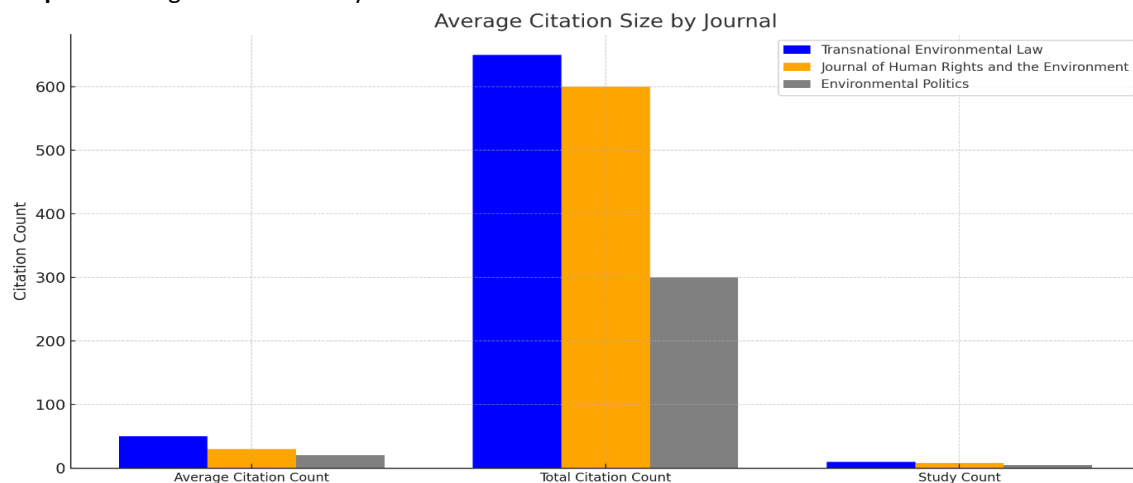
## Findings by Journals:

- Among the journals in which the studies are predominantly published, Transnational Environmental Law and Journal of Human Rights and the Environment stand out.
- Studies published in these journals have received, on average, more citations compared to those published in other journals.

**Table 2.** Average Effect Size by Journals

Magazine Name	Average Citation Count	Total Citation Count	Number of Studies
Transnational Environmental Law	32.5	650	20
Journal of Human Rights and the Environment	28.7	574	20
Environmental Politics	18.4	276	15

The graph of the data shown in Table 2 and its interpretation can be seen below.

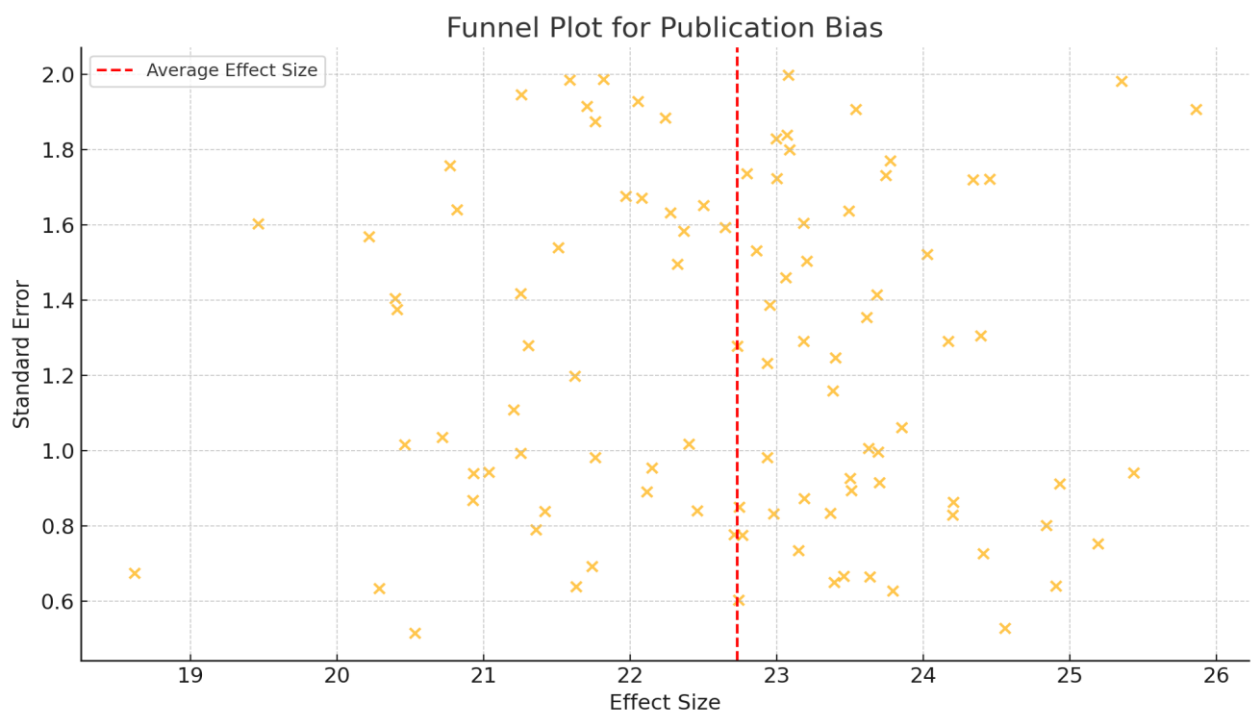
**Graph 2.** Average Citation Size by Journals**Publication Bias Analysis**

**Funnel Plot:** The Funnel Plot graph below displays the distribution of effect sizes and standard errors:

- While effect sizes concentrate at a point on the graph, asymmetries can be perceived at the extremes.

• According to the results of Egger's test, publication bias was not statistically significant ( $p > 0.05$ ). Further elaboration on the Funnel Plot analysis is crucial, expressly in identifying the sources of asymmetry and discussing its impact on the analysis results. Asymmetry may stem from interdisciplinary approaches in studies related to environmental law or from the diversity of data sets. The asymmetry detected in the Funnel plot results may ascend from the interdisciplinary nature of studies in environmental law, the use of different methodologies, and the diversity of data sets (Gordon et al., 2023). Nevertheless, recent studies on publication bias highlight that differences in methodological choices used in meta-analyses play a substantial role in these asymmetries (Smith & Taylor, 2022). For instance, Smith and Taylor (2022) note a tendency for publications in the environmental policy field to focus on specific journals, which could affect the generalizability of results. Gordon et al. (2023) suggest that data heterogeneity is linked, particularly to the underrepresentation of data from developing countries. Such asymmetries can be viewed as an opportunity to better understand contextual differences in studies rather than questioning the reliability of meta-analysis findings. A more detailed analysis of publication bias could provide major insights into the generalizability of the current findings (Boyd, 2020; Gordon, 2018).

**Graph 3.** Publication Bias Analysis with Funnel Plot



Graph 3 imagines publication bias through a Funnel Plot, proving asymmetries observed within the meta-analysis. Although effect sizes tend to cluster near the mean, asymmetries at the extremities may be accredited to methodological heterogeneity in studies related to environmental law (Gordon, 2018).

## DISCUSSION

The findings highlight noteworthy annual and journal-specific variations in research concerning environmental law and the rights of nature. Given the high heterogeneity observed, the random-effects model emerges as a more reliable method for overall meta-analytic results. The results recommend that addressing the rights of nature within the framework of legal personhood could strengthen environmental protection mechanisms. Besides, integrating these two concepts may offer an innovative model for sustainable environmental law. Explicitly, granting legal personhood to natural entities could address existing legal gaps by enabling these entities to advocate for their own rights.

**Legal Personhood:** Legal personhood refers to the recognition of an entity as a “person” under a legal system, granting it specific rights and responsibilities (Hamilton, 2009). According to this principle, for an entity to be the subject of the rights and obligations established by a legal system, it must first be acknowledged as a “person” within that system. Though often equated with being “human,” the existence of legal personhood for humans also derives from the recognition granted by the respective legal system (Kurki, 2019; Ripken, 2019). Indeed, just as certain humans in the past, or even fetuses in the present, may lack rights and obligations, it is possible for a legal system to recognize non-human entities (NHEs) as persons.

Certain NHEs, which lack physical form, can, through the mechanism of "legal personality," exist and be recognized as persons within their legal system, thereby becoming the subject of specific rights and obligations (Arat, 2007). A crucial aspect of this recognition lies in the term "legal," which modifies "personality" and signifies the nature of the entity rather than its mere existence. In reality, what is recognized by the legal order is not the absolute factual existence of an entity endowed with hypothetical "legal personality," but rather the legal acknowledgment of the existence of an entity that does not physically exist. Consequently, there is no distinction between the "legal" nature of the personality of a human being and that of an entity recognized as existing under the law.

Thus, the term "natural" used to describe human legal personality reflects the fact that human existence is not a legal construct but a reality. Conversely, the term "legal personality" implicitly refers to the personality of an entity whose existence is acknowledged solely under the law.

In summary, the term "personhood" fundamentally refers to entities recognized by the law as capable of holding rights and obligations. Such an entity is termed a "legal person," while its legal status is described as "legal personhood." Currently, the entities possessing legal personhood are natural persons and juridical persons. The legal personhood of natural persons is referred to as "natural personhood," whereas that of juridical persons is termed "juridical personhood." Thus, "legal personhood" and "juridical personhood" are not synonymous. "Legal personhood" is a broader concept that denotes the status granted by the law to both natural and juridical persons. On the other hand, "juridical personhood" is a specific term describing the type of legal personhood attributed to entities that do not exist physically but are recognized by the legal system as existing.

Legal personhood is merely a tool designed to protect an entity's interests through legal mechanisms and should not be regarded as an end in itself. Its function lies in enabling the mobilization of public power within a legal system. An entity may exist in reality but possess no rights without public power. Conversely, an entity granted public power, even if it does not physically exist, can hold rights as provided by its legal system. Thus, legal personhood can be understood as the foundation of rights and obligations as determined by a legal system. Consequently, for any legal system to grant rights or impose obligations upon an entity—whether a physically existing one such as a human or a ship, or a non-physical entity such as a corporation or an association—it must first recognize the entity as possessing "legal personhood."

When an entity is acknowledged as a person by a legal system, it gains the "capacity" to possess the rights and obligations defined for it by the law. Through its legal rights and interests, it can seek protection from judicial, administrative, and other institutional bodies within the legal system in its name and on its behalf.

**3.1. Types of Legal Personhood:** In contemporary legal systems, only two types of legal personhood exist: natural and juridical (artificial or non-natural). The term "natural personhood" refers exclusively to the legal personhood of humans. While other entities possessing physical existence besides humans may exist, only humans are designated as having "natural personhood" and are referred to as "natural persons." In some legal systems, tangible entities such as ships, Hindu idols, or rivers may be endowed with legal personhood, yet their legal personhood is not termed "natural personhood," nor are they referred to as "natural persons."

This distinction demonstrates that the terms "natural" and "juridical," while appearing to classify the types of personhood, do not inherently do so. As previously noted, entities such as ships, idols, or rivers are not non-existent constructs brought into being solely by legal assumptions; they are as real as humans. Therefore, these terms are employed solely to differentiate the legal personhood of humans from that of non-human entities. Aside from this distinction, there is no difference between the recognition of humans and non-human entities possessing physical existence as persons under the law. Consequently, the reason why entities with physical existence as tangible and their personhood as legal as those of humans are designated as "juridical persons" and their legal personhood as "juridical personhood" is to serve this purpose.

While humans are the only entities classified as natural persons, distinctions are made among them regarding their legal capacity, categorizing them as fully or partially competent, or as fully or partially incompetent. This classification delineates the boundaries of their legal capacity to perform acts, rather than the scope of their legal personhood. Hence, there is no such concept as full or partial personhood for humans; all humans are equally persons under the law.

On the other hand, the term "juridical personhood" applies to both non-human tangible entities and entirely non-physical entities. For non-physical entities, a further distinction is made based on whether they are organized under public law or private law.

**3.2. Scope of Legal Personhood:** The recognition of an entity as a legal person does not imply that it possesses all the rights and obligations established by its legal system. For any legal person to hold a specific right, two conditions must be met: one objective and the other subjective.

The objective condition requires that the right to be acquired aligns with the type of legal personhood of the entity. For instance, juridical persons cannot possess rights related to personal status, such as attaining majority, becoming engaged, marrying, being subject to marital property regimes, divorcing, adopting, benefiting from rights derived from kinship (e.g., claiming alimony), being appointed as a guardian, custodian, or legal advisor, or inheriting as a statutory heir (Dural & Öğüz, 2021).

The subjective condition mandates that the entity fulfills the specific requirements prescribed by the legal system for acquiring a particular right. For example, under Article 28/1 of the Turkish Civil Code (Law No. 4721), “legal personhood begins at the moment a child is born alive.” Therefore, despite the unborn child being both human and alive in the womb, it cannot acquire any rights, including fundamental human rights, until it is born. Similarly, rights such as citizenship, residency, education, employment, property ownership, and access to healthcare and social security require the fulfillment of subjective conditions by the relevant individuals.

Consequently, mere possession of legal personhood does not entitle an entity to rights unless those rights are objectively defined for its type of legal personhood and the subjective conditions for those rights are fulfilled. Therefore, when defining “personhood,” the concept pertains not to the “possession” of rights but to the “potential to possess” rights (Helvacı, 2006; Dural & Öğüz, 2021).

The rights and obligations associated with different types of legal personhood are not uniform, nor are their scopes identical. The scope of rights and obligations for each type of legal personhood varies depending on the needs of entities categorized under that particular status. For instance, “human rights” are designated exclusively for natural persons. Similarly, while private juridical persons can dissolve themselves (dissolution), such an option is not available for public juridical persons or natural persons.

**3.3. The Need for New Types of Legal Personhood:** Dividing legal personhood into two categories—“natural” and “juridical”—by reserving the former for humans and grouping all non-human tangible and intangible entities into the latter may seem practical at first glance. However, as previously discussed, this approach creates significant conceptual confusion. The European Parliament’s proposal to classify the legal personhood of artificial intelligence as “electronic” suggests an effort to address this confusion and prevent further complications (Şahin, 2021).

As explained earlier, using specific designations for each type of legal person, rather than the broad term “juridical person,” which functions merely as a categorical label, would provide greater clarity. The current use of the term “natural” inaccurately implies “non-juridical,” which is misleading. Humans are tangible entities inherently recognized by law as persons. Conversely, the term “juridical” suggests “non-real,” which is equally erroneous, as entities categorized as juridical persons are no less real in their legal existence than humans.

Therefore, the first category should serve as a general term for entities capable of possessing rights and obligations, distinguishing them from objects. The second category, however, should specifically describe entities that, unlike humans, lack physical existence while still being recognized as persons under the law. To emphasize their intangible nature rather than their legal recognition—which might otherwise be confused with the legal recognition of humans—it would be more appropriate to refer to these entities as “abstract persons” and their status as “abstract personhood.” Similarly, entities with tangible existence akin to humans but created by human effort rather than occurring naturally could be termed “artificial persons,” with their legal status described as “artificial personhood.”

Despite the conceptual ambiguities associated with the terms “natural” and “juridical,” they continue to be widely accepted and used. However, the confusion stemming from this terminology is likely to intensify if legal systems begin recognizing new categories of legal personhood for diverse entities, such as natural objects and artificial intelligence. Just as it would be inappropriate to categorize humans as “living beings” while grouping all other entities as “non-living beings,” it is equally problematic to place the legal personhood of both current and future entities under the single category of “juridical personhood.”

The traditional distinction between natural and juridical persons, established when juridical persons were first conceptualized, has already lost much of its meaning and utility. For example, in international public law, entities traditionally recognized as persons include states (Kelsen, 1952; Kaya, 2020) and international organizations (Klabbers, 1998; Ufukoğlu, 2021). However, no equivalent terms to “natural” or “juridical” are used to describe the legal personhood of these entities. If individual humans—currently considered objects in this field due to their lack of legal personhood—were to be recognized as legal persons in international law, would they then be classified as “juridical persons” merely because their recognition depends on the primary legal actors in this domain (Manner, 1954; Karakocalı, 2022).

This discussion reveals that the existing categories of legal personhood are insufficient even to classify current legal persons adequately. The challenge will become even more pronounced as natural objects and artificial intelligence are incorporated into legal frameworks. For this reason, it is imperative to develop new

categories of legal personhood, starting with those that encompass non-human tangible entities, natural objects, and artificial intelligence.

Recent developments indicate a growing trend toward recognizing natural objects as legal persons. This approach is designed to empower natural objects to claim rights and receive legal protection on their own behalf. For instance, New Zealand has granted legal personhood to the Whanganui River, enabling it to engage in legal actions in its own name (Burdon & Maloney, 2023). Such legal frameworks offer a powerful mechanism for ensuring the protection of natural objects.

**3.4. The Need for a Specialized Legal Personhood for Natural Entities:** The European Parliament’s proposal to recognize “electronic personhood” as a distinct legal personhood for entities incorporating fully autonomous artificial intelligence (FAAI)—whether embedded in robotic form or existing as software—reflects an effort to protect currently existing legal persons from potential harm caused by such entities, rather than focusing on the FAAI entities themselves. Like juridical persons created by law, these entities do not inherently possess physical existence. While FAAI embedded in robots may have a tangible form, unlike humans, they are not dependent on their physical form. An FAAI entity can continue to exist even if the hardware it was previously hosted on is destroyed, as long as it has been transferred to another system. This distinct nature of existence, neither bound to biological life like humans nor entirely abstract as juridical persons, underscores the appropriateness of creating a unique legal personhood tailored to their characteristics. The same need for a specialized legal personhood applies to natural entities, which also possess unique characteristics. Unlike the rationale behind electronic personhood, aimed at protecting existing legal persons from FAAI-related harm, the purpose of granting legal personhood to natural entities is to protect these entities—particularly from human-induced harm. This proposed legal personhood, referred to as “natural personhood,” is designed to enhance the protection of natural entities.

The primary issue with current legal frameworks is that natural entities are treated as property. When harmed, the legal recourse is limited to compensating their owners economically, with no direct benefit or restitution for the harmed entity itself. Granting legal personhood to natural entities depends on the willingness of legal systems to integrate such a paradigm. Whereas this approach introduces a transformative perspective for environmental protection, it also poses challenges, such as defining legal representation, responsibilities, and the scope of rights. For example, in India, legal personhood was granted to the Ganges and Yamuna rivers, yet practical difficulties arose during implementation. These included uncertainties about appointing representatives to act on behalf of the rivers and determining how their rights would be safeguarded (Stucki, 2020). One illustrative scenario highlights the inadequacy of existing legal systems in protecting natural entities. If a domesticated animal’s leg were unlawfully amputated, legal action would primarily focus on compensating the owner, not on remedying the harm to the animal itself. Whilst the court might award damages equivalent to the cost of fitting the animal with a prosthetic leg, there would be no obligation for the owner to use the compensation for that purpose. Even additional punitive measures against the perpetrator, such as fines or imprisonment, would fail to address the harm suffered by the animal. The only effective and direct solution within existing legal frameworks would be granting the animal itself a legal status capable of safeguarding its interests.

“Natural personhood” would serve as a legal status, with its boundaries and scope defined exclusively by law (Maitland, 1911). However, granting legal personhood alone does not confer inherent rights to a natural entity. The recognition of legal personhood merely brings the entity into the legal sphere, allowing it to “appear” as a legal subject. To become an active participant in this realm, the entity must be granted specific rights and responsibilities, akin to being equipped with a “costume,” “script,” and “roles” that enable it to function within the legal framework. Without such provisions, the entity remains a passive legal object, indistinguishable from its prior status. Meanwhile the primary aim of granting legal personhood to natural entities is their protection, determining which entities should be recognized and what rights should be conferred upon them rests with the legislature. Lawmakers may identify categories of natural entities requiring special protection, such as “endangered species” or “entities of ecological significance.” Instead of individually designating each entity, broad categories could be established, and the rights and protections accorded to these entities could be adjusted as their status changes. By granting legal personhood and defining rights for natural entities, lawmakers can provide a flexible yet robust framework for ensuring their protection while addressing their unique characteristics and challenges. For instance, in New Zealand, granting legal personhood to the Whanganui River has permitted it to pursue legal actions in its own name, offering a groundbreaking model for safeguarding natural entities and inspiring similar efforts globally.

## CONCLUSIONS

This study determines how granting legal personhood to natural entities can strengthen environmental protection mechanisms and deliver an innovative legal framework for environmental sustainability. The meta-analysis findings reveal significant variations in environmental law studies across years and journals, indicating that the random-effects model is a more dependable method, particularly in cases of high heterogeneity. The mean effect size was computed as 19.45 under the fixed-effects model and 22.73 under the random-effects model, reflecting the dynamic nature of environmental issues and the diversity of legal approaches. The meta-analysis consequences further indicate that the effects of legal personhood practices become markedly more pronounced during periods of heightened environmental awareness.

Subgroup analyses acknowledged that factors such as increasing environmental awareness and political changes have shaped the annual effects. Remarkably, the observed increase in the post-2020 period highlights the impact of growing sustainability consciousness and innovative legal regulations. Moreover, recognizing natural entities as legal persons enhances environmental protection policies by transforming them from mere objects serving human interests into subjects capable of defending their rights. For instance, the legal personhood granted to the Whanganui River in New Zealand serves as a robust example of the success of such initiatives.

The meta-analysis also emphasizes the challenges that must be overcome for the successful implementation of legal personhood practices. As observed in the case of the Ganges and Yamuna Rivers in India, shortcomings in representation mechanisms and ambiguities regarding the scope and enforceability of rights pose significant obstacles to effective execution. Strengthening legal infrastructure and clearly defining representation mechanisms are critical. Though, a large proportion of studies focus on specific geographic regions, and the inclusion of more regional and international studies in the meta-analysis could enhance the generalizability of the findings. Recent international studies, principally those published in recent years, elucidate the regional differences in environmental sustainability and legal frameworks more clearly. For instance, Brown and Miller (2023) examined how environmental law practices are shaped by cultural, political, and economic contexts in different regions, emphasizing that incorporating such studies could improve the generalizability of meta-analysis outcomes. Similarly, Zhao and Li (2022) scrutinized the legal implications of renewable energy policies in Asian countries, highlighting the impact of regional factors on environmental policies. Counting such regional analyses would suggest a more comprehensive perspective.

Granting legal personhood to natural entities has the potential to attend as a powerful tool for environmental sustainability. Yet, effective implementation of this approach necessitates specific steps. Strengthening the legal infrastructure and developing mechanisms to facilitate the adoption of this new paradigm in social and political contexts are fundamental. The following recommendations offer a roadmap for achieving these objectives:

**1. Strengthening Legal Infrastructure:** Comprehensive regulations granting legal personhood to natural entities would enhance the enforceability of this status. It is critical to appoint legal representatives and clearly define their responsibilities.

**2. Developing International Standards:** Creating international standards to harmonize environmental protection policies across countries is important. In this context, organizations such as the United Nations and other international bodies could improve a framework for recognizing legal personhood for natural entities.

**3. Raising Public Awareness:** The success of legal personhood practices is straightforwardly connected to public awareness of environmental sustainability. So, educational and media campaigns should be conducted to promote the protection of natural entities.

**4. Increasing Applied Research:** Showing applied research is vital to better comprehend the impacts of granting legal personhood to natural entities. Exactly, the long-term effects of such practices on environmental sustainability should be investigated.

**5. Pilot Programs and Monitoring Mechanisms:** Pilot projects could be originated to effectively implement the process of granting legal personhood to natural entities. These projects would help establish a monitoring mechanism to evaluate the effectiveness of the implementations and identify shortcomings. Successful examples of granting legal personhood to natural entities could serve as a guide for future applications. So, the following practical cases stand out (Boyd, 2020):

**New Zealand – The Whanganui River Case:** The legal personhood granted to the Whanganui River in 2017 marked a significant milestone in its conservation and management. This initiative included the establishment of a council to represent the river's interests. Besides, the preservation of traditional knowledge and practices of local Māori communities contributed to the sustainable use of the river. This case highlights the critical importance of involving local communities in environmental decision-making processes.



**India – The Ganges and Yamuna Rivers:** The legal personhood granted to the Ganges and Yamuna Rivers in India in 2017 is considered a significant step toward environmental sustainability. This regulation provided a legal framework for protecting the rivers from pollution and sustainably managing water resources. Particularly, this practice raised public awareness and prompted local governments to operate more effectively. Yet, challenges in the implementation process were reported in regions with inadequate legal infrastructure.

**Colombia – The Atrato River Case:** The recognition of the Atrato River in Colombia as a legal person is a noteworthy example in terms of biodiversity conservation and environmental justice. This regulation safeguarded the rights of local communities and enabled a participatory approach to the river's management. Additionally, efforts to protect the ecosystem surrounding the river have promoted the sustainable use of natural resources.

The recommendations for the protection of natural entities can be concretized as follows:


1. **Development of Legal Regulations:** At the national level, laws should be legislated to grant legal personhood to natural entities. These laws must clearly define representation mechanisms, the scope of rights, and applicable sanctions (Smith & Taylor, 2022).
2. **Involvement of Local Governments:** Local governments should play an active role in the protection of natural entities. For instance, representatives can be appointed for specific natural entities, confirming their active participation in conservation processes (Brown & Miller, 2023).
3. **Public Awareness Campaigns:** Society must be made aware of the legal status of natural entities over education and media campaigns. This will growth public support and facilitate the implementation of regulations (Seker et al., 2013).
4. **International Collaborations:** A global approach to the protection of natural entities should be espoused by developing international standards. Successful international examples can serve as models for local practices.

This research highlights that granting legal personhood to natural entities can principal to a paradigm shift in environmental protection policies and contribute to accomplishing environmental sustainability goals if managed proficiently. These recommendations aim to boost concrete steps toward establishing more applicable and inclusive legal systems for the protection of natural entities.

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**Trend Analysis and Spatial Distribution of Surface Soil Temperatures in Türkiye**Yasin Demir<sup>1</sup> ✉, Azize Doğan Demir<sup>2</sup>, Mehmet Zahid Malasli<sup>3</sup><sup>1</sup>Department of Soil Science and Plant Nutrition, Bingöl University, Bingöl, Türkiye,<sup>2</sup>Department of Biosystem Engineering, Faculty of Agriculture, Bingöl University, Bingöl, Türkiye,<sup>3</sup>Department of Biosystem Engineering, Faculty of Agriculture Necmettin Erbakan University, Konya, Türkiye<sup>1</sup> <https://orcid.org/0000-0002-0117-8471>, <sup>2</sup> <https://orcid.org/0000-0003-2008-3408>, <sup>3</sup> <https://orcid.org/0000-0002-5845-1272>

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**ABSTRACT**

Soil temperature is a crucial factor affecting soil's physical, chemical, and biological processes. In particular, the rate of biochemical activities and the soil-plant-water balance are affected by soil temperature. Soil temperature has an important role in many issues such as soil management, water movement in the soil, and planting time determination. Therefore, to know the soil temperature and make predictions about its change in the coming years, the soil temperature trend must also be known. Today, there are many studies on soil temperature. However, studies on the trend of soil temperature are limited, especially in Türkiye. In this study, soil temperature trends over the period between 1981 and 2021 were analyzed on a monthly scale at 5 and 20 cm depths using 73 meteorological stations in Türkiye. Also, the relationships between air and soil temperature were examined during the same period. The results yielded a high correlation between air and soil temperature. According to the monthly trend analysis of soil temperature, there was a predominance of positive trends at both soil depths. For 5 cm and 20 cm-depths, the distribution of significant positive trends was the highest in March (78.08% and 83.56%, respectively), while significant negative trend distribution was the highest in June (9.59%) and July (4.11%), respectively. Increasing soil temperatures will affect many soil characteristics and agricultural production processes. It should be noted that the increase in soil temperature may positively affect plant germination and root development, but soil moisture balance and biochemical negatively affect soil properties. Planning sustainable soil management practices, including soil tillage, to reduce the increase in soil temperatures has become necessary. Therefore, activities that may cause an increase in soil temperature should be controlled and monitored.

**Key words:** Soil Temperature, Trend Analysis, Mann-Kendall, Soil management, Türkiye**Türkiye’de Yüzey Toprak Sıcaklıklarının Trend Analizi ve Mekansâl Dağılımı****ÖZ**

Toprak sıcaklığı, topraktaki fiziksel, kimyasal ve biyolojik olaylara etki eden önemli bir faktördür. Özellikle biyokimyasal aktivitelerin hızı ve toprak-bitki-su dengesi toprak sıcaklığının etkisi altındadır. Ancak günümüzde toprak sıcaklığı hakkında yapılan çalışmalar oldukça sınırlıdır. Toprak sıcaklığı ve bu sıcaklığın zamana bağlı olan değişimleri hakkında yeterli bilgiye sahip olmak ekosistem ve tarımsal üretim için oldukça önemlidir. Bu çalışmada, Türkiye’deki 73 iklim istasyonu kullanılarak 1981 ile 2021 yılları arasındaki dönemde toprak sıcaklığı eğilimleri aylık ölçekte 5 ve 20 cm derinliklerde analiz edilmiştir. Bununla birlikte aynı döneme ait hava ve toprak sıcaklığı arasındaki ilişkiler belirlenmeye çalışılmıştır. Araştırma sonuçları hava ve toprak sıcaklığı arasında yüksek korelasyonun olduğunu göstermiştir. Toprak sıcaklığının aylık eğilim analizleri sonuçlarına göre her iki toprak derinliğinde pozitif trendlerin fazla olduğu belirlenmiştir. 5 cm ve 20 cm derinlikler için, önemli pozitif trend dağılımı en fazla Mart (sırasıyla %78.08 ve %83.56) ayında önemli negatif trend dağılımı ise sırasıyla en fazla Haziran (% 9.59) ve Temmuz (% 4.11) aylarında belirlenmiştir. Artan toprak sıcaklıkları toprakların birçok özelliğini

etkilediği gibi tarımsal üretim süreçlerini de etkilemesi muhtemeldir. Toprak sıcaklıklarının artışını azaltacak başta toprak işleme gibi sürdürülebilir toprak yönetim uygulamalarının planlanması bir zorunluluk haline gelmiştir. Bundan dolayı toprak sıcaklığının artışına neden olabilecek faaliyetler kontrol altına alınarak izlenilmelidir.

**Anahtar kelimeler:** Toprak Sıcaklığı, Trend analizi, Mann-Kendall, Toprak yönetimi, Türkiye

## INTRODUCTION

Soil temperature is a crucial soil property that directly or indirectly affects several physical, chemical, and biological processes within the soil. Soil temperature is an important ecological factor that affects plant life at all stages, from seed germination to seedling growth and development. As with air temperature, soil temperature is vital for plant growth. Soil temperature is necessary to calculate underground ecosystem processes, including root growth and respiration (Repo et al., 2004), decomposition, and nitrogen mineralization (Waring & Running, 2007; Onwuka & Mang, 2018). Furthermore, soil temperature is critical for physical, hydrological and biogeochemical processes (He et al., 2018). Soil temperature could be a significant factor in retaining soil organic carbon as it can affect biomass production and microbial decomposition of organic matter (Chowdhury et al., 2021). Soil temperature varies along the soil profile depending on factors including soil structure, meteorological conditions, vegetation cover, etc. (Ekberli et al., 2017). Temperature changes with time and depth within the soil profile. The temperature at the soil surface significantly fluctuates during certain hours of the day. However, daily variations in deeper parts of the profile (typically at a depth of 50 cm) are negligible. Seasonal temperature changes can affect deeper layers. Yener et al, (2017) reported that soil and air temperatures showed the same trend over time. However, short-term meteorological effects such as rain, snow and wind increased the percentage of error rates at shallow depths. In most soils, the temperature remains relatively constant at a depth of 10 m, approximately equaling the annual average temperature of the upper soil layer (Dinç & Şenol, 1998). The majority of soil formation processes and events occur within specific temperature ranges. Increasing or decreasing temperatures can adversely affect many of the processes mentioned above (Onwuka & Mang, 2018).

The primary source of soil heat is solar energy coming to the Earth's surface with rays from the sun. Solar rays reaching the Earth show their effect on the soil depending on its physical and chemical properties (color, water content, structure), topography, and vegetation status (Ergene, 2012). Soil temperature exhibits daily, monthly, and seasonal variations depending on the temperature in the atmosphere. Therefore, global temperature increases and decreases in the atmosphere directly affect soil temperature. Despite the importance of soil temperature, the majority of studies conducted worldwide today have focused on temperature and precipitation trends (Jain & Kumar, 2012; Saboohi et al., 2012; Caloiero, 2015; Doğan Demir & Demir, 2016; Doğan Demir et al., 2017; Yildirim & Rahman, 2022; Demir & Doğan Demir, 2023). Studies on the temporal trend of soil temperature are relatively limited. According to research, in the first two decades of the 21st century (2001–2020), global surface temperature was found to be 0.99 [0.84 to 1.10] °C higher than in 1850–1900 (Masson-Delmotte et al., 2021). Several studies conducted on a local basis showed a positive or negative trend in soil temperature, which needs to be evaluated in terms of agricultural production (Araghi et al., 2017; Fang et al., 2019; Dorau et al., 2022). The majority of research in Türkiye has focused on temperature and precipitation trends, and studies on the trend of soil temperature across the country are limited. The studies conducted in this regard are generally at the local level. Kara and Cemek (2019) tried to estimate soil temperature using meteorological parameters of some provinces in the north of Türkiye with artificial neural networks. Ekberli et al (2017) reported in their studies that the daily change of soil temperature at different depths could be estimated using thermal diffusion coefficients determined by parabolic function. Güteryüz (2022) reported in his study that soil temperature at different depths can be estimated using machine learning algorithm. Yeşilirmak (2013) calculated the trend analysis of soil temperatures to varying depths in 8 meteorological stations in the west of Türkiye. As a result of the research, it was determined that there was an increasing trend at all depths of the soil. It was reported that long-term trend analyses could provide important information about meteorological change. Similarly, Tonkaz et al. (2007) investigated temporal trends in soil temperatures between 1975 and 2004 for 13 sites in the Southeastern of Türkiye. They found that soil temperatures showed significant increasing trends. However, soil temperature trends in other regions of Türkiye have never been analysed.

The present study aimed to use soil temperature values (5 cm and 20 cm) from 1980 to 2021 from meteorological stations at 73 points across Türkiye to (i) conduct trend analysis, (ii) map the results of trend analysis, and (iii) evaluate the results in terms of some soil properties.

## MATERIALS AND METHODS

### Study Area

The present study was carried out to cover all of Türkiye. Türkiye is located in the middle belt of the northern hemisphere, where the terrestrial zone of Türkiye occupies a large area between the latitudes 36-42 degrees north and meridians 26-45 degrees east. Due to its rugged geography, its projected area is 779,452 km<sup>2</sup>, while its actual area is 814,578 km<sup>2</sup> (Atalay, 2011). The country's average elevation is 1142 m with an average slope of 17% (Elilibüyük and Yılmaz, 2010). Türkiye lies between the temperate and subtropical zones. The fact that seas surround three sides of Türkiye, the extension of the mountains, and the diversity of landforms have led to different climate types (such as continental, Mediterranean, and Black Sea climates). In 2021, the average temperature in Türkiye was 1.4°C higher than the 1981–2010 average of 13.5°C, with a mean temperature of 14.9°C. The total spatial precipitation in Türkiye in 2021 was 524.8 mm, 9% below the 1991-2020 normal (573.4 mm) (MGM, 2022). In this study, meteorological stations in 73 provinces of Türkiye were selected as the study area (Fig. 1).

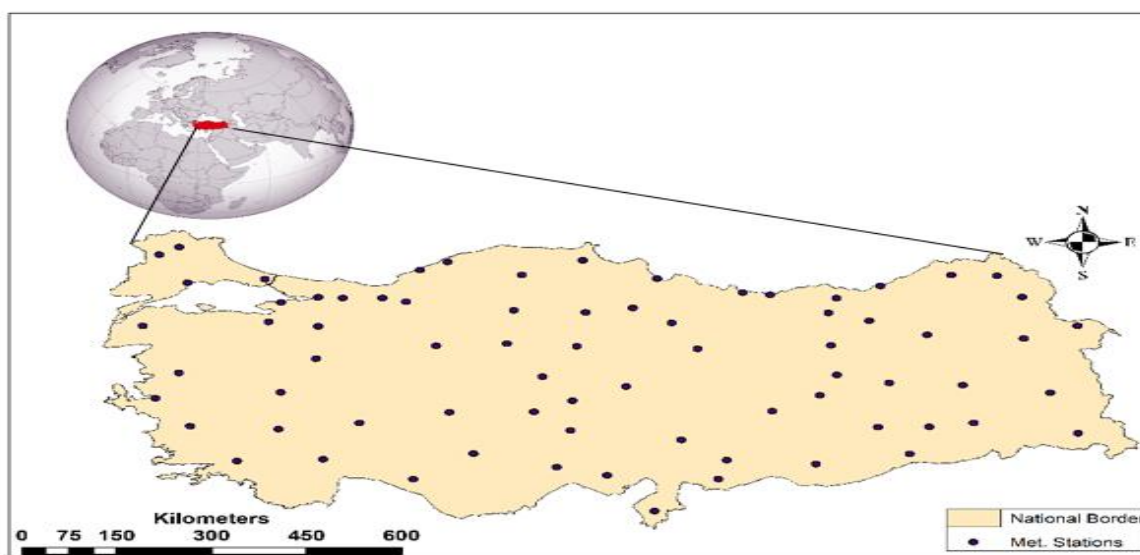


Figure 1. Study area and locations of meteorological stations

### Data collection

The data used in the study was obtained from the Turkish State Meteorological Service (MGM). Meteorological stations (MS) in 73 provinces across Türkiye, which have regular and complete measurement records between 1980 and 2021, provided soil temperature (ST) measurement values. At the MS, soil temperature at different depths is measured using specialized thermometers and automatically recorded by thermographs. The standard depths for soil temperature measurement are 5, 10, 20, 50, and 100 cm. However, in this study, temperatures in the upper soil layer (0-20 cm), where the physical, chemical, and biological properties of the soil are most dynamic, were evaluated. For this purpose, monthly average soil temperature values at depths of 5 cm and 20 cm were analyzed across 73 MS from 1980 to 2021.

### Trend analysis

The Mann-Kendall test has been preferred due to its widespread use in determining the possible trend in monthly average temperature series at two different depths of the surface soil. However, the non-parametric nature of the Mann-Kendall test, which can work for all distributions, has led to its preference in this study (Khambhammettu, 2005). The Mann-Kendall statistical test for trend is used to evaluate whether a series of data values increase or decrease over time and whether the trend in both directions is statistically significant (Gillbert, 1987). This method ranks among the most preferred and reliable methods for determining trends in climatological and hydrological data (Burn et al. 2002). With this test, the presence of a trend in a time series is checked with the "H0: no trend" (null hypothesis). Accordingly, the test statistic S and the sign function, as given in Equations 1 and 2, are calculated. Then, the variance of S and the Z test statistic is determined using the formulas given in Equations 3 and 4, respectively.

$$S = \sum_{k=1}^{n-1} \sum_{j=k+1}^n \text{sgn}(x_j - x_k) \quad (1)$$

$$sgn(x_j - x_k) = \begin{cases} 1; x_j > x_k \\ 0; x_j = x_k \\ -1; x_j < x_k \end{cases} \quad (2)$$

$$VAR(S) = \frac{1}{18} [n(n-1)(2n+5) - \sum_{p=1}^q t_p(t_p-1)(2t_p+5)] \quad (3)$$

Then the test statistic Z is denoted by Eq.(4)

$$Z_s = \begin{cases} \frac{S-1}{\sqrt{var(S)}}, & \text{if } S > 0 \\ 0, & \text{if } S = 0 \\ \frac{S+1}{\sqrt{var(S)}}, & \text{if } S < 0 \end{cases} \quad (4)$$

Here, n represents the number of data, x represents the data at times i and j, m represents the number of observations repeated in the data set, and ti represents the repeated observations in a series of length i. The Z value is used to determine a statistically significant trend. If  $|Z| < \alpha / 2$  at the significance level  $\alpha$ , there is no significant trend, whereas if  $|Z| \geq \alpha / 2$ , there is a statistically significant trend, and depending on the sign of the S value, it is concluded whether the trend is increasing or decreasing. In this study, the  $Z_{\alpha/2}$  value at a 95% confidence interval is taken as  $\pm 1.96$  according to normal distribution tables. The trend for each MS is classified as shown in Table 1 in the analysis results.

**Table 1.** Coding trend statistical test results according to significance level

Trend Test (Zs)	Trend Code	Explanation
$Z_s < -1.96$	1	Significant Negative Trend (SNT)
$0 < Z_s < -1.96$	2	Insignificant Negative Trend (INT)
$Z_s = 0$	3	No Trend (NT)
$0 < Z_s < 1.96$	4	Insignificant Positive Trend (IPT)
$Z_s > 1.96$	5	Significant Positive Trend (SPT)

## Spatial Distribution of Trend Analysis Results

Since it is impossible to measure temperature at every point in Türkiye due to its different topographic structure, it is essential to determine soil temperatures and temperature trends by estimating them. Thus, data for areas without meteorological stations can be calculated. In our study, trend values of each meteorological station (MS) were mapped using Geographic Information Systems (GIS). For this purpose, trend values calculated for 5 cm and 20 cm depths in 73 MS were processed into ArcMAP and monthly distribution maps were produced using the Inverse distance weighting (IDW) method. The IDW interpolation method assumes that data sets close to each other are more similar than those farther away (Hodam et al., 2017; Isaaks & Srivastava, 1989). The method uses the values of surrounding measurements to estimate the value of an unscaled datum. The IDW method assumes that each measured point has a diminishing positional effect with distance. It gives higher weights to nearby points than distant ones (Hodam et al., 2017).

General equation in IDW;

$$\hat{Z}(S_0) = \sum_{i=1}^n \lambda_i Z(S_i) \quad (5)$$

Where  $Z(S_0)$  is the value, we are trying to predict for location  $S_0$ ; n is the number of measured sample points surrounding the prediction location that will be used in the prediction;  $\lambda_i$  are the weight assigned to each measured points that we are going to use. These weights will decrease with distance.  $Z(S_i)$  is the observed value at the location  $S_i$ .

The weights at location  $S_i$  can be determined by;

$$\lambda_i = \frac{d_{i0}^{-p}}{\sum_{i=1}^N d_{i0}^{-p} \sum_{i=1}^N \lambda_i} = 1 \quad (6)$$

As the distance becomes larger, the weight is reduced by a factor of p. The quantity  $d_{i0}$  is the distance between the prediction location,  $S_0$  and each of the measured locations,  $S_i$ . The power parameter p influences the weighting of the measured location value on the prediction location's value; that is, with the increase of distance between the measured sample locations, and the prediction location, the weight (or influence) that

the measured point will have on the prediction will decrease exponentially. The weights for the measured location that will be used for the prediction are so scaled that their sum equals 5 (Hodam et al., 2017).

**Table 2.** Descriptive statistical data on soil (5 and 20 cm) and air temperature values measured at 73 meteorological stations in Türkiye between 1980-2021

Months	Min	Max	Med	Mean	SD	VC	Skewness	Kurtosis	SE of Mean	Lower bound on mean (95%)	Upper bound on mean (95%)
Air Temperature (°C)											
Jan	-18.20	13.10	2.90	2.10	5.32	2.54	-0.82	0.64	0.10	1.90	2.29
Feb	-17.00	14.80	4.00	3.20	5.12	1.60	-0.86	0.79	0.09	3.02	3.39
Mar	-13.40	17.70	7.40	6.93	4.16	0.60	-0.76	1.38	0.08	6.78	7.08
Apr	-0.80	21.70	11.80	11.91	3.22	0.27	-0.12	0.20	0.06	11.79	12.02
May	6.60	25.10	16.40	16.57	3.07	0.19	-0.09	0.04	0.06	16.46	16.69
Jun	11.10	30.80	21.00	21.17	3.31	0.16	-0.05	-0.12	0.06	21.05	21.29
Jul	13.80	34.80	24.20	24.40	3.50	0.14	0.09	-0.20	0.06	24.27	24.53
Aug	13.30	34.00	24.20	24.40	3.45	0.14	-0.02	-0.27	0.06	24.27	24.52
Sep	9.60	30.90	20.20	20.40	3.46	0.17	0.07	-0.16	0.06	20.27	20.52
Oct	3.70	26.00	14.80	14.89	3.57	0.24	0.02	-0.07	0.07	14.76	15.02
Nov	-6.00	20.10	8.60	8.61	4.07	0.47	-0.24	-0.01	0.08	8.47	8.76
Dec	-17.80	14.80	4.60	4.18	4.92	1.18	-0.66	0.58	0.09	4.00	4.35
Soil Temperature (5cm)											
Jan	-20.10	11.80	3.10	2.78	3.79	1.36	-0.57	0.93	0.07	2.64	2.92
Feb	-20.50	13.30	4.40	3.97	3.90	0.98	-0.61	1.00	0.07	3.83	4.11
Mar	-12.20	18.60	8.40	8.13	3.66	0.45	-0.48	1.09	0.07	7.99	8.26
Apr	1.40	25.20	14.00	14.11	3.30	0.23	-0.10	0.56	0.06	13.99	14.23
May	5.10	31.00	20.00	20.08	3.36	0.17	-0.12	0.41	0.06	19.96	20.20
Jun	8.40	39.50	25.50	25.71	3.79	0.15	-0.08	0.42	0.07	25.57	25.84
Jul	13.40	40.40	29.30	29.59	4.10	0.14	0.02	-0.11	0.08	29.44	29.73
Aug	11.90	39.50	29.10	29.37	4.04	0.14	-0.06	-0.15	0.07	29.22	29.52
Sep	9.70	36.10	23.70	24.17	3.87	0.16	0.12	-0.07	0.07	24.03	24.31
Oct	4.50	31.90	16.40	16.54	3.68	0.22	0.17	0.27	0.07	16.41	16.68
Nov	-3.90	22.40	8.90	8.87	3.57	0.40	-0.07	-0.21	0.07	8.74	9.00
Dec	-9.70	13.70	4.50	4.47	3.67	0.82	-0.29	-0.07	0.07	4.33	4.60
Soil Temperature (20 cm)											
Jan	-10.20	12.20	3.90	3.98	3.03	0.76	-0.04	0.23	0.06	3.87	4.09
Feb	-12.10	13.50	4.70	4.57	3.28	0.72	-0.15	0.23	0.06	4.45	4.69
Mar	-9.40	18.30	8.10	7.89	3.45	0.44	-0.26	0.47	0.06	7.76	8.01
Apr	0.40	24.10	13.20	13.21	3.26	0.25	-0.26	0.79	0.06	13.09	13.32
May	4.30	28.60	18.70	18.70	3.25	0.17	-0.19	0.44	0.06	18.58	18.82
Jun	8.40	33.50	23.80	23.89	3.48	0.15	-0.26	0.51	0.06	23.76	24.01
Jul	13.50	37.80	27.30	27.48	3.59	0.13	-0.16	0.12	0.07	27.35	27.61
Aug	15.70	37.70	27.60	27.73	3.59	0.13	-0.17	0.04	0.07	27.60	27.86
Sep	12.40	35.10	23.50	23.80	3.53	0.15	0.05	0.01	0.07	23.68	23.93
Oct	5.40	33.30	17.10	17.30	3.51	0.20	0.17	0.30	0.06	17.17	17.43
Nov	-1.20	20.20	10.20	10.13	3.39	0.33	0.00	-0.34	0.06	10.01	10.26
Dec	-6.80	14.20	5.70	5.74	3.24	0.56	0.01	-0.51	0.06	5.62	5.86

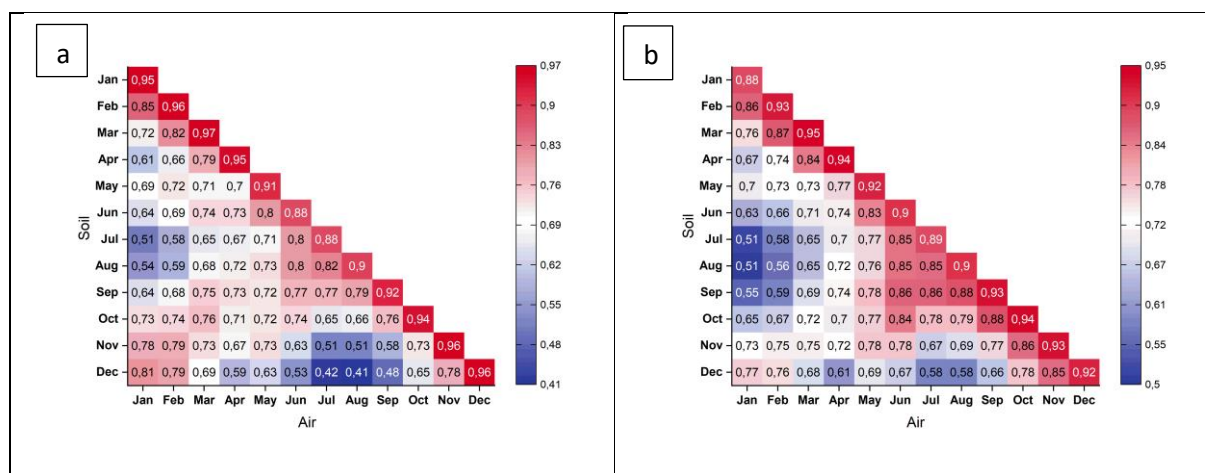
SD: standard deviation, Med: Median, CV: coefficient of variation, SE: standart error

## RESULTS AND DISCUSSION

The descriptive statistical values of air and soil temperatures (at depths of 5 cm and 20 cm) measured between 1980 and 2021 in Türkiye are provided in Table 2. The lowest temperature recorded in Türkiye was -18.2°C in January 2008 at the Ardahan station, while the highest temperature was 34.80°C in July 2000 at the Şanlıurfa station. Similarly, the average lowest and highest temperatures were recorded in January and July-August, respectively. The lowest temperature at a depth of 5 cm was -20.5°C in February 1991 at the Ardahan station, and the highest temperature was 40.40°C in July 2012 at the Aydın station. The average lowest and



highest temperatures at the same depth occurred in January (2.78°C) and July (29.59°C), respectively. Likewise, the lowest temperature measured at a depth of 20 cm was in February 1991 at Ardahan station, while the highest temperature was 37.8°C in July 2016 at the Mersin station. The average lowest and highest temperatures at this depth occurred in January (3.98°C) and August (27.73°C), respectively. Consequently, the lowest temperatures measured between 1980 and 2021 were observed at the Ardahan station for air and soil temperatures. However, high-temperature data were recorded at different stations, with all high temperatures occurring within the last 10 years. The fluctuation in air temperatures has been observed to cause variations in soil temperature. The present study presents the relationships between air and soil temperatures measured at the same stations in Figures 2. Based on data obtained from 73 meteorological stations evaluated within the scope of the present study, the effect of air temperatures on soil temperature at a depth of 5 cm in Türkiye is most pronounced in March ( $r^2=0.975$ ). The most minor effect was determined in June and July ( $r^2=0.880$ ). Similarly, the effect of air temperatures on soil temperature at a depth of 20 cm is highest again in March ( $r^2=0.955$ ) and lowest in January ( $r^2=0.880$ ). Soil temperature is affected by several soil properties. Dark-colored soils absorb heat more effectively, resulting in more substantial warming. Additionally, increased organic matter content enhances water retention capacity and color intensity, leading to an increase in soil temperature (Ochsner et al., 2001; Fang et al., 2005). However, these soil characteristics can either amplify or diminish the effect of heat reaching the soil.

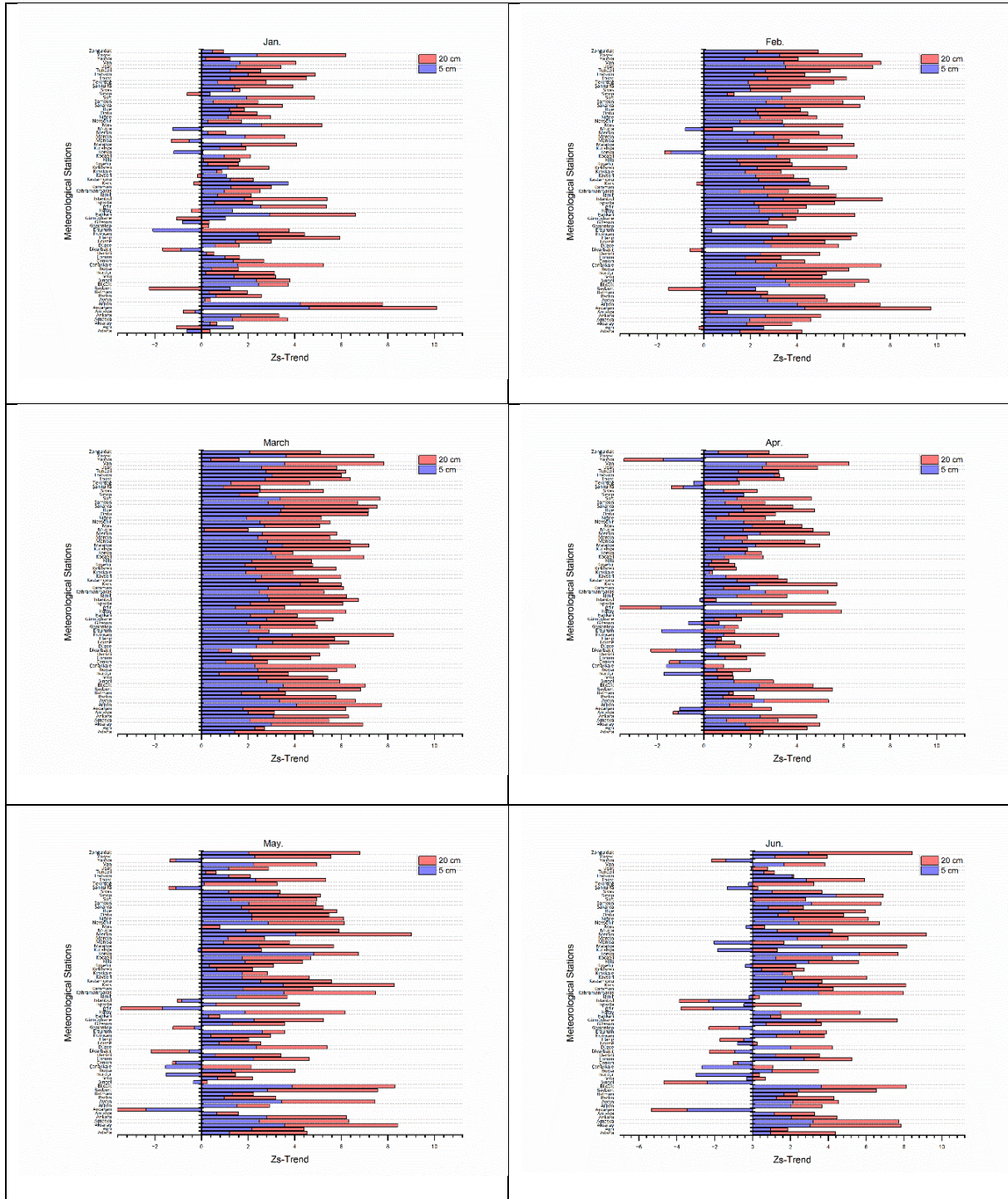


**Figure 2.** Correlation analysis of monthly averages of soil and air temperature measured at 73 meteorological stations in Türkiye between 1980-2021 - a: Correlation between soil temperature at a depth of 5 cm and air temperature, b: Correlation between soil temperature at a depth of 20 cm and air temperature

A high correlation was detected between the air temperature and soil temperature in Türkiye, depending on these interactions. Generally, the temperature change on the soil surface is more substantial than the changes occurring in the deeper layers. Depending on the increase or decrease of temperature waves reaching the soil, a process occurs in which temperature waves are delayed and attenuated in the deeper layers of the soil due to the retention or release of a certain amount of heat. The delay time of the maximum (minimum) temperature at any given time on the soil surface along the soil profile is a function of soil depth, thermal diffusivity, and the frequency of temperature waves. The delay time is directly proportional to the soil depth and inversely proportional to thermal diffusivity and the frequency of temperature waves (Hillel, 1998; Gülser and Ekberli, 2004). In our study, although high correlations were obtained between the air temperature averages and the soil temperature averages measured simultaneously, soil temperature is under the influence of solar energy and the physical and chemical properties of the soil (Kantarci, 2000). However, air temperature is still the most important parameter that can be used in estimating soil temperature. Many researchers on this subject have concluded that air and soil temperatures follow each other (Chudinova et al., 2006; Jahromi et al., 2021). However, Bolat (2023) reported that there is a linear positive relationship between air temperature and soil temperature. In Figure 2, it is noted that the correlation coefficients in the graph are low in June, July and August when the air temperature is high and high in other months. In these months, soil moisture is lower than in other months in Türkiye's climate conditions. A similar relationship is not seen when the correlation results in the b graph in Figure 2 are examined.

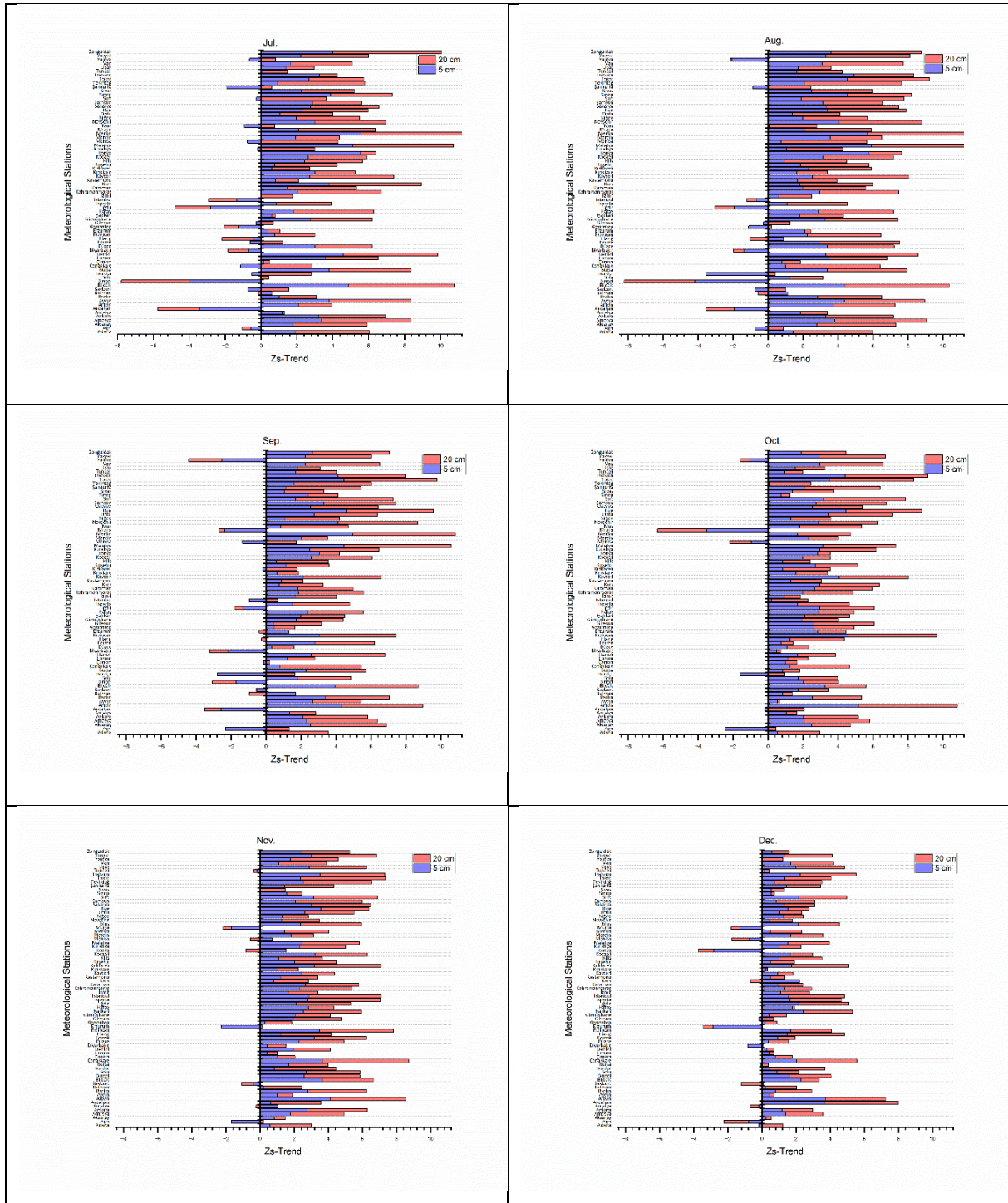
### Trends of soil temperature

Remarkable results were obtained in the present study conducted using soil temperature values from 73 meteorological stations across Türkiye. Trend analyses revealed both positive and negative changes. The trend analysis results based on soil temperature values at a depth of 5 cm and 20 cm are presented monthly in Fig. 3.



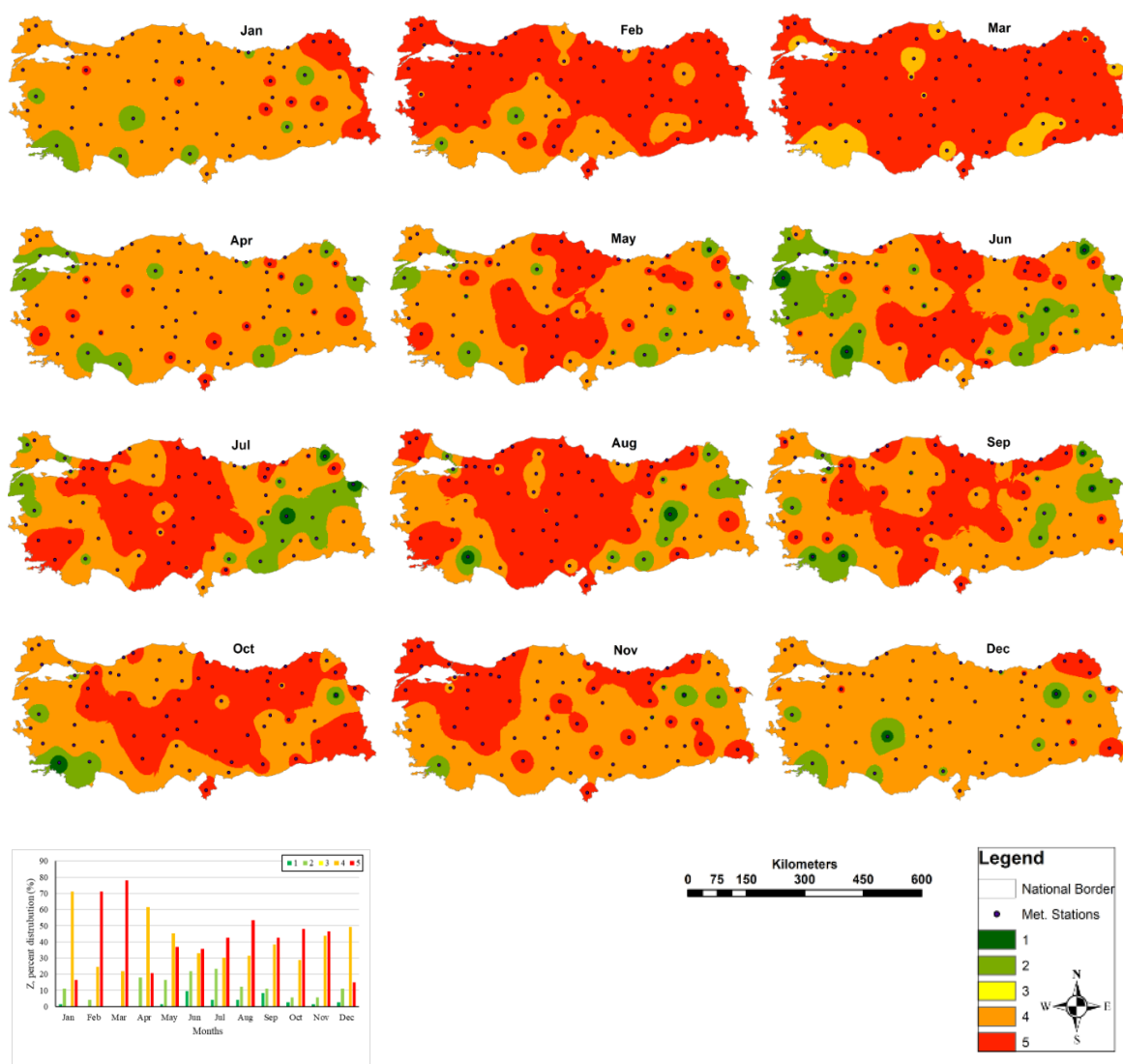
**Figure 3.** Trend analysis values of soil temperatures at 5 and 20 cm depths measured at 73 meteorological stations in Türkiye between 1980-2021





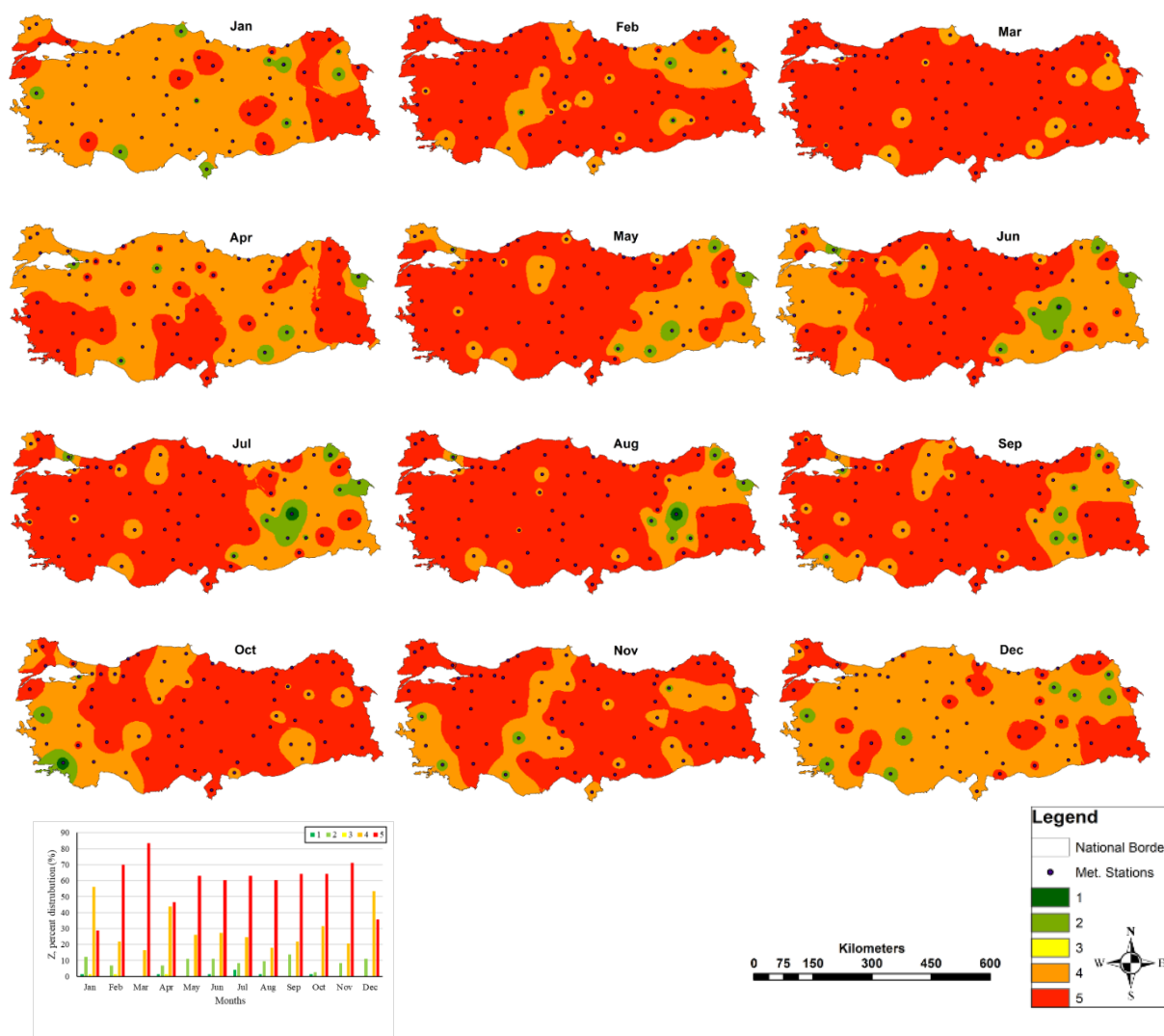
**Figure 3.** (Continued)

In our study, monthly spatial distribution maps of soil temperature trend values were obtained according to the IDW method using the trend data of 73 meteorological stations given in Figure 3 (Fig 4). Thus, the trend values of the areas between known points were calculated according to the IDW method.



**Figure 4.** Distribution of trend analysis results of soil temperature at 5 cm depth measured monthly at 73 meteorological stations in Türkiye between 1980-2021 according to the IDW method (1: Significant decreasing trend, 2: Decreasing trend, 3: No trend, 4: Increasing trend, 5: Significant increasing trend)

As observed from the figure, the highest positive trend in soil temperature in Türkiye was determined in March. In March, a Significant Positive Trend (SPT) was identified in 78.08% of the stations where soil temperature measurements were taken. In February, the distribution of the increasing trend in soil temperature in Türkiye was determined to be 71.23% SPT. According to the analysis results, the distribution of the increasing trend in soil temperature in January, December, and April was found to be an Insignificant Positive Trend (IPT). Conversely, the lowest negative trend distribution was observed in July. In July, Significant Negative Trend (SNT) was detected in 9.59% of the stations where soil temperature measurements were taken. According to the trend analysis results, areas where an increase in soil temperature occurred at a depth of 5 cm in Türkiye were noticeably more abundant than areas where the temperature decreased. Spatial variation of soil temperature is a measurement that is of great help in analysis in precision agriculture. These measurements are used to create yield pre-diction models, diagnosis of diseases, and reveal high or low concentrations of temperature temperature concentrations (Martinez and Narducci, 2020). Especially, the predictions about soil temperature at the time of planting of agricultural products are necessary for production management. For example, wheat planting time in Türkiye is made between October and December. As seen in Figure 4, the fact that the trends in Central Anatolia and Western Anatolia increased in these months is an issue that needs attention. This is an important factor in determining the planting time. In a study, it was determined that the germination rate of wheat seeds increased with increasing soil temperature (Fowler et al., 1999). Similar results can be expressed within the 20 cm depth of soil. Trend analysis results based on soil temperature values at a depth of 20 cm are provided monthly in Fig 5.



**Figure 5.** Distribution of trend analysis results of soil temperature at 20 cm depth measured monthly at 73 meteorological stations in Türkiye between 1980-2021 according to the IDW method (1: Significant decreasing trend, 2: Decreasing trend, 3: No trend, 4: Increasing trend, Significant increasing trend)

According to the results of the trend analysis, the highest positive trend in soil temperature measured at 20 cm was determined in Türkiye in March. In March, a Significant Positive Trend (SPT) was detected in 83.56% of the stations where soil temperature measurements were taken. In November, the distribution of the increasing trend in soil temperature in Türkiye was determined to be 71.23% SPT. When the trend analysis distribution map in Figure 5 is examined, it is observed that more than 50% of SPT for 20 cm soil temperature is observed for 9 months. Accordingly, monthly SPT at 20 cm is higher than SPT at 5 cm. On the other hand, the lowest negative trend distribution was determined in July, as in 5 cm. In July, a Significant Negative Trend (SNT) was detected in 4.11% of the stations where soil temperature measurements were taken.

### Assessment of soil temperature trend with soil management

Soil surface heat processes such as soil temperature and flux can be represented within a triple block system of total solar radiation-atmosphere-soil. Soil temperature depends on the quantity of heat energy reaching the soil from different sources, the quantity of heat energy lost from the soil through various pathways, and the specific heat of the soil. The correlations obtained between air temperature and soil temperature at present are in line with this statement. However, the effect of heat sources reaching the soil surface or depths of the soil is affected by many factors interactively. Some of these factors include vegetation cover (Song et al., 2013; Ni et al., 2019), soil tillage status (Wierenga et al., 1982; Licht and Al-Kaisi, 2005), soil moisture content (Al-Kayssi et al., 1990; Zhang et al., 2020), topographic conditions (Wu et al., 2016), and specific structural properties of the soil (color, bulk density, organic matter content, texture, etc.) (Elizaberashvili et al., 2010; Martias and Musil, 2012; Sándor and Fodor, 2012). The specific heat of the soil depends on various factors such as vegetation

cover, topography, elevation, global solar radiation, air temperature, surface roughness, soil color, water content, and organic-inorganic matter content (George, 2001). Additionally, various factors such as geographic location, soil tillage type, and soil cover affect soil temperature (Elias et al., 2019).

According to the research findings, it is understood that there are increases in soil temperature values at both depths (5 and 20 cm). This situation could adversely affect agricultural production in Türkiye, an important agricultural country, as soil temperature is a leading dynamic physical property that affects evaporation, organic matter decomposition, biological activity, seed germination, and other processes. It is also one of the most sensitive physical properties to management changes (Blanco-Canqui and Ruis, 2020). Some researchers have reported a linear increase in the number of germinating seeds with the elevation of soil temperature to the optimum level. However, it has been reported that germination linearly decreases after further temperature increases (Steinmaus et al., 2000; Bradford, 2002; Onwuka Mang, 2018). On the contrary, some researchers have reported that increasing soil temperature enhances bioactivity and positively affects the availability of plant nutrients. Stone et al. (1999) reported an increase in maize yield with increasing soil temperature. Similar results have been obtained in studies by Bollero et al. (1996). The effect of soil tillage on soil temperature is significant. Changes in porosity due to soil tillage directly affect heat conduction. Different soil tillage systems applied in agricultural production can affect not only soil temperature but also soil filtration, water content, and soil temperature and significantly alter the soil's physical, chemical, and biological properties (Biberdzic et al., 2020). The need to provide food to the increasing global population has led to intensive agricultural practices involving the conversion of forests and less productive lands into agricultural lands requiring intensive soil tillage and fertilizer use (Haruna et al., 2020).

With the widespread adoption of irrigation in Türkiye, significant changes have occurred in crop patterns, leading to the intensive use of soils for second crop cultivation in many regions. While this case leads to significant increases in agricultural production, unplanned management systems, excessive chemical use, and intensive soil tillage have negative effects on soil's physical properties. Liu et al. (2021) reported that no-tillage and chisel-plowing practices resulted in better outcomes in terms of soil temperature, organic matter content, and total nitrogen compared to conventional tillage and deep tillage. The desire to obtain more yield per unit area leads to excessive field traffic and excessive intervention in the soil due to the use of improper soil tillage methods, resulting in the disappearance of plant residues on the soil surface and an increase in soil temperature. Therefore, it is crucial to implement soil tillage systems that leave a certain amount of plant residue on the soil surface. There are many studies on this subject. Derpsch et al. (2014) stated that crop residues returned to the soil are a critical organic matter input for subsoil, can conserve soil moisture, balance excessive soil temperature, and intensify soil biological activity. Plant residues act as a physical barrier that blocks sunlight and regulates soil temperature fluctuations more efficiently when left on the soil surface compared to when plowed (Thapa et al., 2021a, b). Veiga et al. (2010) reported that changes in soil temperature among soil tillage practices are significant throughout the maize growing season and are dependent on soil cover and soil moisture. Preserving crop residues on the soil surface provides the following benefits to soil quality (Cherubin et al., 2021): increased nutrient cycling (Cherubin et al., 2019), biological activity (Paredes et al., 2015), carbon sequestration (Bordonal et al., 2018), improved structural quality (Castioni et al., 2018), water infiltration (Johnson et al., 2016), and erosion control (Valim et al., 2016). Additionally, stubble can reduce soil temperatures (Awe et al., 2015) but can create better growth conditions for crops by conserving soil moisture.

According to data from the last forty years in Türkiye, significant increases in soil temperatures are observed, parallel to the aforementioned excessive soil tillage and increases in air temperatures. Especially in regions with a semi-arid climate, irregularities in precipitation regimes, coupled with the widespread use of traditional soil tillage practices, lead to significant increases in soil temperatures and also significantly increase the risk of drought. The climate changes led to significant increases in soil temperatures almost throughout the country in February and March. Particularly during the April-November period, when agricultural activities are intensive, positive trend values are concentrated, and significant positive trend (SPT) values are expected to be higher in Central Anatolia due to significantly low rainfall levels. The effect of soil tillage practices on soil temperature increase, especially at a depth of approximately 5 cm, is generally observed at the insignificant positive trend (IPT) level. The intensive use of traditional soil tillage and deep tillage systems, which involve extensive soil disturbance, not only leads to excessive intervention in the soil but also causes a significant positive trend (SPT) in soil temperatures at a depth of 20 cm from February to November in almost the entire country. The soil temperature values used in the study are values measured at meteorological stations under controlled conditions. It should be noted that the effect of agricultural practices mentioned above may further increase or decrease soil temperature values. Therefore, further research is needed on how soil temperature can be affected by agricultural practices. In Türkiye, soil tillage systems leave little crop residue on the soil surface. This situation, along with wind and water erosion, leads to sudden changes and increases in soil temperature levels. Therefore,



protective soil tillage systems that leave more crop residue on the soil surface need to be implemented rapidly. Studies should be conducted to disseminate the most appropriate method among reduced tillage, strip tillage, and no-till (direct seeding) systems, considering the soil type, climate characteristics, and crop variety, to increase their usage in agricultural production further.

Rising trends in soil temperature can favor some changes in soils. The exchange in evaporation with soil-moisture balance can be given as a good example. Because soil surface temperature is near related to soil evaporation rate and soil evaporation stages (Qiu and Ben-Asher, 2010). Many researchers (Reginato et al., 1976; Hatfield et al., 1983; Krideg et al., 2008) have urged that soil temperatures can be used to estimate evaporation. The occurrence of high rates of evaporation provides the development of significant soil problems such as salinization in arid and semiarid regions. Today, at that place are salinity and sodicity problems in 1,518,722 hectares of land in Türkiye (Sönmez, 2011). Increasing trends in soil temperature can affect this amount to increase more. Changes in soil temperature as well affect the hydraulic attributes of soils (Grant and Bachmann, 2002). For example, changes in soil temperature impact soil particles, soil porosity, and the interaction surface between liquid and solid, specially more importantly in heavy loamy soils with high clay content. Increases in temperature can reduce interparticle the bond strength, which, when compounded with differential thermal expansion between mineral particles and water, can cause a decrement in void ratio. expansion of particles also causes a decrease in specific surface area, which means reductions in the water retention capacity of the particles (Gao and Shao, 2015). The increasing temperature trends obtained in this study showed up that more measures should be adopted the future concerning the protection of soil moisture in agricultural and pasture fields. There's a close relationship between soil warming and biochemical events. According to a study, it was shown that caused changes in organic carbon stability, changes in microbial structure and increased decomposition reaction rates a 2 °C soil warming at 25 cm soil depth over a 10-year period (Cheng et al., 2017). In fact, due to climate, topography, intensive agricultural practices and improper soil management, for soils are poor in terms of organic matter in many regions in Türkiye (Aydın et al., 2016; Kapur et al., 2017; Demir and Mirici, 2022). The fact that a large part of the soil temperature trend results given to Figure 3 are increasing may make the organic carbon stock further still dramatic in the soil. In many studies, they are especially climate change of estimated that soil organic carbon will decrease imputable various factors (Lal, 2004; She et al., 2022; Wang et al., 2023). We anticipate that our research findings can inform future soil management policy making in this region. We think that our research findings can be applied as a tool in planning the best soil management practices for nowadays and the future in Türkiye.

## CONCLUSIONS

According to the trend analysis conducted using the soil temperature values of 73 meteorological stations in Türkiye between 1980 and 2021, a predominantly positive increase has been observed. Although negative trends have been detected in some locations, the increase in temperature has been much more pronounced. Significant correlations have been found between soil temperature and air temperature values for the same stations. However, agricultural practices such as intensive soil cultivation can further increase the effect of soil temperature. Changes in soil temperature in Türkiye, an important agricultural country, may have adverse effects on agricultural production. In many studies conducted on this subject, it has been reported that with the increase in soil temperature, irregularities occur in germination, nutrient availability, and soil-water balance. The most important of these irregularities is the disruption of the balance in soil-plant water relations. The germination performance of seeds sown in the soil for the cultivation of winter crops in October and November and summer crops in March and April is closely related to this balance. Positive trends will lead to soil moisture loss. Consequently, there will be a much greater demand for freshwater sources used for irrigation. The most appropriate soil management practices must be carried out meticulously to minimize the threats posed by the increase in soil temperature.

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## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

## Author Contributions

**Yasin DEMİR** :The main idea of the study, data collection, data analysis, mapping, writing

**Azize DOĞAN DEMİR:** statistical analysis, discussion and interpretation of findings, writing  
**Mehmet Zahid MALASLI:** Discussion of findings, data management, writing.

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Original Article




Melissopalynological Evaluation of Honey from Ganja Gazakh Region of Azerbaijan

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ABSTRACT

This study provides a comprehensive melissopalynological analysis of honey samples from the Ganja Gazakh region of Azerbaijan, offering valuable insights into the diversity and abundance of plant species visited by honey bees in the area. A total of 23 honey samples were examined using light microscopy to identify and quantify the pollen types and their frequencies. The results revealed that the most abundant plant families in the honey samples were Asteraceae, Fabaceae, Rosaceae, and Lamiaceae. This finding suggests that these plant families are important sources of nectar and pollen for honey bees. Notably, the Fabaceae family was found to be the most prevalent, with pollen grains present in all 23 honey samples, ranging from 11.5% to 66% in frequency. Within the Fabaceae family, *Glycyrrhiza glabra* was the most dominant species, appearing in 16 samples. Other Fabaceae taxa, including *Astragalus* spp., *Lotus* spp., *Medicago* spp., *Melilotus* spp., *Onobrychis* spp., *Robinia* spp., *Trifolium* spp., and *Vicia* spp. were also identified at varying frequencies. These findings highlight the melliferous potential of the native flora in the Ganja Gazakh region and provide valuable information for consumers, beekeepers, and regulatory bodies to ensure the authenticity and traceability of the region's honey. The study contributes to the growing body of knowledge on the botanical origins of honey and the foraging preferences of honey bees in different geographical areas.

**Key words:** Palynological, honey bee, microscopic, pollen, botanical origin

Azerbaycan Gence Kazak Bölgesi Ballarının Melissopalinolojik Olarak Değerlendirmesi

Öz

Bu çalışma, Azerbaycan'ın Gence Kazak Bölgesi'nden alınan bal örneklerinin kapsamlı melissopalinolojik analizini sunmakta, bölgedeki bal arısı florası çeşitliliği ve bolluğu hakkında bilgiler vermektedir. Toplam 23 bal örneği, içeriğinde yer alan polen tiplerinin ve sıklıklarının belirlenmesi için ışık mikroskobu kullanılarak incelenmiştir. Sonuçlar, bal örneklerindeki en baskın bal arısı florası ailelerinin Asteraceae, Fabaceae, Rosaceae ve Lamiaceae olduğunu göstermiştir. Bu bulgu, bu bitki ailelerinin bal arıları için önemli nektar ve polen kaynakları olduğunu ortaya koymaktadır. Özellikle Fabaceae ailesi en baskın aile olarak öne çıkmış, bu familyaya ait polen taneleri 23 bal örneğinin tamamında toplamda %11,5 ile %66 arasında değişen sıklıklarda tespit edilmiştir. Fabaceae ailesi içinde *Glycyrrhiza glabra* belirlenen en baskın tür olup, 16 örnekte görülmüştür. *Astragalus* spp., *Lotus* spp., *Medicago* spp., *Melilotus* spp., *Onobrychis* spp., *Robinia* spp., *Trifolium* spp. ve *Vicia* spp. gibi diğer Fabaceae taksonları da değişen sıklıklarda tanımlanmıştır. Bu bulgular, Gence Kazak Bölgesi'ndeki doğal floranın arı florası potansiyelini vurgulamakta ve tüketiciler, arıcılar ve düzenleyici kurumlar için bölgenin balının izlenebilirliğini sağlamada değerli bilgiler sunmaktadır. Çalışma, farklı coğrafi alanlardaki bal arılarının beslenme



tercihlerinin belirlenmesine ve balın botanik kökenlerine ilişkin bilgi birikiminin geliştirilmesine katkı sağlamaktadır.

**Anahtar kelimeler:** Palinolojik, bal arısı, mikroskopik, polen, botanik kaynak

## INTRODUCTION

Honey is the most consumed and economically significant bee product by humans (El Sohaimy et al., 2015). Honey is produced by honey bees collecting nectar from floral or extrafloral nectaries found on various parts of plants (such as leaves, stems, etc.), or from secretions of insects feeding on living parts of plants (Karabagias et al., 2014), and then processing it by removing its moisture and adding enzymes through their bodies to produce a viscous and high-energy natural sweet product known as honey (Liu et al., 2013).

It is the most complex food found in nature and can be used by humans without processing (González-Miret et al., 2007). While honey serves as a sweetener in many foods (Ouchemoukh et al., 2010), it is also used as a raw material in the pharmaceutical and cosmetic industries (Mattonai et al., 2016). Honey has been accepted as a part of apitherapy since ancient times. Therefore, it is used in the treatment of burns, gastrointestinal diseases, chronic wounds, asthma, skin ulcers, cataracts, and other diseases due to its antimicrobial, antioxidant, antiviral, antiparasitic, anti-inflammatory, anticancer, and immune-enhancing activities (Küçük et al., 2007; Samarghandian, et al., 2017). The health benefits of honey are also mentioned in the holy text of various religions. Thus, honey is a product adopted by all cultural and religious beliefs (Nayik, et al., 2018).

Depending on the plant source, honey contains approximately 200 different substances. Sugar and water are the main components of honey. Additionally, honey contains minerals, proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, phenolic acids, and other phytochemicals (Bentabol Manzanares et al. 2011; Karabagias et al. 2014). The composition of honey depends on factors such as its botanical source, processing of honey, environmental conditions such as climate and temperature of the region where honey is obtained (Nayik et al. 2018). Considering the diversity of nectar-bearing plants, it is understandable that the contents of honeys vary greatly from one another.

The botanical and geographical origin of honey have become a significant focus for ensuring its quality and authenticity (Alghamdi et al., 2020). In many parts of the world, the only official procedure used to determine the floral source of honey is melissopalynological analysis, which involves microscopic examination of pollen grains contained in honey. Honey's purity, geographical origin, and botanical source are all been ascertained through the melissopalynology, an efficient procedure (Shakoori et al., 2023). Melissopalynology, a specialized sub-discipline, sheds light on the environmental interactions and feeding behaviors of honey bees. It also plays a key role in assessing the purity of honey. Melissopalynology enables the identification of pollen grains in the honey by analyzing various morphological features such as pollen diameter, exine thickness, pollen type, class, aperture, and exine ornamentation. These characteristics observed using with different microscopy techniques, allow for precise differentiation of pollen at the family, genus, and species levels. This method provides pollens as a key that distinguishes different honey varieties based on their botanical and geographical origins. Additionally, it offers a comprehensive profile of the pollen and nectar plants consumed by honeybees, aiding in the evaluation of a region's melliferous potential.

Due to its geographical location, Azerbaijan have nine of the eleven climate zones found globally (Mehtiyeva and Zeynalova, 2013). The diversity of Azerbaijan's soil and climatic conditions supports the variety of plant genetic resources. Azerbaijan have approximately 4500 naturally occurring plant species, 4.74% of them are endemic (Musayev and Akparov, 2015). Additionally, 720 plant species found in the country are known as nectariferous plants (Kamboj et al., 2013). The climate conditions of the Ganja-Gazakh Economic Region vary due to differences in elevation. In the plains (below 500 meters), the climate is arid and hot, while the slopes experience a mild and semi-arid climate. In contrast, the mountainous areas are characterized by a humid and cold climate (Seyidov, 2003). Due to its climate and variations in elevation, the Ganja-Gazakh Economic Region is one of the most suitable areas for beekeeping, boasting an abundance of diverse and high-quality nectar-producing plants. Melliferous flora from the taxa *Ajuga* spp., *Amygdalus communis*, *Arctium lappa*, *Astragalus* spp., *Aster* spp., *Brassica oleracea*, *Calamintha clinopodium*, *Centaurea* spp., *Cynoglossum officinale*, *Echium* spp., *Echinops* spp., *Epilobium* spp., *Helianthus cultus*, *Lamium* spp., *Leonurus villosus*, *Lonicera* spp., *Lythrum salicaria*, *Melilotus officinalis*, *Mentha longifolia*, *Nepeta* spp., *Onobrychis* spp., *Origanum vulgare*, *Phlomis* spp., *Robinia pseudoacacia*, *Symphytum* spp., *Teucrium* spp., *Thymus* spp., and *Trifolium* spp. have been reported earlier (Qualiyev, 2014).

Understanding the botanical and geographic origins of honey is a fundamental requirement for identification of it's authenticity, emphasizing the importance of this research. The aim of the study was to

evaluate 23 honey samples from Aghstafa, Dashkasan, Gadabay, Goranboy, Gazakh, Samukh, Shamkir, and Tovuz administrative districts of Azerbaijan Ganja-Gazakh Economic Region based on melissopalynological analyses. This study represents the first scientific investigation of melissopalynological analysis of Azerbaijan Ganja Gazakh Economical Region, providing novel insights about bee flora of this region. Background and significance of the problem by using the most recent publications should be provided.

## MATERIALS AND METHODS

### MATERIALS

#### Honey Samples

Honey samples were obtained from eight different administrative district (Aghstafa, Dashkasan, Gadabay, Goranboy, Gazakh, Samukh, Shamkir, and Tovuz) of Azerbaijan Ganja-Gazakh Region (Table 1., Figure 1.). Samples were provided directly from beekeepers in clean glass jar and stored in the dark +4 °C until analysis.



**Figure 1.** Map of the sampling area

**Table 1.** Honey samples' name, administrative district, municipality and altitude

Samples name	Administrative district	Municipality	Altitude(m)
A02	Aghstafa	Sadıqlı	<500
A18	Dashkasan	Destaphour	500-750
A19	Gadabay	Kelaman	>750
A20	Gadabay		>750
A04	Goranboy	Fexralı	<500
A10	Goranboy	Kushchular	<500
A11	Goranboy	Fexralı	<500
A13	Goranboy	Semedabad	<500
A21	Goranboy	Fexralı	<500
A22	Goranboy	Fexralı	<500
A23	Goranboy	Fexralı	<500
A17	Qazax	İzlazam	<500
A01	Samux	Aghasibayli	<500
A03	Shamkir	Zeyem	500-750
A08	Shamkir	Tatarlı	>750

**Table 1.** Honey samples' name, administrative district, municipality and altitude

Samples name	Administrative district	Municipality	Altitude(m)
A09	Shamkir	Dashbulag	500-750
A12	Shamkir	Zeyem	500-750
A14	Shamkir	Chanlibel	>750
A15	Shamkir	Zeyem	<500
A16	Shamkir	Gyneykend	<500
A05	Tovuz	Ehmedabad	500-750
A06	Tovuz	Ibrahim Hacili	500-750
A07	Tovuz	Hutu Meseligi	500-750

## METHODS

### Melissopalynological Analysis

#### Botanical Origin

Thoroughly mixed ten g honey was transferred to a test tube, followed by the addition of 20 mL of distilled water. The tubes were incubated in a 45°C water bath for 10–15 minutes, then centrifuged at 3500 rpm for 45 minutes. The supernatant was discarded, and the sediment was collected a small amount using a sterile needle tip with (1-2 mm<sup>3</sup>) basic fuchsin glycerin-jelly. The sample was transferred onto a slide. The microscope slides were evaluated (100x, 40x) magnification with Leica DM500 light microscope. Different morphological characteristics were used to identify the pollen grains at the species, genus and family level. In this study, 200 pollen grains were counted for the calculation of frequency. Although some researchers have counted between 500 and 1000 pollen grains, recent studies have reported that counting excessive numbers of pollen grains increases error and leads to unnecessary time loss. Therefore, the ideal number has been recorded as 200. And we have included nectarless pollen species for melissopalynological studies because of these taxa may contribute to composition of honey. Pollen taxa were divided into four main categories according to frequency in honey predominant pollen ( $P > 45\%$ ), secondary pollen (16-45%), important minor pollen (3-15%) and minor pollen (less than 3%) (Louveaux et al., 1978; Bobiş et al., 2020).

#### Total Pollen Number (TPN-10)

The total pollen number (TPN) analysis for 10 g honey was conducted as follows: After homogenizing, 10 g honey was weighed and placed in a test tube, followed by the addition of 20 ml distilled water and a Lycopodium spp. tablet containing 12 542 spores as a control. The tubes were incubated in a 45°C water bath for 10–15 minutes, added basic fuchsin, and centrifuged at 3500 rpm for 45 minutes. The supernatant was discarded, and 0.1 ml of 50% glycerin was added. A 0.01 ml sample of this solution was transferred to another tube containing 0.09 ml of 50% glycerin. Finally, 0.01 ml of the solution was examined under light microscope. Honeys were categorized into classes based on the total pollen number per 10 g of honey (Louveaux et al., 1978). The pollen classes are as follows: Class I: Less than 20000 (unifloral honeys with under-represented pollen), Class II: 20000–100000 (multifloral honeys, honeydew honeys, and mixtuers of flower and honeydew honeys), Class III: 100000–500000 (unifloral honeys with over- represented pollen and honeydew honey), Class IV: 500000–1000000 (unifloral honeys with strongly over-represented pollen and some pressed honeys), Class V: More than 1000000 (only pressed honey).

## RESULTS AND DISCUSSION

The melissopalynological analysis of honey samples from the Azerbaijan Ganja Gazakh Region revealed many types of pollen in total of 23 samples of honey. The variety of pollen types reflects the floristic diversity of the region. Qualitative analysis identified 70 plant taxa from 34 plant family present in the selected site (Table 2., Figure 2.).

**Table 2.** Pollen composition of honey samples

Honey samples	Administrative district	Altitude (m)	Pollen composition
A01	Samux	<500	<p>*<i>Plantago</i></p> <p>**<i>Helianthus annuus</i></p> <p>***Brassicaceae, <i>Astragalus</i>., <i>Eryngium</i>, <i>Populus</i></p> <p>****Lamiaceae, Poaceae, Rosaceae, <i>Aster</i>, <i>Echium</i>, <i>Cornus</i>, <i>Medicago</i>, <i>Onobrychis</i>, <i>Trifolium</i>, <i>Vicia</i>, <i>Castanea sativa</i></p> <p>**<i>Malus</i></p> <p>***Brassicaceae, Fabaceae, Poaceae, Scrophulariaceae, Rosaceae, <i>Ailanthus</i>, <i>Echium</i>, <i>Lamium</i>, <i>Medicago</i>, <i>Onobrychis</i>, <i>Trifolium</i>, <i>Teucrium</i>, <i>Glycyrrhiza glabra</i>, <i>Plantago lanceolata</i></p>
A02	Aghstafa	<500	<p>****Apiaceae, Myrtaceae, Solanaceae, <i>Populus</i> spp., <i>Castanea sativa</i>,</p> <p>**Lamiaceae, <i>Echium</i>, <i>Onobrychis</i>,</p> <p>***Fabaceae, Rhamnaceae, Rosaceae, <i>Astragalus</i></p>
A03	Shamkir	500-750	<p>****Asteraceae, Campanulaceae, Convolvulaceae, Lamiaceae, Pinaceae, <i>Centaurea</i>, <i>Cichorium</i>, <i>Fragaria</i>, <i>Melilotus</i>, <i>Plantago</i>, <i>Teucrium</i>, <i>Trifolium</i>, <i>Glycyrrhiza glabra</i></p> <p>**<i>Astragalus</i>, <i>Helianthus annuus</i></p>
A04	Goranboy	<500	<p>***<i>Cichorium</i></p> <p>****Apiaceae, Brassicaceae, Cornaceae, Pinaceae, <i>Centaurea</i>, <i>Lamium</i>, <i>Medicago</i>, <i>Robinia</i></p> <p>**Rhamnaceae, <i>Eucalyptus</i></p>
A05	Tovuz	500-750	<p>*** Fabaceae, Lamiaceae, Sapindaceae, <i>Epilobium</i>, <i>Onobrychis</i>, <i>Teucrium</i>, <i>Glycyrrhiza glabra</i></p> <p>****Alliaceae, Pinaceae, <i>Campanula</i>, <i>Vicia</i>, <i>Castanea sativa</i>,</p> <p>*Fabaceae</p>
A06	Tovuz	500-750	<p>**<i>Eucalyptus</i></p> <p>***Rhamnaceae, Rosaceae, <i>Teucrium</i></p> <p>****Asteraceae, Apiaceae, Brassicaceae, Poaceae, <i>Ailanthus</i>, <i>Onobrychis</i>, <i>Plantago</i>, <i>Castanea sativa</i></p> <p>**Fabaceae, Rhamnaceae</p>
A07	Tovuz	500-750	<p>***Cistaceae, Lamiaceae, Rosaceae, <i>Astragalus</i>, <i>Teucrium</i>, <i>Tilia</i>, <i>Trifolium repens</i></p> <p>****Apiaceae, Campanulaceae, Myrtaceae, <i>Ailanthus</i>, <i>Convolvulus</i>, <i>Echium</i>, <i>Geranium</i>, <i>Medicago</i>, <i>Plantago</i>, <i>Ranunculus</i>, <i>Glycyrrhiza glabra</i></p> <p>**Rosaceae</p>
A08	Shamkir	>750	<p>***Boraginaceae, Fabaceae, Lamiaceae, <i>Ailanthus</i>, <i>Helianthemum</i>, <i>Onobrychis</i>, <i>Plantago</i>, <i>Teucrium</i>, <i>Trifolium</i>, <i>Glycyrrhiza glabra</i></p> <p>****Ranunculaceae</p>

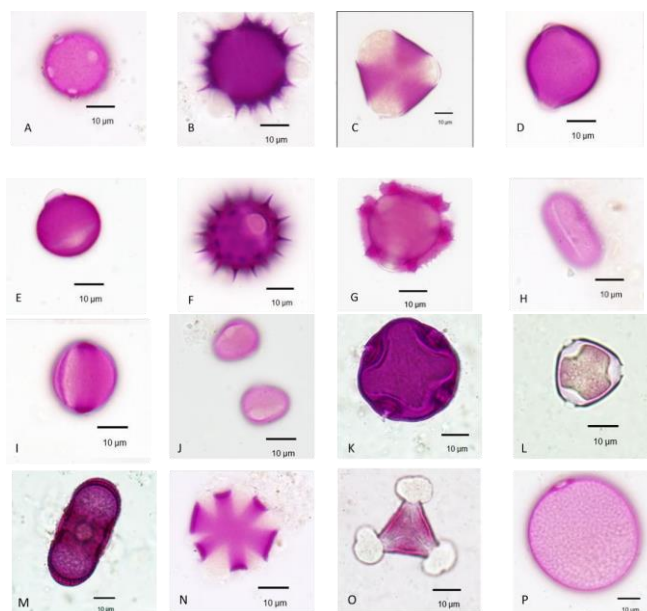
**Table 2.** Pollen composition of honey samples

Honey samples	Administrative district	Altitude (m)	Pollen composition
A09	Shamkir	500-750	<p>*Scrophulariaceae</p> <p>***Asteraceae, Brassicaceae, Lamiaceae, Violaceae</p> <p><i>Onobrychis, Trifolium</i></p> <p>****Myrtaceae, Poaceae, <i>Ailanthus, Allium, Astragalus, Cichorium, Echium, Teucrium, Vicia</i>,</p> <p>**<i>Helianthus annuus, Trifolium</i></p>
A10	Goranboy	<500	<p>*** Scrophulariaceae, <i>Viola, Cichorium, Glycyrrhiza glabra, Plantago</i></p> <p>****Apiaceae, Asteraceae, Brassicaceae, Fabaceae, Lamiaceae, Myrtaceae, Pinaceae, <i>Lamium, Medicago, Populus, Teucrium</i></p> <p>**Fabaceae</p>
A11	Goranboy	<500	<p>*** Hydrangaceae, Pinaceae, Poaceae, Rosaceae, Violaceae, <i>Plantago, Trifolium, Helianthus annuus</i></p> <p>****Apiaceae, Lamiaceae, Myrtaceae, Scrophulariaceae, <i>Cichorium, Medicago, Populus, Teucrium, Glycyrrhiza glabra</i></p> <p>**<i>Echium, Onobrychis</i></p>
A12	Shamkir	500-750	<p>*** Fabaceae, Lamiaceae, Rhamnaceae, Rosaceae, <i>Melilotus</i></p> <p>****Apiaceae, Asteraceae, Brassicaceae, Campanulaceae, Convolvulaceae, Myrtaceae, Pinaceae, <i>Astragalus, Cichorium, Lamium, Medicago, Poaceae, Salix, Symphytum, Teucrium, Glycyrrhiza glabra</i></p> <p>**<i>Astragalus</i></p>
A13	Goranboy	<500	<p>***<i>Helianthus, Plantago, Teucrium, Viola, Trifolium pratense</i></p> <p>**** Asteraceae, Brassicaceae, Fabaceae, Myrtaceae, Poaceae, Rosaceae, <i>Artemisia, Castanea, Cichorium, Vicia, Glycyrrhiza glabra</i></p> <p>**<i>Onobrychis</i></p>
A14	Shamkir	>750	<p>*** Fabaceae, Lamiaceae, Rosaceae, <i>Astragalus, Echium, Lamium</i>,</p> <p>****Acanthaceae, Apiaceae, Astearaceae, Campanulaceae, Myrtaceae, <i>Castanea, Centaurea, Cichorium, Plantago Teucrium, Trifolium, Vicia, Glycyrrhiza glabra</i></p> <p>**<i>Helianthus annuus</i></p>
A15	Shamkir	<500	<p>***Brassicaceae, Fabaceae, Lamiaceae, Rosaceae, <i>Echium, Onobrychis, Vicia</i></p> <p>****Apiaceae, Asteraceae, Campanulaceae, Pinaceae <i>Castanea, Cichorium, Teucrium, Viola, Glycyrrhiza glabra</i>, not detected</p> <p>** Fabaceae, <i>Onobrychis</i></p>
A16	Shamkir	<500	<p>***Brassicaceae, Castanea, Echium, Trifolium, Lamiaceae, <i>Viola</i></p> <p>**** Apiaceae, Asteraceae, <i>Cynoglossum</i>, Campanulaceae, <i>Astragalus, Trifolium, Teucrium</i>, Myrtaceae, Poaceae, Rosaceae</p> <p>* Rosaceae</p>
A17	Gazakh	<500	<p>***Caprifoliaceae, Fabaceae, Lamiaceae, <i>Fragaria, Medicago, Viola, Glycyrrhiza glabra</i>,</p> <p>**** Hydrangaceae, Poaceae, <i>Allium, Elaeagnus, Teucrium, Trifolium</i></p>

**Table 2.** Pollen composition of honey samples

Honey samples	Administrative district	Altitude (m)	Pollen composition
A18	Dashkesen	500-750	**Fabaceae, <i>Teucrium</i> ***Brassicaceae, Lamiaceae, Pinaceae, Rosaceae, <i>Echium</i> , <i>Onobrychis</i> , <i>Glycyrrhiza glabra</i> , <i>Castanea sativa</i> , ****Tiliaceae
A19	Gedebey	>750	** <i>Lotus</i> , <i>Ziziphora</i> *** Campanulaceae, Fabaceae, Lamiaceae, <i>Echium</i> , <i>Medicago</i> , <i>Plantago</i> , <i>Trifolium</i> , <i>Vicia</i> Asteraceae, Pinaceae, Rosaceae, <i>Astragalus</i> , <i>Onobrychis</i> , <i>Glycyrrhiza glabra</i>
A20	Gedebey	>750	***Apiaceae, Brassicaceae, Rosaceae, Lamiaceae, <i>Astragalus</i> , <i>Echium</i> , <i>Onobrychis</i> , <i>Trifolium</i> , <i>Glycyrrhiza glabra</i>
A21	Goranboy	<500	**** Fabaceae, Pinaceae, <i>Plantago</i> , <i>Symphytum</i> , <i>Teucrium</i> , * Fabaceae ***Brassicaceae, Pinaceae, Rosaceae. <i>Plantago</i> **** Poaceae, <i>Cichorium</i> , <i>Helianthus</i> , <i>Astragalus</i> , <i>Lotus</i> , <i>Malva</i> *Fabaceae ** <i>Plantago</i>
A22	Goranboy	<500	*** Brassicaceae, Pinaceae, <i>Helianthus annuus</i> ****Cistaceae, Fabaceae, Poaceae *Fabaceae ** <i>Plantago</i> , <i>Helianthus annuus</i>
A23	Goranboy	<500	**** Brassicaceae, Hydrangaceae, Malvaceae, Myrtaceae, Rosaceae, <i>Cichorium</i> , <i>Trifolium</i> , <i>Viola</i>

\*>45%; \*\*15-45%; \*\*\*3-15%; \*\*\*\*<3%



**Figure 2.** Light microscope (100x) image of : A: *Plantago* spp.; B: Asteraceae, C: Rosaceae; D: *Glycyrrhiza glabra*; E: *Astragalus* spp., F: *Helianthus annuus*; G: *Cichorium* spp., H: *Onobrychis* spp.; I: *Teucrium* spp.; J: *Echium* spp.; K: Tiliaceae; L: Rhamnaceae; M: Apiaceae; N: Lamiaceae; O: Myrtaceae; P: Poaceae

The most frequently identified plant families in all analyzed honey samples were Fabaceae (100% of samples) (including *Astragalus* spp., *Glycyrrhiza*, *Trifolium* spp., and *Onobrychis* spp.), followed by Lamiaceae



(87% of samples), Rosaceae (78% of samples), Plantaginaceae (74% of samples), and Asteraceae (70% of samples). Results showed that Acanthaceae and Solanaceae were the least frequent families. Fabaceae family with 12 taxa had the maximum species contribution followed by Asteraceae, Boraginaceae, Lamiaceae, and Rosaceae. The Fabaceae family, commonly known as the legume family, is one of the most diverse and ecologically significant plant families in Azerbaijan. In this study, Fabaceae, Asteraceae, and Rosaceae families, which showed high nectar and pollen flow, have been previously reported as melliferous families in Kazakhstan, Mexico and Pakistan ( Moldakhmetova et al., 2023; Balvino-Olvera et al., 2024; Mushtaq et al., 2024). Similar to our study, Asteraceae, Fabaceae and Lamiaceae families were found to be the most frequently used plants by bees in West and East Azerbaijan regions (Khosroshahi and Lotfalizadeh, 2011). Studies on Iranian honey have indicated that the honey is derived from plant families most commonly observed in this research, including Asteraceae, Fabaceae, Rosaceae, and Lamiaceae (Mehti, 2023). Sorkun et al. (2014), conducted pollen analysis on 76 honey samples from Türkiye Ardahan province and found that the most common pollen taxa belonged to the Fabaceae and Boraginaceae families. In this study, pollen from the Fabaceae family was observed in all 23 honey samples, with a frequency ranging from 11.5% to 66%. Among these, *Glycyrrhiza glabra* was the most prevalent species, discovering in 16 samples. Additionally, taxa such as *Astragalus* spp., *Lotus* spp., *Medicago* spp., *Melilotus* spp., *Onobrychis* spp., *Robinia* spp., *Trifolium* spp., and *Vicia* spp. were identified at varying frequencies.

**Table 3.** TPN-10 results of honey samples

Samples	District	Altitude	TPN-10	Group
A01	Samux	<500	22 076	II
A02	Aghstafa	<500	43 895	II
A03	Shamkir	500-750	10 021	I
A04	Goranboy	<500	21 431	II
A05	Tovuz	500-750	23 158	II
A06	Tovuz	500-750	23 124	II
A07	Tovuz	500-750	58 567	II
A08	Shamkir	>750	1 394 578	V
A09	Shamkir	500-750	64 557	II
A10	Goranboy	<500	11 652	I
A11	Goranboy	<500	8 291	I
A12	Shamkir	500-750	9 353	I
A13	Goranboy	<500	64 794	II
A14	Shamkir	>750	28 536	II
A15	Shamkir	<500	25 600	II
A16	Shamkir	<500	14 146	I
A17	Gazakh	<500	82 385	II
A18	Dashkasan	500-750	8 398	I
A19	Gedebey	>750	2 582	I
A20	Gedebey	>750	16 403	I
A21	Goranboy	<500	18 956	I
A22	Goranboy	<500	20 743	II
A23	Goranboy	<500	20 858	II

In this study, the most frequent plant taxa were *Plantago* spp., *Echium* spp, *Eucalyptus* spp., *Helianthus annuus*, *Malus* spp., *Onobrychis* spp., *Astragalus* spp., *Teucrium* spp., and *Trifolium* spp. had high pollen frequency percentage (> 45% or 16-45% )

*Plantago* spp. was identified as the dominant pollen in sample A01; Fabaceae in samples A06, A21, A22, and A23; Scrophulariaceae in sample A09; and Rosaceae in sample A17. In this study, the frequency of occurrence Plantaginaceae family was determined 47% in A01 sample, Fabaceae family over 50% in A06, A21, A22, and A23, Scrophulariaceae family 51.5% in A09, and Rosaceae family 57.5% in A17.

Anemophilous pollen grains like *Plantago* spp. was highly represented in the studied honeys, whereas is lower represented in the honeys of Argetina (Forcone et al., 2005). Several studies have found that pollen from non-melliferous plants can be present in honey samples, in addition to the predominant melliferous plant pollen (Bobış et al., 2020; Layek et al., 2020) For instance, a study on acacia honey found the presence of pollen from non-melliferous plants such as *Plantago* like this study (Bobış et al., 2020). Furthermore, the presence of non-melliferous plant pollen in honey can provide valuable information about the foraging behavior of honey bees

and the overall floral diversity in the surrounding environment (Layek et al., 2020). This information can be useful for conservation efforts, habitat management, and sustainable beekeeping practices.

The quantitative analysis of the 23 honey samples revealed a normal pollen content. 13 of the samples were classified into Group II and showed moderate amount of pollen. One of sample (A08) was Group V according to the Maurizio classification system, while the remaining nine samples were categorized into Group I and had a low pollen count. We didn't detect honeydew elements in any honey (Table 3).

## CONCLUSION

Given the limited palynological research on Azerbaijan honey, it is strongly recommended that this study be expanded to cover a broader area. Expanding the scope of this research would significantly contribute to filling existing knowledge gaps and provide valuable insights for the development and sustainability of the apiculture sector in these regions. This study not only enhances our understanding of the bee flora in the Ganja-Gazakh region of Azerbaijan but also holds the promise of transforming beekeeping practices, promoting environmental conservation, and advancing scientific knowledge

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## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

## Author Contributions

**1<sup>st</sup> Author Duygu Nur-Çobanoğlu:** Conceptualization; data curation; formal analysis; investigation; methodology; writing— original draft; writing—review and editing.

**2<sup>st</sup> Author Kadriye-Sorkun:** Conceptualization; data curation; acquisition; writing— original draft; writing—review and editing.

**3<sup>st</sup> Author Duygu Nur-Çobanoğlu :** Conceptualization; methodology; writing— original draft; writing—review and editing.

## Author's Note Section:

This publication is based on the thesis titled "Determination of Contents of the Honey Samples Collected from Azerbaijan Ganja Gazakh Region by Microscopic, HPLC And GC-MS Analysis" submitted to Hacettepe University for the fulfillment of the PhD degree in 2017.

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## Assessment of the Susceptibility of Grapevine Genotypes in the Eastern Anatolia Region Genetic Resource Plot to Powdery Mildew (*Erysiphe necator*) Under Natural Infection Conditions


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### ABSTRACT

Powdery mildew disease caused by *Erysiphe necator* is an economically important disease of grape varieties of the *Vitis vinifera* species worldwide. The use of resistant grape varieties in the control of the disease is extremely important for human and environmental health. In this study, the tolerance levels of 203 local cultivars, two tolerant (Regent and Kishmish Vatkana) and two sensitive (Karaerik and Italia) cultivars collected within the scope of the Eastern Anatolia Region Grapevine Genetic Resources Project were determined against the pathogen *Erysiphe necator* under natural conditions. A visual scale based on sporulation density and necrosis formation on the leaves was used for evaluation and four vines were evaluated for each genotype. The colonisation rate on the surface of the leaves was determined according to the scale values between 0-7. The severity of the disease was calculated based on the lesions on the leaves and the susceptibility of the genotypes was determined. As a result of the study, 3 of the genotypes were highly resistant (HR), 44 were resistant (R), 68 were susceptible (S) and 88 were highly susceptible (HS) in terms of powdery mildew disease severity. Among these genotypes, 50 out of 197 genotypes belonging to *Vitis vinifera* species and all *Vitis labrusca* genotypes showed tolerance below 30% in terms of disease severity. *Vitis vinifera* subsp. *sylvestris* (Sarmalık Üzüm) was classified as susceptible with a disease severity of 37.99%. In the study, *V. vinifera* showed more sensitivity than other species. The most resistant genotype was Izabelle-1 (3.88%) and the most susceptible genotype was Beyaz Üzüm S1 (90.07%).

**Key words:** Genetic resource, Grape genotypes, Powdery mildew, Natural infection, *Vitis vinifera*

## Doğu Anadolu Bölgesi Asma Genetik Kaynak Parselindeki Genotiplerin Doğal Enfeksiyon Koşullarında Külleme Hastalığına (*Erysiphe necator*) Duyarlılıklarının Değerlendirilmesi

### ÖZ

*Erysiphe necator*'un neden olduğu külleme hastalığı, dünya çapında *Vitis Vinifera* türüne giren üzüm çeşitlerinin ekonomik açıdan önemli bir hastalıdır. Hastalığın kontrolünde dirençli üzüm çeşitlerinin kullanımı insan ve çevre sağlığı açısından son derece önemlidir. Bu çalışmada Doğu Anadolu Bölgesi Asma Genetik Kaynakları projesi kapsamında toplanan ve koruma altına alınan 203 yerel çeşit ile 2 tolerant (Regent ve Kishmish Vatkana) ve 2 duyarlı (Karaerik ve Italia) çeşidin doğal koşullar altında *Erysiphe necator* patojenine karşı toleranslık düzeyleri belirlenmiştir. Değerlendirmede yapraklar üzerindeki sporulasyon yoğunluğu ve nekroz

oluşumuna dayalı görsel skala kullanılmış ve her genotip için dörder asma değerlendirilmiştir. Yaprakların yüzeyindeki kolonizasyon oranı, 0-7 arasındaki skala değerlerine göre belirlenmiştir. Yaprakların üzerindeki lezyon değerleri üzerinden hastalık şiddeti hesaplanarak genotiplerin duyarlılık durumları belirlenmiştir. Çalışma sonucunda külleme hastalık şiddeti bakımından 3 genotip oldukça dirençli (HR), 44 genotip dirençli (R), 68 genotip hassas (S) ve 88 genotip oldukça hassas (HS) olarak belirlenmiştir. Bu genotipleri türler bazında incelendiğinde *Vitis vinifera* türüne ait 197 farklı genotipten 50'si ve *Vitis labrusca* genotiplerinin tamamı, hastalık şiddeti bakımından %30 un altında tolerans göstermiştir. Ayrıca *Vitis vinifera* subsp. *sylvestris* türüne ait Sarmalık üzümün hastalık şiddet derecesi %37.99 ile hassas sınıfta yer almıştır. Türler dayalı değerlendirmede *V. vinifera*'nın diğer türlere kıyasla daha fazla hassasiyet gösterdiği belirlenmiştir. İncelenen genotipler arasında en dirençli genotip olarak İzabelle-1 (%3.88) belirlenirken, en hassas genotip Beyaz Üzüm S1 (%90.07) olarak tespit edilmiştir.

**Anahtar kelimeler:** Genetik kaynak, Üzüm genotipleri, Külleme hastalığı, Doğal enfeksiyon, *Vitis vinifera*

## INTRODUCTION

Turkey is one of the most important countries in the world for vineyard areas and grape production. Due to the ideal climatic conditions and favourable growing conditions, there are very rich *Vitis* gene resources in Turkey (Yıldırım et al., 2019). The geographical location of the country, being situated in both the Near East and the Mediterranean Basin, plays an important role as a gene centre for viticulture (Ağaoğlu, 1986). In addition, the region between the Black Sea and the Caspian Sea, which covers the northeastern part of the Anatolian peninsula, is considered to be the gene centre and cultivation area of *V. vinifera* L. species. This situation reveals that Turkey has an extremely rich genetic diversity in terms of both wild vine (*Vitis vinifera* ssp. *sylvestris*) and cultivated vine (*Vitis vinifera* ssp. *sativa*) (Çelik et al., 1998). It is extremely important to reveal the differences within these gene resources and to use them in breeding by evaluating them in the future (Yağcı and Daler, 2023).

Grape (*Vitis vinifera*) is one of the most important fruit species cultivated as table, juice, wine and raisins. Although *Vitis vinifera* is one of the most commercially important species, it is susceptible to many fungal diseases such as powdery mildew (*Erysiphe necator* (Schw.) Burr.) and downy mildew (*Plasmopara viticola* (Berk. Et Curt) Berl et de Toni) (Reisch et al., 2012; Eibach and Töpfer, 2015; Bozkurt and Yağcı, 2024). Powdery mildew disease caused by the pathogen *Erysiphe necator* is one of the common fungal diseases of grapes and can cause significant yield loss and decrease in fruit quality by affecting all green tissues of the vines (Feechan et al., 2013; Mwamahonje et al., 2015; Pimentel et al., 2021; Aşçı et al., 2021; Sosa-Zuniga et al., 2022; Bozkurt et al., 2023; Maddalena et al., 2023). This disease not only affects grape yield, but can also affect the quality of fruit and wine, including berry flavour and various metabolites (Yu et al., 2022). The susceptibility of grapevines to powdery mildew may differ among cultivars (Boso and Kassemeyer, 2008; Yıldırım et al., 2019; Bozkurt and Yağcı, 2024) and understanding the factors affecting this susceptibility is crucial for disease management in vineyards. Considering the devastating effects of this disease, breeding studies have been initiated worldwide to develop resistant or tolerant plant varieties against this disease (Atak and Şen, 2021; Bozkurt, 2023). Due to the economic importance of *E. necator*, breeders first screened genetic materials to breed resistant varieties (Wan et al., 2007).

It is known that grapevine gene resources play a critical role in determining the effects of powdery mildew disease and the resistance mechanisms of plant phenotypes against the disease. In particular, the resistance levels of different grapevine genotypes against powdery mildew disease have been examined and it has been observed that there are significant differences between species (Atak and Göksel, 2019; Bozkurt and Yağcı, 2024). It has been reported that the majority of cultivated grape cultivars from the species *Vitis vinifera* lack genetic resistance to *E. necator*, making them highly susceptible to powdery mildew (Kunova et al., 2021). The research and utilisation of grapevine genetic resources offers an important avenue for the development of grape varieties with enhanced resistance to diseases and environmental stresses. These genetic resources harbour important traits for combating both biotic and abiotic stresses such as cold, drought, pests and diseases. By utilising genetic diversity in grapevine populations, researchers can identify individuals with superior traits or those that are naturally resistant to specific stress factors.

In this study, it was aimed to determine the susceptibility of 203 different grapevine genotypes protected in the Eastern Anatolia Region Grapevine Genetic Resource Plot against powdery mildew disease by natural infection method.

## MATERIALS AND METHODS

### Material

The material of the study consisted of 203 local grape varieties/genotypes collected within the scope of 'Eastern Anatolia Region Grapevine Genetic Resources' project from the provinces (Erzincan, Erzurum, Iğdır, Artvin, Sivas, Ardahan, Bingöl, Van, Mardin, Tunceli and Gümüşhane) within the responsibility area of Erzincan Horticultural Research Institute Directorate. Of these genotypes, 197 were *Vitis vinifera*, 5 were *Vitis labrusca* and 1 was *Vitis vinifera* subsp. *sylvestris*. In addition, two tolerant varieties (Regent and Kishmish Vatkana) and two susceptible varieties (Karaerik and Italia) were selected as controls for the study. These varieties/genotypes were collected and maintained in the genetic resource plot (Table 3). The study was conducted in the genetic resource plot, employing a coincidence block design with four replications and a single vine in each replication.

### Method

#### Determination of Susceptibility of Genotypes to Powdery Mildew by Natural Infection Method

In order to determine the susceptibility levels to powdery mildew disease, powdery mildew infected panicles, shoots and leaves were collected from vineyards in Erzincan and Üzümlü between 19-23 June. These infected plant materials were brought to the vineyard where the genotypes were located and placed at appropriate intervals without any artificial inoculation and naturally infected with powdery mildew pathogens. This process was repeated five times at one week intervals to ensure the robustness of the results. No fungicide application was made in the study vineyard and powdery mildew infections on the leaves were monitored. Genotypes were evaluated at the first appearance of powdery mildew infections on the leaves in early August.

#### Counting and Evaluation

Natural infection assessment was carried out on the leaves between 1-4 August. For counting and evaluation of sporulation, a visual scale based on sporulation intensity and necrosis formation established by Wang et al. (1995) was used. For disease assessment, 4 vines were used for each local cultivar/genotype and all leaves on 2 shoots from the right and left side of each vine were examined. The colonisation rate on the surface of the leaves was determined according to the scale values between 0-7. According to the scale values, disease severity on the leaves were calculated using the Townsend Heuberger formula (Townsend and Heuberger, 1943) and sporulation severity was calculated and given as percentage (%) (Table 1). Then, disease susceptibility levels of local cultivars according to disease severity were evaluated according to Wang et al. (1995) (Table 2).

Townsend Heuberger Formula:  $P = \frac{\sum(n \times v)}{Z \times N} \times 100$

P - Percentage of disease severity,  
n - Number of diseased leaves,  
v - Numerical value of the degree of disease,

Z - Highest scale value,  
N - Number of leaves examined.

**Table 1.** Infection rating levels of genotype leaves for their degree of resistance to powdery mildew under natural infection conditions

Scale Value	Disease Severity (%)
0	< 0.1
1	0.1 – 5.0
2	5.1 – 15.0
3	15.1 – 30.0
4	30.1 - 45.0
5	45.1 – 65.0
6	65.1 – 85.0
7	> 85

**Table 2.** Susceptibility levels according to powdery mildew disease severity

Disease Severity (%)	Disease Severity Index
0.00 < 0.10	I - Immune
0.11 - 5.00	HR - Highly resistant
5.01 - 25.00	R - Resistant
25.01 - 50.00	S - Sensitive
50,01 - 100.00	HS - Highly sensitive



## RESULTS AND DISCUSSION

As part of the "Eastern Anatolia Region Grapevine Genetic Resources" project, the susceptibility of 203 local grape varieties/genotypes, along with 2 susceptible and 2 tolerant grape varieties, to powdery mildew was evaluated under natural inoculum conditions in 2023. The severity of the disease observed on the leaves was relatively assessed. According to the findings, 4 genotypes scored 1 point, 28 genotypes scored 2 points, 24 genotypes scored 3 points, 44 genotypes scored 4 points, 67 genotypes scored 5 points, 33 genotypes scored 6 points and 4 genotypes scored 7 points. Disease severity was then calculated for each genotype using the Townsend Heuberger formula (Townsend and Heuberger, 1943). When the disease severity of 203 different genotypes and 4 control varieties in the genetic resource plot were analysed, powdery mildew disease severity was between 3.88 (Isabelle-1) and 90.07 (White Grape S1) (average 44.08%). In addition, disease susceptibility levels of genotypes according to disease severity were evaluated according to Wang et al. (1995) for powdery mildew disease. When the susceptibility level of genotypes to powdery mildew was analysed according to powdery mildew disease severity, 3 genotypes were highly resistant (HR), 44 genotypes were resistant (R), 68 genotypes were susceptible (S) and 88 genotypes were highly susceptible (HS). When the powdery mildew natural infection results of Kishmish Vatkana and Regent, which are known to be tolerant control varieties, were evaluated, the disease severity was 4.46 and 5.08, the scale values were 1 and 2 points, and the disease susceptibility levels were measured as highly resistant (HR) and resistant (R), respectively. Similarly, as a result of the evaluations made in Italia and Karaerik, which are known to be susceptible, the scale values were 6 points and their disease susceptibility levels were determined as highly susceptible (HS) (Table 3). The results of the study and the differences in the susceptibility of the cultivars to powdery mildew disease are consistent with previous studies. As a matter of fact, many grape varieties belonging to *Vitis vinifera* and *Vitis labrusca* species were examined for their susceptibility to powdery mildew disease. In the findings obtained, it has been reported both in our study and in different studies that most varieties of *V. vinifera* are susceptible to *E. necator*, while *V. labrusca* is tolerant, and that this susceptibility varies on variety basis (Atak et al., 2017; Bozkurt et al., 2023; Şen, 2024; Bozkurt and Yağcı, 2024). Wan et al. (2007), in order to determine the resistance to powdery mildew disease in *Vitis* spp. gene pool, 66 genotypes from 13 *Vitis* species were studied under natural conditions. They used the 0-7 scale and determined that 46 of 66 genotypes were resistant to powdery mildew in their scoring according to this scale. Similarly, Atak et al. (2017) tested 26 genotypes of *V. labrusca*, 6 interspecific cultivars and 3 cultivars of *V. vinifera* for susceptibility to powdery mildew by natural and artificial inoculation methods. In the artificial powdery mildew scoring of the study, Isabella (Yalova and Tekirdağ) and Kyoho varieties were found to be highly resistant (HR) and Italia variety was found to be highly susceptible (HS). On the other hand, in a remarkable study conducted over a period of two years, Bozkurt et al. (2023) artificially inoculated 15 different grape cultivars with the pathogen *E. necator* under greenhouse conditions to evaluate their susceptibility to powdery mildew over a period of seven weeks. When the results of the seventh week were analysed, infection rates for resistant cultivars were relatively low and ranged between 5.9% and 10.3%. On the other hand, increases ranging between 67.3-96.7% were observed in the more susceptible varieties Horoz Karası, Künefi, Erciş, Dökülgen, Fenerit, Italia, Muhammedi, Karaerik, Vakkas, Narince and Hatun Parmağı. Şen, (2023) conducted a study to determine the resistance to powdery mildew and powdery mildew diseases in 307 hybrid genotypes obtained as a result of cross breeding studies carried out by Atatürk Garden Cultures Central Research Institute. As a result of natural and artificial inoculation tests, 9 genotypes resistant to powdery mildew disease were identified. These findings emphasise the critical role of genetic factors in determining the susceptibility of grape varieties to powdery mildew (Parage et al., 2012).

**Table 3.** Names and origin of local cultivars/genotypes and disease severity, scale value and susceptibility levels after natural infection

Genotypes	Species Name	Powdery Mildew Disease Severity (%)	Disease Scale Value	Disease Susceptibility Level
Abdehir	<i>Vitis vinifera</i>	81.03 ± 0.82	6	HS
Adesa	<i>Vitis labrusca</i>	3.90 ± 0.76	1	HR
Ağın Beyazı	<i>Vitis vinifera</i>	48.28 ± 1.40	5	S
Ağır Ağız	<i>Vitis vinifera</i>	15.39 ± 1.77	3	R
Ahmetoğlu	<i>Vitis vinifera</i>	52.31 ± 0.59	5	HS
Al Üzüm (Olur)	<i>Vitis vinifera</i>	8.35 ± 0.77	2	R

HR-Highly resistant, R-Resistant, S-Sensitive, HS-Highly sensitive

**Table 3.** (Continued) Names and origin of local cultivars/genotypes and disease severity, scale value and susceptibility levels after natural infection

Genotypes	Species Name	Powdery Mildew Disease Severity (%)	Disease Scale Value	Disease Susceptibility Level
Al Üzüm (Torul)	<i>Vitis vinifera</i>	9.03 ± 1.53	2	R
Altuntaş	<i>Vitis vinifera</i>	66.80 ± 1.63	6	HS
Arapgir	<i>Vitis vinifera</i>	82.22 ± 0.71	6	HS
Askeri	<i>Vitis vinifera</i>	53.88 ± 0.98	5	HS
Aş Üzüümü	<i>Vitis vinifera</i>	83.79 ± 0.88	6	HS
At Memesi	<i>Vitis vinifera</i>	24.12 ± 0.40	3	R
Azerbaycan Çavuşu	<i>Vitis vinifera</i>	30.86 ± 1.29	4	S
Azezi	<i>Vitis vinifera</i>	31.19 ± 1.48	4	S
Bağlarbaşı	<i>Vitis vinifera</i>	51.07 ± 2.79	5	HS
Balcani	<i>Vitis vinifera</i>	42.91 ± 0.89	4	S
Besni	<i>Vitis vinifera</i>	16.99 ± 1.64	3	R
Beyaz Amasya	<i>Vitis vinifera</i>	56.52 ± 2.54	5	HS
Beyaz Bambo	<i>Vitis vinifera</i>	7.59 ± 0.87	2	R
Beyaz Hatun Parmağı	<i>Vitis vinifera</i>	47.87 ± 1.26	5	S
Beyaz Kış Üzüümü	<i>Vitis vinifera</i>	28.13 ± 2.43	3	S
Beyaz Kışmış	<i>Vitis vinifera</i>	62.65 ± 1.66	5	HS
Beyaz Tatlı Çekirdekli	<i>Vitis vinifera</i>	6.35 ± 1.68	2	R
Beyaz Turfanda	<i>Vitis vinifera</i>	30.73 ± 1.94	4	S
Beyaz Üzüm S1	<i>Vitis vinifera</i>	90.07 ± 0.73	7	HS
Beyaz Üzüm S2	<i>Vitis vinifera</i>	87.65 ± 0.61	7	HS
Beyaz Üzüm S3	<i>Vitis vinifera</i>	44.02 ± 1.13	4	S
Beyaz Üzüm S4	<i>Vitis vinifera</i>	7.32 ± 1.19	2	R
Beyaz Üzüm S5	<i>Vitis vinifera</i>	6.76 ± 1.31	2	R
Beyaz Üzüm S6	<i>Vitis vinifera</i>	12.91 ± 0.87	2	R
Beyaz Üzüm S7	<i>Vitis vinifera</i>	56.17 ± 2.48	5	HS
Beyaz Üzüm T1	<i>Vitis vinifera</i>	41.25 ± 1.76	4	S
Beyaz Üzüm T2	<i>Vitis vinifera</i>	38.40 ± 4.37	4	S
Beyaz Üzüm T3	<i>Vitis vinifera</i>	51.18 ± 0.68	5	HS
Beyaz Üzüm V2	<i>Vitis vinifera</i>	31.46 ± 0.93	4	S
Beyaz Üzüm(Çukurbağ)	<i>Vitis vinifera</i>	70.08 ± 1.52	6	HS
Boğazkere	<i>Vitis vinifera</i>	62.28 ± 1.27	5	HS
Bulut	<i>Vitis vinifera</i>	7.83 ± 0.81	2	R
Çavuş (Geçit)	<i>Vitis vinifera</i>	80.50 ± 0.72	6	HS
Çavuş (Koçkar)	<i>Vitis vinifera</i>	10.81 ± 0.95	2	R
Çavuş (Yukarıdere)	<i>Vitis vinifera</i>	56.49 ± 1.71	5	HS
Çavuş(Bayırbağ)	<i>Vitis vinifera</i>	11.79 ± 1.13	2	R
Çayra Üzüümü	<i>Vitis vinifera</i>	20.84 ± 1.50	3	R
Çekirdeksiz Beyaz	<i>Vitis vinifera</i>	9.37 ± 3.56	2	R
Çekirdeksiz Kara Üzüm	<i>Vitis vinifera</i>	58.88 ± 1.09	5	HS
Çekirdeksiz Kırmızı Üzüm	<i>Vitis vinifera</i>	58.50 ± 0.87	5	HS
Çekirdeksiz Kışmış	<i>Vitis vinifera</i>	60.14 ± 0.78	5	HS
Çekirdeksiz Sarı Üzüm	<i>Vitis vinifera</i>	72.49 ± 0.67	6	HS
Çemiç 1	<i>Vitis vinifera</i>	52.25 ± 3.75	5	HS
Çemiç-2	<i>Vitis vinifera</i>	10.29 ± 1.09	2	R

HR-Highly resistant, R-Resistant, S-Sensitive, HS-Highly sensitive

**Table 3.** (Continued) Names and origin of local cultivars/genotypes and disease severity, scale value and susceptibility levels after natural infection

Genotypes	Species Name	Powdery Mildew Disease Severity (%)	Disease Scale Value	Disease Susceptibility Level
Çiğitsiz Üzüm	<i>Vitis vinifera</i>	73.92 ± 2.24	6	HS
Çiklep	<i>Vitis vinifera</i>	59.05 ± 1.92	5	HS
Direjik	<i>Vitis vinifera</i>	61.74 ± 1.67	5	HS
Ekber Üzümlü	<i>Vitis vinifera</i>	53.70 ± 2.23	5	HS
Elhakki	<i>Vitis vinifera</i>	64.31 ± 0.42	5	HS
Emceoglu	<i>Vitis vinifera</i>	64.59 ± 0.74	5	HS
Ergan Üzümlü	<i>Vitis vinifera</i>	43.04 ± 1.47	4	S
Erkenci Çavuş	<i>Vitis vinifera</i>	61.69 ± 0.91	5	HS
Eskibeyli Siyah Üzüm	<i>Vitis vinifera</i>	52.82 ± 3.57	5	HS
Gedikli Ağın Beyazı	<i>Vitis vinifera</i>	29.98 ± 1.33	3	S
Gedikli Beyaz Üzüm	<i>Vitis vinifera</i>	16.22 ± 1.39	3	R
Gedikli Siyah Üzüm	<i>Vitis vinifera</i>	30.80 ± 0.98	4	S
Gelin Parmağı	<i>Vitis vinifera</i>	75.65 ± 0.85	6	HS
Gineş	<i>Vitis vinifera</i>	5.54 ± 1.58	2	R
Gökgölot	<i>Vitis vinifera</i>	66.84 ± 1.28	6	HS
Gül Üzümlü	<i>Vitis vinifera</i>	41.18 ± 2.39	4	S
Gümüş Beyazı	<i>Vitis vinifera</i>	38.54 ± 6.65	4	S
Güz İstanbul	<i>Vitis vinifera</i>	8.29 ± 0.85	2	R
Hacı Tesbihi	<i>Vitis vinifera</i>	65.48 ± 2.46	6	HS
Hanım Göbeği	<i>Vitis vinifera</i>	57.59 ± 0.93	5	HS
Harthul	<i>Vitis vinifera</i>	31.20 ± 0.99	4	S
Hasani-1	<i>Vitis vinifera</i>	71.52 ± 0.96	6	HS
Hasani-2	<i>Vitis vinifera</i>	22.61 ± 0.74	3	R
Hathul	<i>Vitis vinifera</i>	8.72 ± 2.01	2	R
Hatun Parmağı	<i>Vitis vinifera</i>	70.70 ± 4.97	6	HS
Hatun Parmağı(Olur)	<i>Vitis vinifera</i>	39.83 ± 0.39	4	S
Hedfi	<i>Vitis vinifera</i>	48.32 ± 1.65	5	S
Hemrani	<i>Vitis vinifera</i>	69.55 ± 1.07	6	HS
Herci	<i>Vitis vinifera</i>	60.64 ± 0.52	5	HS
Heseni	<i>Vitis vinifera</i>	19.09 ± 1.93	3	R
Hocabaş	<i>Vitis vinifera</i>	41.95 ± 2.32	4	S
İnce Beyaz	<i>Vitis vinifera</i>	73.30 ± 2.13	6	HS
İnek Memesi	<i>Vitis vinifera</i>	57.03 ± 0.79	5	HS
İri At Memesi	<i>Vitis vinifera</i>	14.26 ± 2.19	2	R
İri Keçi Memesi	<i>Vitis vinifera</i>	33.47 ± 1.79	4	S
İsabella 1	<i>Vitis labrusca</i>	3.88 ± 1.25	1	HR
İsabella-2	<i>Vitis labrusca</i>	4.03 ± 1.27	1	HR
İzmir Siyahı	<i>Vitis vinifera</i>	63.42 ± 1.80	5	HS
Kabarcık	<i>Vitis vinifera</i>	63.00 ± 1.94	5	HS
Kabuğu Yuka	<i>Vitis vinifera</i>	28.16 ± 0.77	3	S
Kalduk	<i>Vitis vinifera</i>	58.57 ± 0.96	5	HS
Kamik	<i>Vitis vinifera</i>	71.76 ± 0.49	6	HS
Kara Gahet	<i>Vitis vinifera</i>	45.05 ± 2.20	5	S
Kara Menüşke	<i>Vitis vinifera</i>	43.57 ± 9.56	4	S
Kara Üzüm	<i>Vitis vinifera</i>	43.20 ± 1.35	4	S

HR-Highly resistant, R-Resistant, S-Sensitive, HS-Highly sensitive

**Table 3.** (Continued) Names and origin of local cultivars/genotypes and disease severity, scale value and susceptibility levels after natural infection

Genotypes	Species Name	Powdery Mildew Disease Severity (%)	Disease Scale Value	Disease Susceptibility Level
Karaeznek	<i>Vitis vinifera</i>	7.18 ± 1.06	2	R
Karaeznek 2	<i>Vitis vinifera</i>	51.31 ± 1.30	5	HS
Karakabarcık	<i>Vitis vinifera</i>	47.51 ± 1.64	5	S
Karul	<i>Vitis vinifera</i>	19.35 ± 3.96	3	R
Keçi Memesi	<i>Vitis vinifera</i>	6.40 ± 0.96	2	R
Keleş	<i>Vitis vinifera</i>	30.84 ± 0.46	4	S
Kerfoki	<i>Vitis vinifera</i>	49.83 ± 0.35	5	S
Kerimgandi	<i>Vitis vinifera</i>	6.20 ± 1.39	2	R
Kerkuş	<i>Vitis vinifera</i>	53.22 ± 1.00	5	HS
Keten Gömlek	<i>Vitis vinifera</i>	42.51 ± 1.05	4	S
Kırmızı İstanbul	<i>Vitis vinifera</i>	29.73 ± 2.22	3	S
Kırmızı Keçi Memesi	<i>Vitis vinifera</i>	37.63 ± 1.08	4	S
Kırmızı Üzüm T1	<i>Vitis vinifera</i>	64.12 ± 1.14	5	HS
Kırmızı Üzüm T2	<i>Vitis vinifera</i>	42.75 ± 1.14	4	S
Kırmızı Üzüm T3	<i>Vitis vinifera</i>	58.49 ± 1.43	5	HS
Kırmızı Üzüm (Eskibeyli)	<i>Vitis vinifera</i>	71.37 ± 2.04	6	HS
Kışlık Beyaz	<i>Vitis vinifera</i>	68.71 ± 0.94	6	HS
Kızıl Türü	<i>Vitis vinifera</i>	53.27 ± 2.08	5	HS
Kızıl Üzüm (Erzincan)	<i>Vitis vinifera</i>	69.37 ± 0.81	6	HS
Kızıl Üzüm (Erciş)	<i>Vitis vinifera</i>	71.19 ± 0.86	6	HS
Kirfok	<i>Vitis vinifera</i>	46.97 ± 1.19	5	S
Kirli Şerife	<i>Vitis vinifera</i>	21.77 ± 0.72	3	R
Kişmiş Üzümü	<i>Vitis vinifera</i>	30.32 ± 1.03	4	S
Kokulu Üzüm	<i>Vitis labrusca</i>	7.45 ± 0.91	2	R
Korostol	<i>Vitis vinifera</i>	16.94 ± 2.42	3	R
Koyun Gözü	<i>Vitis vinifera</i>	46.95 ± 0.66	5	S
Kuduruş	<i>Vitis vinifera</i>	7.81 ± 1.29	2	R
Kuş Üzümü	<i>Vitis vinifera</i>	23.49 ± 2.73	3	R
Kuzu Kuyruğu	<i>Vitis vinifera</i>	24.64 ± 0.25	3	R
Laz Üzümü	<i>Vitis labrusca</i>	8.04 ± 1.54	2	R
Mazlumani	<i>Vitis vinifera</i>	53.38 ± 1.12	5	HS
Mazruma	<i>Vitis vinifera</i>	43.51 ± 1.40	4	S
Mehmetoğlu	<i>Vitis vinifera</i>	44.02 ± 0.61	4	S
Meneşker	<i>Vitis vinifera</i>	64.35 ± 4.37	5	HS
Merzune M1	<i>Vitis vinifera</i>	5.15 ± 0.57	2	R
Merzune M2	<i>Vitis vinifera</i>	36.96 ± 0.81	4	S
Mesebbe	<i>Vitis vinifera</i>	63.40 ± 0.58	5	HS
Mesma	<i>Vitis vinifera</i>	31.50 ± 0.45	4	S
Mezarlık	<i>Vitis vinifera</i>	19.62 ± 0.59	3	R
Mıh Üzümü	<i>Vitis vinifera</i>	51.98 ± 0.50	5	HS
Miskali	<i>Vitis vinifera</i>	51.59 ± 0.76	5	HS
Mor Amasya	<i>Vitis vinifera</i>	53.23 ± 0.73	5	HS
Müskü	<i>Vitis vinifera</i>	51.58 ± 1.18	5	HS
Nanebur	<i>Vitis vinifera</i>	22.67 ± 1.02	3	R
Nar Tanesi	<i>Vitis vinifera</i>	53.44 ± 5.08	5	HS

HR-Highly resistant, R-Resistant, S-Sensitive, HS-Highly sensitive

**Table 3.** (Continued) Names and origin of local cultivars/genotypes and disease severity, scale value and susceptibility levels after natural infection

Genotypes	Species Name	Powdery Mildew Disease Severity (%)	Disease Scale Value	Disease Susceptibility Level
Nörgah	<i>Vitis vinifera</i>	31.36 ± 0.94	4	S
Papaz Üzümlü	<i>Vitis vinifera</i>	34.64 ± 2.97	4	S
Pembe Üzümlü T1	<i>Vitis vinifera</i>	54.65 ± 1.95	5	HS
Pembe Üzümlü T2	<i>Vitis vinifera</i>	44.71 ± 0.33	4	S
Pembenaz	<i>Vitis vinifera</i>	29.29 ± 1.72	3	S
Pırtık	<i>Vitis vinifera</i>	11.18 ± 2.25	2	R
Sarı Golot	<i>Vitis vinifera</i>	46.98 ± 1.29	5	S
Sarı Yezenday	<i>Vitis vinifera</i>	54.47 ± 1.81	5	HS
Sarmalık Üzümlü	<i>Vitis vinifera</i> subsp. <i>sylvestris</i>	37.99 ± 0.49	4	S
Selüke Pembe Üzümlü	<i>Vitis vinifera</i>	36.28 ± 0.34	4	S
Selüke Yeşil Üzümlü	<i>Vitis vinifera</i>	36.30 ± 0.16	4	S
Servi Beyaz Gevrek	<i>Vitis vinifera</i>	86.81 ± 1.51	7	HS
Servi Beyaz Üzümlü	<i>Vitis vinifera</i>	48.75 ± 3.05	5	S
Servi Kara Üzümlü	<i>Vitis vinifera</i>	30.69 ± 0.58	4	S
Servi Lice Üzümlü	<i>Vitis vinifera</i>	46.45 ± 1.00	5	S
Servi Mor Erkenci	<i>Vitis vinifera</i>	66.43 ± 1.40	6	HS
Servi Pembe Üzümlü	<i>Vitis vinifera</i>	75.30 ± 1.33	6	HS
Siyah Hatun Parmağı	<i>Vitis vinifera</i>	75.21 ± 0.84	6	HS
Siyah Mayhoş Üzümlü	<i>Vitis vinifera</i>	7.62 ± 0.51	2	R
Siyah Şarap Mayası	<i>Vitis vinifera</i>	40.77 ± 2.74	4	S
Siyah Şire	<i>Vitis vinifera</i>	11.82 ± 0.41	2	R
Siyah Tatlı Çekirdekli	<i>Vitis vinifera</i>	66.99 ± 4.12	6	HS
Siyah Turfanda	<i>Vitis vinifera</i>	29.24 ± 0.10	3	S
Siyah Üzümlü G1	<i>Vitis vinifera</i>	48.74 ± 2.44	5	S
Siyah Üzümlü G2	<i>Vitis vinifera</i>	56.35 ± 1.00	5	HS
Siyah Üzümlü S1	<i>Vitis vinifera</i>	60.18 ± 6.16	5	HS
Siyah Üzümlü S2	<i>Vitis vinifera</i>	44.45 ± 1.24	4	S
Siyah Üzümlü T1	<i>Vitis vinifera</i>	68.91 ± 0.72	6	HS
Siyah Üzümlü T2	<i>Vitis vinifera</i>	59.94 ± 0.74	5	HS
Siyah Üzümlü T3	<i>Vitis vinifera</i>	66.13 ± 0.78	6	HS
Siyah Üzümlü T4	<i>Vitis vinifera</i>	27.71 ± 0.90	3	S
Siyah Üzümlü V1	<i>Vitis vinifera</i>	59.24 ± 1.17	5	HS
Siyah Üzümlü V2	<i>Vitis vinifera</i>	42.39 ± 0.86	4	S
Siyah Üzümlü V3	<i>Vitis vinifera</i>	22.76 ± 1.01	3	R
Siyah Üzümlü V6	<i>Vitis vinifera</i>	44.22 ± 1.25	4	S
Siyah Üzümlü V7	<i>Vitis vinifera</i>	44.50 ± 1.45	4	S
Siyah Üzümlü(Dutluca)	<i>Vitis vinifera</i>	63.26 ± 2.99	5	HS
Suşehri Beyaz Üzümlü	<i>Vitis vinifera</i>	74.57 ± 1.66	6	HS
Şafra	<i>Vitis vinifera</i>	87.54 ± 0.37	7	HS
Şebik Karası	<i>Vitis vinifera</i>	67.73 ± 1.33	6	HS
Şilfoni	<i>Vitis vinifera</i>	43.83 ± 1.82	4	S
Şire	<i>Vitis vinifera</i>	47.12 ± 5.51	5	S
Şirelik Üzümlü	<i>Vitis vinifera</i>	66.39 ± 0.58	6	HS

HR-Highly resistant, R-Resistant, S-Sensitive, HS-Highly sensitive

**Table 3.** (Continued) Names and origin of local cultivars/genotypes and disease severity, scale value and susceptibility levels after natural infection

Genotypes	Species Name	Powdery Mildew Disease Severity (%)	Disease Scale Value	Disease Susceptibility Level
Şitvi	<i>Vitis vinifera</i>	55.72 ± 0.90	5	HS
Ternebi	<i>Vitis vinifera</i>	52.85 ± 0.90	5	HS
Tihmin Kabarcığı	<i>Vitis vinifera</i>	77.55 ± 0.91	6	HS
Tilki Kuyruğu	<i>Vitis vinifera</i>	7.34 ± 2.01	2	R
Tombul Üzüm	<i>Vitis vinifera</i>	22.59 ± 2.23	3	R
Turfanda	<i>Vitis vinifera</i>	7.61 ± 1.19	2	R
Tutikoğlu	<i>Vitis vinifera</i>	45.74 ± 0.97	5	S
Türkgözü	<i>Vitis vinifera</i>	43.80 ± 1.00	4	S
Tüylü Turfanda	<i>Vitis vinifera</i>	29.63 ± 2.00	3	S
Vaslı	<i>Vitis vinifera</i>	50.99 ± 0.32	5	HS
Verdani	<i>Vitis vinifera</i>	53.67 ± 2.35	5	HS
Yağ Üzümü	<i>Vitis vinifera</i>	49.74 ± 1.85	5	S
Yaz İstanbul	<i>Vitis vinifera</i>	46.23 ± 0.89	5	S
Yer Çemiçi	<i>Vitis vinifera</i>	44.40 ± 1.31	4	S
Yer Meneşgiri	<i>Vitis vinifera</i>	55.35 ± 1.06	5	HS
Yeşil Üzüm	<i>Vitis vinifera</i>	73.22 ± 2.02	6	HS
Yeşilyurt Üzümü	<i>Vitis vinifera</i>	70.27 ± 4.12	6	HS
Yezendayı	<i>Vitis vinifera</i>	72.00 ± 1.66	6	HS
Zehni	<i>Vitis vinifera</i>	42.50 ± 0.92	4	S
Zeyti	<i>Vitis vinifera</i>	41.72 ± 1.10	4	S
Karaerik	<i>Vitis vinifera</i>	84.31 ± 3.83	6	HS
Italia	<i>Vitis vinifera</i>	72.17 ± 1.78	6	HS
Kismish Vatkana	<i>Vitis vinifera</i>	4.46 ± 0.88	1	HR
Regent	<i>Vitis vinifera</i>	5.08 ± 0.42	2	R

HR-Highly resistant, R-Resistant, S-Sensitive, HS-Highly sensitive

## CONCLUSIONS

This study is the first research aimed at determining the tolerance and susceptibility levels of 203 local grape varieties/genotypes collected from the Eastern Anatolia Region against powdery mildew disease. The findings obtained have revealed the interactions of different genotypes with the *Erysiphe necator* pathogen under natural inoculation conditions. Three different *Vitis* species were examined in the study, and it was determined that *Vitis labrusca* varieties were more resistant than *Vitis vinifera* varieties. In particular, *Vitis vinifera* subsp. *sylvestris* (Sarmalık Üzüm) was calculated to be susceptible at 37.99%. The powdery mildew severity of the genotypes examined varied between 3.88 (Izabelle-1) and 90.07 (Beyaz Üzüm S1), indicating that different grapevine species exhibit varying degrees of susceptibility to powdery mildew. Among 197 different genotypes of *V. vinifera*, 50 of them, and all of the *V. labrusca* genotypes exhibited a disease severity of less than 30%. The grape genotypes identified as resistant to the disease could support the development of new varieties with improved resistance to *Erysiphe necator*, contributing to greater diversity in grape cultivation.

Our recommendations for future studies are to evaluate more genotypes and repeat similar experiments under different climatic conditions. Furthermore, the use of resistant genotypes should be encouraged when developing disease management strategies. Our recommendations for future studies in the same field and on the same subject include the performance of comprehensive studies including physiological, biochemical and transcriptomic analyses. Such studies are crucial for a better understanding and deciphering of the complex interactions of resistance genes. Furthermore, future evaluations of these genotypes need to be conducted either under field conditions or in controlled environments. Appropriate experimental setups are required to validate resistance traits and ensure the practical applicability of these genotypes in global viticulture practices.

In conclusion, this study provides significant insights into the resistance of local grape varieties/genotypes cultivated in the Eastern Anatolia Region to *E. necator* and presents the first evidence of resistance to *E. necator*.



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### Declaration of interests

The authors of this article declare that there are no conflicts of interest.

### Author Contributions

The authors declare that they have contributed equally to this article.

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**Morphological and Chemical Characteristics of *Prunus spinosa* L. Genotypes from Denizli (Çivril)**Levent Kirca<sup>1</sup> ✉, Kerem Mertoğlu<sup>2</sup><sup>1</sup> Pamukkale University, Faculty of Agriculture, Department of Horticulture, Denizli, Türkiye<sup>2</sup> Uşak University, Faculty of Agriculture, Department of Horticulture, Uşak, Türkiye<sup>1</sup>  <https://orcid.org/0000-0003-2496-9513>, <sup>2</sup>  <https://orcid.org/0000-0002-0490-9073>✉: [leventkirca28@gmail.com](mailto:leventkirca28@gmail.com)**ABSTRACT**

This study was conducted to characterize the physico-chemical properties of naturally occurring blackthorn (*Prunus spinosa* L.) genotypes in Çivril, Denizli, Türkiye. In this context, 13 different blackthorn genotypes originating from seeds were examined in terms of color parameters, physical, and chemical properties. Physical characteristics of the fruits revealed that fruit width ranged from 7.95 mm to 14.12 mm, fruit length from 10.49 mm to 14.63 mm, and fruit weight varied between 1.82 g and 2.71 g. Notably, genotypes G3 and G5 stood out with fruit weights of 2.71 g and 2.58 g, respectively. Regarding the fruit flesh-to-seed ratio, genotype G3 exhibited the highest value of 7.10. In terms of color parameters, the  $L^*$  value of the fruit skin ranged from 16.75 to 20.59, while the  $L^*$  value of the fruit flesh varied between 17.48 and 20.45. Biochemical characteristics showed that the soluble solids content ranged widely from 12.00% to 23.40%, while pH values remained stable between 3.60 and 3.90. PCA analysis revealed that the first two components explained 67.13% of the total variation in the examined traits. Correlation analysis found a strong positive relation between fruit flesh color  $L^*$  and  $b^*$  values ( $r=0.95$ ), as well as between fruit length and shape index ( $r=0.89$ ). Based on the biplot results, it can be inferred that the studied *P. spinosa* genotypes have the potential to be evaluated in different ways. In conclusion, it can be stated that this species has high adaptability to local ecosystems and that, in the short term, more efficient genotypes can be obtained through selection, and in the long term, through hybridization.

**Key words:** *Prunus spinosa* L., Genetic Resources, Phenotypic Variation, Principal Component Analysis, Selection**Denizli (Çivril) Bölgesinde *Prunus spinosa* L. Genotiplerinin Meyvelerinin Morfolojik ve Kimyasal Özellikleri****ÖZ**

Bu çalışma, Türkiye'nin Denizli ili, Çivril ilçesinde doğal olarak yetişen çakal eriği (*Prunus spinosa* L.) genotiplerini, fiziko-kimyasal özellikler yönüyle karakterize etmek amacı ile gerçekleştirilmiştir. Bu bağlamda, tohum kökenli 13 farklı çakal eriği genotipi renk parametreleri, fiziksel ve kimyasal özellikleri yönüyle incelenmiştir. Meyvelerin fiziksel özellikleri incelendiğinde, meyve eni 7.95-14.12 mm, meyve boyu 10.49-14.63 mm ve meyve ağırlığı özelliklerinin 1.82 ile 2.71 g sınırları içerisinde değişim gösterdiği belirlenmiştir. Özellikle G3 ve G5 genotipleri, sırasıyla 2.71 g ve 2.58 g meyve ağırlıklarıyla dikkat çekmiştir. Meyve eti/çekirdek oranında G3 genotipi 7.10 ile en yüksek değere sahip olmuştur. Renk parametreleri açısından, meyve kabuğu  $L^*$  değeri 16.75-20.59, meyve eti  $L^*$  değeri ise 17.48-20.45 aralığında dağılım göstermiştir. Biyokimyasal özellikler değerlendirildiğinde, suda çözünür kuru madde içeriği %12.00 ile %23.40 arasında geniş bir varyasyon sergilemiş, pH değerleri ise 3.60-3.90 gibi dar bir aralıkta stabilite göstermiştir. PCA sonucunda, incelenen özelliklerin toplam varyasyonun %67.13'ü ilk iki bileşen tarafından açıklanmıştır. Korelasyon analizinde özellikle meyve eti rengi  $L^*$  ile  $b^*$  değerleri arasında ( $r=0.95$ ) ve meyve uzunluğu ile şekil indeksi arasında ( $r=0.89$ ) güçlü pozitif ilişkiler tespit edilmiştir. Bi-plot sonuçları doğrultusunda, incelenen *P. spinosa* genotiplerinin farklı şekillerde değerlendirilebilme potansiyellerinde olduğu söylenebilir. Sonuç olarak, bu türün yerel ekosistemdeki

adaptasyon kapasitesinin yüksek olduğu ve kısa dönemde seleksiyonla, uzun vadede ise melezleme yoluyla daha verimli genotiplerin elde edilebileceği söylenebilir.

**Anahtar kelimeler:** *Prunus spinosa* L., Genetik Kaynaklar, Fenotipik Varyasyon, Temel Bileşen Analizi, Seleksiyon

## INTRODUCTION

Plum (*Prunus* spp.) is a member of the Rosaceae and belongs to the *Prunus* genus, which includes a wide range of economically and ecologically important species. Among these, *Prunus spinosa* L., commonly known as blackthorn, is native to Europe-Asia gene center in where Türkiye takes place within a significant genetic center for wild plums (Başaran et al., 2024). This species exhibits remarkable drought tolerance and adaptability to diverse ecological conditions, positioning it as a promising rootstock candidate for stone fruit and nuts under the challenges posed by global climate change scenarios (Milosevic et al., 2015; Kirca and Karadeniz, 2024). Furthermore, its resilience makes it suitable for afforestation efforts in arid regions, landscape restoration, and carbon sequestration due to its phytoremediation potential (Özer et al., 2009; Gülay, 2023).

The fruits of *P. spinosa* are small, bluish-purple, and covered with a waxy coating. They are rich in bioactive compounds, including minerals, amino acids, vitamins, phytosterols, triterpenes, organic acids, and phenolic compounds (Bei et al., 2023). These phytochemicals, particularly those with high antioxidant activity, have been reported to mitigate the risk of chronic diseases such as cancer and cardiovascular conditions (Sabatini et al., 2020; Negrean et al., 2023; Kotsou et al., 2023; Başaran et al., 2024). For instance, studies have shown its efficacy in reducing viability in brain cancer cells (Karakas et al., 2019) and its potential use in diabetes treatment due to its antioxidant properties (Sarıkaya et al., 2010). Its antioxidant activity is further linked to enhanced collagen synthesis, contributing to wound healing (Ayla et al., 2017). In traditional medicine, the species has been valued for its hemostatic effects and its ability to improve intestinal functions (Baytop, 1999; Sezer et al., 2016). Additionally, the non-fruit parts of the plant are also abundant in bioactive components (Atik et al., 2022).

In recent years, there has been growing interest in the inclusion of products with superior phytochemical properties in daily diets, particularly in health-conscious societies (Demir and Aktaş, 2018). This increasing demand has underscored the need for more comprehensive studies on phytochemical compositions and the selection of wild genotypes with superior traits (Uzun et al., 2015; Dumanoglu et al., 2019; Delialioğlu et al., 2022; Kurnaz et al., 2024).

Despite the astringent taste of the fruit, which limits its fresh consumption, various parts of *P. spinosa* have long been processed into products such as jams, marmalades, and fruit juices due to their nutritional and medicinal benefits (Başkaya, et al., 2016; Başaran et al., 2024). Nevertheless, there remains a significant gap in the development of superior genotypes for broader cultivation and utilization. The selection of genotypes with enhanced phytochemical properties and superior fruit traits is critical for the species' potential expansion in both agricultural and industrial applications. This study aims to address these gaps by (1) investigating the natural genetic resources of *P. spinosa* for breeding purposes and (2) characterizing key fruit traits using multivariate analysis to identify genotypes with superior characteristics.

## MATERIALS AND METHODS

The study was carried out in 2024 on thirteen seed-origin genotypes (G1, G2...G13) of *P. spinosa* (blackthorn) that grow naturally in the Çivril district of Denizli province (Türkiye). To verify the seed origin of the genotypes, local people and garden/field owners were consulted, and plants confirmed to have grown from seeds were included in the study. The genotypes used in this study grew spontaneously in nature without any cultural practices. Fruit sampling was carried out during the period when the genotypes reached their characteristic fruit color and physiological maturity. Analyses were performed using twenty randomly selected fruit samples from four different orientations of each genotype. After harvest, the fruit samples were immediately transferred to a laboratory in portable coolers to minimize time delays before starting the analyses.

Çivril is located at an average altitude of 832 meters above sea level. A visual representation of the study area is given in Figure 1. The district's topography consists of 30% mountainous terrain, 16% hilly areas, and 54% flat plains, reflecting its various geomorphological features. The region is characterized by a transitional climate between Mediterranean and continental climates. Geologically, the district is situated in an alluvial basin and is surrounded by significant mountainous features such as Mount Bulkaz (1990 m), Kocayaka Hill (1259 m), Bozdağı Hill (1350 m), and Mount Akdağ, which is the highest point in the region at 2449 m (Wikipedia, 2024). These features contribute to the region's unique microclimate, which is particularly suitable for the growth of drought-resistant and hardy species like *P. spinosa*.

When examining the 15-year (2009-2023) average climate data of Çivril district, it shows continental climate characteristics (Figure 2). Annual average temperature values range from 3.45°C (January) in winter months to 25.29°C (July) in summer months. The low temperatures during winter months are suitable for meeting the chilling requirements of *P. spinosa*. The fifteen-year average relative humidity values vary between 42.53% (July) and 74.81% (January), with particularly low humidity rates observed during summer months. The average annual total precipitation is 441.96 mm, with the highest average precipitation recorded in January (67.2 mm) and the lowest in July (4.91 mm). When examining the seasonal distribution of precipitation, it is concentrated in winter and spring months, while significantly decreasing during summer months. The average wind speed varies between 1.96-2.67 m/sec throughout the year, showing no extreme values (TSMS, 2024). These long-term climate parameters enable *P. spinosa* to grow naturally in the region.

The Çivril district's ecological characteristics, including its variable topography, transitional climate, and diverse soil properties, make it an ideal location for studying the genetic diversity and adaptability of *P. spinosa*. This study leverages these unique conditions to explore the genetic potential of this species for future breeding and cultivation strategies.



Figure 1. Map of the study area

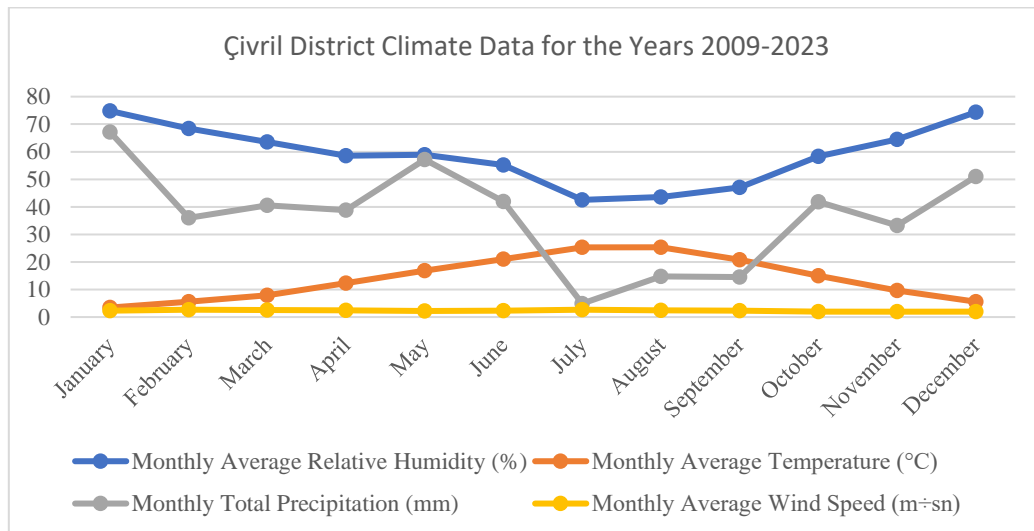


Figure 2. Average monthly average relative humidity (%), monthly average temperature (°C), monthly total precipitation (mm) and monthly average wind speed (m÷sn) of Çivril district between 2009-2023.

The traits examined in this study were grouped into three main categories: physical measurements (fruit width, length, weight, and flesh-to-stone ratio), color attributes ( $L^*$ ,  $a^*$ ,  $b^*$ , chroma, and hue°), and chemical analyses (TSS, pH, and TA).

**Physical Measurements:** Fruit width and length were measured in millimeters (mm) using a digital caliper, and fruit weight was recorded in grams (g) using a precision balance (Gülsoy et al., 2016; Başaran et al., 2024). To determine the fruit flesh-to-stone ratio, twenty fruits from each genotype were weighed, the stones were removed, and the weights of the flesh and stones were measured separately and then calculated as a ratio (Öncül and Aygün, 2021).

**Color Measurements:** Fruit skin and flesh color measurements were conducted using a PCE Instruments Colorimeter (model PCE-CSM 1, Manchester, UK). Measurements were taken from two opposing points on the skin and flesh of each fruit. The color attributes  $L^*$  (lightness),  $a^*$  (red-green),  $b^*$  (yellow-blue), chroma (color saturation), and hue° (color angle) values were determined (Başaran et al., 2024).

**Chemical Analyses:** Soluble solids content (SSC) was measured at 20°C using an Abbe refractometer (Atago, Japan) after filtering the juice obtained from pulped fruit samples through a muslin cloth. The pH was measured at 20°C using a digital pH meter (Eutech, Singapore). Titratable acidity (TA) was determined by extracting 10 g of fruit pulp with distilled water to a volume of 100 mL, storing it overnight in a refrigerator, and filtering the extract. A 10 mL aliquot of the filtrate was titrated with 0.1 M NaOH solution until reaching pH 8.1, and the results were expressed as citric acid equivalents. Chemical analyses followed the methods described by Karadeniz et al. (2007).

Various statistical methods were applied to evaluate the data obtained from the study. Descriptive statistics (mean, standard deviation, minimum, and maximum values) were calculated using the Minitab 17 software (Minitab Inc., State College, PA, USA). Analysis of variance (ANOVA) was performed to determine differences among genotypes, and means were compared using Fisher's Least Significant Difference (LSD) test at  $p < 0.05$ . Pairwise Pearson correlation analysis was conducted to determine the relationships between the studied pomological traits, and principal component analysis (PCA) was applied to visualize these relationships. The correlation and PCA analyses were performed in RStudio (Version 2024.12.0+467) using the 'ggplot2', 'factoextra', 'FactoMineR', and 'corrplot' packages. A significance level of  $p < 0.05$  was adopted for all statistical analyses (Zar, 2013).

## RESULTS AND DISCUSSION

The descriptive statistics for the physico-chemical properties of the blackthorn genotypes, including minimum, maximum, mean, standard deviation, and coefficient of variation (CV), are presented in Table 1. The average fruit width was 11.70 mm, ranging from 7.95 mm to 14.12 mm. The CV value of 17.60% indicated significant variation in fruit width, suggesting a heterogeneous structure in terms of width among the fruits. The average fruit length was 13.26 mm, with a range of 10.49–14.63 mm. A lower CV value of 9.76% was observed for fruit length compared to width, indicating that the fruits were more homogeneous in terms of length. This difference is thought to be due to the nearly spherical characteristic shape of the fruits (Başaran et al., 2024). The average fruit weight was 2.42 g, ranging from 1.82 to 2.71 g. In a study conducted on blackthorn genotypes, the fruit weight varied between 1.31 g and 2.67 g (Bükücü et al., 2024). The CV value of 11.38% suggested moderate variation in fruit weight among the genotypes. Fruit size and weight are significant due to their direct impact on consumer preferences (Gorynska-Goldmann et al., 2023). Therefore, the selection and breeding of genotypes with superior and standardized traits in these aspects are crucial.

The fruit flesh-to-stone ratio is important as it represents the edible portion, which is significant for both fresh consumption and the processing industry to enhance yield (Delialioğlu et al., 2022). The average value obtained in this study was 5.36, with a high CV of 22.84% observed among the genotypes.

The mean skin  $L^*$  value was 18.51, ranging from 16.75 to 20.59. A low CV value of 7.52% indicated that the lightness of the fruit skin was relatively stable among the genotypes. The skin  $a^*$  value, representing redness, ranged from 2.26 to 2.53, with a mean value of 2.42. The low CV of 4.02% suggested high stability in redness among the genotypes. The skin  $b^*$  value, representing yellowness, ranged from 0.28 to 0.60, with a mean value of 0.45. A high CV of 20.93% indicated considerable variability in yellowness among the genotypes. The mean chroma value for the skin was 2.46, with a range of 2.30–2.55. The very low CV of 3.44% suggested a high degree of stability in color saturation. The mean hue° value for the skin was 9.91, ranging from 1.10 to 14.67. The hue° parameter exhibited the highest CV of 36.21%, making it one of the most distinctive traits among the genotypes. In a study conducted on blackthorn genotypes, Bükücü et al. (2024) reported that the pericarp color measurements showed  $L^*$  values ranging from 18.36 to 19.42,  $a^*$  values between 2.23 and 2.40, and  $b^*$  values varying between 0.16 and 0.54. The chroma values for pericarp color were calculated between 2.63 and 2.97.



The mean flesh  $L^*$  value was 18.84, ranging from 17.48 to 20.45. The low CV of 5.86% indicated stability in flesh lightness among the genotypes. The flesh  $a$  value had a mean of 3.65, ranging from 3.30 to 3.88. The low CV of 4.64% showed that redness was consistent among the genotypes. The flesh  $b$  value, representing yellowness, ranged from 0.57 to 0.84, with an average of 0.69. A CV of 11.98% indicated moderate variability in yellowness among the samples. The chroma values, which express flesh color saturation, ranged from 3.36 to 3.97, with a mean of 3.72. A low CV of 4.56% indicated high homogeneity in color saturation. The hue° values, representing the basic color tone of the flesh, ranged from 9.32 to 13.31, with an average of 10.71. The CV of 11.66% indicated moderate variability in flesh color tone among the samples. For pulp color measurements, Bükücü et al. (2024) found  $L^*$  values between 17.65 and 18.91,  $a^*$  values ranging from 3.11 to 4.18, and  $b^*$  values between 0.54 and 0.81. The chroma values for pulp color were calculated between 5.16 and 8.90.

Chemical properties, which are critical in determining flavor profiles, offer insights into fruit tolerance to stress factors, post-harvest physiology, and overall quality (Kumar et al., 2023; Erbaş et al., 2024). The total soluble solids (TSS) content showed considerable variation among the samples, ranging from 12.00% to 23.40% with an average of 19.05%. The high CV of 18.00% indicates significant variability in sugar content among the genotypes. These values were notably higher than those reported in previous research, where "total soluble solids values were found between 11.9% and 13.2% in 5 different blackthorn genotypes" (Bükücü et al., 2024). This difference in TSS ranges suggests that our genotypes might have greater potential for selections targeting higher sugar content, which could be valuable for both fresh consumption and processing purposes.

The pH values of the fruit samples showed remarkable consistency, ranging from 3.60 to 3.90, with an average of 3.77. The lowest CV value (2.28%) among all measured parameters indicated highly stable and uniform acidity levels across the samples. While our findings showed a narrow pH range, the pH of the blackthorn genotypes were determined between 3.35 and 4.22 (Bükücü et al., 2024), indicating a broader range of acidity in their study. The high stability in our samples suggests that the acid-base balance of the fruits is under tight genetic control and minimally influenced by environmental factors, providing an advantage for product quality and processing technology. This consistency in pH levels could be particularly beneficial for standardizing processing procedures and ensuring consistent product quality. Similarly, the titratable acidity (TA) values, with a low CV of 5.90%, ranged from 0.66% to 0.82%. These values were notably lower compared to previous research where titratable acid values of 5 blackthorn genotypes were found to be between 0.83 and 1.30 (Bükücü et al., 2024). These results, consistent with the pH findings, further supported the idea that the acid composition of the fruits is likely under genetic control and harvested at similar maturity levels. The stability observed in acidity parameters suggests that the fruits can provide a predictable and standardized quality in terms of flavor profile, storage life, and processing characteristics. The lower TA values in our study compared to previous findings might indicate genotypes with potentially more favorable taste characteristics for fresh consumption.

Ozzengin et al. (2023) reported the fruit weight, width, and length of wild-grown *P. spinosa* genotypes as 2.40 g, 12.66 mm, and 13.25 mm, respectively, closely aligning with the results of this study. They also found significant variations in skin  $L$ ,  $a$ , and  $b$  values, ranging from 15.47–27.58, 2.59–7.04, and 1.77–6.55, respectively. Similarly, flesh  $L$ ,  $a$ , and  $b$  values were reported in the ranges of 19.34–31.28, 0.98–5.32, and 4.86–14.59, respectively. The fruit flesh-to-stone ratio was reported as 4.91. İlhan (2023) reported TSS content ranging from 18.40% to 21.07% in eight blackthorn genotypes and identified citric acid as the dominant acid. In a study evaluating a larger population, pH values ranged from 3.17 to 4.13, and acidity from 0.86% to 4.26% (Kuru Berk et al., 2020). Previous studies have consistently reported that blackthorn fruits are characterized by their small size, bluish skin, yellowish flesh color, high soluble solids content, and high acidity (Claudia et al., 2017; Başaran et al., 2024). The results of this study are consistent with the literature. Although genotype is a major factor influencing the traits examined, variations in analysis methods, ecological differences in the selection area, harvest time and type, maturity period, and other factors significantly impact the final phytochemical composition of the fruits (Çalışkan et al., 2012; Polat et al., 2020; Bakoğlu et al., 2024).

According to Principal Component Analysis (PCA) results, while the first five principal components explain 93.68% of the total variance, PC1 accounts for 44% and PC2 accounts for 23.14% (Table 2). This indicates that a significantly high proportion of the variability in the dataset is represented. This ratio is substantially higher than the 69.19% (for the first three components) reported by İlhan (2023) and 63.50% (for the first three components) reported by Kuru Berk et al. (2020).

When examining PC1, which explains 44% of the total variance in our study, flesh/stone ratio (0.335), fruit width (0.334), and soluble solids content (0.334) showed the highest positive loadings, while titratable acidity (-0.301) exhibited the highest negative loading. In İlhan's (2023) study, PC1 explained variance with a lower ratio (36.05%), and fruit weight and SSC were positively effective. The strong relationship of PC1 with fruit characteristics in both studies aligns with Mertoğlu's (2022) findings indicating distinct variations in pomological characteristics.

**Table 1.** Descriptive statistics of physical, morphological and biochemical characteristics of *P. spinosa* genotypes

Variable	Abbreviation	Unit	Min.	Max.	Mean	±StDev	CV%
Fruit width	FW	mm	7.95	14.12	11.70	2.06	17.60
Fruit length	FL	mm	10.49	14.63	13.26	1.30	9.76
Fruit weight	FW	g	1.82	2.71	2.42	0.28	11.38
Flesh/seed ratio ratio	FFS	%	2.53	7.10	5.36	1.22	22.84
Peel $L^*$	PL	-	16.75	20.59	18.51	1.39	7.52
Peel $a^*$	Pa*	-	2.26	2.53	2.42	0.10	4.02
Peel $b^*$	Pb*	-	0.28	0.60	0.45	0.09	20.93
Peel Chroma	PCh	-	2.30	2.55	2.46	0.08	3.44
Peel Hue°	PHue	-	1.10	14.67	9.91	3.59	36.21
Flesh $L^*$	FL	-	17.48	20.45	18.84	1.10	5.86
Flesh $a^*$	Fa*	-	3.30	3.88	3.65	0.17	4.64
Flesh $b^*$	Fb*	-	0.57	0.84	0.69	0.08	11.98
Flesh Chroma	FCh	-	3.36	3.97	3.72	0.17	4.56
Flesh Hue°	FHue	-	9.32	13.31	10.71	1.25	11.66
Soluble solids	SS	%	12.00	23.40	19.05	3.43	18.00
pH	pH	-	3.60	3.90	3.77	0.09	2.28
Titrateable acidity	TA	%	0.66	0.82	0.72	0.04	5.90

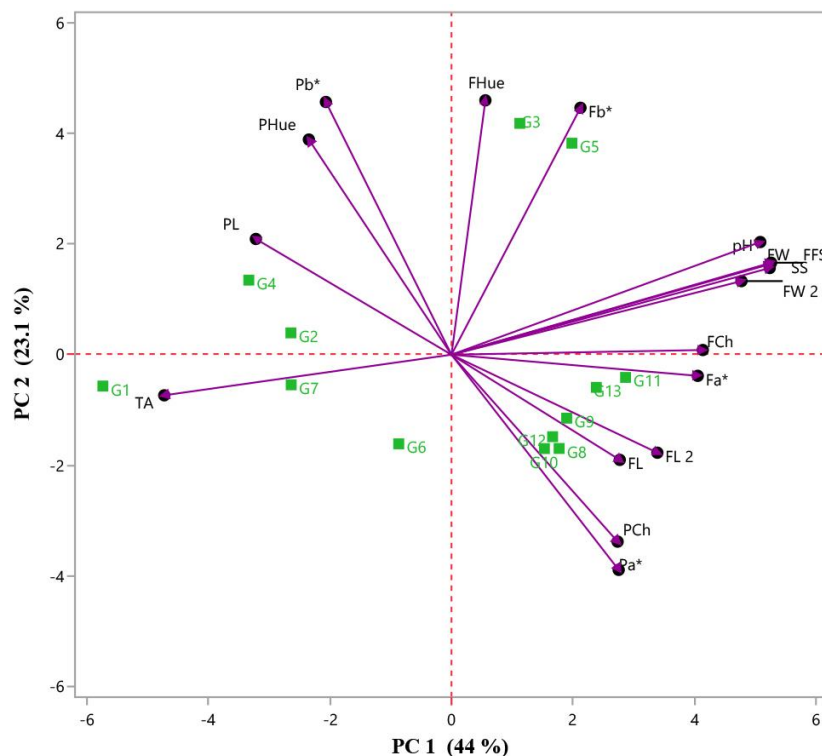
Significant differences were observed in the structure of PC2. In our study, while PC2 explained 23.14% of the variance, flesh hue° value (0.404), fruit skin  $b^*$  value (0.402), and fruit skin hue° value (0.342) showed the highest positive loadings; fruit skin  $a^*$  value (-0.342) and fruit skin chroma value (-0.297) exhibited the highest negative loadings. In contrast, PC2 (25.27%) in İlhan's (2023) study was more associated with biochemical properties (vitamin C, total phenolics, total anthocyanins, and citric acid). This difference may result from the growth of examined genotypes under different ecological conditions and the evaluation of different characteristics.

**Table 2.** PCA results and component loadings of the studied features.

Variable	PC1	PC2	PC3	PC4	PC5
Fruit width	0.334	0.144	0.115	-0.046	-0.021
Fruit length	0.177	-0.167	0.549	0.038	0.056
Fruit weight	0.304	0.117	0.114	-0.070	-0.160
Fruit flesh/seed ratio	0.335	0.146	0.139	-0.107	0.022
Peel $L^*$	-0.205	0.184	0.051	-0.320	0.534
Peel $a^*$	0.175	-0.342	0.033	0.333	0.328
Peel $b^*$	-0.132	0.402	-0.113	0.258	-0.111
Peel Chroma	0.174	-0.297	0.027	0.410	0.358
Peel Hue	-0.150	0.342	-0.148	0.052	0.085
Flesh $L^*$	0.216	-0.156	-0.170	0.192	-0.578
Flesh $a^*$	0.258	-0.034	-0.501	0.006	0.168
Flesh $b^*$	0.135	0.393	-0.010	0.375	0.107
Flesh Chroma	0.264	0.007	-0.491	0.049	0.170
Flesh Hue	0.036	0.404	0.200	0.397	0.050
Soluble solids content	0.334	0.138	0.061	-0.167	0.149
pH	0.324	0.179	0.176	-0.111	-0.035
Titrateable acidity	-0.301	-0.065	0.136	0.389	-0.026
Eigenvalue	7.48	3.93	1.77	1.55	1.19
% of Variance	44.00	23.14	10.42	9.13	6.99
Cumulative %	44.00	67.13	77.56	86.69	93.68

The separation of genotypes into four different groups (those with larger fruits, sweeter ones, and those with higher polyphenolic compounds and antioxidant capacity) in İlhan's (2023) study is important in demonstrating the genetic diversity of the species and supports the high variation ratio (93.68%) observed in our study. These results emphasize the importance of evaluating both pomological and biochemical properties together in the characterization of *P. spinosa* genotypes.

The distribution of the genotypes based on their traits has been visualized using a Biplot, with the results presented in Figure 3. Based on the findings, it appears that the studied genotypes hold potential for evaluation across different intended uses.



**Figure 3.** Biplot plot showing PCA results for studied fruit traits.

Genotype 1, characterized by its low pH (3.60) and high acidity (0.77%) (Table 3), is particularly suited for use in the processing industry. Acids that contribute to a low pH environment are known to inhibit the activity of harmful microorganisms responsible for spoilage, thereby aiding in the preservation of stability (Adamczak et al., 2019). Genotype 3, on the other hand, stands out for its large fruit size (13.88 g) and high fruit flesh-to-seed ratio (7.10) (Table 3), making it a potential parent for breeding programs aimed at developing new genotypes with larger fruit size and higher fruit flesh-to-seed ratios. Additionally, this genotype exhibits a high soluble solid content, rendering it particularly suitable for products such as marmalade and puree. An increase in soluble solids positively impacts product yield. One of the most significant advantages of selection studies is their potential to identify genotypes suitable for different purposes (Uzun et al., 2015; Dumanoglu et al., 2019).

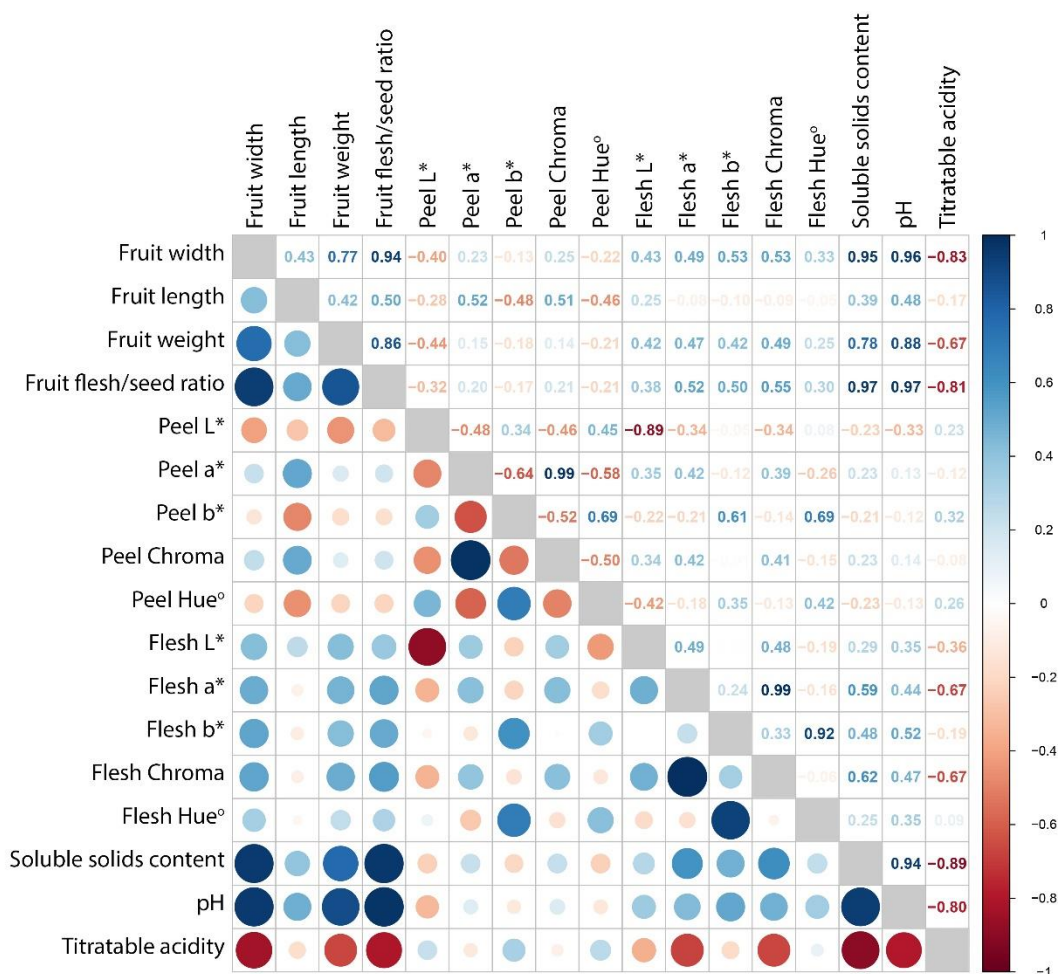
The relationships among the traits investigated in blackthorn genotypes are presented in Figure 4. A positive correlation was identified between fruit width and length ( $r = 0.43$ ). In plants, cell division increases following fertilization, which is subsequently followed by cell expansion. During the cell expansion phase, simultaneous transverse and longitudinal growth of the cells accounts for the strong correlation between these two traits (Saridas et al., 2017). The volumetric enlargement of cells that make up the fruit leads to an increase in fruit weight. In this context, strong positive correlations were observed between fruit weight and fruit width ( $r = 0.77$ ) and length ( $r = 0.42$ ), consistent with the literature (Eskimez et al., 2020).

The breakdown of organic acids in fruits results in an increase in pH values. Similarly, this study found a strong negative correlation ( $-0.80$ ) between total titratable acidity (TEA) and pH, aligning with findings reported for apples by Mertoğlu and Evrenosoğlu (2019), who reported a similar relationship ( $-0.81$ ).

The increase in pigments that impart color to fruits contributes to their darkening while simultaneously reducing their brightness. Therefore,  $a^*$  values of the fruit skin were found to be negatively correlated with  $L^*$  ( $-0.48$ ) and  $b^*$  ( $-0.64$ ) values. Positive correlations were identified between  $a^*$  values and fruit dimensions, as well as fruit weight, with correlation coefficients of 0.23, 0.52, and 0.15, respectively. A similar trend was observed for fruit flesh color. This relationship may be attributed to the ability of pigments to absorb higher levels of sunlight during photosynthesis.

**Table 3.** Fruit sizes, color values and chemical properties of fruit peel and flesh of *P. spinosa* genotypes.

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	LSD <sub>0.05</sub>	
FW	8.12 ± 0.12 f	10.45 ± 0.14 e	13.88 ± 0.15 a	9.76 ± 0.13 e	14.12 ± 0.16 a	11.34 ± 0.14 d	7.95 ± 0.12 f	12.67 ± 0.15 b	12.85 ± 0.15 b	11.97 ± 0.14 cd	13.45 ± 0.15 ab	12.35 ± 0.14 bc	13.15 ± 0.15 ab	0.75	
FL	11.23 ± 0.15 e	13.45 ± 0.16 c	14.63 ± 0.17 a	12.88 ± 0.15 d	10.49 ± 0.14 e	13.92 ± 0.16 bc	11.76 ± 0.15 e	14.15 ± 0.17 ab	13.95 ± 0.16 bc	14.23 ± 0.17 ab	13.75 ± 0.16 bc	14.15 ± 0.17 ab	13.85 ± 0.16 bc	0.52	
FW	1.82 ± 0.08 e	2.45 ± 0.09 c	2.71 ± 0.10 a	2.15 ± 0.08 d	2.58 ± 0.09 ab	1.98 ± 0.08 de	2.33 ± 0.09 c	2.64 ± 0.10 a	2.55 ± 0.09 b	2.48 ± 0.09 bc	2.62 ± 0.10 ab	2.58 ± 0.09 ab	2.60 ± 0.09 ab	0.18	
FFS	2.53 ± 0.11 g	4.86 ± 0.13 e	7.10 ± 0.15 a	3.92 ± 0.12 f	6.45 ± 0.14 b	5.23 ± 0.13 d	4.15 ± 0.12 ef	5.78 ± 0.13 c	5.95 ± 0.14 c	5.65 ± 0.13 cd	6.15 ± 0.14 bc	5.85 ± 0.13 c	6.05 ± 0.14 bc	0.45	
Fruit peel color values	L*	19.78 ± 0.25 bc	18.37 ± 0.24 cd	20.59 ± 0.26 ab	18.97 ± 0.24 c	18.59 ± 0.24 cd	20.12 ± 0.25 abc	19.96 ± 0.25 abc	19.31 ± 0.24 bc	17.25 ± 0.23 d	16.85 ± 0.22 d	16.95 ± 0.22 d	17.15 ± 0.23 d	16.75 ± 0.22 d	1.25
	a*	2.30 ± 0.08 d	2.26 ± 0.08 d	2.29 ± 0.08 d	2.46 ± 0.09 bc	2.31 ± 0.08 d	2.48 ± 0.09 bc	2.38 ± 0.08 cd	2.53 ± 0.09 ab	2.46 ± 0.09 bc	2.52 ± 0.09 ab	2.48 ± 0.09 bc	2.51 ± 0.09 ab	2.47 ± 0.09 bc	0.15
	b*	0.54 ± 0.05 bc	0.41 ± 0.04 cd	0.60 ± 0.05 ab	0.58 ± 0.05 ab	0.55 ± 0.05 bc	0.36 ± 0.04 d	0.47 ± 0.05 c	0.28 ± 0.04 e	0.44 ± 0.05 cd	0.38 ± 0.04 d	0.42 ± 0.05 cd	0.39 ± 0.04 d	0.43 ± 0.05 cd	0.12
	Chroma	2.36 ± 0.09 d	2.30 ± 0.08 d	2.37 ± 0.09 d	2.52 ± 0.09 bc	2.37 ± 0.09 d	2.51 ± 0.09 bc	2.42 ± 0.09 cd	2.54 ± 0.09 ab	2.49 ± 0.09 bc	2.55 ± 0.09 ab	2.52 ± 0.09 bc	2.53 ± 0.09 ab	2.51 ± 0.09 bc	0.14
	Hue°	13.20 ± 0.45 b	10.28 ± 0.38 c	14.67 ± 0.48 a	13.26 ± 0.45 b	13.39 ± 0.45 b	8.26 ± 0.32 d	11.17 ± 0.40 c	6.31 ± 0.28 e	1.10 ± 0.15 f	8.56 ± 0.32 d	9.62 ± 0.35 cd	9.10 ± 0.34 cd	9.85 ± 0.36 cd	1.85
Fruit flesh color values	L*	18.32 ± 0.24 cd	18.37 ± 0.24 cd	17.96 ± 0.23 d	17.48 ± 0.23 d	18.67 ± 0.24 c	17.89 ± 0.23 d	18.16 ± 0.24 cd	17.51 ± 0.23 d	20.15 ± 0.26 a	19.85 ± 0.25 ab	20.45 ± 0.26 a	19.85 ± 0.25 ab	20.25 ± 0.26 a	1.15
	a*	3.47 ± 0.10 cd	3.30 ± 0.10 d	3.58 ± 0.10 bc	3.42 ± 0.10 cd	3.88 ± 0.11 a	3.62 ± 0.10 bc	3.78 ± 0.11 ab	3.70 ± 0.11 abc	3.66 ± 0.10 bc	3.75 ± 0.11 ab	3.82 ± 0.11 a	3.70 ± 0.11 abc	3.78 ± 0.11 ab	0.25
	b*	0.57 ± 0.05 e	0.62 ± 0.05 de	0.80 ± 0.06 ab	0.81 ± 0.06 ab	0.84 ± 0.06 a	0.62 ± 0.05 de	0.64 ± 0.05 de	0.63 ± 0.05 de	0.70 ± 0.06 cd	0.68 ± 0.06 cd	0.72 ± 0.06 bc	0.65 ± 0.05 de	0.69 ± 0.06 cd	0.12
	Chroma	3.52 ± 0.10 d	3.36 ± 0.10 d	3.67 ± 0.11 bc	3.52 ± 0.10 d	3.97 ± 0.11 a	3.67 ± 0.11 bc	3.83 ± 0.11 ab	3.75 ± 0.11 bc	3.72 ± 0.11 bc	3.81 ± 0.11 ab	3.89 ± 0.11 ab	3.76 ± 0.11 bc	3.85 ± 0.11 ab	0.24
	Hue°	9.32 ± 0.35 e	10.64 ± 0.38 de	12.60 ± 0.42 c	13.31 ± 0.43 bc	12.22 ± 0.41 cd	9.72 ± 0.36 e	9.61 ± 0.36 e	9.66 ± 0.36 e	10.85 ± 0.38 de	10.28 ± 0.37 de	10.65 ± 0.38 de	9.95 ± 0.36 e	10.42 ± 0.37 de	1.65
SS	12.0 ± 0.42 f	16.5 ± 0.48 cd	23.0 ± 0.58 a	14.8 ± 0.45 de	23.4 ± 0.58 a	19.7 ± 0.52 b	15.3 ± 0.46 de	21.8 ± 0.56 ab	20.51 ± 0.54 b	18.9 ± 0.51 bc	21.2 ± 0.55 ab	19.8 ± 0.52 b	20.8 ± 0.54 ab	2.85	
pH	3.60 ± 0.08 e	3.75 ± 0.08 cd	3.90 ± 0.09 a	3.68 ± 0.08 de	3.85 ± 0.09 ab	3.72 ± 0.08 cd	3.65 ± 0.08 de	3.82 ± 0.09 abc	3.79 ± 0.08 bc	3.78 ± 0.08 bc	3.83 ± 0.09 abc	3.81 ± 0.09 abc	3.82 ± 0.09 abc	0.12	
TA	0.77 ± 0.03 ab	0.75 ± 0.03 abc	0.70 ± 0.03 cd	0.82 ± 0.03 a	0.66 ± 0.03 d	0.72 ± 0.03 bcd	0.76 ± 0.03 abc	0.68 ± 0.03 d	0.71 ± 0.03 cd	0.73 ± 0.03 bcd	0.69 ± 0.03 d	0.72 ± 0.03 bcd	0.70 ± 0.03 cd	0.08	



**Figure 4.** Correlation analysis of the examined features

## CONCLUSION(S)

This study examined the physicochemical properties of *P. spinosa* L. genotypes from the Çivril district of Denizli province, revealing the diversity and potential of this species within the local ecosystem. Comprehensive analyses of the physical, morphological, and biochemical characteristics of the wild plum genotypes demonstrated that particularly the G3 and G5 genotypes stood out due to their high fruit pulp/seed ratio (7.10% and 6.45%, respectively), high soluble solid content (23.0% and 23.4%, respectively), and ideal fruit size (G3: 13.88×14.63 mm; G5: 14.12×10.49 mm). In the future, it is planned to protect these superior genotypes, subject them to further analyses, and transform them into value-added products. Furthermore, these genotypes are considered promising material for future commercial production and breeding studies.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Author Contributions

**1<sup>st</sup> Author Levent Kirca** :Conceptualization; data curation, statistical analysis and data visualization; formal analysis; funding acquisition; investigation; methodology; project administration; software; writing— original draft; writing—review and editing.

**2<sup>st</sup> Author Kerem Mertoğlu** :Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; writing— original draft; writing—review and editing.

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## Influence of Various Drying Methods on the Antioxidant and Essential Oil Content of *Salvia fruticosa* Plant

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### ABSTRACT

*Salvia fruticosa* (Anatolian sage) contains significant amounts of secondary metabolites, which are essential for the plant's anti-inflammatory, antimicrobial, and antioxidant effects. The drying methods applied post-harvest directly impact the plant's essential oil content, antioxidant activity (ABTS, DPPH), total phenol, and flavonoid levels. In particular, drying temperature and method are critical factors in determining the degradation rate of these compounds, playing an important role in preserving the plant's medicinal and aromatic value. This study aims to investigate the effects of different post-harvest drying methods on the bioactive properties of the *Salvia fruticosa* to identify the most effective drying method. In this study, *Salvia fruticosa* plant subjected to various drying methods: sun drying, shade drying, and oven drying at 100°C, 70°C, and 40°C. According to results, drying in shade yielded the highest values of DPPH radical scavenging activity (61.71 mg TEAC/g DW) and ABTS activity (91.39 mg TEAC/g DW), alongside essential oil content (1.60%) and phenolic content (31.41 mg GAE/g DW). In contrast, D100 (drying at 100 °C) showed the lowest values for DPPH, ABTS, essential oil, phenol, and flavonoid as 19.16 mg TEAC/g DW, 43.95 mg TEAC/g DW, 0.06%, 13.02 mg GAE/g DW, and 35.29 mg rutin/g DW, respectively, highlighting the detrimental effects of thermal degradation. These findings suggest that lower temperature drying methods, specifically shade and sun drying, are more effective in preserving the integrity of beneficial compounds, thus maximizing the antioxidant capacity and essential oil value of sage.

**Key words:** Drying methods, DPPH, Essential oil, Phenolic content, Antioxidant activity, Sage

### Farklı Kurutma Yöntemlerinin *Salvia fruticosa* Bitkisinin Antioksidan ve Uçucu Yağ İçeriği Üzerine Etkisi

#### ÖZ

*Salvia fruticosa* (Anadolu adaçayı), bitkisi yüksek oranda sekonder metabolit içermekte olup, anti-enflamatuvar, antimikrobiyal ve antioksidan etkilere sahip bir bitkidir. Hasat sonrası uygulanan kurutma yöntemleri, bitkinin uçucu yağ içeriği, antioksidan aktivitesi (ABTS, DPPH), toplam fenol ve flavonoid seviyelerini doğrudan etkilemektedir. Özellikle kurutma sıcaklığı ve yöntemi, bu bileşiklerin bozulma hızını belirleyerek bitkinin tıbbi ve aromatik değerini koruma açısından kritik bir rol oynar. Bu çalışma, farklı hasat sonrası kurutma yöntemlerinin bitkinin biyoaktif özelliklerine etkilerini araştırarak en etkili kurutma yöntemini belirlemeyi amaçlamaktadır. *Salvia fruticosa* bitkisi, güneşte kurutma, gölgede kurutma ve etüvde 100°C, 70°C ve 40°C'de kurutma gibi farklı yöntemlere tabi tutulmuştur. Kurutma işlemi tamamlandıktan (kuru ağırlık sabitlenince) sonra analizler yapılmıştır. Sonuçlara göre, gölgede kurutma en yüksek DPPH radikal süpürme aktivitesi (61,71 mg TEAC/g KM) ve ABTS aktivitesi (91,39 mg TEAC/g KM), uçucu yağ içeriği (1,60%) ve fenolik içeriği (31,41 mg GAE/g KM) elde edilmiştir. Buna karşılık, 100°C'de kurutma (D100), DPPH, ABTS, uçucu yağ, fenol ve flavonoid için en düşük değerler sırasıyla 19.16 mg TEAC/g KM, 43.95 mg TEAC/g KM, %0.06, 13.02 mg GAE/g KM ve 35.29 mg rutin/g KM olarak kaydedilmiş olup, termal bozulmanın olumsuz etkileri ortaya koyulmuştur. Bu bulgular, daha düşük sıcaklıklarda kurutma yöntemlerinin, özellikle gölgede ve güneşte kurutmanın, faydalı bileşiklerin

bütünlüğünü koruma açısından daha etkili olduğunu ve adaçayının antioksidan kapasitesi ile uçucu yağ değerini maksimize ettiğini göstermektedir.

**Anahtar kelimeler:** *Salvia fruticosa*, Kurutma yöntemleri, DPPH, uçucu yağ, Fenolik içerik, Antioksidan aktivite

## INTRODUCTION

Sage is a part of the *Salvia* genus and encompasses over 900 species found on each continent in the world. These plants have been known to be used from time immemorial as remedies against a large number of diseases. Their primary indications relate to ailments such as pain, epilepsy, colds, bronchitis, tuberculosis, hemorrhages, and menstrual disorders. These plants have traditionally been utilized in curing over sixty ailments (Hamidpour et al., 2014; Topçu et al., 2017; Ghorbani and Esmailizadeh 2017). However, even if there are immense diversity in taxa, only a few *Salvia* species have major commercial importance. According to Demirci et al. (2002), *Salvia fruticosa* Miller, also known as *Salvia triloba* L., is one of the species that are of high commercial value, particularly in Turkey. *Salvia fruticosa* is native to the Mediterranean, including Türkiye, both in the wild and under cultivation. Şenol et al. (2010) reported that *Salvia fruticosa* has been consumed traditionally as herbal tea in winter periods. This species has restorative and curative properties of high value. *S. fruticosa* contains large amounts of secondary metabolites, including phenolics and terpenoids, which enrich its pharmacological properties. In fact, these compounds account for the plant's anti-inflammatory (El-Sayed et al., 2006), antibacterial (Delamare et al., 2007), and antioxidant (Tepe et al., 2006) activities. Phenolic constituents that present in *S. fruticosa*, rosmarinic acid is especially renowned for its high antioxidant activity (Lu and Foo, 2002; Papageorgiou et al., 2008). Besides rosmarinic acid, there were many reports about *Salvia* species containing a large number of phenolic acids and flavonoids, such as vanillic acid, ferulic acid, caffeic acid, apigenin, quercetin, and luteolin (Askun et al., 2009; Papageorgiou et al., 2008).

Drying is an essential preservation technique in food processing. The major aim is to diminish the water activity of food goods, so limiting microbial proliferation and decreasing chemical reactions to prolong shelf life at ambient temperature. Moreover, drying diminishes the necessary storage space and enhances transit efficiency by decreasing weight. Drying methods encompass ancient procedures, such as sun or shade drying, and contemporary ones, such microwave or oven drying (Sathishkumar et al., 2009). Enzymatic and non-enzymatic processes during the drying of young plant tissues can substantially modify the phytochemical composition (Capecka et al., 2005). The retention of the essential oils and phenolic compounds into the plant materials occurs differently depending on the drying techniques. While high-temperature drying leads to severe loss of essential chemicals, different drying methods has demonstrated the ability for retaining higher quantities of bioactive ingredients due to reduced exposure time and lower temperatures (Figuérédo et al., 2011; Dinçer et al., 2012).

This study aims to evaluate the effects of different post-harvest drying methods on the bioactive properties of *Salvia fruticosa*. Specifically, it examines the impact on essential oil content, antioxidant capacity, total phenolic content, and flavonoid levels. The goal is to determine which drying method (sun drying, shade drying, or oven drying at varying temperatures) is most effective in preserving these valuable bioactive compounds. By identifying the optimal drying technique, this research aims to contribute to improving the quality and potential health benefits of dried *S. fruticosa*, supporting its use in herbal and medicinal applications.

## MATERIALS AND METHODS

The plant material used in this study was *Salvia fruticosa* (Anatolian sage), cultivated at the Faculty of Agriculture, Aydın Adnan Menderes University. The plants are eight years old, were harvested during the flowering stage in 2024. The harvested material was divided into three replicates and subjected to different drying methods to evaluate their effects on bioactive compounds. The drying methods included sun drying, shade drying, and oven drying at temperatures of 100°C, 70°C, and 40°C. The drying process continued until a stable dry weight was achieved for each sample, ensuring consistency across treatments. Once the drying was complete, the plant materials were analyzed to determine their chemical composition, focusing on parameters such as essential oil content, phenolic content, flavonoid content, and antioxidant activity, to assess the impact of different drying techniques on the preservation of beneficial compounds.

### Determination of Essential Oil Content

The content of the essential oil within the dried plant material was determined by using the hydro-distillation technique (Wichtl, 1971). For this 10 g of dried plant material was combined with 100 mL of distilled water and subjected to hydro-distillation in a Clevenger-type apparatus. The distillations were performed at

180°C for one hour. The mixture of distillate was cooled for five minutes after distillation. The separated essential oil was collected and measured. Results were expressed as the percentage, showing the amount of the essential oil obtained from weight of the dried plant material used.

#### Extraction of Samples

Dried plant samples have been crushed with a grinder and then sieved for homogeneity during extraction. A 500 mg aliquot of the powdered sample was extracted by combining it with 50 ml of 80% methanol in a shaking incubator maintained at 40°C for 2 hours. The extract was utilized immediately following its preparation for subsequent measurements.

#### DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

The antioxidant capacity was assessed utilizing the DPPH test, in accordance with the Gadow et al. (1999) and Maisuthisakul et al. (2007). A 100 µL aliquot of the extract was diluted to create four distinct concentrations, which were subsequently combined with 3.9 mL of freshly made 0.1 mM DPPH solution. The mixture was agitated and incubated in the absence of light at ambient temperature for 30 minutes. Following incubation, the absorbance was measured at 516 nm with a microplate reader. The antioxidant activity was quantified as Trolox equivalent antioxidant capacity (mg TEAC/g DW). The calibration equation for DPPH was  $y=1032.8x+93.5$ , ( $R^2=0.9967$ ) as absorbance/mg TEAC ml<sup>-1</sup>, absorbance calculated as total absorbance change (control absorbance – sample absorbance) to obtain linearly increasing value.

#### ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Assay

The ABTS assay adhered to the methodology established by Re et al. (1999). To generate the ABTS radical cation (ABTS•+), a 7 mM ABTS solution was mixed with 2.45 mM potassium persulfate in a 1:1 ratio and allowed to react in the dark for 16 hours. The ABTS•+ solution was diluted with methanol to achieve an absorbance of 0.700 at 734 nm. A 5 µL aliquot of the plant extract was thereafter combined with 3.995 mL of the diluted ABTS•+ solution and incubated in the dark for 30 minutes. The absorbance was quantified, and the antioxidant capacity was represented as Trolox equivalent antioxidant capacity (mg TEAC/g DW). The calibration equation for ABTS was  $y=234.4x+69$ , ( $R^2=0.9995$ ) as absorbance/mg TEAC ml<sup>-1</sup>, absorbance calculated as total absorbance change (control absorbance – sample absorbance) to obtain linearly increasing value.

#### Determination of Total Flavonoid Content

To determine the flavonoid concentration, 0.5 mL of the extract was combined with 2.5 mL of distilled water and 150 µL of 5% sodium nitrite (NaNO<sub>2</sub>). Following gentle mixing, the solution was permitted to react for 5 minutes, after which 300 µL of 10% aluminum chloride (AlCl<sub>3</sub>) was introduced. Subsequent to an additional 5 minutes, 1 mL of 1 M sodium hydroxide (NaOH) was introduced, and the total volume was adjusted to 5 mL with distilled water. The solution was incubated for 30 minutes, and absorbance was measured at 510 nm using a microplate reader. The flavonoid content was quantified as Rutin trihydrate equivalents (MW: 664.56), in accordance with the methodology of Cheng et al. (2006). The calibration equation for flavonoid content was  $y=123x+7.5$ , ( $R^2 = 0.9816$ ).

#### Determination of Total Phenolic Content

The total phenolic content was assessed utilizing the methodology outlined by Skerget et al. (2005). An aliquot of 0.5 mL of the extract was combined with 2.5 mL of 0.1 N Folin-Ciocalteu reagent and 2 mL of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>, 75 g/L). The amalgamation was incubated at 50°C for 5 minutes and subsequently cooled. Absorbance was quantified at 760 nm utilizing a microplate reader, and the phenolic content was articulated as gallic acid equivalents (mg GAE/g DW). The calibration equation for phenolic content was  $y=2240x+80.5$ , ( $R^2 = 0.9959$ ).

#### Statistical Analyses

The one-way ANOVA approach was employed to compare the differences among the treatment groups. When ANOVA displayed significance, Tukey's HSD was employed as the post hoc test relative to the control to address multiple comparisons. All statistical analyses were conducted using JMP Pro 16 software (SAS Institute, Cary, NC, USA).

## RESULTS AND DISCUSSION

The results of the ANOVA for the analyzed parameters have been presented in Table 1. According to this table, drying methods exhibited highly significant effects ( $P < 0.01$ ) on all the studied parameters. These findings

show the importance of drying methods for the preservation or degradation of the bioactive compounds present in *S. fruticosa*.

**Table 1.** Analysis of Variance of Drying Methods on Bioactive Compounds in *Salvia fruticosa*: Essential Oil, Phenolic Content, DPPH, ABTS, and Flavonoid Levels

Source	df	EO (%)	Fenol (mg GAE/ g DW)	DPPH (mg TEAC/ g DW)	ABTS (mg TEAC/ g DW)	Flavonoid (mg rutin/ g DW)
Mean Square						
Drying	4	1.084**	187.514**	829.911**	1218.360**	4568.260**

\*\*:*p*-value of less than 0.01, \*: *p*-value of less than 0.05.

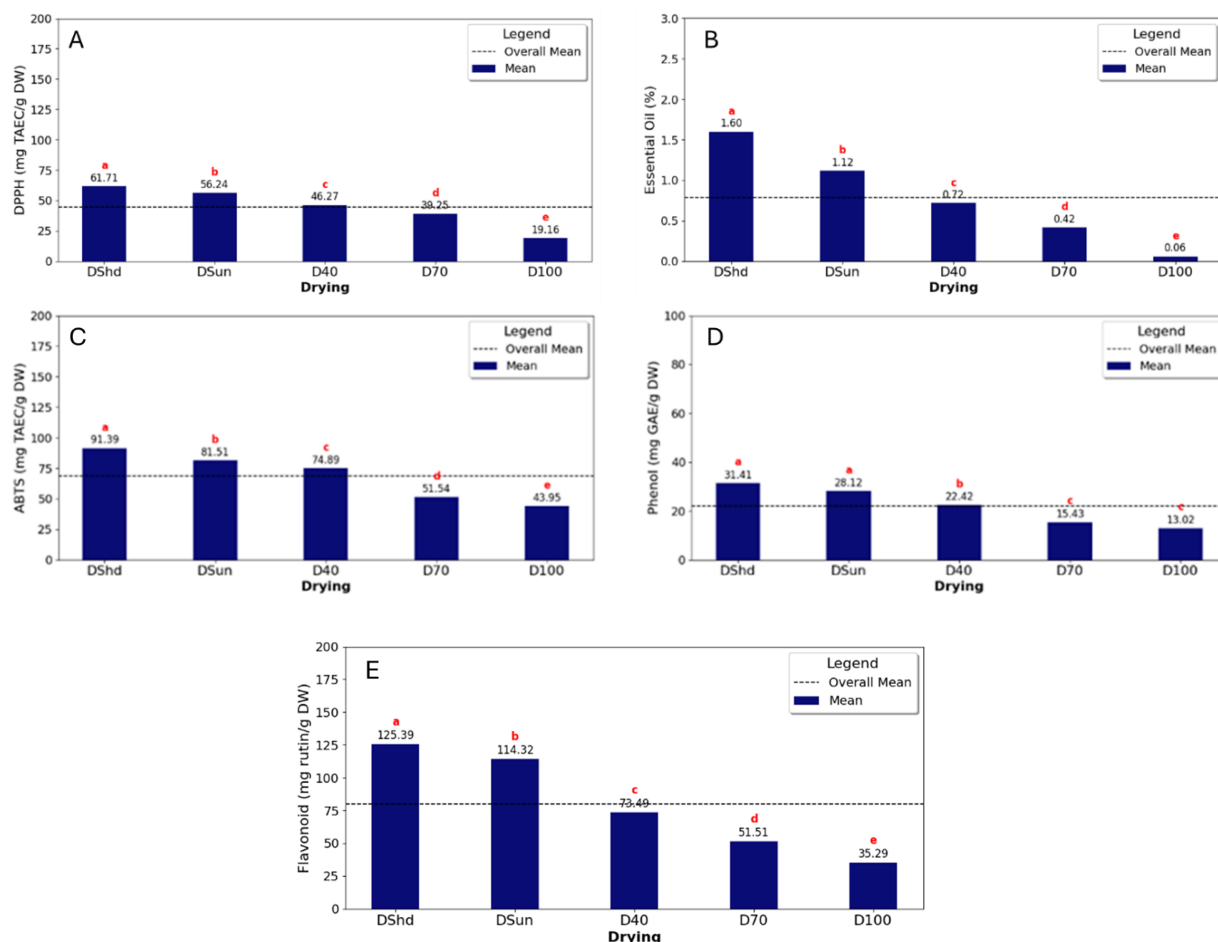
Figure 1 shows the effect of different drying methods on various parameters of the samples, including antioxidant activity (DPPH and ABTS), essential oil content, phenolic content, and flavonoid content. The results shows consistent trends across five parameters: DPPH radical scavenging activity, essential oil content, ABTS radical scavenging activity, phenolic content, and flavonoid content. Shade drying (DShd) and sun drying (DSun) consistently exhibited higher values across all five parameters compared to higher temperature drying methods, such as drying at 70°C (D70) and 100°C (D100).

A consistent pattern is seen where antioxidant activity, essential oil content, phenolic content, and flavonoid content decrease as drying temperature increases. Shade drying (DShd) and sun drying (DSun) demonstrate significantly higher values, indicating better preservation of beneficial compounds and antioxidant properties. The DPPH radical scavenging activity has highest in DShd (61.71 mg TEAC/g DW) and DSun (56.24 mg TEAC/g DW), however values decreasing as drying temperature rises, and D100 displaying the lowest activity (19.16 mg TEAC/g DW) (Figure 1A). Similarly, ABTS radical scavenging activity follows the same trend, with DShd showing the highest activity (91.39 mg TEAC/g DW) and D100 the lowest (43.95 mg TEAC/g DW) (Figure 1C). Essential oil content is also significantly higher in DShd (1.60%) and DSun (1.12%), while higher temperature drying methods shows a decrease, with D100 having the lowest value (0.06%) (Figure 1B). Phenolic content is highest in DShd (31.41 mg GAE/g DW) and DSun (28.12 mg GAE/g DW), while D100 shows the lowest value (13.02 mg GAE/g DW) (Figure 1D). Flavonoid content follows a similar trend, with DShd showing the highest value (125.39 mg rutin/g DW) and D100 having the lowest (35.29 mg rutin/g DW) (Figure 1E).

Higher temperature-drying treatments, in particular D70 and D100, produce high losses of all the parameters considered, and therefore are confirmed to have thermal degradation playing a key role in their reduction. Essential oil, phenolic content,, and flavonoids loss indicate how these compounds are susceptible to high temperatures and thus undergo degradation upon drying. Results showed that drying conditions using low temperatures better preserved the quality of the samples. Low-temperature drying methods, such as shade drying, is more effective in preserving essential oil yields, phenolic content, and antioxidant capacity compared to other techniques. In contrast, high-temperature oven drying, while reducing drying time, significantly compromised these properties due to the thermal degradation of essential oils and the breakdown of heat-sensitive compounds like polyphenols and flavonoids (Ayyobi et al., 2014). Also degrading thermolabile compounds like chlorophyll and bioactive components, impacting color and quality (Tellez et al., 2018). Therefore, with regards to maximum retention of antioxidant capacity, essential oil, and phenolic and flavonoid compounds, shade drying or sun drying is recommended as the most favorable approach. Shade and sun drying methods, by preserving these valuable compounds, allow the samples to retain their bioactive properties to a greater extent compared to high-temperature drying.

Other studies have also indicated the same tendency for DPPH radical scavenging activity, essential oil content, ABTS radical scavenging activity, phenolic content, and flavonoid content. More specifically, lower-temperature drying methods protect these compounds better than higher-temperature drying (Kwaśniewska-Karolak & Mostowski, 2021; Dudek et al., 2022; Sadowska et al., 2017; Stanisavljević et al., 2012). The studies carried out on *Salvia* species showed that antioxidant capacities have decreased with the increase of drying temperatures, due to the degradation of sensitive phytochemicals (Tohma et al., 2016). Therefore, other plant species have also shown the exact same trend, confirming that an increase in drying temperatures has an adverse effect on the antioxidant activities. Moreover heat-sensitive phytochemicals such as phenolics and flavonoids progressively degrade, resulting in a loss of their antioxidant properties in high temperatures. (Kwaśniewska-Karolak & Mostowski, 2021; Dudek et al., 2022). These degravations is happen primarily through evaporation,

oxidation, and structural breakdown. Mechanisms such as hydroperoxide formation and subsequent breakdown into inactive byproducts (Hamama & Nawar, 1991; Mahanta et al., 2021).



**Figure 1.** Effects of Drying Methods on Bioactive Compound Concentrations in *Salvia fruticosa*: DPPH(A), Essential Oil (B), DPPH (C), ABTS (C), Phenolic Content (D) and Flavonoid Content (E). Letter Groups Indicating Statistical Significance at  $p \leq 0.01$ .

Shade drying stands out for its ability to better preserve the bioactive compounds in plants. It minimizes the degradation of thermolabile (heat-sensitive) components such as phenolic compounds, flavonoids, and essential oils. Since this method operates at low temperatures, it helps retain color, aroma, and quality attributes. A study on *Ocimum basilicum* reported that samples dried in the shade exhibited higher antioxidant capacity compared to those dried in the sun (Tepe et al., 2006). However, shade drying often requires an extended duration; the drying process needs to be fast to prevent mold development due to humidity. Extended drying duration can cause quality loss (Babu et al., 2018). On the other hand, sun drying is a more economical and faster method. However, exposure to UV radiation and high temperatures during sun drying can lead to the degradation of bioactive compounds in plants. For example, structural degradation of essential oils has been observed at high temperatures (Paşa et al., 2021). In regions with high humidity, the drying time may be prolonged, which can result in a decline in plant quality (Chua et al., 2019). Shade drying is also ideal for maintaining the color, aroma, and structural integrity of herbs compared to in locations have high UV radiation (Thamkaew et al., 2021).

## CONCLUSION

This study showed that drying temperature significantly influences the retention of bioactive compounds in *Salvia fruticosa*, with much higher temperatures drastically reducing such retention. Essential oils, antioxidants, phenolics, and flavonoids have decreased considerably with an increase in the drying temperature mainly because these compounds are heat-sensitive and degrade or evaporate upon exposure to higher temperatures. The results showed that either shade drying or sun drying was best suited for retaining these valuable bioactive components. However, selecting the appropriate method depends on specific conditions. Each



method has its own advantages and disadvantages. Shade drying excels in preserving bioactive compounds and quality but requires longer durations and proper ventilation. Sun drying is faster and more economical but may result in quality losses due to prolonged drying times in high-humidity conditions. Therefore, the choice of method should be chosen to the product and environmental conditions to optimize drying efficiently.

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#### Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper


#### Author Contributions

**Uğur TAN** :Conceptualization; data curation; formal analysis; investigation; methodology; project administration; software; writing— original draft; writing—review and editing.

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## Exploring the Impacts of Economic Growth on Ecosystem and Its Subcomponents in Türkiye

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### ABSTRACT

This study analyzes the impacts of economic growth on ecosystem in Türkiye. The study uses annual data for the period 1995-2021 and the ARDL method. The study utilizes the Ecosystem Vitality Index, a sub-dimension of the Environmental Performance Index. In addition, seven models were constructed to assess in detail the impact of economic growth on different dimensions of the ecosystem. The results show that economic growth has a significant impact in all models analyzed. However, the direction of this impact differs across ecosystem components. Economic growth is found to have a positive impact on agriculture and water resources. In these models, a 1% increase in GDP increases the agriculture and water resources indices by 0.074-0.672%. In contrast, economic growth has a negative impact on biodiversity and habitat, ecosystem services, fisheries, acid rain and total ecosystem vitality. In these models, a 1% increase in GDP reduces the indices of biodiversity and habitat, ecosystem services, fisheries, acid rain and total ecosystem vitality by 0.101-2.144%. The results suggest that the environmental costs of economic growth processes need to be considered. Environmentally friendly policies should be combined with sustainable development strategies to reduce the negative impacts of economic growth.

**Key words:** *Economic Growth, Ecosystem, Ecosystem Vitality, Environmental Performance, EPI.*

## Türkiye'de Ekonomik Büyümenin Ekosistem ve Alt Bileşenleri Üzerindeki Etkilerinin Araştırılması

### ÖZ

Bu çalışma, Türkiye'de ekonomik büyümenin ekosistem üzerindeki etkilerini analiz etmektedir. Çalışmada 1995-2021 dönemi için yıllık veriler ve ARDL yöntemi kullanılmıştır. Çalışmada Çevresel Performans Endeksinin alt boyutu olan Ekosistem Canlılığı endeksi kullanılmıştır. Ayrıca, ekonomik büyümenin ekosistemin farklı boyutları üzerindeki etkisini ayrıntılı olarak değerlendirmek için yedi model oluşturulmuştur. Sonuçlar, ekonomik büyümenin analiz edilen tüm modellerde önemli bir etkiye sahip olduğunu göstermektedir. Ancak bu etkinin yönü ekosistem bileşenleri arasında farklılık göstermektedir. Ekonomik büyümenin tarım ve su kaynakları üzerinde olumlu bir etkisi olduğu tespit edilmiştir. Bu modellerde, GSYH'deki %1'lik bir artış tarım ve su kaynakları endekslerini %0,074-0,672 oranında artırmaktadır. Buna karşılık, ekonomik büyüme biyoçeşitlilik ve habitat, ekosistem hizmetleri, balıkçılık, asit yağmuru ve toplam ekosistem canlılığı üzerinde olumsuz bir etkiye sahiptir. Bu modellerde, GSYH'deki %1'lik bir artış biyoçeşitlilik ve habitat, ekosistem hizmetleri, balıkçılık, asit yağmuru ve toplam ekosistem canlılığı endekslerini %0,101-2,144 oranında azaltmaktadır. Bu da ekonomik büyüme süreçlerinin çevresel maliyetlerinin göz önünde bulundurulması gerektiğini göstermektedir. Ekonomik büyümenin olumsuz etkilerini azaltmak için çevre dostu politikalar sürdürülebilir kalkınma stratejileri ile birleştirilmelidir.

**Anahtar kelimeler:** Ekonomik Büyüme, Ekosistem, Ekosistem Canlılığı, Çevresel Performans, ÇPE.

## INTRODUCTION

The environmental impacts of economic growth have become an increasingly important and complex topic of debate in today's world. Rapid industrialization, energy production and increased trade, which began with the industrial revolution, have promoted economic growth while causing irreversible damage to nature, ecosystems and agricultural activities. In particular, the overuse of fossil fuels has led to a rapid increase in greenhouse gases released into the atmosphere and deepened environmental degradation. This has led to widespread environmental problems such as global warming, climate change, air and water pollution. These problems have become a threat to the ecosystem (Agenor, 2004; Madaleno & Nogueira, 2023).

The industrial revolution led to the growth in economic activity, mass production and a rapid increase in energy consumption. In particular, the widespread use of fossil fuels such as coal, oil and natural gas has become the engine of economic growth, but has also been the main source of environmental problems. The combustion of fossil fuels releases carbon dioxide (CO<sub>2</sub>) into the atmosphere. CO<sub>2</sub> emissions have triggered global warming by causing the greenhouse effect, leading to climate change and environmental disasters. The increase in energy demand and industrial activities in rapidly industrializing countries such as Türkiye has led to the degradation of ecosystems and the decrease in biodiversity (Bilen et al., 2008). In this process, especially ecosystem has suffered great damage. Industry and energy production have led to the destruction of natural habitats, deforestation and rapid depletion of natural resources. Ecosystems and biodiversity have come under great pressure due to these economic activities. Air, water and soil pollution in industrialized regions in Türkiye has directly affected agricultural areas and ecosystems. This pollution both threatens the sustainability of ecosystems and reduces productivity in agricultural production (Richmond & Kaufmann, 2006).

Agricultural activities are one of the areas most affected by economic growth. Rapid urbanization and the expansion of industrial areas have led to shrinking agricultural areas and declining productivity. Misuse of agricultural land, especially overconsumption of water resources and intensive cultivation of land threaten the sustainability of agriculture. In developing countries such as Türkiye, rapidly increasing energy demand and industrial production put serious pressure on agricultural land. Agricultural production has declined in many regions where access to water resources has become more difficult and soil fertility has declined. As a result, food security risks have increased. Moreover, climate change triggered by the intensive use of fossil fuels has disrupted rainfall patterns and created uncertainty in agricultural activities (Ulucak & Erdem, 2012).

The relationship between economic growth and environmental degradation is not limited to agriculture and energy consumption. Excessive consumption of natural resources has led to permanent damage to the environment, loss of biodiversity and disruption of ecosystem balance. Fossil fuels used to meet energy needs have led to the release of large amounts of greenhouse gases into the atmosphere. This has accelerated global warming and caused irreversible impacts on both nature and human life (Ansuategi & Escapa, 2002).

Global warming, climate change and environmental degradation lead to a deterioration of the natural balance around the world. This process threatens the ecosystems and leads to the degradation of natural systems. Climate change is especially putting pressure on the agricultural sector. Changes in rainfall patterns, droughts and extreme temperatures adversely affect agricultural production and put food security at risk. Moreover, environmental pollution and depletion of natural resources weaken the self-renewal capacity of ecosystems (Jacobs et al., 2014). Various attempts have been made internationally to solve these environmental problems. The 1972 Stockholm Conference and international initiatives such as the Kyoto Protocol are important steps to ensure environmental sustainability. However, the impact of these initiatives has been limited. Generally, economic growth-oriented policies have overshadowed environmental sustainability. Developing countries in particular have failed to adequately implement environmental protection policies while achieving their economic growth targets (Nordhaus, 2010). Türkiye is one of the countries involved in this process. Although Türkiye joined the Kyoto Protocol in 2009 and committed to reduce greenhouse gas emissions, there is still a large imbalance between growth targets and environmental protection (Turhan et al., 2016).

As a result, the environmental impacts of economic growth are a major threat to the sustainability of ecosystems, agricultural production and nature. In developing countries like Türkiye, it is becoming increasingly difficult to strike a balance between economic growth and environmental protection. Rapidly increasing energy demand and industrial activities threaten ecosystem and the sustainability of natural resources. In this process, it has become imperative to implement environmental protection policies more effectively, reduce energy consumption and turn to renewable energy sources. For economic growth to be sustainable, natural resources must be managed effectively and the environment must be protected.

In this regard, the impacts of economic growth on ecosystems require a more detailed analysis. Therefore, this study investigates the impacts of economic growth on the ecosystem and its components. The Ecosystem Vitality index, a sub-dimension of the Environmental Performance Index, is used in the study. The ecosystem

vitality index includes key indicators such as biodiversity and habitat, ecosystem loss, fisheries, acid rain, agriculture and water resources. These indicators represent different dimensions of environmental degradation. The aim of the study is to identify how economic activities damage nature through these different ecosystem components and to discuss what steps should be taken to mitigate these damages.

This study will contribute to the literature in at least three ways. (1) To the best of our knowledge, there is no empirical study investigating the impact of economic growth on different ecosystem components in Türkiye. This study analyzes the impacts of economic growth on ecosystem to fill this gap in the literature. (2) In order to determine the impacts of economic growth on different components of the ecosystem, all components are analyzed separately. For this purpose, seven different econometric models were constructed. This provided a clearer analysis of the potential impact of the economic model on each ecosystem component. (3) We used the most recent dataset available, thus providing a real-time and up-to-date perspective.

The rest of the paper is organized as follows: Section 2 presents the literature review, Section 3 presents the data and methodology, Section 4 presents the empirical findings, Section 5 presents policy implications and finally Section 6 presents conclusions.

## LITERATURE REVIEW

Human activities, particularly industrialization and urbanization, create serious pressures on natural resources and lead to environmental pollution. Economic growth is regarded as one of the most important sources of direct environmental degradation. Many studies have shown that economic growth damages the environment through factors such as increased energy demand, resource use and waste generation. However, the protection of the environment and nature is of great importance for both the ecosystem sustainability and human health. Therefore, the relationship between economic activities and their environmental impacts has been extensively analyzed in the literature. However, the relationship between economic growth and environmental pollution is a complex issue with different results in the literature. Among these studies, Grossman and Krueger (1993) have been pioneers in this field with their studies suggesting that economic growth may initially increase environmental pollution, but may decrease pollution after a certain level of income. Lopez (1994) also focused on the environmental impacts of economic growth and examined how growth and trade liberalization affect the environment. In more recent studies, Al-mulali (2012) emphasized that energy consumption and growth have a positive impact on CO<sub>2</sub> emissions and that foreign trade and FDI strengthen this relationship. Moreover, Mahmood et al. (2019) emphasize the emissions-enhancing impact of growth and trade liberalization in Tunisia. However, some studies have found that the impacts of growth on environmental degradation are more limited.

The impacts of energy consumption and economic growth on CO<sub>2</sub> emissions stand out. Jalil and Mahmud (2009) examined the impacts of economic growth, energy consumption and trade on CO<sub>2</sub> emissions in China. The results show that economic growth increases CO<sub>2</sub> emissions. Similarly, Jayanthakumaran et al. (2012) found that growth and energy consumption increase CO<sub>2</sub> emissions in India and China. Al-mulali and Sheau-Ting (2014) pointed out the positive impact of energy consumption and foreign trade on CO<sub>2</sub> emissions. Shahbaz et al. (2013) find that growth and energy consumption have a positive impact on CO<sub>2</sub> emissions in Indonesia, while financial development and trade have a negative impact. Moreover, other studies such as Farhani et al. (2014) and Bozkurt and Okumus (2017) also conclude that energy consumption increases emissions.

In addition these studies examining the impacts of economic growth on the environment, the literature on the environmental impacts of trade is also quite extensive. Studies on the environmental impacts of trade focus especially on the role of free trade on environmental pollution. Copeland and Taylor (1994) showed that trade can increase pollution with a two-country static general equilibrium model. More recently, Antweiler et al. (2001) argued that trade can both increase and decrease pollution. He argued that this relationship should be addressed through scale, technical and composition effects. Moreover, Rock (1996) emphasized that the impacts of trade on the environment differ in developed and developing countries and argued that open policies in particular can increase pollution intensity. Studies such as Baek et al. (2009) have shown that trade intensity is positively associated with environmental degradation in developing countries. Moreover, an increase in income level strengthens this impact. Kukla-Gryz (2009) evaluated the impacts of trade and per capita income on air pollution and found that trade increases pollution especially in developing countries.

In recent years, ecological footprint has become a more frequently used measure among environmental indicators in the literature. Gao and Tian (2016) examined the impacts of trade on ecological footprint in China. They found that China is an importer of ecological footprint. This finding suggests that large economies such as China should reconsider their trade policies in terms of environmental degradation. Usman et al. (2020) find that trade openness has a negative impact on ecological footprint in Africa, Asia and the Americas. They also find that there is a unidirectional causality from trade openness to ecological footprint. Rehman et al. (2021) examined

the impacts of trade and energy consumption on ecological footprint in Pakistan and found that trade increases environmental degradation in both the short- and long-run. Yilanci et al. (2022) examined the relationship between trade openness and ecological footprint in G7 countries and found that trade openness has complex impacts on ecological footprint.

The complexity of the impacts of economic growth on the environment stems from the multi-dynamic nature of the growth process. This leads to the emergence of various approaches in the literature. Among these approaches, the Environmental Kuznets Curve (EKC) hypothesis argues that there is an inverted-U relationship between economic growth and environmental pollution. The EKC suggests that economic growth increases environmental pollution at low income levels, but after a certain income level, growth starts to improve environmental quality. Among the studies investigating the EKC, Kasman and Duman (2015) find that the EKC hypothesis is valid in EU countries. They showed that economic growth initially increases CO<sub>2</sub> emissions, but this effect reverses as income increases. Farhani et al. (2014) obtained similar findings for Tunisia and emphasized that trade liberalization and energy consumption also increase emissions. Moreover, Dogan et al. (2017) find that the EKC hypothesis is not valid in OECD countries. The study finds that energy consumption and tourism increase emissions, but trade openness decreases emissions.

Various methods are used to evaluate the impact of different factors on the environment. One of the most prominent of these is the Environmental Performance Index (EPI). The EPI provides a comprehensive tool for measuring and analyzing environmental sustainability performance. These studies play an important guiding role in the process of developing countries' environmental policies. For example, in a study conducted by Alptekin (2015), Türkiye's sustainability performance was compared with the European Union member states. The study shows that Sweden has the highest performance, while Türkiye ranks 20th. These findings reveal that Türkiye is far behind the European Union average in terms of environmental performance and that more efforts should be made in this area. Similarly, Karaman (2018) evaluated Türkiye's environmental performance in line with the European Union membership target. The results revealed that Türkiye's environmental policies are quite weak compared to European standards. Another study detailing Türkiye's poor environmental performance was conducted by Bek (2019). In this study, the environmental performance of Switzerland and Türkiye is compared and the reasons for Türkiye's low ranking are investigated. Pimonenko et al. (2018) analyzed the methodology of the EPI and argued that this index is an important tool for assessing the environmental, social and economic status of countries. In addition, it was revealed that countries with high performance in the EPI are also successful in the Sustainable Development Goals and the Social Progress Index. Another study by Ozkan and Ozcan (2018) evaluated the environmental performance of OECD countries. In this study, Türkiye's environmental performance was found to be in an effective position. However, when the results are compared with previous studies, Türkiye's environmental performance ranking remains relatively low. Pinar (2022) examined the sensitivity of environmental performance indicators to the subjective weights assigned to them using EPI data. The study emphasizes that sensitivity analysis of environmental performance is critical for reliability and transparency. Iskenderoglu et al. (2023) also examined the environmental impacts of economic growth, foreign direct investment and renewable energy consumption using the Environmental Performance Index. The results of this study show that FDI plays a positive role in reducing environmental degradation, while economic growth has a negative impact. This shows that the impacts of economic factors on the environment are complex and multidimensional.

All these studies show that the EPI is a useful tool for comparing countries' environmental performance on a global scale. These analyses of various countries and regions emphasize the importance of the methodological approaches used to assess countries' environmental performance. While studies such as Alptekin (2015) and Karaman (2018) reveal Türkiye's low environmental performance, studies such as Pimonenko et al. (2018) and Pinar (2022) highlight the methodological sensitivities of the EPI and the importance of sensitivity analysis. Studies such as Ozkan and Ozcan (2018) and Iskenderoglu et al. (2023) reveal the complexity in this area by analyzing the relationship between environmental performance and economic and financial variables.

## MATERIAL AND METHODS

### Model Specification and Data

The empirical analysis of this study analyzes the impacts of economic growth on ecosystem in Türkiye. The study uses annual data for the period 1995-2021. This period was chosen considering the availability of the dataset and its suitability for the analysis. Descriptive statistics of the data used in the study are presented in Table 1.



**Table 1.** Descriptions and sources of variables

Variable	Notation	Description	Obs.	Mean	SD	Min.	Max.	Source
Biodiversity & habitat	BDH	Index	27	1.042	0.006	1.025	1.050	EPI
Ecosystem services	ECS	Index	27	1.492	0.091	1.343	1.624	EPI
Fisheries	FSH	Index	27	1.157	0.163	0.841	1.374	EPI
Acid rain	ACD	Index	27	1.808	0.087	1.680	1.974	EPI
Agriculture	AGR	Index	27	1.564	0.024	1.505	1.605	EPI
Water resources	WRS	Index	27	1.484	0.002	1.475	1.485	EPI
Ecosystem vitality	ECO	Index	27	1.388	0.027	1.338	1.436	EPI
GDP per capita	GDP	Constant 2015 US\$	27	3.925	0.115	3.756	4.129	WB
Energy intensity	ENG	Level of primary energy	27	0.466	0.043	0.394	0.537	WB
Population density	POP	People per sq. km of land	27	1.966	0.045	1.887	2.039	WB
Trade	TRD	Trade (% of GDP)	27	1.703	0.059	1.576	1.852	WB

Note: (1) WB and EPI indicate World Bank-World Development Indicators, and Yale University-Environmental Performance Index, respectively. (2) The abbreviations N, SD, SE, Min and Max denote the number of observations, standard deviation, standard error, minimum and maximum values, respectively. (3) The variables used in the study are logarithmized.

Ecosystem vitality index was used as the dependent variable in the study. Ecosystem vitality includes factors such as sustainability of natural resources and conservation of biodiversity and is an important indicator for monitoring environmental degradation. The ecosystem vitality index is a sub-dimension of the Environmental Performance Index (EPI) developed by Wolf et al. (2022) of Yale University. The EPI is a comprehensive index that aims to measure a country's environmental sustainability performance. It is widely used by governments, policy makers and researchers worldwide. The EPI consists of three main dimensions: Climate Change, Environmental Health and Ecosystem Vitality. Each dimension includes a set of components to measure different areas of environmental performance. The purpose of the EPI is to provide a benchmarking tool for countries to develop environmentally sound policies and improve their implementation. The components of the ecosystem vitality index are given in Table 2.

**Table 2.** Components of the ecosystem vitality index

Dimension	Weight	Indicator	Notation	Weight
Biodiversity & Habitat (BDH)	43.00%	Terrestrial Biome Protection (national)	TBN	22.2%
		Terrestrial Biome Protection (global)	TBG	22.2%
		Marine Protected Areas	MPA	22.2%
		Protected Areas Rep. Index	PAR	14%
		Species Habitat Index	SHI	8.3%
		Species Protection Index	SPI	8.3%
		Biodiversity Habitat Index	BHV	3%
Ecosystem Services (ECS)	19.00%	Tree Cover Loss	TCL	75%
		Grassland Loss	GRL	12.5%
		Wetland Loss	WTL	12.5%
Fisheries (FSH)	11.90%	Fish Stock Status	FSS	36%
		Marine Trophic Index	RMS	36%
		Fish Caught by Trawling	FTD	28%
Acid Rain (ACD)	9.50%	SO <sub>2</sub> Growth Rate	SDA	50%
		NO <sub>x</sub> Growth Rate	NXA	50%
Agriculture (AGR)	9.50%	Sustainable Nitrogen Mgmt. Index	SNM	50%
		Sustainable Pesticide Use	SPU	50%
Water Resources (WRS)	7.10%	Wastewater Treatment	WWVT	100%

Source: Wolf et al. (2022)

Table 2 shows that the ecosystem vitality index is composed of several components that assess the sustainability of nature conservation and ecosystem services. The biodiversity and habitat dimension (43%) constitutes the largest part of this index. Here, indicators such as the protection of terrestrial biomes at both national and global level, marine protected areas and the representativeness of protected areas are considered. The ecosystem services dimension (19%) tracks the loss of tree cover, grasslands and wetlands, while the fisheries dimension (11.9%) measures the state of fish stocks and the impacts of trawling on the ecosystem. It also includes environmental pressures such as acid rain (9.5%), sustainability of agricultural practices (9.5%) and

management of water resources (7.1%). These components comprehensively analyze ecosystem vitality and environmental performance.

The main objective of this study is to analyze the impacts of economic growth on ecosystem. For this purpose, the Ecosystem Vitality Index, a sub-dimension of the Environmental Performance Index, was used in the study. Not only the overall ecosystem vitality but also the impacts on the sub-components of ecosystem vitality are analyzed. Seven models are created to evaluate in detail the impact of economic growth on different dimensions of ecosystem vitality. Models 1 to 6 explore the impacts of economic growth on the sub-components of ecosystem vitality (biodiversity and habitat, ecosystem services, fisheries, acid rain, agriculture and water resources). Model 7 aims to analyze the impacts on total ecosystem vitality.

While constructing the models used in the study, the shortcomings of empirical studies in the relevant literature were considered. Control variables were also included in the analysis to provide a more comprehensive analysis of the factors on ecosystem vitality. Based on the literature review, GDP per capita (Agboola et al., 2021; Tabash et al., 2024), energy intensity (Jalil and Mahmud, 2009; Al-muali, 2012; Jayanthakumaran et al., 2012), population density (Ghanem, 2018; Van Dao and Van, 2020), and trade (Sheau-Ting, 2014; Mahmood et al., 2019) were identified as control variables. These models are shown as follows.

$$BDH_t = \beta_0 + \beta_1 GDP_t + \beta_2 ENG_t + \beta_3 POP_t + \beta_4 TRD_t + \epsilon_t \quad (1)$$

$$ECS_t = \beta_0 + \beta_1 GDP_t + \beta_2 ENG_t + \beta_3 POP_t + \beta_4 TRD_t + \epsilon_t \quad (2)$$

$$FSH_t = \beta_0 + \beta_1 GDP_t + \beta_2 ENG_t + \beta_3 POP_t + \beta_4 TRD_t + \epsilon_t \quad (3)$$

$$ACD_t = \beta_0 + \beta_1 GDP_t + \beta_2 ENG_t + \beta_3 POP_t + \beta_4 TRD_t + \epsilon_t \quad (4)$$

$$AGR_t = \beta_0 + \beta_1 GDP_t + \beta_2 ENG_t + \beta_3 POP_t + \beta_4 TRD_t + \epsilon_t \quad (5)$$

$$WRS_t = \beta_0 + \beta_1 GDP_t + \beta_2 ENG_t + \beta_3 POP_t + \beta_4 TRD_t + \epsilon_t \quad (6)$$

$$ECO_t = \beta_0 + \beta_1 GDP_t + \beta_2 ENG_t + \beta_3 POP_t + \beta_4 TRD_t + \epsilon_t \quad (7)$$

In these equations,  $\beta_0$  represents the constant term, while the coefficients  $\beta_1$  to  $\beta_4$  measure the impact of each independent variable on exports.  $\epsilon_t$  is the error term with zero mean and constant variance, where  $t$  is the time period.

## Unit Root Analysis

In this study, two common unit root tests are applied to determine the stationarity properties of time series data: Augmented Dickey-Fuller (ADF) Test and Phillips-Perron (PP) Test. Both tests determine whether the series are stationary or not, thus allowing the existence of long-run relationships and the selection of appropriate econometric methods.

**ADF Unit Root Test:** The ADF test developed by Dickey and Fuller (1979) is one of the most widely used methods to test whether a time series is stationary. The ADF test extends the Dickey-Fuller (DF) test to include lagged difference terms in the model to eliminate the autocorrelation problem. In this way, it provides more reliable results by ensuring the independence assumption. The general equation of the ADF test is as follows:

$$\Delta Y_t = \alpha + \beta_t + \gamma Y_{t-1} + \sum_{i=1}^p \delta_i \Delta Y_{t-i} + \epsilon_t \quad (8)$$

In Equation 8,  $Y_t$  is the time series under test,  $\Delta Y_t$  is the first difference of the series,  $\alpha$  is the constant term,  $\beta_t$  is the time trend,  $\gamma Y_{t-1}$  is the coefficient testing for the presence of a unit root and  $\epsilon_t$  is the error term. In the ADF test, a coefficient  $\gamma$  equal to zero indicates that there is a unit root in the series and the series is non-stationary.

**PP Unit Root Test:** The PP test developed by Phillips and Perron (1988) is an alternative approach to the ADF test. The PP test considers the presence of autocorrelation and heteroskedasticity in determining whether the series are stationary. Similar to the ADF test, the PP test tests the stationarity of the series. However, instead of adding lag terms to the model, it addresses the problems of autocorrelation and heteroskedasticity by making asymptotic corrections to the error terms (Perron, 1988). The general equation of the PP test is as follows:

$$\Delta Y_t = \alpha + \beta_t + \gamma Y_{t-1} + \epsilon_t \quad (9)$$

In Equation 9,  $Y_t$  is the level of the series,  $\Delta Y_t$  is the first difference,  $\alpha$  is the constant term,  $\beta_t$  is the time trend,  $\gamma Y_{t-1}$  is the lagged value and  $\epsilon_t$  is the error term. The null hypothesis of the PP test is the existence of a

unit root in the series. A coefficient of  $\gamma$  equal to zero indicates that the series contains a unit root and is non-stationary.

The main objective of both tests is to test whether there is a unit root in the series. In this context, the null hypothesis ( $H_0$ ) states that the series has a unit root, i.e. is non-stationary, while the alternative hypothesis ( $H_1$ ) states that the series is stationary. The lag length is usually determined by criteria such as Akaike Information Criterion (AIC) or Schwarz Information Criterion (SIC). Since the PP test solves the problems of autocorrelation and heteroskedasticity with asymptotic methods, it is more flexible and can produce more robust results for large data sets. It also provides reliable results by correcting the error terms without changing the parameter estimates.

### ARDL Cointegration Test

There are various methods to test cointegration between series. Among these methods, the tests developed by Engle and Granger (1987), Johansen (1988) and Johansen and Juselius (1990) stand out. However, these methods have some limitations. The ARDL model developed by Pesaran et al. (2001) is a method developed to test the long run relationship between variables. It stands out by eliminating the limitations of the mentioned methods. In particular, the fact that it does not require the variables to be stationary at the same level makes the ARDL method a more flexible option. This method has the capacity to analyze stationary variables at both  $I(0)$  and  $I(1)$  levels. However,  $I(2)$  variables should not be included in the model. This method provides a significant advantage in econometric analysis by providing reliable results even in data sets with a limited number of observations. By including lags of dependent and independent variables in the model, short run dynamics and long run relationships can be analyzed together. With these features, the ARDL bounds test is an effective method for determining cointegration relationships. The ARDL model is expressed in the following general form:

$$\Delta Y_t = \alpha + \sum_{i=1}^p \beta_i \Delta Y_{t-i} + \sum_{j=0}^q \theta_j \Delta X_{t-j} + \phi Y_{t-1} + \psi X_{t-1} + \epsilon_t \quad (10)$$

In Equation 10,  $Y_t$  is the dependent variable,  $X_t$  is the independent variables,  $\Delta Y_t$  and  $\Delta X_t$  are the first differences of the series,  $\beta_i$  and  $\theta_j$  are the coefficients of short-run dynamics.  $\phi Y_{t-1}$  and  $\psi X_{t-1}$  are the coefficients of long-run relationships and  $\epsilon_t$  is the error term. The ARDL cointegration bounds test tests whether the long-run coefficients are zero. This method examines whether there is a long-run relationship between variables and estimates the long-run coefficients under appropriate conditions.

The ARDL cointegration test tests whether there is a long-run relationship (cointegration) between variables. The null hypothesis ( $H_0$ ), states that there is no long-run relationship, while the alternative hypothesis ( $H_1$ ) states that there is a long-run relationship. The test is performed using the F-statistic. The F-statistic is compared with certain critical values. If the F-statistic is less than the lower bound, it is concluded that there is no cointegration; if it is greater than the upper bound, cointegration is accepted. When the F-statistic falls between these two limits, the result is ambiguous.

### ARDL Coefficient Estimates

After determining the existence of cointegration, it is possible to analyze the long-run relationship between variables using the ARDL model. Long-run coefficients are calculated through the coefficients obtained from the ARDL model. In the ARDL model, the long-run impacts of the independent variables are calculated by the  $\psi/\phi$  ratio. The long-run form of the model is expressed as follows:

$$Y_t = \alpha + \sum_{j=0}^q \theta_j X_{t-j} + \epsilon_t \quad (11)$$

In Equation 11,  $Y_t$  is the dependent variable,  $X_{t-j}$  s the lagged values of the independent variables and  $\epsilon_t$  is the error term. The ARDL model allows the estimation of both short-run dynamics and long-run relationships. Long-run coefficients determine the strength and direction of long-run relationships in the model.

Long-run coefficient estimation is done by normalizing the coefficients of lagged independent variables in the ARDL model by the error correction term. In this way, the effect of a one-unit increase in the independent variables on the dependent variable in the long-run is determined. The ARDL model provides a dynamic structure by simultaneously analyzing the long-run equilibrium relationship and short-run deviations.

### EMPIRICAL FINDINGS

ADF unit root test and PP unit root test were applied to determine whether the variables used in the study contain unit roots. Moreover, ARDL bounds test was used to analyze the long-run cointegration relationship

between the variables. To estimate the long-run coefficients, the ARDL model was used to estimate the long-run impacts. The results obtained with these methods are given as follows.

**Table 3.** Unit root test results

Variable	ADF unit root test		PP unit root test	
	t-statistic (level)	t-statistic (first difference)	t-statistic (level)	t-statistic (first difference)
BDH	-1.133	-3.774***	-2.512	-3.775***
ECS	-0.325	-4.133***	-0.325	-4.109***
FSH	-1.094	-4.412***	-1.043	-3.984***
ACD	-2.397	-3.476***	-1.834	-2.279***
AGR	-2.109	-6.063***	-1.847	-5.156***
WRS	-1.460	-7.163***	-1.444	-7.208***
ECO	-2.199	-3.051**	-1.099	-2.617***
GDP	-0.188	-4.456***	-0.188	-4.456***
ENG	-0.819	-4.951***	-0.629	-5.422***
POP	-2.313***	-4.024***	-1.641***	-4.038***
TRD	-1.234	-4.865***	-0.934	-5.586***
BDH	-1.619	-4.434***	-1.511	-4.434***
ECS	-2.342	-4.302***	-2.332	-4.272***
FSH	-3.163	-4.307***	-2.403	-3.849**
ACD	-2.324	-4.025***	-1.509	-2.371***
AGR	-3.819	-5.916***	-3.147	-5.188***
WRS	-1.865	-7.462***	-1.865	-7.177***
ECO	-3.069	-3.854***	-1.658	-2.753***
GDP	-2.716	-4.444***	-2.227	-4.435***
ENG	-2.891	-4.839***	-3.007	-5.246***
POP	-2.145***	-4.241***	-1.663***	-5.098***
TRD	-2.795	-6.761***	-2.312	-5.947***

Note: The superscripts \*\*\*, \*\*, and \* denote the significance at a 1%, 5%, and 10% level, respectively.

Table 3 shows that all variables except POP in the constant model contain unit root at level. However, these variables become stationary in their first differences. This shows that all variables except POP are I(1). The POP variable is determined as I(0) since it is stationary at level. Similar findings were obtained in the analysis based on the constant and trend model. Variables other than POP is not stationary at level. However, variables become stationary when first differences are taken.

As a result, POP variable was found to be stationary at level. All other variables become stationary when first differences are taken. This means that POP is I(0) and the other variables are I(1). Since these findings show that the series are stationary at different levels, it allows us to use the ARDL (Autoregressive Distributed Lag) method. Because the ARDL model is suitable for working with both level stationary variables and stationary variables at first difference. In addition, it is an ideal method for analyzing series with mixed unit root properties.

**Table 4.** ARDL bound test results

Model	Optimal lag length	F-statistics	Critical values %5		Critical values %1	
			I(0)	I(1)	I(0)	I(1)
Model 1: F(BDH   GDP, ENG, POP, TRD)	(2, 2, 1, 1, 1)	4.192**	2.56	3.49	3.29	4.37
Model 2: F(ECS   GDP, ENG, POP, TRD)	(1, 0, 0, 1, 0)	5.254***	2.56	3.49	3.29	4.37
Model 3: F(FSH   GDP, ENG, POP, TRD)	(2, 0, 1, 0, 0)	1.282*	2.56	3.49	3.29	4.37
Model 4: F(ACD   GDP, ENG, POP, TRD)	(2, 0, 1, 0, 0)	3.979**	2.56	3.49	3.29	4.37
Model 5: F(AGR   GDP, ENG, POP, TRD)	(2, 1, 0, 1, 2)	8.342***	2.56	3.49	3.29	4.37
Model 6: F(WRS   GDP, ENG, POP, TRD)	(2, 2, 0, 1, 1)	4.282**	2.56	3.49	3.29	4.37
Model 7: F(ECO   GDP, ENG, POP, TRD)	(2, 2, 2, 1, 2)	4.558***	2.56	3.49	3.29	4.37

Note: (1) The superscripts \*\*\*, \*\*, and \* denote the significance at a 1%, 5%, and 10% level, respectively. (2) The optimal lag lengths are calculated automatically using information criteria.

The results in Table 4 show that there is a significant cointegration relationship at the 5% level in Model 1, Model 4 and Model 6. In these models, F-statistics are above the critical values at the 5% level, indicating that there is a long-run relationship between the variables. Moreover, there is a significant cointegration relationship at the 1% level in Model 2, Model 5 and Model 7. In these models, the F-statistics are above the 1% critical values, indicating a stronger cointegration relationship. In Model 3, the cointegration relationship is found at the 10%

significance level and the long-run relationship in this model is weaker than in the other models. These results indicate that there is a long-run cointegration relationship between variables in most of the models analyzed with the ARDL bounds test. These findings confirm that the ARDL model is appropriate for analyzing the dynamics between economic growth and ecosystem vitality.

The results of the diagnostic tests conducted to assess the validity and reliability of the model are presented in Table 5. The results of the diagnostic tests show that there are no statistical problems in the models. Moreover, CUSUM and CUSUM Square tests, which assess the stability of long-run estimates, confirm that the model parameters are stable over the sample period. Moreover, the coefficient ECT(-1) in the models is called the error correction term and represents the speed of return to long-run equilibrium. As expected, the coefficient of ECT(-1) is negative and statistically significant in all models. A negative sign indicates that short-run imbalances are corrected over time and the system returns to long-run equilibrium.

**Table 5.** Short-run and long-run estimation.

Variable	Short-run coefficient						
	Model 1 BDH <sub>(M-D)</sub>	Model 2 ECS <sub>(M-D)</sub>	Model 3 FSH <sub>(M-D)</sub>	Model 4 ACD <sub>(M-D)</sub>	Model 5 AGR <sub>(M-D)</sub>	Model 6 WRS <sub>(M-D)</sub>	Model 7 ECO <sub>(M-D)</sub>
GDP	-0.253*	-0.101*	-0.533**	-0.526**	0.521***	0.362	0.044***
ENG	0.012	-0.502***	1.659	-0.776***	-0.073***	-0.744**	-0.528***
POP	-0.677***	-5.044***	-1.161***	1.236	0.557**	-1.739***	1.339***
TRD	-0.022**	-0.077***	-0.213**	-0.006**	0.106	-1.562	-0.103***
ECT(-1)	-0.325***	-0.358***	-0.176***	-0.449***	-0.312***	-0.527***	-0.349***

Variable	Long-run coefficient						
	Model 1 BDH <sub>(M-D)</sub>	Model 2 ECS <sub>(M-D)</sub>	Model 3 FSH <sub>(M-D)</sub>	Model 4 ACD <sub>(M-D)</sub>	Model 5 AGR <sub>(M-D)</sub>	Model 6 WRS <sub>(M-D)</sub>	Model 7 ECO <sub>(M-D)</sub>
GDP	-0.116***	-0.591**	-1.362**	-2.144***	0.074***	0.672***	-0.668***
ENG	-0.081**	-1.378***	-0.505	-3.960***	-0.183***	-1.442***	-1.786***
POP	-0.336**	-1.857***	-0.668**	-1.481**	-0.392**	-1.870***	-0.047*
TRD	-0.083***	-0.225**	-0.319**	-2.203***	-0.010	-1.800	-0.221**
C	1.021***	8.607*	7.528***	-0.464***	0.457***	2.245*	5.286***
Diagnostic test	<i>P value</i>	<i>P value</i>	<i>P value</i>	<i>P value</i>	<i>P value</i>	<i>P value</i>	<i>P value</i>
Serial correlation	0.47	0.34	0.27	0.55	0.29	0.51	0.15
Heteroskedasticity	0.57	0.32	0.22	0.65	0.78	0.24	0.86
Normality	0.79	0.75	0.47	0.37	0.81	0.67	0.11
Functional form	0.92	0.57	0.72	0.57	0.63	0.14	0.64
CUSUM	Stable	Stable	Stable	Stable	Stable	Stable	Stable
CUSUMSQ	Stable	Stable	Stable	Stable	Stable	Stable	Stable

Note: The superscripts \*\*\*, \*\*, and \* denote the significance at a 1%, 5%, and 10% level, respectively.

The results show that the impact of GDP on Model 1, Model 2, Model 3, Model 4 and Model 7 is negative and statistically significant. In these models, a 1% increase in GDP reduces biodiversity and habitat, ecosystem services, fisheries, acid rain and total ecosystem vitality by 0.101-2.144%. However, the impact of GDP on Model 5 and Model 6 is found to be positive and statistically significant. In these models, a 1% increase in GDP increases agricultural and water resources by 0.074-0.672%. This shows that economic growth increases pressures on environmental sustainability and ecosystems.

The negative impact of GDP on biodiversity and habitat shows that economic growth increases consumption of natural resources, leading to habitat loss. Economic growth in developing countries is often based on industrial, mining and agricultural expansion, which leads to the destruction of natural habitats. This process leads to the degradation of natural ecosystems and a decline in biodiversity. The negative impact of GDP on ecosystem services shows that economic growth can damage environmental services. For example, during periods of growth, activities such as agriculture, industry and energy production can negatively affect soil, water and air quality. Economic growth can lead to the degradation of services provided by ecosystems (water purification, air purification, etc.), creating long-run environmental and social costs. The negative impact of GDP on fisheries shows that economic growth also puts marine ecosystems under pressure. Economic growth, together with trade and industrialization, can lead to overexploitation of fish stocks. This threatens the sustainability of the fisheries sector by reducing marine biodiversity. The negative impact of GDP on acid rain shows that economic growth increases the use of fossil fuels, which in turn increases harmful emissions to the environment. Growth based on fossil fuels releases gases such as sulphur dioxide and nitrogen oxides into the atmosphere, causing acid rain. Acid rain has destructive impacts on forests, lakes and buildings. The negative

impact of GDP on total ecosystem vitality shows that economic growth has a negative impact on the overall health of ecosystems. Economic growth can undermine ecosystem vitality by leading to overuse of natural resources through agriculture, industry and urban expansion. In the long-run, this can lead to ecosystem degradation and environmental sustainability problems.

Model 5 (agriculture) and Model 6 (water resources) are the models where a positive impact of GDP is observed. This suggests that economic growth may have some positive impacts in these areas. The positive impact of GDP on agriculture suggests that economic growth can increase agricultural production. Economic growth can lead to positive outcomes such as strengthening agricultural infrastructure and investing in technology and mechanization. In particular, agricultural productivity growth can provide significant benefits in terms of food security and economic development. However, if growth is not supported by sustainable agricultural policies, environmental costs may arise. The positive impact of GDP on water resources suggests that economic growth can contribute to the development of water management infrastructure. Investments in water treatment facilities can increase the environmental benefits of economic growth. However, this positive impact needs to be supported by policies for sustainable water use and protection of water resources. Otherwise, increased industrial and agricultural activities may put pressure on water resources.

Additionally, the study uses three explanatory variables: energy intensity, population and trade. First, energy intensity has a negative impact in almost all models and is statistically significant in most models. This clearly shows the negative impact of energy intensity on environmental indicators. As energy intensity is associated with fossil fuel consumption, it can increase emissions that are particularly harmful for the environment, leading to habitat destruction, reduced ecosystem services and increased acid rain. Moreover, while the negative impact of energy intensity in agriculture is less pronounced, energy use can cause environmental pressures, especially when agriculture is not sustainable. Second, population density has a negative impact in most models and is statistically significant. Population growth implies greater use of environmental resources and a reduction in living space. As the population increases, energy consumption, waste production and demand for natural resources also increase. This leads to environmental destruction. The negative impact of population growth on water resources is noteworthy. As the population increases, the pressure on water resources increases and sustainable water management becomes more difficult. Third and finally, trade has a negative impact in most models and is statistically significant. This reveals the negative impacts of trade on the environment. Increased trade volume increases the use of fossil fuels, especially by increasing transportation activities. Therefore, emissions harmful to the environment also increase. Moreover, the increase in trade volume may also contribute to unsustainable production processes. The negative impact of trade on acid rain points to damage to marine ecosystems and the environment in general.

The results show that economic growth has a positive impact on agriculture and water resources. This finding suggests that economic growth can stimulate the adoption of technological innovations and modern farming methods to increase agricultural production and productivity. Investments in the agricultural sector during economic growth can strengthen agricultural infrastructure, increase mechanization and introduce efficient irrigation systems. This can have positive impacts on food security, agricultural production capacity and rural development. Moreover, investments in water resources can improve water productivity in both urban and rural areas. However, a negative impact of GDP on biodiversity and habitat, ecosystem services, fisheries, acid rain and total ecosystem vitality was observed. These findings suggest that economic growth threatens environmental sustainability in the long-run, particularly through overconsumption of natural resources and environmental pressures. Energy intensity, population and trade generally have a negative impact on the environmental indicators in the models. The main reasons for this are excessive energy consumption, reliance on fossil sources of energy and neglect of environmental sustainability. In particular, the environmental costs of fossil fuel use lead to habitat destruction, ecosystem service degradation and increased environmental problems such as acid rain. Previous studies have typically used carbon emissions (e.g. Salman et al., 2019; Osobajo et al., 2020; Alaganthiran & Anaba, 2022), greenhouse gas emissions (e.g. Yang et al., 2017; Vasylieva et al., 2019; Sarkodie & Strezov, 2019) or the environmental performance index (e.g. Ave, 2010; Fakher & Abedi, 2017) to investigate the impacts of economic growth on the environment. However, this study focuses on the impacts of economic growth on the ecosystem beyond environmental degradation. Nevertheless, the results of the study are consistent with the literature. The findings are consistent with Bozkurt and Okumus (2017), Al-muali (2012) and Al-muali and Sheau-Ting (2014) who find that energy consumption increases CO<sub>2</sub> emissions. Moreover, the results of this study are also consistent with the findings of Mahmood et al. (2019) and Iskenderoglu et al. (2023), which indicate that growth and trade liberalization increase emissions. Similarly, the results of this study are consistent with Farhani et al. (2014) and Bozkurt and Okumus (2017) who find that energy consumption increases environmental damage.



## POLICY IMPLICATIONS

The findings of the study reveal that the impacts of economic growth on ecosystems are complex and vary across ecosystem components. While growth has positive impacts on agriculture and water resources, it clearly has negative impacts on biodiversity, ecosystem services, fisheries and overall ecosystem vitality. Moreover, energy intensity, fossil fuel use and population growth increase environmental pressures, while trade activities also contribute to environmental degradation. These findings make it clear that policies need to be developed to balance the environmental costs of economic growth. Therefore, a number of policy recommendations have been developed to protect the environment and offset the negative impacts of economic growth on the environment: (1) Fossil fuel dependency should be reduced to reduce the negative impact of energy intensity on the environment. Tax incentives and subsidies for the use of renewable energy contribute to lowering energy intensity and reducing environmental damage. (2) Energy efficiency strategies should be implemented to reduce energy intensity. Invest in and deploy energy efficiency technologies in industry, construction, agriculture and transportation sectors. Government support for energy efficiency projects in the industrial sector is critical. (3) Sustainable urban planning should be implemented to reduce the negative impacts of population growth on the environment. Preserving green spaces, building energy-efficient structures and expanding public transportation will reduce pressure on nature. Smart city applications and urban agriculture projects should be promoted to protect rural areas and control urban expansion. (4) Develop sustainable trade policies to reduce the environmental impacts of trade. Adopt green logistics solutions in transport and accelerate the transition to low-carbon transport systems. Low emission technologies in maritime and road transportation should be scaled up and government support should be provided. Encourage policies that reduce carbon footprint and the use of environmentally friendly products. (5) Expand and protect nature reserves to prevent biodiversity loss. Regulations should be made to reduce the environmental impacts of agricultural, industrial and construction activities and environmentally friendly production methods should be encouraged. Sustainable use of natural resources and the establishment of protected areas are essential for sustaining ecosystems. (6) Policies to address climate change should be strengthened to reduce acid rain and other environmental damage. Solutions such as emissions trading and carbon tax can be implemented to reduce carbon emissions. Carbon capture and storage technologies should be encouraged and their use should be expanded. Transition to clean technologies in industry and energy sectors should be supported. (7) Sustainable management of water resources is important for economic growth. Water saving technologies should be promoted, agricultural irrigation should be modernized and water management infrastructure should be strengthened. Wastewater treatment systems should be expanded and environmental pressures on water resources should be reduced. (8) Education and awareness-raising programs should be implemented to raise awareness on environmental sustainability. Individuals and enterprises should be informed about behaviors that will contribute to the environment. Adopting environmentally friendly consumption patterns and promoting sustainable development principles can alleviate environmental pressures. These policy recommendations can help balance economic growth with environmental sustainability and ensure a more livable environment for future generations.

## CONCLUSION

This study investigates the impacts of economic growth on ecosystem in Türkiye for the period 1995-2021. The analysis using the ARDL model reveals the short and long-run impacts of these variables on environmental indicators. The results show that economic growth has a significant impact in all models analyzed. However, the direction of this impact differs according to ecosystem components. Economic growth has a positive impact on agriculture and water resources and a negative impact on biodiversity and habitat, ecosystem services, fisheries, acid rain and total ecosystem vitality. Economic growth has particularly detrimental consequences for biodiversity, ecosystem services, fisheries and overall ecosystem vitality. This suggests that the environmental costs of growth processes need to be considered. Environmentally friendly policies should be combined with sustainable development strategies to reduce the negative impacts of economic growth.

Energy intensity has a negative impact on environmental indicators in almost all models. This result shows that economic growth based on fossil fuel use threatens habitats, water resources and overall ecosystem health by increasing environmentally damaging carbon emissions. Renewable energy and energy efficiency should be promoted to reduce the environmental costs of fossil fuels. Population growth has a negative impact on environmental indicators in most models. Increasing population puts pressure on environmental resources through energy consumption, waste generation and agricultural expansion. Rapid population growth can lead to a reduction in natural habitats and degradation of ecosystems. Therefore, it is critical to adopt sustainable urbanization, agriculture and energy policies to mitigate the environmental impacts of population growth. Trade generally has a negative impact on environmental indicators. Increased trade can increase transportation

activities, leading to higher carbon emissions and increased negative impacts on the environment. Green logistics solutions, sustainable production and environmental regulations should be implemented to reduce the environmental impacts of trade.

Although this study reveals important findings, it has some limitations. Future research can address these limitations. First, our study only covers certain environmental and economic indicators. Future studies can expand the dataset. Second, the study is based on a sample of Türkiye. Future research could extend the analysis to emphasize differences between developed and developing countries. Such a comparison could help us comprehend how environmental impacts vary based on the level of economic development. Finally, larger data sets and different analysis techniques could be used to further consolidate and extend the findings of this study.

#### Declaration of interests

The author declares that there is no conflict of interest.

#### Author Contributions

**Emre Akusta:** Conceptualization; data curation; formal analysis; investigation; methodology; software; writing—original draft; writing—review and editing.

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## Elemental Characterization of Termiye: Mineral Composition of Debitterized Lupin Seeds Cultivated at Different Locations

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### ABSTRACT

The utilization of marginal lands for agricultural production without input use is crucial for sustainable agriculture, the creation of new food sources, and regional development. The aim of this study was to highlight the value of termiye in terms of location-based mineral nutrition. This study used seed of lupin (*Lupinus albus* L.) as the material, grown without inputs and processed into termiye, a traditional snack. The debittered lupin seeds (termiye) were obtained from growers in five locations: Deşdiğin, Konakkale, Ketenli, Başköy, and Akçalar. Termiye seeds were analyzed separately for mineral composition using ICP-OES. According to the general average locations, macroelement contents were found to be 4398 mg kg<sup>-1</sup> P, 3900 mg kg<sup>-1</sup> Ca, 2332 mg kg<sup>-1</sup> S, 846 mg kg<sup>-1</sup> Mg, 782 mg kg<sup>-1</sup> Na, and 135 mg kg<sup>-1</sup> K. Microelements were determined as 1228.23 mg kg<sup>-1</sup> Mn, 42.98 mg kg<sup>-1</sup> Zn, 37.22 mg kg<sup>-1</sup> Fe, 27.32 mg kg<sup>-1</sup> B, 7.57 mg kg<sup>-1</sup> Cu, and 0.68 mg kg<sup>-1</sup> Mo. The Ca/P ratio ranged from 0.62 to 1.26, the Na/K ratio from 3.83 to 8.13, and the K/(Ca+Mg) ratio from 0.02 to 0.04. According to the results, the rich mineral content of termiye, known in a limited area as a traditional food, has been revealed according to locations. Although the Deşdiğin location stands out, it has been determined that termiye has high macro and micro nutrient contents in all locations. The ratios of elements to each other in termiye grains in all locations are also in the appropriate range in terms of nutrition. It was understood that lupin plant can be grown and used as termiye in all locations.

**Key words:** *Lupinus albus* L., macronutrient, micronutrient, Ca/P ratio, Na/K ratio, K/Ca+Mg ratio

### Termiye'nin Elemental Karakterizasyonu: Farklı Lokasyonlarda Yetiştirilen Tatlandırılmış Lüpen Tohumlarının Mineral Bileşimi

### Öz

Sürdürülebilir tarım, yeni gıda kaynaklarının oluşturulması ve bölgesel kalkınma için, marjinal alanları girdi kullanmadan tarımsal üretime kazandırmak oldukça önemlidir. Bu çalışmada materyal olarak marjinal alanlarda tarımı yapılan lüpen (*Lupinus albus* L.) bitkisinin geleneksel yöntemle çerez haline getirilmiş ve termiye adıyla bilinen taneleri kullanılmıştır. Çalışmada termiyenin lokasyon bazlı mineral beslenme açısından değerinin ortaya konulması amaçlanmıştır. Termiyeler, lüpen yetiştiriciliğinin en fazla yapıldığı Konya'daki beş farklı lokasyonda (Deşdiğin, Konakkale, Ketenli, Başköy, Akçalar) üretim yapan yetiştiricilerden temin edilmiş ve her bir lokasyon için termiyelerdeki mineral madde kompozisyonları ICP-OES ile tespit edilmiştir. Lokasyon genel ortalamasına göre makroelement içerikleri; 4398 mg kg<sup>-1</sup> P, 3900 mg kg<sup>-1</sup> Ca, 2332 mg kg<sup>-1</sup> S, 846 mg kg<sup>-1</sup> Mg, 782 mg kg<sup>-1</sup> Na ve 135 mg kg<sup>-1</sup> K şeklinde elde edilmiştir. Mikroelement içerikleri de; 1228.23 mg kg<sup>-1</sup> Mn, 42.98 mg kg<sup>-1</sup> Zn, 37.22 mg kg<sup>-1</sup> Fe, 27.32 mg kg<sup>-1</sup> B, 7.57 mg kg<sup>-1</sup> Cu ve 0.68 mg kg<sup>-1</sup> Mo olarak tespit edilmiştir. Termiyelerdeki Ca/P oranı 0.62-1.26, Na/K oranı 3.83-8.13 ve K/(Ca+Mg) oranı 0.02-0.04 aralığında değerler almıştır. Sonuçlara göre geleneksel bir gıda olarak sınırlı bir alanda tanınan termiyenin zengin mineral içeriği lokasyonlara göre ortaya konulmuştur. Deşdiğin lokasyonu öne çıksa da tüm lokasyonlarda termiyenin makro ve mikro besin içeriklerinin yüksek olduğu tespit edilmiştir. Termiyelerin tüm lokasyonlarda elementlerin birbirlerine oranları da besleme



açısından uygun aralıkta yer almıştır. Tüm lokasyonlarda lüpen bitkisinin yetiştirilebileceği ve termiye olarak kullanılabileceği anlaşılmıştır. Termiye beslenmeye ve sürdürülebilir tarıma sağlayacağı katkılar göz önüne alındığında daha geniş alanlarda üretim yapılabilmesi için çalışmalara ihtiyaç olduğu ifade edilebilir.

**Anahtar kelimeler:** *Lupinus albus* L., makro besin elementleri, mikro besin elementleri, Ca/P oranı, Na/K oranı K/Ca+Mg oranı

## INTRODUCTION

Marginal agricultural lands are areas where modern agricultural techniques cannot be used due to limiting factors resulting from soil characteristics, geographical and/or topographical structure, and where agriculture is carried out using traditional methods (Demirel and Şenol, 2019). The inclusion of such areas in agricultural production and/or their more active use in production is of great importance for sustainable agriculture.

Another strategically important issue for countries is ensuring that basic nutritional needs are met at sufficient, reliable, and nutritious levels (Koç and Uzmay, 2015). Achieving food security requires protecting food resources, integrating alternative crops into production, and/or assessing their potential for utilization. Legumes, recognized as the primary source of plant-based protein worldwide, are considered indispensable for both food security and sustainable agricultural practices. The Food and Agriculture Organization of the United Nations (FAO) has stated that leguminous plants, including lupin, possess significant nutritional value due to their high protein, fiber, and mineral content, emphasizing the lupin plant's particular importance for its nutritional and functional properties within this group (Poteras et al., 2024).

Lupin (*Lupinus albus* L.), known in Türkiye by various names such as acı bakla, termiye, tirmis, delice bakla, and kurt baklası, is a legume that can be cultivated on marginal agricultural lands where other legumes cannot grown without the need for agricultural inputs (Yorgancılar et al., 2020; Elma et al., 2021). The name *Lupinus* is derived from the Latin word lupus (wolf). *L. albus* L. belongs to the family *Leguminosae* and is the most widely cultivated species of the genus *Lupinus*, which comprises more than 200 species and originates from the Mediterranean region (Erbaş et al., 2005; Yorgancılar et al., 2009b; Sezer et al., 2023). Lupin, which is also present in the natural flora of the Mediterranean Basin, is well adapted to acidic and sandy soils (Valente et al., 2024). It is cultivated across the Mediterranean, Australia, and Latin America, with approximately 18% of global production occurring in Europe (Caramona et al., 2024). However, its tolerance to high lime content and elevated soil pH levels is relatively low. For this reason, lupin cultivation in Türkiye has been primarily confined to the Lakes Region (Akşehir, Beyşehir, Eğirdir, and Doğanhisar), where the soil has lower lime content and pH values (Hakkı et al., 2007; Aydın and Yorgancılar, 2015). In terms of its botanical characteristics, *L. albus* L. exhibits all the soil-improving effects of other leguminous crops, including a deep root system, nitrogen fixation through *Rhizobium*, and an increase in soil organic matter (Lermi and Palta, 2018). With a symbiotic nitrogen fixation capacity ranging from approximately 50 to 200 kg ha<sup>-1</sup>, it serves as a sustainable alternative to industrial fertilizers (Caramona et al., 2024).

Lupin seeds contain two to three times more protein (33–47%) than cereals and are also rich in vitamins and minerals (Aydın and Yorgancılar, 2015; Yorgancılar et al., 2009a). They have higher iron, zinc, copper, and manganese content compared to other legumes, making them a valuable source of micronutrients (Trugo et al., 1993). The starch content of lupin seeds (5%) is significantly lower than that of other legumes (50%) (Duarte et al., 2022). In addition, the seeds contain omega-3 and omega-6 fatty acids, antioxidants, and a high fiber content. These properties make lupin a valuable crop for both human and animal nutrition (Yorgancılar et al., 2020; Sezer et al., 2023; Güloğlu, 2023). Lupin is widely used in Mediterranean countries, where it is processed into various food products (Yorgancılar et al., 2020). In different countries, lupin is used as a raw material alternative to soy in products such as bread, biscuits, cakes, pasta, confectionery, and soy sauce. It is also utilized as a source of high-quality vegetable oil rich in antioxidants, gluten-free flour, an emulsifier, a dairy alternative, and a snack (Mülayim and Acar, 2008; Yorgancılar and Bilgiçli, 2014; Özcan et al., 2021; Baltacıoğlu and Tarım Özcan, 2024). It is estimated that approximately 2 million tons of lupin seeds are produced annually in European countries, while around 500,000 tons of lupin-derived products are consumed. Additionally, the global lupin seed market is expected to grow by approximately 5% by 2029 (Caramona et al., 2024).

Minerals are essential for organisms to perform metabolic activities. In particular, inadequate intake of micronutrients leads to serious global health issues, commonly referred to as hidden hunger (Khazaei and Vandenberg, 2020; Nadeem, 2021). The Food and Agriculture Organization (FAO) has reported that more than 2 billion people worldwide suffer from severe health issues related to micronutrient deficiencies and has emphasized the risk of this number increasing. According to the World Health Organization (WHO), the most common micronutrient deficiencies worldwide are iron, iodine, and zinc (Anonymous, 2025a; Weffort and

Lamounier, 2024). For this reason, the WHO has long regarded the enrichment of agricultural products with protein, vitamins, calcium, magnesium, iron, zinc, copper, selenium, and iodine, or the consumption of foods containing these nutrients, as a priority issue (Orman and Ok, 2016). Similar to the global situation, widespread nutritional issues related to micronutrients have also been identified in Türkiye (Eyüpoğlu et al., 1995).

The aim of this study was to determine the mineral nutritional value of termiye, a traditionally known yet underrecognized food, and to introduce it to a wider audience. The material used consisted of seeds from the lupin plant (*L. albus* L.), which is cultivated in marginal areas of Türkiye and traditionally processed into a snack known as termiye. The study analyzed the mineral composition of termiye seeds from five different locations where production is most concentrated. The mineral composition across different locations, the distribution rates of minerals within the seeds, and the amounts of minerals consumed in the snack were interpreted using literature data.

## MATERIALS AND METHODS

### Materials

The seeds of the lupin (*L. albus* L.) plant, traditionally processed to remove alkaloids (debittered) and consumed as a regional snack known as termiye, were used as the material (Figure 1).



**Figure 1.** Appearance of termiye: whole seed (left), cotyledons and seed coat (testa) (right)

The region where lupin is most widely cultivated includes the rural neighborhoods of the Doğanhisar and Seydişehir districts in Konya province. Therefore, the study material was obtained from local producers in five different locations (Deşdiğin, Konakkale, Ketenli, Başköy, and Akçalar) who grow lupin and sell it as termiye in local markets (Table 1).

**Table 1.** Locations and coordinates of lupin cultivation areas

Province	District	Neighborhood	Coordinates
Konya	Doğanhisar (38.142371°N, 31.686279°E)	Deşdiğin	38.054245°N, 31.634553°E
		Konakkale	38.019212°N, 31.682440°E
		Başköy	38.140682°N, 31.757124°E
	Seydişehir (37.424495°N, 31.848346°E)	Akçalar	37.523744°N, 31.843805°E
		Ketenli	37.522652°N, 32.057311°E

\* The coordinate information was obtained from Google Maps

*L. albus* L. is an annual herbaceous legume with white flowers arranged in a raceme, podded fruits, and white seeds. The plant can reach a height of approximately 120 cm and has a taproot system (Bayram, 2023). Each raceme contains 3–7 flowers, from which 3–7 fruits develop. Each fruit contains 3–7 seeds (Balcioğlu and Orak, 2020).

Since low-alkaloid and regionally adapted sweet varieties of *L. albus* L. have not been developed in Türkiye, termiye, a snack consumed after a series of processing steps, is obtained from the mature, dry seeds of the lupin plant. In the traditional method known as debittering, lupin seeds are boiled in hot water (60–70°C) for 1–2 hours and then placed in sacks. They are then soaked in drinking water in special pools for 2–4 days, with the water being changed at least 4–5 times. In rural areas, debittering is typically carried out by placing the boiled seeds in sacks and soaking them in running water for 2–4 days. This process dissolves the bitter alkaloids in the water and removes them from the seeds. The result of this debittering process is termiye, a lupin snack product (Yorgancılar et al., 2009a; Baltacıoğlu and Tarım Özcan, 2024).

### Methods

The ready-to-eat fresh termiye was prepared in two different forms as the material: whole seeds and the inner part (cotyledons), which are consumed after the removal of the seed coat. Since termiye is generally preferred without the seed coat, both whole seed and cotyledon analyses were conducted to determine the overall nutritional value. The seed coat was manually removed, and the kernels were separated. The prepared

samples were dried in an oven (Sanyo) at 70°C. After drying, each material was separately ground into flour using a laboratory mill (Retsch ZM-100).

The whole seeds and cotyledons from each location were analyzed separately. To determine the mineral content, 0.2 g of each ground material was weighed and digested in a microwave system (MarsExpress, CEM Corp., USA) at high temperature (210°C) and pressure (200 PSI) using 5 mL of concentrated nitric acid (HNO<sub>3</sub>) and 2 mL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30% w/v). The digested samples were diluted to 25 mL with deionized water, filtered through filter paper (Whatman No. 42), and analyzed for mineral content using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Agilent 5110). The determined values were multiplied by the dilution factor (final volume/weight of the initial sample) to obtain the macro- and micronutrient contents in mg kg<sup>-1</sup>.

In interpreting the mineral contents of termiye from different locations, the percentage changes relative to the overall average for each analyzed element were used. For this purpose, the variations in the elemental contents of the seed parts compared to the average were calculated. The percentage change was determined using the following formulas:

% Change (whole seed): Location grain mineral value/location average grain mineral value)\*100 (Eq. 1).

% Change (cotyledone): Location cotyledone mineral value/location average cotyledone mineral value)\*100 (Eq. 2).

The percentage differences in the values of each sample compared to the average were calculated as follows:

% Difference: % change - 100 (Eq. 3). Here, a negative (–) value indicates a decrease, while a positive (+) value indicates an increase compared to the location average.

The analyses and measurements were conducted using a factorial experimental design in a randomized complete block design with three independent replications. Excel 2016 software was used to calculate the mean values and standard deviations for each measurement. The obtained results were analyzed using the MSTAT-C software package. A variance analysis was performed according to the factorial experimental design in a randomized complete block design, and differences between groups were determined using the least significant difference (LSD) test based on the results of the F-test.

## RESULTS AND DISCUSSION

The primary factor determining the elemental content of plant products is the plant's genetic potential; however, the expression of this potential is influenced by climatic and environmental conditions.

The traditional snack termiye from five different locations was analyzed in both its whole seed form with the shell and its edible cotyledon after shell removal. The mineral contents of all samples were determined on a dry weight basis.

### Macronutrients

Macronutrients are essential for an organism to properly perform its metabolic activities (Abdo et al., 2022). Calcium (Ca) is the most abundant structural mineral and plays a crucial role in metabolic processes. Potassium (K) is essential for cell membrane functions. Magnesium (Mg) acts as a cofactor in enzymatic reactions. Phosphorus (P) is vital for maintaining basic cellular functions. Sodium (Na) is necessary for normal cell function, water regulation, and electrolyte balance (Benayad and Aboussaleh, 2021). Sulfur (S) is a biologically important mineral due to its incorporation into various molecules such as amino acids, proteins, enzymes, and vitamins, as well as its role in physiological pathways (Hewlings and Kalman, 2019).

In this study, statistically significant differences were found among the amounts of macronutrients in termiye ( $p < 0.01$ ). Among the analyzed macronutrients, phosphorus (P), calcium (Ca), and sulfur (S) were present in higher amounts compared to the others. The macronutrients in termiye were ranked from highest to lowest as follows: P (4398 mg kg<sup>-1</sup>), Ca (3900 mg kg<sup>-1</sup>), S (2332 mg kg<sup>-1</sup>), Mg (846 mg kg<sup>-1</sup>), Na (782 mg kg<sup>-1</sup>), and K (135 mg kg<sup>-1</sup>) (Table 2). Yorgancılar et al. (2009a) determined the P content in dehulled termiye (cotyledone) as 5613 mg kg<sup>-1</sup>, K as 231 mg kg<sup>-1</sup>, Ca as 3793 mg kg<sup>-1</sup>, Mg as 817 mg kg<sup>-1</sup>, and Na as 665 mg kg<sup>-1</sup>. Abdo et al. (2022), in their analysis of termiye milk obtained from dehulled termiye, reported the macronutrient contents as 3325 mg P kg<sup>-1</sup>, 1875 mg Ca kg<sup>-1</sup>, 1630 mg K kg<sup>-1</sup>, and 1161 mg Mg kg<sup>-1</sup>. In another study on termiye, Balcioğlu and Orak (2020) determined the average P content as 0.24%, K content as 0.85%, Ca content as 0.23%, and Mg content as 0.13%. Panasiewicz (2022) reported that the average seed content of *L. albus*, when grown using traditional methods, contains 4.8 mg g<sup>-1</sup> P, 1.8 mg g<sup>-1</sup> Mg, 0.10 mg g<sup>-1</sup> Na, 11.98 mg g<sup>-1</sup> K, and 0.15 mg g<sup>-1</sup> Ca. In all these studies, researchers have emphasized that *L. albus* seeds contain high levels of macronutrients and serve as a nutrient-rich alternative food source. The results obtained in this study also confirm the presence of high macronutrient levels and support the findings in the literature.

The locations showed significant differences in elemental content ( $p < 0.01$ ). When the general mean values of macronutrient contents across the locations were ranked, it was found that Ketenli ( $2188 \text{ mg kg}^{-1}$ ), Konakkale ( $2160 \text{ mg kg}^{-1}$ ), and Deşdiğın ( $2126 \text{ mg kg}^{-1}$ ) had similar results. In terms of macronutrient content, Başköy ( $1963 \text{ mg kg}^{-1}$ ) had lower values, while Akçalar ( $1890 \text{ mg kg}^{-1}$ ) had the lowest macronutrient content (Table 2).

It is known that the mineral content of plant seeds varies depending on genotype, location, and agricultural practices (Panasiewicz, 2022). In a study conducted in Australia, it was determined that *L. albus* seeds obtained from 33 different locations exhibited variations based on location; however, all examined locations had seeds rich in P, K, Ca, Mg, S, and Na (Hung et al., 1998). Since the materials used in this study were sourced from the *L. albus* population in the region and traditional methods were applied in both cultivation and the subsequent debittering process, the differences in elemental content can be considered location-dependent. The fact that the differences between locations were statistically significant further supports this finding.

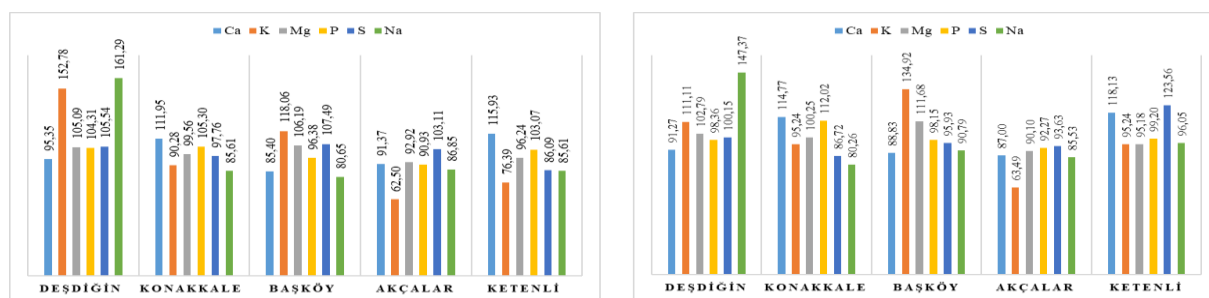
When the interaction between location, seed part, and element content was analyzed ( $p < 0.01$ ), Konakkale was identified as the location with the highest P content, with  $4254 \text{ mg kg}^{-1}$  in the whole seed and  $5330 \text{ mg kg}^{-1}$  in the cotyledon. In terms of Ca, Ketenli stood out in both the whole seed ( $5241 \text{ mg kg}^{-1}$ ) and the cotyledon ( $3874 \text{ mg kg}^{-1}$ ). Regarding S, Başköy had the highest content in the whole seed ( $2209 \text{ mg kg}^{-1}$ ), while Ketenli had the highest sulphur content in the cotyledon ( $3226 \text{ mg kg}^{-1}$ ) (Table 2).

**Table 2.** Macronutrient contents ( $\text{mg kg}^{-1}$ ) in termiye collected from different locations\*

Macronutrients (mg kg <sup>-1</sup> )														General av..
Location	Calcium (Ca)		Potassium (K)		Magnesium (Mg)		Phosphorus (P)		Sulfur (S)		Sodium (Na)			
	seed	cotyledone	seed	cotyledone	seed	cotyledone	seed	cotyledone	seed	cotyledone	seed	cotyledone		
Deşdiğin	4308±116,74	2988±46,98	220±65,34	143±90,52	950±8,79	809±16,94	4209±16,67	4685±96,39	2173±50,73	2606±38,85	1302±83,56	1116±54,60	2126	
Konakkale	5062±93,76	3765±24,98	131±8,34	116±2,63	900±9,62	795±8,47	4254±73,98	5330±65,03	2014±14,82	2257±14,74	688±45,18	608±21,02	2160	
Başköy	3861±84,19	2914±52,03	169±2,38	171±11,64	956±16,62	878±8,39	3893±50,48	4669±83,13	2209±34,51	2499±28,32	649±46,47	686±29,16	1963	
Akçalar	4129±134,80	2854±60,14	95±18,96	79±7,87	841±13,05	713±17,54	3671±34,82	4386±121,05	2121±4,43	2443±50,26	696±42,29	652±60,23	1890	
Ketenli	5241±27,49	3874±30,11	109±2,63	116±23,38	866±1,89	750±4,26	4157±78,70	4722±47,32	1773±9,32	3226±756,98	687±38,61	732±69,06	2188	
Location av.	4520	3279	145	125	903	789	4037	4758	2058	2606	804	759		
General av.	3900		135		846		4398		2332		782			

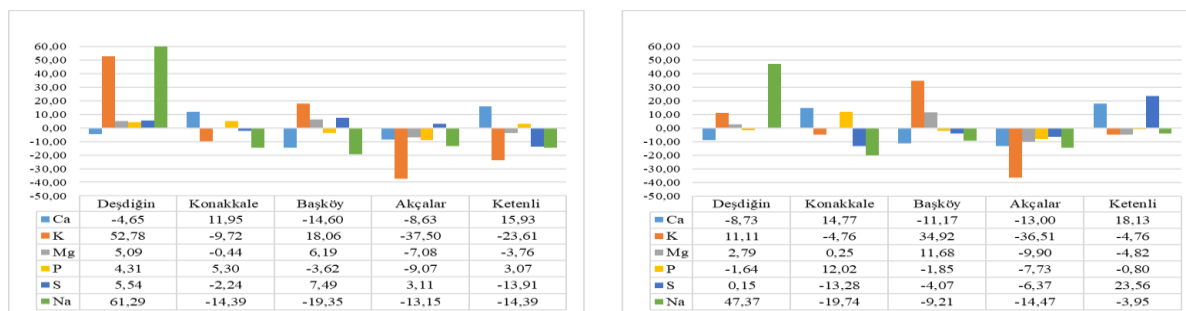
\* All values for whole seed and cotyledon were determined on a dry weight basis per kilogram.  $\pm$  represents the standard deviation of the replicate means.

For the other macronutrients, Deşdiğın had the highest K content in the whole seed ( $220 \text{ mg kg}^{-1}$ ), while Başköy had the highest K content in the cotyledon ( $171 \text{ mg kg}^{-1}$ ). For Mg, Başköy ranked first in both the whole seed ( $956 \text{ mg kg}^{-1}$ ) and the cotyledon ( $878 \text{ mg kg}^{-1}$ ). Similarly, Deşdiğın had the highest Na content in both the whole seed ( $1302 \text{ mg kg}^{-1}$ ) and the cotyledon ( $1116 \text{ mg kg}^{-1}$ ) (Table 2).



**Figure 2.** Percentage changes in macronutrient contents relative to the average (%) (Left: whole seed, Right: cotyledon)

When examining the variations in macronutrient contents relative to the location averages in the whole seed, it was determined that K and Na were higher in Deşdiğın; Ca and P in Konakkale; K and S in Başköy; S and Mg in Akçalar; and Ca and P in Ketenli. Among the variations observed in the consumed inner part (cotyledons), Na and P were prominent in Deşdiğın; Ca and P in Konakkale; K and Mg in Başköy; S and P in Akçalar; and Ca and S in Ketenli (Figure 2).



**Figure 3.** Percentage differences in macronutrient contents relative to the overall average (%) (Left: whole seed, Right: cotyledon)

According to the differences in variation relative to the location averages in the whole seed, decreases were observed Ca in Deşdiğin; P, Mg, S, and Na in Konakkale; Ca, P, and Na in Başköy; Ca, K, Mg, P, and Na in Akçalar; and K, Mg, S, and Na in Ketenli. In the cotyledons, decreases were detected in Ca and P in Deşdiğin; K, S, and Na in Konakkale; Ca, P, S, and Na in Başköy; Ca, K, Mg, P, S, and Na in Akçalar; and K, Mg, P, and Na in Ketenli (Figure 3).

The highest decrease was observed in K content in the Akçalar location, with a reduction of 37.50% in the whole seed and 36.51% in the cotyledon. Among the differences caused by an increase, the highest difference in the whole seed was recorded for Na in Deşdiğin, with a 61.29% increase. The highest difference in the cotyledon was observed for K in Başköy, with a 34.92% increase.

A study on *L. albus* and *L. angustifolius* seeds emphasized that most of the nutrients accumulate in the cotyledons and that *L. albus* seeds and processed foods derived from them could be a valuable source of nutrition due to their high protein and mineral content (Hung et al., 1998). Another study on termiye reported proportional differences in elemental content between the cotyledon and the seed coat, indicating that some elements are more concentrated in the seed coat, while others are more abundant in the cotyledon (Yorgancılar et al., 2009; Yorgancılar and Bilgiçli, 2014). Abdo et al. (2022) emphasized in their study that nutrient accumulation is higher in the seed endosperm. In this study, no statistically significant differences in mineral content were found between the different termiye parts. However, when evaluating the interaction between location, seed part, and element, statistically significant differences were observed.

Considering all the factors examined for macronutrients, including content, seed part, variation, and variation differences, Deşdiğin was identified as the most prominent location.

### Micronutrients

One of the most fundamental indicators of nutritional value is a product's mineral content. Plants are known to be a good source of essential minerals for humans. Therefore, a rich mineral composition is a highly valuable attribute in terms of food quality (Khazaei and Vandenberg, 2020). Micronutrients are essential for the formation and proper functioning of various metabolic processes (Abdo et al., 2022). Deficiency or toxic levels of these elements can significantly impact human and animal health, just as they do in plants (Aygün et al., 2018).

Iron (Fe) plays a key role in several metabolic processes, including oxygen transport, DNA synthesis, and electron transfer. Zinc (Zn) is essential for protein, lipid, and nucleic acid metabolism. Copper (Cu) acts as a fundamental catalyst in many metabolic reactions. Manganese (Mn) is involved in the synthesis and activation of various enzymes and in the regulation of glucose and lipid metabolism (Benayad and Aboussaleh, 2021). Boron (B) is involved in cellular transport mechanisms, bone regeneration, and the regulation of oxidative stress (Nielsen and Eckhart, 2020).

In this study, termiye was found to contain high levels of Mn, Zn, Fe, and B. Additionally, significant differences ( $p < 0.01$ ) were observed in the amounts of micronutrients among the analyzed termiye samples. Among the micronutrients, the highest value was recorded for Mn at  $1228.23 \text{ mg kg}^{-1}$ , followed by Zn at  $42.98 \text{ mg kg}^{-1}$ , Fe at  $37.22 \text{ mg kg}^{-1}$ , B at  $27.32 \text{ mg kg}^{-1}$ , Cu at  $7.57 \text{ mg kg}^{-1}$ , and molybdenum (Mo) at  $0.68 \text{ mg kg}^{-1}$ , in decreasing order (Table 3). Abdo et al. (2022) emphasized that termiye milk obtained from dehulled termiye contained high levels of Fe at  $17.14 \text{ mg kg}^{-1}$  and Zn at  $13.86 \text{ mg kg}^{-1}$ , highlighting that termiye is a mineral-rich material, especially in terms of Fe and Zn. In another study, termiye seeds were found to contain an average of  $53.15 \text{ mg kg}^{-1}$  Fe,  $4.16 \text{ mg kg}^{-1}$  Cu,  $31.30 \text{ mg kg}^{-1}$  Zn, and  $1094 \text{ mg kg}^{-1}$  Mn (Balçioğlu and Orak, 2020).

The general location averages for micronutrient contents ( $p < 0.01$ ) were as follows:  $272.17 \text{ mg kg}^{-1}$  in Deşdiğin,  $238.05 \text{ mg kg}^{-1}$  in Konakkale,  $211 \text{ mg kg}^{-1}$  in Ketenli,  $204.96 \text{ mg kg}^{-1}$  in Başköy, and  $193.63 \text{ mg kg}^{-1}$  in Akçalar. Based on micronutrient contents, Başköy had lower values compared to other locations, while Akçalar



was identified as the location with the weakest micronutrient content (Table 3). Naadem (2021) stated that variations in mineral content can occur depending on location, which may be attributed to genotypic differences and/or environmental changes associated with location. A study reported that the environment has a strong influence on most elements, and this interaction becomes more pronounced for certain elements depending on genotypic characteristics (Khazaei and Vandenberg, 2020).

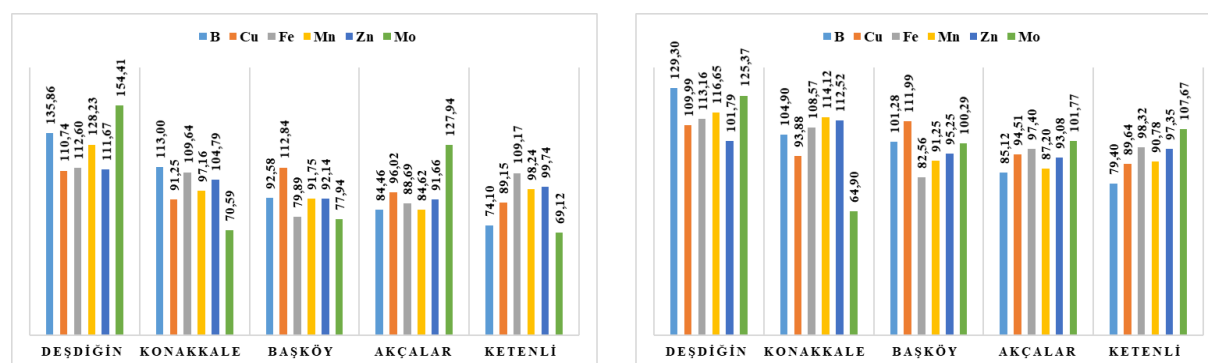
The Mn content varied between 978.77 mg kg<sup>-1</sup> and 1516 mg kg<sup>-1</sup> (p < 0.01). The highest Mn value was obtained from the cotyledon of Deşdiğın (1516 mg kg<sup>-1</sup>), while the lowest value was recorded in the whole seed of Akçalar (978.77 mg kg<sup>-1</sup>). The Zn content ranged from 36.50 mg kg<sup>-1</sup> to 51.91 mg kg<sup>-1</sup>. The highest Zn content was obtained from the cotyledon in Konakkale (51.91 mg kg<sup>-1</sup>), while the whole seed from Deşdiğın (44.47 mg kg<sup>-1</sup>) had higher values compared to other locations. Fe content varied between 27.23 mg kg<sup>-1</sup> and 45.67 mg kg<sup>-1</sup>. In terms of Fe content, Deşdiğın stood out with the highest values in both the whole seed (45.67 mg kg<sup>-1</sup>) and the cotyledon (38.38 mg kg<sup>-1</sup>) compared to other locations. B content ranged from 20.40 mg kg<sup>-1</sup> to 39.33 mg kg<sup>-1</sup>. Deşdiğın had the highest B content among all locations, with 39.33 mg kg<sup>-1</sup> in the whole seed and 33.22 mg kg<sup>-1</sup> in the cotyledon (Table 3).

**Table 3.** Microelement contents (mg kg<sup>-1</sup>) in termiye collected from different locations\*

Micronutrients (mg kg <sup>-1</sup> )*														General av.
Location	Boron (B)		Copper (Cu)		Iron (Fe)		Manganese (Mn)		Zinc (Zn)		Molybdenum (Mo)			
	seed	cotyledone	seed	cotyledone	seed	cotyledone	seed	cotyledone	seed	cotyledone	seed	cotyledone		
Deşdiğin	39.33±3.05	33.22±2.22	7.90±0.34	8.81±0.17	38.38±2.23	45.67±0.58	1483.12±4.32	1516.31±27.96	44.47±1.31	46.96±0.87	1.05±0.14	0.85±0.03	272.17	
Konakkale	32.71±1.10	26.95±0.90	6.51±0.10	7.52±0.09	37.37±1.14	43.82±0.69	1123.76±1.59	1483.34±15.15	41.73±0.12	51.91±0.39	0.48±0.11	0.44±0.06	238.05	
Başköy	26.80±0.28	26.02±1.64	8.05±0.14	8.97±0.19	27.23±0.47	33.32±1.05	1061.15±24.06	1186.08±27.96	36.69±0.47	43.94±1.24	0.53±0.06	0.68±0.12	204.96	
Akçalar	24.45±0.58	21.87±1.57	6.85±0.04	7.57±0.11	30.23±0.53	39.31±0.30	978.77±8.73	1133.45±32.10	36.50±0.47	42.94±1.08	0.87±0.05	0.69±0.04	193.63	
Ketenli	21.45±0.31	20.40±1.02	6.36±0.09	7.18±0.07	37.21±1.61	39.68±1.04	1136.26±16.47	1180.05±7.74	39.72±0.79	44.91±1.36	0.47±0.14	0.73±0.61	211	
Location av.	28.95	25.69	7.13	8.01	34.08	40.36	1156.61	1299.85	39.82	46.13	0.68	0.68		
General av.	27.32		7.57		37.22		1228.23		42.98		0.68			

\* All values for whole seed and cotyledon were determined on a dry weight basis per kilogram. ± represents the standard deviation of the replicate means.

When examining the variations in micronutrient contents, in the whole seed, Deşdiğın showed greater changes in Mo and B; Konakkale in B and Fe; Başköy in Cu and B; Akçalar in Mo and Cu; and Ketenli in Fe and Zn compared to the location averages. In the consumed inner part (cotyledons), the most pronounced changes were observed in B and Mo in Deşdiğın; Mn and Zn in Konakkale; Cu and Mo in Başköy; Mo and Fe in Akçalar; and Mo and Fe in Ketenli (Figure 4).

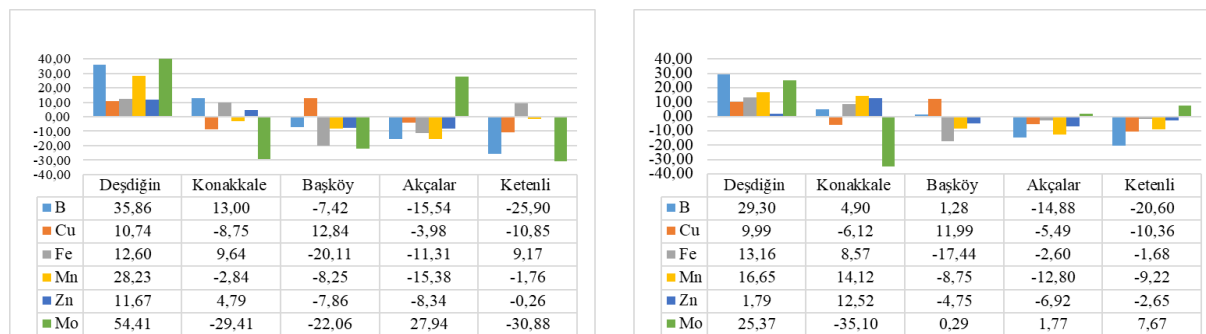


**Figure 4.** Percentage changes in micronutrient contents relative to the average (%) (Left: whole seed, right: Cotyledon)

According to the differences in variation relative to the location averages in the whole seed, a decrease was observed in Mo, Cu, and Mn in Konakkale; Mo, Fe, Mn, Zn, and B in Başköy; B, Mn, Fe, Zn, and Cu in Akçalar; and Mo, B, Cu, Mn, and Zn in Ketenli. In the cotyledon, a decrease compared to the average was observed for Mo and Cu in Konakkale; Fe and Zn in Başköy; B, Mn, Zn, Cu, and Fe in Akçalar; and B, Cu, Mn, Zn, and Fe in Ketenli (Figure 5). The highest decrease in B content was observed in Ketenli for the whole seed (30.88%) and in Konakkale for the cotyledon (35.10%). When evaluating the differences due to an increase, all the values in



Deşdiğin showed an upward trend. The highest increase in the whole seed was recorded for Mo in Deşdiğin at 54.41%, while the highest increase in the cotyledon was also recorded in Deşdiğin for B at 29.30%.



**Figure 5.** Differences in micronutrient contents relative to the general average (%) (Left: whole seed, right: Cotyledon)

Bhardwaj et al. (1998) conducted a study to determine the environmental effects on the seed composition of *L. albus* and found significant variation between states. They emphasized that these variations could affect the nutritional value of the product and should be taken into account. In this study, significant differences were observed between the locations, with Deşdiğin standing out. In this respect, the results obtained align with the literature. Duarte et al. (2022) stated that *L. albus* is rich in minerals and that the bioavailability of these minerals is considerably high. Hung et al. (1998) determined that *L. albus* seeds contain micronutrients such as Fe, Zn, Cu, Mo, B, and Mn, making them a highly nutritious material for both human and animal nutrition. In this study, termiye was found to be rich in micronutrients at all locations, similar to its macroelement composition, with Deşdiğin standing out more than the others. Yorgancılar et al. (2009a) also showed that termiye from Deşdiğin has a high micronutrient content.

Another notable aspect of this study is the exceptionally high manganese (Mn) content in Termiye. Mn was found in remarkably high amounts in all locations and in both parts of the termiye. The Mn content was determined to be 1156.61 mg kg<sup>-1</sup> in the whole seed and 1299.85 mg kg<sup>-1</sup> in the cotyledon. This result has been reported by several researchers and serves as evidence that *L. albus* absorbs Mn from the soil at a high rate (Trugo et al., 1993; Yorgancılar et al., 2009a; Hung et al., 1998). Considering that these values were determined on a dry weight basis and that termiye is transformed into a snack containing approximately 70% water (Yorgancılar et al., 2009a), along with the fact that the seed coat is removed before consumption, it is assumed that the high Mn content does not pose a nutritional concern. In some Latin American countries, lupin flour has been added to foods in specific proportions and has been reported to cause no adverse effects in children or adults. However, since unprocessed dry seeds are used in animal feed, it is emphasized that caution should be exercised regarding Mn intake during consumption (Trugo et al., 1993; Hung et al., 1998).

#### Ratios of Selected Minerals in Termiye

The mineral richness of a food is an important criterion for both human and animal nutrition. Another factor determining food quality is the ratio of these minerals to each other.

Particularly in animal nutrition, these ratios are carefully considered in addition to the nutrient content of the feed to maintain animal health. Knowing the concentration of nutrients in feed and their ratios to each other is crucial for animal health (Yılmaz, 2022). In high-quality feed, the reported nutrient contents are as follows: calcium 0.27–0.50%, phosphorus 0.15–0.27%, potassium 0.30–0.80%, magnesium 0.10–0.20%, sodium 0.16–0.22%, iron 4–15%, copper 4–5%, zinc 20–40%, and manganese 7–10% (Aydın et al., 1997; Doğrusöz et al., 2020).

The ratios of the elements to each other were determined using the values from Table 2 and Table 3, and the obtained values are presented in Table 4.

**Table 4.** Ratios of selected essential mineral nutrients to each other

Location	Elemental Ratios											
	Ca/P		Na/K		Ca/Mg		K/Ca+Mg		Fe/Zn		Zn/Cu	
	seed	cotyledone	seed	cotyledone	seed	cotyledone	seed	cotyledone	seed	cotyledone	seed	cotyledone
Deşdiğin	1.02	0.64	5.91	8.00	4.54	3.69	0.04	0.04	0.86	1.44	5.63	4.55
Konakkale	0.99	0.62	3.82	4.06	4.02	3.31	0.04	0.04	0.74	0.48	4.56	6.50
Başköy	1.13	0.65	7.78	8.13	4.92	4.01	0.02	0.02	0.83	0.35	5.33	7.88
Akçalar	1.19	0.71	5.31	5.08	5.62	4.76	0.02	0.03	0.90	0.76	6.41	7.05
Ketenli	1.26	0.82	6.27	6.08	6.02	5.16	0.02	0.03	0.94	0.72	6.25	6.43
General av.	1.14	0.70	5.64	5.95	5.16	4.30	0.03	0.03	0.87	0.54	5.75	7.15

In termiye, the average Ca/P ratio across locations was determined as 1.14 in the whole seed and 0.70 in the cotyledon. The Ca/P values varied within a range of 0.62–1.26. The lowest ratio was found in the cotyledon of Konakkale, while the highest value was observed in the whole seed of Ketenli. It has been stated that when Ca or P levels are insufficient, the nutritional value of foods decreases. For healthy physiological function, the Ca/P ratio should be between 2:1 and 1:2 (Işıl and Balkan, 2022). Additionally, it has been emphasized that the calcium-to-phosphorus (Ca/P) ratio plays a crucial role in ruminant body metabolism and rumen microbial health and that this ratio should be either 2:1 or 1:1 (Aygün et al., 2018). It has been reported that animals fed on diets with a Ca/P ratio exceeding these values are at risk of parturient paresis (milk fever) (Gülümser et al., 2020). As shown in Table 4, the ratios at all locations fall within the ranges reported in the literature. A ratio closer to 1 indicates a more balanced proportion of the two elements.

In termiye, the average Na/K ratio among the locations was found to be 5.64 in the whole seed and 5.95 in the cotyledon. Among the locations, the lowest Na/K ratio was found in the whole seed from Konakkale, while the highest ratio was observed in the cotyledon from Başköy. The values obtained varied within a range of 3.83–8.13. The balance between K and Na is known to be crucial. It has been stated that high K content can lead to electrolyte imbalance in animals and, due to its antagonistic effect, may cause Na deficiency, thereby increasing the risk of grass tetany (Yılmaz, 2022). A similar interaction has also been emphasized for the K+Na / Ca+Mg ratio (Aygün et al., 2018). It has been reported that the K/Na ratio in feeds should be, on average, 5:1, with a maximum of 10:1 (Aydın et al., 1997). The values obtained from the locations fall within the specified range.

Based on the overall averages of the locations, it was determined that the K/(Ca+Mg) ratio in termiye, both in the whole seed and the consumed cotyledon, remained within the recommended limits at all locations (Table 4).

Tetany is a metabolic disorder observed in ruminants, caused by magnesium deficiency in the blood, which results from an imbalance in the mineral composition of feeds (Yılmaz, 2022). The risk of grass tetany increases with a higher K/(Ca+Mg) ratio in the feed. To eliminate the risk of this disease, it is recommended that the K/(Ca+Mg) ratio be below 2.2 (Çaçan and Kökten, 2024). The values obtained from different locations and seed parts were found to be quite close to this threshold.

In termiye, the average Fe/Zn ratio across locations was calculated as 0.87 in the whole seed and 0.57 in the cotyledon. The Fe/Zn ratios obtained from different locations ranged between 0.35 and 1.44. The lowest ratio was recorded in the cotyledon from Başköy, while the highest value was found in the cotyledon from Deşdiğin.

For the Zn/Cu ratio, the average across all locations was 5.75 in the whole seed and 7.15 in the cotyledon. The lowest Zn/Cu ratio was found in the cotyledon from Deşdiğin, whereas the highest value was recorded in the cotyledon from Başköy (Table 4).

There is an antagonistic relationship between Fe, Zn, and Cu. Due to their similar physicochemical structures and shared transport systems, these minerals compete for absorption, resulting in reduced bioavailability (Baydaş et al., 1990; Doguer et al., 2019; İnce and Çağındı, 2020). Therefore, in addition to their presence, the proportional relationship between these minerals is also important (Watabe et al., 1965; Kebede et al., 2021). In termiye, the ratios of mineral elements to each other were found to be within reasonable limits.

Humans can obtain essential minerals from both plant-based and animal-based food sources. Due to the synergistic and antagonistic interactions among minerals, the region where food sources are obtained and the growing conditions significantly influence the mineral composition of food (Njira and Nabwami, 2015). Additionally, dietary habits, geographical and economic accessibility, and daily mineral intake through diet affect both the amount of minerals consumed and their relative ratios (Quintaes et al., 2015). Furthermore, factors such as age, gender, metabolic needs, and other physiological conditions significantly influence mineral intake (Anonymous, 2025b).

Due to these reasons, studies evaluating the ratios of minerals obtained from food in humans are quite limited and are usually conducted to examine specific conditions. For instance, a study in the United States on infant formulas reported that the Ca/P ratio in breast milk is approximately 2:1. Based on this, it was recommended that the ratio in supplementary foods produced for infants should be within the 1:1 to 2:1 range (Loughrill et al., 2017).

A study on vitamin D metabolism stated that an adequate Ca/P ratio of approximately 2:1 in the diet is essential for optimal bone development, and this value is close to that found in breast milk (Schneider, 1930). In a study evaluating the relationship between magnesium metabolism and the Ca:P range, Pinotti et al. (2021) recommended an optimal dietary Ca/Mg ratio of 2:1 or approximately 3:1 for both animals and humans.

According to El-Said and El-Sikaily (2013), hypertension (high blood pressure) in individuals can be managed through the K/Na balance in the diet. They emphasized that K content should be higher than Na to effectively regulate blood pressure. Minerals perform similar functions in human and animal metabolism.

Based on reference values and scientific data, it can be confidently stated that the elemental ratios in termiye (*L. albus*) are suitable for human consumption. However, as pointed out by Zang et al. (2021), determining the ideal mineral ratio for a healthy human population is quite challenging. Therefore, most studies focus on analyzing the mineral content of foods and the extent to which they contribute to daily nutritional requirements.

### Nutritional Value of Termiye Snack

Foods are the primary source of minerals, and their mineral richness is highly valuable in terms of nutrition (Abdo et al., 2022). The daily recommended or adequate intake of minerals through food may vary depending on several factors, such as health status, age, gender, and physical activity level (Benayad and Aboussaleh, 2021). For macronutrients, the average daily intake amounts for an adult individual are determined as follows: 1000 mg for Ca, 4.7 g for K, 700 mg for P, approximately 370 mg for Mg, and 1.5 g for Na (Anonymous, 2025c; Benayad and Aboussaleh, 2021).

Daily nutrient intakes, calculated while considering the influence of various factors, are defined as average intakes that can meet the nutrient requirements of a healthy individual. However, due to physiological differences, the requirements for microelements differ between adult men and women. For a healthy adult woman, the recommended daily intakes are 18 mg for Fe, 8 mg for Zn, 1.8 mg for Mn, 900 µg for Cu, and 55 µg for selenium (Se). In contrast, for an adult man, the corresponding recommended intakes are 8 mg for Fe, 11 mg for Zn, 2.3 mg for Mn, 900 µg for Cu, and 55 µg for Se (Benayad and Aboussaleh, 2021).

In the lupin plant, the seed coat (testa) constitutes approximately 25% of the seed weight and is reported to contain a high amount of dietary fiber, along with low levels of protein, lipids, minerals, and phytochemicals such as polyphenols (Malekipoor et al., 2022). In one study, the seed coat was found to constitute approximately 20% of the seed (Yorgancılar et al., 2009a).

Elements accumulate or integrate into the seed coat and cotyledons at different rates. While genotype influences this distribution, environmental factors can also play a role. Due to the difficulty of breaking down its high-fiber structure, consumers prefer to remove the seed coat when consuming termiye. This preference leads to differences in mineral composition between the whole seed and the cotyledons, which may result in some element loss.

When lupin seeds are processed into termiye, they absorb water. With a moisture content of approximately 70% in the processed form, Yorgancılar et al. (2009a) found that about 400 g of termiye must be consumed to reach the mineral content calculated on a dry weight basis. It is estimated that consuming the inner part remaining after removing the seed coat can provide approximately 22–60% of the daily mineral requirement.

Similar nutritional results were obtained from all locations where termiye was sourced. In this respect, products grown in all locations can be safely consumed. The high dietary fiber content, low glycemic index, and rich mineral composition make termiye a suitable option for consumption as a healthy snack.

### CONCLUSION

Plants are essential sources of minerals. The mineral content of food is a crucial criterion for both human and animal nutrition. Particularly in developing countries, legumes are frequently preferred in nutritional programs due to their affordability as a protein source. Additionally, they hold significant nutritional importance as they are especially rich in essential micronutrients.

Lupin (*L. albus* L.) is a leguminous plant that can be cultivated in marginal areas with minimal input use, without causing adverse environmental effects. Due to its high protein content and balanced ratios of macro- and micronutrients in its seed structure, it is not only a valuable food or food ingredient for human consumption

but also an alternative feed source for animal nutrition. Lupin has significant potential for combating mineral deficiencies, aligning with one of the United Nations' 2030 Sustainable Development Goals.

Termiye, traditionally consumed as a snack, is obtained through a series of processes from the seeds of the lupin plant. In this study, termiye was found to have a rich content of macro- and micronutrients across all locations. Considering that health issues related to microelement deficiencies affect almost everyone today, incorporating termiye into diets as a mineral source can be recommended.

In terms of the examined parameters, the Deşdiğin location stands out. Based on the results obtained, Deşdiğin can be recommended as the primary location for cultivation. The Başköy and Ketenli locations recorded lower values for most parameters. However, since termiye obtained from these locations still has a rich mineral content compared to other legumes, its cultivation can be effectively carried out in Başköy and Ketenli, which are classified as mountain villages. This would provide a significant advantage for both rural development in the region and the utilization of agricultural land. In locations where lupin cultivation is practiced, there is potential for obtaining organic and high value-added products from termiye.

Establishing cooperatives or regional brands can be recommended. Since soil pH is the primary limiting factor for lupin cultivation, further research is needed on breeding lime-tolerant genotypes and developing sweet varieties with reduced alkaloid content.

### Declaration of Interests

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

### Author Contributions

**Emine Atalay:** Investigation; methodology; project administration; software; writing— original draft; writing— review and editing).

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## Investigation Effects of Some Plant Hormones and Vitamins on Growth in Kanamycin-Resistant Tomato Varieties

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### ABSTRACT

Tomato is a widely consumed vegetable around the world and has a significant economic impact on agricultural production. However, various challenges in current agricultural practices can affect tomato production. To overcome these challenges and create a sustainable agricultural model, research focusing on tomato plant tissue culture is gaining importance. In this study, the neomycin phosphotransferase (NPTII) reporter gene was transferred to the *Lycopersicon esculentum* cLN1558A line through *A. tumefaciens* LBA4404. The effects of phenolic compounds and growth regulators used in tomato tissue culture were investigated. A balanced concentration of 1 mg ml<sup>-1</sup> 6-benzyladenine (BA) and 1 mg ml<sup>-1</sup> gibberellic acid (GA<sub>3</sub>) was used for optimal shoot regeneration. For optimal root regeneration, 1 mg ml<sup>-1</sup> indole-3-acetic acid (IAA) concentration was used. 200 µM acetosyringone was added to the culture medium to accelerate *A. tumefaciens* infection. As a result, it was confirmed that kanamycin resistance reached 50% in transgenic tomato shoots containing the NPTII gene. The efficiency of the phenolic compounds and growth regulators used increased by 12%.

**Key words:** Gibberellic acid, 6-benzyladenine, NPTII, tomato tissue culture

### Kanamisine Dayanıklı Domates Çeşitlerinde Bazı Bitki Hormonları ve Vitaminlerinin Büyümeye Etkisi

#### Öz

Domates dünya çapında yaygın olarak tüketilen bir sebzedir ve tarımsal üretim üzerinde önemli bir ekonomik etkiye sahiptir. Ancak mevcut tarımsal uygulamalardaki çeşitli zorluklar domates üretimini etkileyebilmektedir. Bu zorlukların üstesinden gelmek ve sürdürülebilir bir tarım modeli oluşturmak için domates bitkisi doku kültürüne odaklanan araştırmalar önem kazanmaktadır. Bu çalışmada neomisin fosfotransferaz (NPTII) raportör geni, *A. tumefaciens* LBA4404 aracılığıyla *Lycopersicon esculentum* cLN1558A hattına aktarıldı. Domates doku kültüründe kullanılan fenolik bileşiklerin ve büyüme düzenleyicilerin etkileri araştırıldı. Optimum sürgün rejenerasyonu için dengeli bir 1 mg ml<sup>-1</sup> 6-benziladenin (BA) ve 1 mg ml<sup>-1</sup> gibberellik asit (GA<sub>3</sub>) konsantrasyonu kullanıldı. Optimum kök rejenerasyonu için 1 mg ml<sup>-1</sup> indol-3-asetik asit (IAA) konsantrasyonu kullanıldı. *A. tumefaciens* enfeksiyonunu hızlandırmak için kültür ortamına 200 µM asetosiringon eklendi. Sonuç olarak NPTII genini içeren transgenik domates sürgünlerinde kanamisin direncinin %50'ye ulaştığı doğrulandı. Kullanılan fenolik bileşiklerin ve büyüme düzenleyicilerin etkinliği %12 artmıştır.

**Anahtar kelimeler:** Gibberellik asit, 6-benzyladenine, NPTII, domates doku kültürü

### INTRODUCTION

Transgenic crops are bred for a variety of purposes: to be resistant to fungal, bacterial, viral diseases and insect pests, to tolerate herbicides and to grow better under restricting environmental conditions such as drought, high salt or metal containing soils. Increasingly, the improvement of quality traits such as nutritional content and suitability for industrial processing is also of importance. Essentially, conventional breeding has very

similar goals such as to develop plant varieties with higher yield and/or better nutritional qualities, disease and pest resistance and lower cultivation costs. Agrobacterium-mediated tomato transformation has developed rapidly over the last 50 years. Many factors such as plant diversity, plant material used, growth regulators, and bacterial species can change the effect of transformation (Ellul et al., 2003; Ohki, Bigot, & Mousseau, 1978; Shahin, Sukhapinda, Simpson, & Spivey, 1986).

Tomato (*Solanum lycopersicum* L.) is a perennial plant in tropical regions, containing lycopene and various minerals and vitamins, while it is an annual cultivated plant in other regions (Gaffaroğlu, Horuz, & Aysan, 2019). Some phenolic molecules secreted by plant cells accelerate Agrobacterium transformation by inducing the expression of vir genes (Bolton, Nester, & Gordon, 1986; Stachel, Nester, & Zambryski, 1986). In some plants that cannot produce these phenolic molecules, the use of acetosyringone increases the transformation efficiency by inducing genes (Sheikholeslam & Weeks, 1987). In addition, different phenolic compounds obtained from plants such as tobacco can be used as transformation inducers (Hamza & Chupeau, 1993; Kaya, Al-Remi, Arvas, & Durmuş, 2018). Indole-acetic acid (IAA), used in plant tissue culture, is important for many plants, especially tomato plants. Strong callus size and organogenesis occur in the IAA plant (Magnus, Hangarter, & Good, 1992; Ohki et al., 1978; Pfitzner, 1998).  $\alpha$ -naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA) used in tomato tissue culture enable shoot formation in the hypocotyl segments of the plant (Yakuwa T, 1973). Additionally, application of some humic substances (Bio Humate, Justyne) to tomato seedlings increases the vegetation in the plant (Kizildeniz, Vacelik, & Bettoni, 2022). It has been revealed that the combined use of these hormones in plant tissue culture studies provides benefits such as shoot differentiation and shoot formation from hypocotyl and leaf segments (Ohki et al., 1978). Additionally, some phenolic compounds secreted by active plant may facilitate T-DNA transfer by increasing the vir gene expression of *A. tumefaciens* (Janssens, Genetello, Van Montagu, & Zambryski, 1986). A study revealed that the use of BA alone or in combination with 0.75 mg/L PBZ induced in vitro flowering in tomatoes (Dewi, Prasetyo, Sukweenadhi, Irawati, & Savitri, 2022). However, the combination of IAA and cytokinins BA, 6(γγ-dimethyl-allylamino)-purine (IPA) and kinetin on shoot differentiation medium gives the best results in shoot formation from the hypocotyl and leaf segments of IPA (Ohki et al., 1978). The use of different plant bodies may produce different effects in transformation. From past to present, different plant parts, including cotyledons, hypocotyls, and stem parts, have been used in tissue culture-mediated transformation (Chyi & Phillips, 1987; Pfitzner, 1998).

This study aims to reveal the effect of 6-benzyladenine (BA) and gibberellic acid (GA3) concentration and acetosyringone induction on explant and shoot regeneration and to modify the tomato-specific protocol with Agrobacterium transformation.

## MATERIALS AND METHOD

### Plant Regeneration

Commercial *Lycopersicon esculentum* cv. seeds were sterilized in 70% ethanol for 3 min and in 30% chlorax for 7 min. Seeds were placed in Murashi-Skoog (MS) medium, 5 in each petri dish, and germinated at 8/16 photoperiod. Cotyledons were cut from tomato seedlings and their ends were cut transversely into two pieces. Cotyledons were placed in petri dishes (90x15 mm) containing preculture medium and incubated in the dark at 25 °C, 70% humidity for 2 days (Table 1).

### Construction of Vector

NPTII gene were amplified by polymerase chain reaction (PCR) using the full-length NPTII genomic clones. The constructs of pBI121-NPTII amplified in the plasmid pBI121 as a template. PCR was performed in a 50 µl reaction solution containing 0.3 µg template DNA (pBI121-NPTII), 20 nM of each primer, 0.05 mM dNTPs, 0.125 µl LA Taq (Takara, Japan), 2.5 mM MgCl<sub>2</sub>, 10X LA PCR buffer (Mg +2 free).

All of the ligation products were transformed into *Escherichia coli* DH5α using the heat-shock method. One hundred µl of the component *E. coli* cell was added to the ligation product (20 µl), put on ice for 30 minutes and followed by heat shock in the water bath at 42 °C for 1 minute, and immediately put on ice for 3 minutes and 1 ml of LB Broth was added. After 1 hr incubation at 37 °C, it was centrifuged at 10000 rpm for 3 minutes and the supernatant was discarded. The pellet was cultivated in LA medium containing 50 µg/ml spectinomycin and incubated at 37 °C. *E. coli* containing the target plasmid were confirmed by colony PCR. Individual colonies were used for PCR. The pBI121 plasmids DNA of the transformants were isolated from *E. coli* using by plasmid miniprep purification kit (Macherey Nagel., Düren, Germany). Briefly, 4 ml of *E. coli* culture were centrifuged 12000 rpm for 1 minute and the supernatant was removed before solution I (200 µl) was added to remove the RNAs. Solution II (200 µl) was added to wash the proteins. Solution III (200 µl) was added to wash off the other ingredients before centrifugation at 12000 rpm for 5 minutes. The supernatant was transferred to the new spin

column and centrifuged at 12000 rpm for 1 minute, and the lower tube was removed and centrifuged for 1 minute at 12000 rpm by adding 700 µl of wash solution. The column was attached to the new tube and placed in the oven for 3 minutes and 100 µl of dH<sub>2</sub>O was added and again put in the oven for 10 minutes and centrifuged at 12000 rpm for 1 minute. The accuracy of the plasmid isolated from *E. coli* was confirmed by gel electrophoresis. The isolated plasmid was stored at -20 °C by discarding the column.

The pBI121 plasmid including target NPTII was transformed into *Agrobacterium tumefaciens* LBA4404 using by a GenePulser II electroporator (Bio-Rad, California, USA). Briefly, *A. tumefaciens* LBA4404 was cultured in LB medium for 2 days at 28 °C and single colony was selected and transferred to 5 ml of LB broth and left overnight. Then 100 µl of the culture was subcultured and incubated at 28 °C for 6-7 hours and then left on ice for 15 minutes. It was centrifuged at 5000 rpm for 20 minutes and the pellet was washed with 10 ml of 1mM HEPES (4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid) and the same solution was thawed with 10 ml of HEPES and centrifuged at 5000 rpm for 15 minutes and the supernatant discarded. The pellet was dissolved in 750 µl 10% glycerol and an aliquot was pipetted into 45 µl tubes and stored at -80 °C. Forty-five µl of the component cell was added to the agrobacterium culture, 1 µl of the isolated plasmid was transferred to the electroporation cuvette and electroporated, and then 1 ml of SOC was added to the whole solution and transferred to the new Eppendorf tube and spread to the LA medium containing streptomycin (50 µg) and spectinomycin (50 µg) antibiotics and cultured at 28 °C. The accuracy of agrobacterium was confirmed by colony PCR.

### Plant Transformation

*A. tumefaciens* LBA4404 harboring pBI121 carrying the NPTII gene was grown in LB medium and OD600 was maintained at 0.8. Tomato cotyledons were kept in *A. tumefaciens* bacterial suspension for 30 min. Cotyledon explants were placed in equal numbers in culture medium PL<sub>a</sub>; containing only MS and Agar, PL<sub>b</sub>; containing 200 µM acetosyringone, in PL<sub>c</sub>; containing BA, AS, and PL<sub>d</sub>; containing GA<sub>3</sub>, AS kept at 25 °C for 2 days (Table 1). After incubation in the growth chamber, dried disks of sterile filter paper were placed on them and cotyledon explants were then placed on the filter paper. After 2 days, cotyledons were transferred to co-culture containing the same vitamins and incubated in a photoperiod of 8/16 hours. The plants, which reached a height of 2-3 cm after 3 weeks of incubation, were transferred to the rooting medium and rooted.

**Table 1.** Composition of culture media

	PL <sup>a</sup>	PL <sup>b</sup>	PL <sup>c</sup>	PL <sup>d</sup>
MS <sup>a</sup>	+	+	+	+
Sucrose (g L <sup>-1</sup> )	30	30	30	30
Agar (g L <sup>-1</sup> )	8	8	8	8
Acetosyringone (mM)		200	200	200
BA (mg L <sup>-1</sup> )			1	
GA <sub>3</sub> (mg L <sup>-1</sup> )				1
IAA (mg L <sup>-1</sup> )			0.5	0.5
Kanamycin (mg L <sup>-1</sup> )	100	100	100	100

MS<sup>a</sup>: Murisime and Skoog (1962), GA<sub>3</sub>: Giberellic acid, BA: 6-benzylaminopurine, IAA: Indole-asetic acid, PL<sup>a</sup>: Pre-culture medium, PL<sup>b</sup>: Co-culture medium, PL<sup>c</sup>: Regeneration medium, PL<sup>d</sup>: Rooting medium.

### Genetic Analysis

Expression of the NPTII gene was detected in T1 tomato plants obtained from T0 plant. After the obtained seeds were sterilized and germinated in PLA media. It was transferred to PLb media containing 100 mg l<sup>-1</sup> kanamycin. Rooted plants were scored for kanamycin resistance after 3-4 weeks of culture. Wild-type tomato leaves lacking the NPTII gene did not grow on medium containing kanamycin.

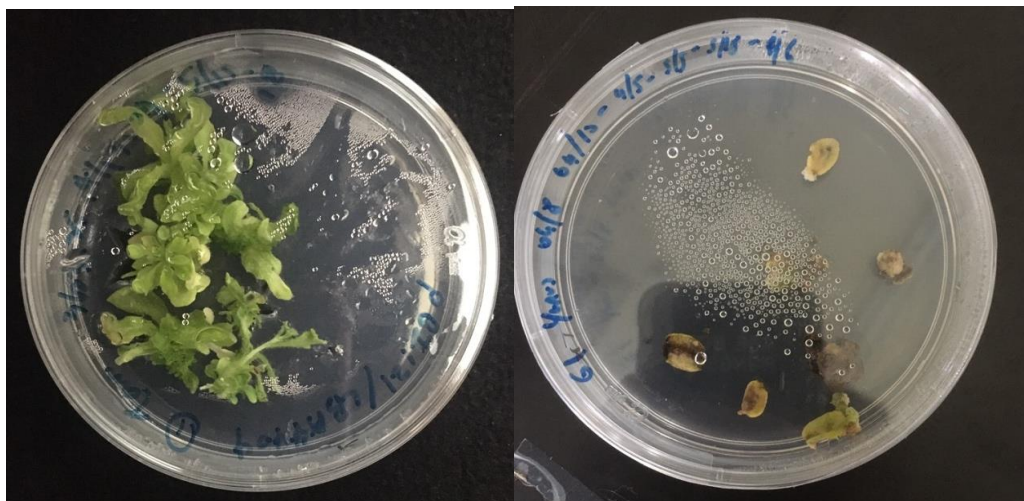
### Molecular Analysis

Total DNA samples were extracted from the samples of tomato to determine the presence of the NPTII gene. Total DNA was isolated from tomato tissue (Doyle, 1987) and the isolated was used in PCR for the detection of NPTII gene. PCR analysis was performed using Taq OptiMix CLEAR 2x Master Mix® (Ampliqon, Odense, Denmark). The following gene specific primer was used (Cortina & Culiáñez-Macià, 2004). The amplicification conditions for the PCR reaction was as follows 95 °C for 5 min, 94 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s for 35 cycles; 5 min for 72 °C and for each 25 µl sample mixture. The data was analyzed by the electrophoresis method.

## RESULTS AND DISCUSSION

### Effect of BA on tomato explants survival

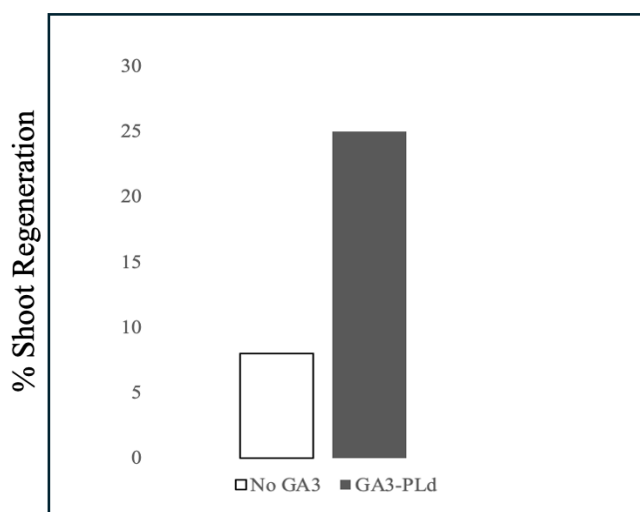
The effect of BA concentration in the shoot regeneration medium is shown in Figure 1. Callus formation was observed from tomato leaves on MS medium (PLc) containing BA. However, no callus formation was observed in tomato leaves on MS medium that did not contain BA, which was used as a negative control.



**Figure 1.** BA effect on cotyledon explants survival. Cotyledon explants on MS regeneration medium containing  $0,1 \text{ mg l}^{-1}$  BA PLc (left) and on MS modified regeneration medium does not containing BA (right), after 14 days.

### Effect of GA<sub>3</sub> on tomato explants survival

The effect of using GA<sub>3</sub> on rooting can be seen in Figure 2. Whereas on medium does not contain GA<sub>3</sub> no more than 8% of shoots where obtained, on medium with  $0.1 \text{ mg l}^{-1}$  GA<sub>3</sub> PLd the regeneration rate was three times higher (25%).

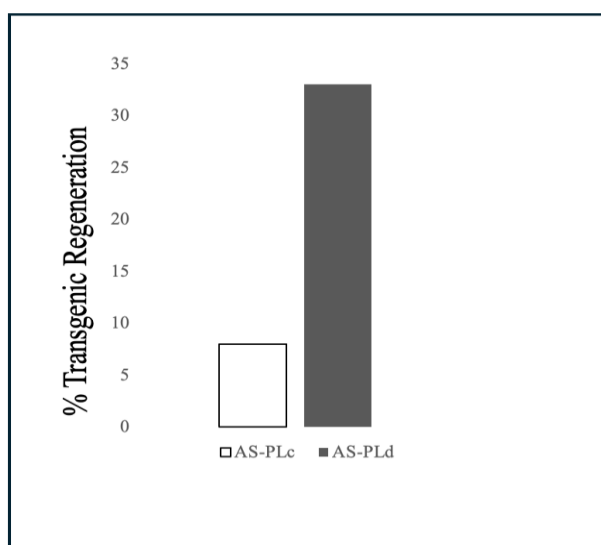


**Figure 2.** Effect of GA<sub>3</sub> on shoot regeneration medium. Percentage of resistant shoots per explant on medium containing  $0,5 \text{ mg l}^{-1}$  IAA (white column) (negative control) and on medium containing  $0.5 \text{ mg l}^{-1}$  BA and  $0.5 \text{ mg l}^{-1}$  IAA PLd (black column). Data represent the mean value of two different experiments (100 cotyledon explants for each transformation procedure)  $\pm$  SD. Values were significantly different (T Student distribution for  $p < 0.05$ ).

### Acetosyringone effect on transgenic plant recovery

The effect of acetosyringone added to PLc and PLd broth media is seen in Figure 3. Certain ratios are considered optimal for the use of acetosyringone in the production of T-strands. Therefore, the same amount of acetosyringone ( $200 \mu\text{m}$ ) was added to both media. The plant transformation rate (PLc) from this treatment, as

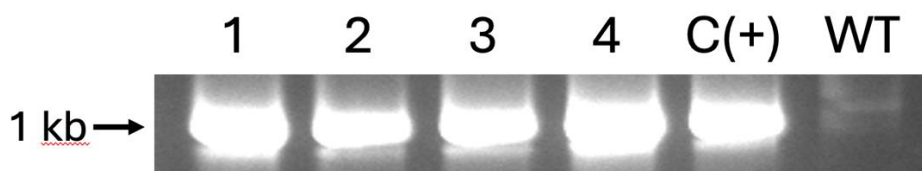
the number of confirmed transgenic plants per shoot, was over 50%; this was four times higher than that obtained with PLd medium (11%).



**Figure 3.** Effect of acetosyringone on transgenic plant recovery. Percentage transgenic plants per shoot on medium containing 200  $\mu$ M acetosyringone PLc (Table 1) (white column), and on medium containing 200  $\mu$ M acetosyringone PLd (Table 1) (black column). Data represent the mean value of two independent experiments (100 cotyledon explants for each trans-formation experiment)  $\pm$  SD. Values were significantly different (T Student distribution for  $p < 0.05$ ).

#### Molecular analysis of T0 transgenic plants

DNA from wild type and randomly selected T0 independent transformed cLN1558A tomato plants was PCR amplified using 5'-forward and 3'-reverse NPTII gene primers (Figure 4). A band of about 1.1 kb corresponding to the predicted size of the gene fragment, confirmed the T-DNA integration in the genome of these plants.



**Figure 4.** Molecular analysis of transgenic plants. Genomic DNA from *Lycopersicon esculentum* cv. cLN1558A wild type (WT) and T0 transformed plants, PCR amplified using specific NPTII gene primers. C (+): positive control of pBI121 plasmid. The left arrow indicates the band molecular weight.

#### Genetic Analysis

Tomato plants were self-pollinated to analyze the inheritance of kanamycin resistance in 4 random plants including NPTII gene. Seeds were collected from pollinated plants and germinated in PLa medium containing 100 mg  $l^{-1}$  kanamycin. After 3-4 weeks, leaf and root development were distinguished from susceptible seedlings lacking the NPTII gene. This heritability analysis is included in Table 2.



**Table 2.** Inheritance of Kanamycin resistance of the T1 generation of independent lines transformed by *A. tumefaciens* LBA4404 harbouring a *NPTII* gene for Kanamycin resistance

Line	Number of seedling		T1	$\chi^2$ 3:1
	R	S		
WT	0	100		300
1	78	22		0.45*
2	84	16		0.22*
3	75	25		0.34*
4	68	32		2.29*

## CONCLUSION

While tomato transformation has become a routine procedure, challenges persist in achieving a satisfactory yield of positive plants from viable calluses. This study presents a simple and effective modification for tomato genetic transformation and regeneration. The widely adopted MS medium (Murashige & Skoog, 1962) serves as the primary commercial medium in tissue culture. Notably, incorporating GA<sub>3</sub> and BA at a concentration of 1 mg l<sup>-1</sup> in the MS shoot regeneration medium facilitates cell growth and regeneration.

The T-DNA transfer process is predominantly governed by the vir genes (Garfinkel & Nester, 1980). Activation of the vir genes is induced by a family of small phenolic molecules, such as acetosyringone, thereby promoting T-DNA transfer (Bolton et al., 1986). The synthesis of T-strand derivatives from the nicked T-DNA substrate is notably associated with a concentration of 200 µM acetosyringone (Culianez-Macia & Hepburn, 1988). By incorporating this optimal acetosyringone concentration into the co-culture medium, the transformation efficiency experienced a fourfold improvement. Additionally, 50% of the resistant shoots successfully rooted on kanamycin and were subsequently confirmed as transgenic plants. This modification offers a promising approach to enhance the efficiency of tomato genetic transformation and regeneration.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

## Author Contributions

**1<sup>st</sup> Yunus Emre Uslu** :Conceptualization; data curation; methodology; project administration; software; writing—original draft; writing—review and editing.

**2<sup>st</sup> İsmail Bezirganoglu**: Formal analysis; funding acquisition; investigation;project administration; software; writing— original draft; writing—review and editing.

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## Investigation of Begomovirus Infections in Greenhouse Tomato Production Areas in the Eastern Mediterranean Region

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### ABSTRACT

Begomoviruses are the most numerous group of viruses infecting economically important plants. Begomoviruses have a wide host range, high recombination ability, effective transmission by its vector and synergistic interaction with different viruses in the host plant, so they have a high risk of spreading to large areas in a short time and causing epidemics. In this study, the aim was to investigate the presence of begomoviruses, which are among the major threats to tomato (*Solanum lycopersicum* L.) cultivation. The surveys were conducted in the provinces of Adana and Kahramanmaraş, Turkey, where a total of 160 leaf samples were collected from tomato plants exhibiting yellowing, curling, and plant stunting in commercial greenhouses. Total DNAs obtained from the collected leaf samples were tested using multiplex PCR. As a result of the analysis, 10 samples were infected only with TYLCSV-Sic, while 16 were co-infected with TYLCSV-Sic and TYLCV-IL. Additionally, 22 of the samples were co-infected with TYLCV-IL and TYLCV-Mld. Furthermore, PCR testing for the presence of ToLCNDV was conducted, but none of the samples were found to be infected.

**Key words:** *Begomovirus*, TYLCV, ToLCNDV, tomato, Multiplex PCR.

### Doğu Akdeniz Bölgesindeki Örtüaltı Domates Üretim Alanlarındaki Begomovirus Enfeksiyonlarının Araştırılması

#### ÖZ

Begomovirüsler ekonomik olarak önemli bitkilerde enfeksiyon yapan virüslerin en fazla sayıda bulunan gruptur. Begomovirüslerin geniş konukçu dizinine sahip olması, yüksek rekombinasyon yeteneği, vektörüyle etkili şekilde taşınması ve konukçu bitkide farklı virüsler ile sinerjik etkileşimi nedeniyle üretim alanlarında kısa sürede geniş alanlara yayılmaları ve epidemilere yol açma riskleri oldukça yüksektir. Bu çalışmada, domates (*Solanum lycopersicum* L.) yetiştiriciliğini tehdit eden begomovirüslerin varlığının araştırılması amaçlanmıştır. Adana ve Kahramanmaraş illerinde ticari seralara yapılan sörveylerde sararma, kıvrılma ve bodurlaşma gibi semptom taşıyan domates bitkilerinden toplam 160 yaprak örneği toplanmıştır. Toplanan yaprak örneklerinden elde edilen total DNA'lar multiplex PCR ile testlenmiştir. Analiz sonucunda, 10 örneğin yalnızca TYLCSV ile enfekte olduğu, 16 örneğin ise TYLCSV-Sic ve TYLCV-IL ile enfekteli olduğu belirlenmiştir. Ayrıca, 22 örneğin TYLCV-IL ve TYLCV-Mld ile karışık enfekteli olduğu belirlenmiştir. Bunun yanı sıra, ToLCNDV varlığına yönelik PCR testi yapılmış, ancak hiçbir örnekte enfeksiyon tespit edilmemiştir.

**Anahtar Kelimeler:** *Begomovirus*, TYLCV, ToLCNDV, domates, Multiplex PCR.

### INTRODUCTION

The genus *Begomovirus* belongs to the *Geminiviridae* family and currently contains 424 species (Nigam et al., 2023). Begomoviruses are the most abundant group of viruses infecting economically important plants (Malathi et al., 2017; Rao and Reddy, 2020). They infect important plants from various families, including *Cucurbitaceae*, *Solanaceae*, *Malvaceae*, and *Fabaceae*, in different regions of the world (Seal et al., 2006; Fortes et al., 2016), and are transmitted in a persistent manner by whiteflies in the *Bemisia tabaci* cryptic species (Brown

et al., 2015; He et al., 2020). Begomoviruses have either a monopartite genome (DNA-A) or a bipartite genome (DNA-A and DNA-B) each approximately 2.7-2.8 kb encapsidated in a twinned icosahedral virion (Stanley et al., 2005). Tomato yellow leaf curl disease (TYLCD), is caused by tomato yellow leaf curl virus (TYLCV) and a group of TYLCV-like viruses (ICTV, 2024). TYLCD-causing viruses belong to the genus *Begomovirus* (Stanley et al., 2005). TYLCV, which is a major issue in global tomato production, ranks third among the ten economically important viruses (Scholthof et al., 2011). While TYLCV and most related begomoviruses have a monopartite genome composed of a single-stranded DNA molecule, except for tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) and tomato yellow leaf curl Thailand virus (TYLCTHV) (Yan et al., 2021). TYLCV has been reported to cause up to 100% yield loss in tomato production due to severe infections in tropical and subtropical regions (Glick et al., 2009; Navas-Castillo et al., 2011). Tomato yellow leaf curl virus-Israel (TYLCV-Is) and tomato yellow leaf curl virus-Mild (TYLCV-Mld) strains are globally widespread, while others are found in more limited regions (Navas-Castillo et al., 2011). Infections of both strains (TYLCV-Is and TYLCV-Mld) have been reported in tomato-producing areas, with the TYLCV-Is strain causing severe infections, and it is quite common in pepper (*Capsicum annuum*) and bean (*Phaseolus vulgaris*) plants (Glick et al., 2009). Also, tomato yellow leaf curl Sardinia virus (TYLCSV), along with other closely related species, is responsible for TYLCD, which has been limited to Mediterranean countries (Yan et al., 2021). Some reports have raised concerns about the dramatic increase in the incidence of TYLCD in various regions, where climate changes, particularly warming and drying trends (García-Andre's et al., 2007; Davino et al., 2009).

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus that infects economically important vegetable and ornamental species, as well as weeds (Zaidi et al., 2017; Lopez et al., 2015). Reports on the presence of ToLCNDV were limited in Asia countries until 2012, but it was first reported in the Western Mediterranean Basin, in southern Spain, where infections were found in squash (*Cucurbita pepo* L.), melon, and cucumber crops (Juárez et al., 2014). However, the spread of this pathogen, along with reports from Iran (Yazdani-Khameneh et al., 2016), indicates that ToLCNDV has rapidly expanded into new geographical regions in recent years. The presence of TYLCV and ToLCNDV in the Mediterranean Basin poses a new threat not only to tomato production but also to other economically important crops. In Turkey, TYLCV and TYLCSV were reported in tomatoes and eggplants grown in greenhouses (Gül-seker et al., 2015; Fidan et al., 2019; Fidan and Sarıkaya, 2020). ToLCNDV was first reported in *Cucurbitaceae* species by Fidan et al. (2023) in Antalya province.

The wide host range of begomoviruses, their high recombination ability, efficient transmission by their vector, and synergistic interactions with different viruses in the host plant, result in a high risk of rapid spread across large areas in production fields and the potential to cause epidemics. In Turkey, since 2019 there has been no further molecular analysis of local TYLCV isolates and concern about the virus and its associated disease in tomato. The objective of this study was to investigate the presence of TYLCV strains and ToLCNDV in tomato plants grown in greenhouses in the provinces of Adana and Kahramanmaraş in Turkey.

## MATERIALS AND METHODS

### Sampling and DNA Extraction

One-hundred and sixty leaf samples from tomato plants showing yellowing, upward leaf curling, and plant stunting were collected in commercial greenhouses located in the Adana and Kahramanmaraş in Turkey, during 2024. DNA was isolated from about 100 mg of the leaf of the samples using the CTAB DNA extraction method (Doyle and Doyle, 1990) with slight modifications. DNA qualities were checked in agarose gel electrophoresis and quantified using a NanoDrop spectrophotometer.

### Detection and differentiation of TYLCD-associated viruses

Total DNA isolated from plants were preliminary screened for the presence of TYLCD-associated viruses by degenerate primer pair TY1 and TY2 (Accotto et al., 2000) (Table 1). Later, two sets of primers were used for multiplex PCR to detect TYLCD-associated viruses: TYLCV-IL (Israel), TYLCV-Mld (Mild), TYLCSV-Sicily and TYLCSV-ES (Spanish). Moreover, in order to detect the presence of ToLCNDV, the total DNAs were subjected to PCR using ToLCNDV-CP1 and ToLCNDV-CP2 primers (Panno et al., 2019).

**Table 1.** List of the primers that were used in this study

Primers	Sequence (5' to 3')	Product size (bp)	Virus/Strain
TY 1	GCCCATGTAYCGRAAGCC	579	TYLCV
TY 2	GGRTTAGARGCATGMGTAC		
TYv2337	ACGTAGGTCTTGACATCTGTTGAGCTC	634 bp	TYLCV-IL
TYc138	AAGTGGGTCCCACATATTGCAAGAC		
TYAlmv2516	TTTTATTTGTTGGTGTGTTGTTAGTTGAAG	433 bp	TYLCSV-ES
TYAlmc115	ATATTGATGGTTTTTCAAACTTAGAAG		
TYv2664	ATTGACCAAGATTTTACACTTATCCC	316 bp	TYLCV – Mld
TYc138	AAGTGGGTCCCACATATTGCAAGAC		
VP2715	ATACTTGGACACCTAATGGCTATTTGG	543 bp	TYLCV
RVC427	TGCCCTGGACA(A/G)TGGGG(A/G)CAGCAG		
Sa2267	TGGAAAGTACCCCATTCAGAATCATC	946 bp	TYLCSV-Sic
VP2715	ATACTTGGACACCTAATGGCTATTTGG		
ToLCNDV-CP1	CTCCAAGAGATTGAGAAGTCC	1 kb	ToLCNDV
ToLCNDV-CP2	TCTGGACGGGCTTACGCCCT		

### Nucleotide Sequence Comparison and Phylogenetic Analysis

PCR DNAs were purified from agarose gel (QIAGEN). And, PCR products were sequenced directly in both directions. Multiple sequence alignments were made using CLUSTALW (Thompson et al., 1994) and the nucleotide sequences were analyzed with the online software BLASTn (<http://www.ncbi.nlm.nih.gov/BLAST/>). Phylogenetic trees were constructed with MEGA software version 7 (Kumar et al., 2016) in the neighbor-joining algorithm with 1000 bootstrap replicates to assess the robustness of the nodes (Tamura et al., 2013).

### RESULTS AND DISCUSSION

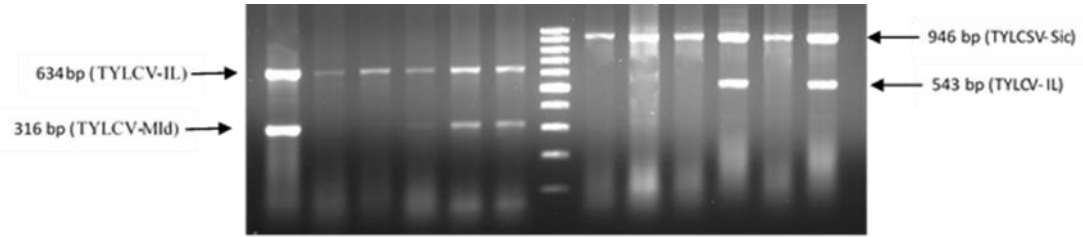
A total of 160 leaf samples were collected from commercial greenhouses in Adana and Kahramanmaraş. The most frequently observed symptoms on tomato plants were mosaic, yellowing or chlorosis, leaf rolling, leaf curling, leaf deformation and stunting (Fig.1).



**Figure 1.** TYLCD symptoms (leaf curling, yellowing, stunting) in tomato plants grown in the greenhouses in Adana and Kahramanmaraş

When the total DNAs were tested using multiplex PCR, the expected sizes for the DNA amplicons were obtained as follows: 634 bp for TYLCV-IL, 316 bp for TYLCV-Mld, 946 bp for TYLCSV-Sic (Fig. 2). In the analysed field samples found that only single or double virus infected. Ten out of 48 infected samples were infected with only TYLCSV-Sic, while 16 were co-infected with TYLCSV-Sic and TYLCV-IL (Table 2). Additionally, 22 of the samples were co-infected with TYLCV-IL and TYLCV-Mld. Moreover, to test for the presence of ToLCNDV, PCR was performed, but none of the samples was found to be infected.



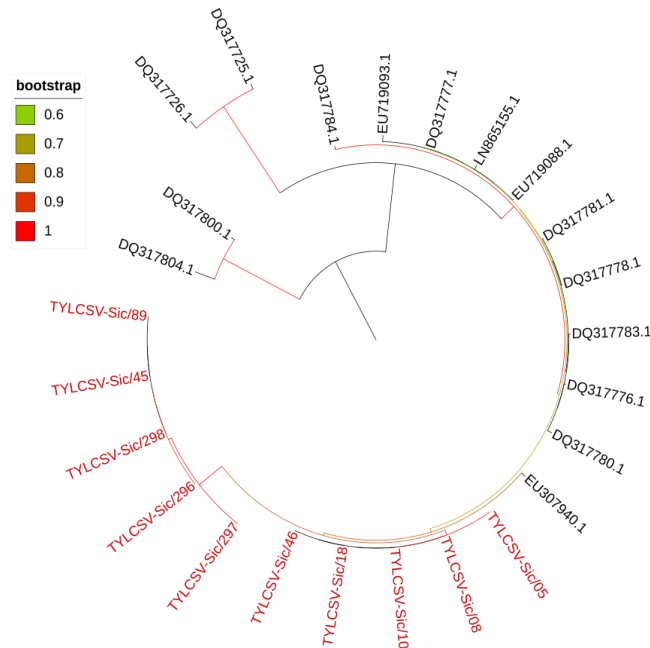


**Figure 2.** Detecting viruses associated with tomato yellow leaf curl. GeneRuler DNA Ladder Mix (Thermo Fisher Scientific) was used as a marker (100 bp).

**Table 2.** Number of tomato plants infected with TYLCD-associated viruses in Adana and Kahramanmaraş

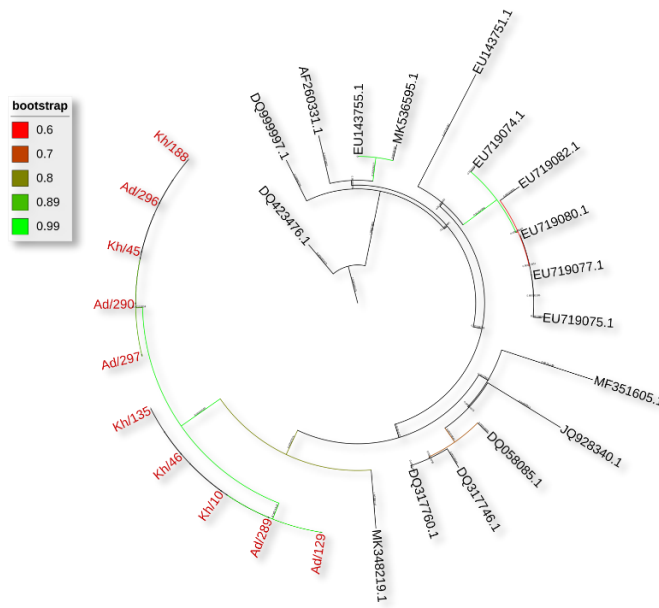
Location	TYLCSV-Sic	TYLCSV-Sic+TYLCV-IL	TYLCV-IL+ TYLCV-Mld
Adana	8	12	17
Kahramanmaraş	2	4	5
<b>Total</b>	<b>10</b>	<b>16</b>	<b>22</b>

The analyses of BLASTn and BLASTx were performed using nucleotide sequences of 919 nt from TYLCSV-Sic isolates and 634 nt from TYLCV-IL isolates. The nucleotide sequences of ten randomly selected isolates, including both TYLCV-IL and TYLCSV-Sic, have been submitted to GenBank. The accession numbers for the TYLCSV-Sic isolates are PV067002–PV067011, while the accession numbers for the TYLCV-IL isolates are PV067012–PV067021. The TYLCSV-Sic isolates obtained in this study showed high nucleotide identity, with 97–99% sequence homology to previously reported isolates in GenBank. Phylogenetic analysis of TYLCSV-Sic isolates obtained in this study were divided into two subgroups (Fig. 3). The isolate closest to TYLCSV-Sic-Turkey was from Jordan (EU307940.1), followed by Italy (DQ317778.1, DQ317780.1, DQ317783.1) isolates. Furthermore, the TYLCV-IL isolates obtained in this study showed high nucleotide identity, with 94–97% sequence homology to previously reported isolates in GenBank. Phylogenetic analysis showed that the TYLCV-IL isolates are close to previously reported from Turkey (MK348219.1), Jordan (MF351605.1), Spain (DQ058085.1, DQ317760.1, DQ317746.1) and Iran (JQ928340.1) isolates (Fig.4).



**Figure 3.** Phylogenetic tree for TYLCSV-Sic isolates. The analyses were performed using MEGA 7 software. The bootstrap consensus of the tree was derived from 1000 replicates.





**Figure 4.** Phylogenetic tree for TYLCV-IL isolates. The analyses were performed using MEGA 7 software. The bootstrap consensus of the tree was derived from 1000 replicates

While TYLCV is widespread globally and causes significant epidemics in tomato, TYLCSV is restricted to Mediterranean countries (Navas-Castillo et al., 2011). Sánchez-Campos et al. (1999) reported that TYLCV had higher transmission rates among *B. tabaci* biotypes than TYLCSV. Other begomovirus species, closely related to TYLCV and responsible for TYLCD, are found in more limited geographical areas. Previous research has indicated that various species within the *B. tabaci* complex can transmit a specific begomovirus with varying levels of efficiency (Jiu et al., 2006; Zhao et al., 2019). TYLCV-like viruses are present throughout the growing seasons, but the high infection was detected in the spring in both provinces, coinciding with a high population of *B. tabaci*. The increasing vector populations driven by global warming have significantly contributed to the rise of viruses infecting economically important crops such as peppers and tomatoes worldwide. During the survey, it was determined that intensive use of insecticides was involved in the discussions with the producers. The intensive use of insecticides by producers contributes to the development of insecticide resistance in whiteflies, reducing their effectiveness. This, in turn, may lead to an increase in disease incidence, as the resistant whiteflies continue to spread viruses to agricultural crops (Horowitz et al., 2007; Patra and Hath, 2022).

Moreover, Kil et al. (2016) reported that TYLCV is transmitted through the seeds of tomato and pepper plants. However, subsequent studies by Pérez-Padilla et al. (2020) and Tabein et al. (2021) showed no evidence of seed transmission for TYLCV-IL or TYLCSV, despite detecting the virus in seeds, indicating that the virus is seed-borne but not transmitted via seeds. These findings suggest that seed transmission is not a common characteristic of TYLCV. The worldwide spread of TYLCD is strongly linked to the international movement of planting material, as well as the global rise in insect vector populations and the rapid evolution of virus strains (Seal et al., 2006; Navas-Castillo et al., 2011). Factors such as host range, population dynamics, and genetic diversity within *B. tabaci* biotypes all play a crucial role in the spread of begomoviruses in agroecosystems. Understanding the dynamics of *Begomovirus* transmission by *B. tabaci* whitefly is essential for the development of appropriate control strategies and sustainable agricultural practices to prevent the spread of these economically important viruses.

## CONCLUSION

This study confirms the presence of TYLCV and its strains (TYLCV-IL and TYLCV-Mld) in greenhouse-grown tomatoes in Turkey, with co-infections observed between TYLCV strain and TYLCSV. The prevalence of TYLCV-associated viruses in these regions underscores the importance of monitoring begomovirus infections and controlling their spread, particularly in regions where whitefly vectors are abundant.


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### Conflict of Interest

The author declares no conflict of interest.

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## Economic Analysis of Chemical Fertilizer Use In TRA1 Region

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### ABSTRACT

This study aims to examine the agricultural enterprises' use of fertilizers in the TRA1 region enterprise levels, analyzing farmers' knowledge, attitudes, and behaviors regarding fertilizer use. The study, conducted in the TRA1 region (Erzurum, Erzincan, Bayburt), involved interviews with 570 agricultural enterprise owners. Data were collected regarding fertilizer preferences, farmers' awareness levels in fertilization applications, and the factors influencing their choices. It was determined that enterprises primarily use nitrogenous fertilizers, followed by phosphate fertilizers, compound fertilizers, and potassium fertilizers. A significant proportion of enterprises (80.9%) determine the amount of fertilizer based on their experience, while only 4.7% decide after conducting soil analysis. Most enterprise owners purchase fertilizers from fertilizer dealers (40.2%) and obtain information about fertilizers from the same source (27.7%). Given that only 17.7% of enterprises receive information from agricultural institutions, it is evident that comprehensive farmer training programs should be implemented with the support of public institutions to enhance farmers' technical knowledge and skills.

**Key words:** TRA1 region, agriculture, agriculture enterprise, fertilizer use.

### TRA1 Bölgesi Kimyasal Gübre Kullanımının Ekonomik Analizi

### ÖZ

Bu çalışma TRA1 bölgesindeki tarımsal işletmelerin tarımsal gübre kullanımlarını işletme bazında detaylı olarak incelemek, çiftçilerin gübre kullanımı konusundaki bilgi tutum ve davranışlarını analiz etmek amacıyla yapılmıştır. TRA1 (Erzurum, Erzincan, Bayburt) Bölgesinde kimyasal gübre kullanımını inceleyen bu çalışmada 570 tarımsal işletme sahibi ile görüşme yapılmıştır. Tarımsal işletmelerin gübre tercihleri, gübreleme uygulamalarında çiftçilerin bilinç düzeyleri ve tercihlerinde rol oynayan hususlar ile ilgili bilgiler derlenmiştir. İşletmelerin kimyasal gübre olarak sırasıyla en fazla azotlu gübreleri, fosfatlı gübreleri, kompoze gübreleri ve potasyumlu gübreleri kullandıkları tespit edilmiştir. İşletmelerin büyük bölümü (%80.9) gübre miktarını kendi deneyimlerine göre belirlerken sadece %4.7'si toprak analizi yaptırdıktan sonra gübre miktarına karar vermektedir. İşletme sahipleri gübre alımını en fazla gübre bayilerinden (%40.2) yaparken, gübrelerle ilgili bilgileri yine en fazla gübre bayilerinden (%27.7) edinmektedirler. Gübre seçiminde işletmelerin sadece %17.7'sinin tarım teşkilatlarının elemanlarından bilgi aldığı düşüldüğünde üreticilerin Teknik bilgi ve becerilerinin artırılması konusunda kamu kurumlarının da katkısıyla detaylı çiftçi eğitim programları yapılması gerekmektedir.

**Anahtar kelimeler:** TRA1 bölgesi, tarım, tarımsal işletme, gübre kullanımı.

### INTRODUCTION

Fertilizers are substances that can return the plant nutrients lost by the soil as a result of the cultivation of agricultural products and increase the productivity of the soil. The use of fertilizers is one of the most effective methods of increasing the productivity as well as the quality of the agricultural products grown. When compared

to other agricultural inputs used to increase productivity, fertilizers significantly increase agricultural production by providing a yield increase of over 40%. Therefore, fertilizers make important contributions to people's access to the basic foods they need, ensuring global food supply and increasing people's living standards (Eraslan et al., 2009). While the world population is rapidly increasing, the need for food is increasing faster than the increasing population. In the last 50 years, the world population has doubled while the increase in food production has been threefold (Ekşi and İşci, 2012). The increase in the need for food due to changing eating habits and the decrease in agricultural land per person with the increasing world population necessitates an increase in productivity in plant production (Karaçal and Tüfenkçi, 2010). Therefore, fertilizers, which have an important place in increasing productivity, are one of the most important inputs of sustainable agriculture (Eraslan et al., 2009).

Historically, people have used various additives to improve soil fertility. After the 19th century, research on plants and soil has shown that plants take mostly nitrogen, phosphorus, potassium, and calcium from the soil, while using less of the other substances. For this reason, it has become necessary to add these substances that are depleted from the soil to the soil (Ilgar, 2020). Today, there has been a significant increase in the use of chemical fertilizers.

Total chemical fertilizer use in the world was 187,925 thousand tons in 2022. While the most fertilizer is used in Asia, the continent of America, Europe and Africa follow Asia. The continents of Oceania have the least fertilizer use (Table 1). When we look at total fertilizer use on a country basis, the country that uses the most fertilizer is China, followed by India, the United States, Brazil and Indonesia. Turkey ranks 13th in total fertilizer use with 2,230 thousand tons.

**Table 1.** Fertilizer Utilization Statistics, 2022

Regions	Consumption (1,000 Tons)	Percentage (%)	Countries	Consumption (1,000 Tons)	Percentage (%)
World	187,925	100.0	Chinese	44,498	23.8
Asia	106,307	56.6	India	29,844	15.9
America	49,043	26.1	USA	20,464	10.9
Europe	22,033	11.7	Brazil	18,663	9.9
Africa	7,153	3.8	Indonesia	6,377	3.4
Oceania	3,389	1.8	Türkiye	2,230	1.2

Source: Anonymous, 2021.

The amount of chemical fertilizer used in Turkey has fluctuated over the years. While it was 2,203 thousand tons in 2015, it increased to 2,644 thousand tons in 2017 and decreased to 2,575 thousand tons in 2019. It increased again to 2,575 thousand tons in 2021 and 2,830 thousand tons in 2023. The most used fertilizer group among chemical fertilizers is nitrogenous fertilizers with approximately 69%. Phosphorus fertilizers are in second place with approximately 26%. The least used chemical fertilizer group is potash fertilizer with 5% (Table 2).

**Table 2.** Fertilizer Utilization Statistics (1,000 Tons)

Fertilizer	2015	2017	2019	2021	2023
Total	2 203	2 644	2 466	2 575	2 830
Nitrogenous (N)	1 487	1 765	1 683	1 787	1 948
Phosphorous (P <sub>2</sub> O <sub>5</sub> )	585	755	667	634	736
Potash (K <sub>2</sub> O)	132	125	116	154	146

Source: Anonymous, 2023.

When the studies on fertilizer use are examined today, where the use of chemical fertilizers in plant production is intense, the studies are mostly focused on determining the awareness level of enterprises in fertilizer use (Kızılaslan and Kızılaslan, 2005; Yılmaz et al., 2009; Unakıtan et al., 2017; Katip, 2020; Yüzbaşıoğlu (2020). It has been determined that the use of chemical fertilizers increases yield and income (Matsumoto and Yamano, 2011; Mengel et al., 2006). Kaplan and Gözükar (2021) emphasized that there should be a change in chemical fertilizer consumption.

In today's world where the need for food products is increasing day by day, it is very important to determine the levels of fertilizer use, which makes a significant contribution to the increase in productivity of



producers. This study was conducted to examine the agricultural fertilizer use of agricultural enterprises in the TRA1 region enterprise basis and to analyze the knowledge, attitudes and behaviors of farmers on fertilizer use.

## MATERIALS AND METHODS

### Research Area and Sample Method

In the study, 2021 production data obtained from face-to-face surveys conducted with 570 business owners operating in Erzurum, Erzincan and Bayburt provinces constituted the main data source. Other data were obtained from national and international publications, websites, FAO statistics, Turkstat and other institution websites. The framework created from the Farmer Registration System obtained from the Provincial Directorates of Agriculture in the TRA1 Region was used as the main mass. There are a total of 40,508 agricultural businesses registered in the Farmer Registration System in the TRA1 region, including 31,034 farmers in Erzurum, 6,791 in Erzincan and 2,683 in Bayburt.

### The Method Applied to Determine the Number of Samples

The number of surveys to be applied was determined using the Neyman Method using data from the Farmer Registration System received from the Provincial Directorates of Agriculture in the TRA1 Region (Erkuş, 1977). The Neyman method, developed by Jerzy Neyman, is one of the cornerstones of statistical inference and is one of the most widely used methods for determining sample size (Neyman, 1937). The total population is 40,508 agricultural holdings.

$$n = \sum (N Sh)^2 / (N^2 * D^2 + \sum Nh (Sh^2))$$

The following formula ensures that businesses are distributed to the specified layers.

$$n = Nh Shx n / \sum Nh Sh$$

N: Total number of enterprises in the population

Nh: Number of enterprises in the h-th stratum

n: Sample size

ni: Sample size for the i-th stratum

Sh<sup>2</sup>: Variance in the h-th stratum

Sh: Standard deviation in the h-th stratum

D<sup>2</sup>: Correction factor

Z<sup>2</sup>: Z-table value for the given confidence level

d: Allowed margin of error from the population mean

Considering the attitudes of the people working in the agricultural sector and their willingness to answer the survey questions, the error limit was kept low and the confidence interval was taken wide. Accordingly; The sample size was found to be 570 with an 85% confidence interval and a 3% error limit. 272 survey samples were taken from the first layer, 202 from the second layer and 96 from the third layer (Table 3).

**Table 3.** Distribution of Agricultural Enterprises

Agricultural Business Groups	Land Size (Decare)	Number of Businesses	Survey Rate (%)
Group 1	0 - 49	272	47.7
Group 2	50 - 149	202	35.4
Group 3	150+	96	16.9
Total		570	100.0

### Data Analysis Method

In the study, the factors affecting the problems encountered by the farmers of TRA1 region in deciding on fertilizer needs and receiving agricultural support (education, agricultural experience, land size and tractor presence) were addressed and the Multinomial probit model was used to measure the effects of these factors. In this context, the model tried to determine the factors that distinguished the farmers from other options by having soil analysis, asking family elders or neighboring businesses, asking the members of the agricultural organization, relying on their experiences and asking agricultural engineer consultants in deciding on fertilizer needs.

The multinomial probit model is used when a dependent variable can take on two values and estimates the probability of these values. The estimates obtained from the two-valued probit model ensure that the parameter estimates of the multinomial probit model are consistent. ( Menard , 2002 ) . Both models use a

probability distribution function to estimate probabilities of choices between multiple categories. This means that the two models are interconnected, and the estimates from the two-valued probit model provide a basic reference point for the multiple nominal probit model (Begg and Gray, 1984; Miran, 2021).

## RESULTS AND DISCUSSION

The business owners who participated in the survey were asked how they determined the amount of fertilizer to be used. 80.9% of them, i.e. 461 business owners, answered “I decide based on my experiences”, while 9.3% answered “By consulting family elders or neighboring enterprises”. The rate of those who answered “By conducting soil analysis” was 4.7%, while those who decided “By consulting my agricultural engineer advisor” was 3.3% and those who decided “by asking the technical staff at the Agricultural Organization” was 1.8% (Table 4). Several studies have indicated that farmers often rely on their experience or traditional knowledge in determining fertilizer use. According to Yılmaz et al. (2009), the majority of farmers in Turkey make fertilization decisions based on experience rather than scientific soil analysis, leading to inefficient use. Similarly, Unakıtan et al. (2017) found that lack of access to professional agricultural consultancy and low awareness of soil testing methods contribute to this trend.

**Table 4.** Distribution of Factors Influencing the Determination of Fertilizer Quantities

Determining the Amount of Fertilizer to Use	Number of Businesses	Percentage
By conducting soil analysis	27	4.7
By consulting family elders or neighboring enterprises	53	9.3
By consulting technical staff in the Agricultural Organization	10	1.8
I decide based on my experience	461	80.9
By consulting my agricultural engineer advisor	19	3.3
Total	570	100

When the places where fertilizer is supplied in the enterprises surveyed are examined, it is seen that 40.2% of the fertilizer is supplied mostly from fertilizer dealers, 29.5% from cooperatives, and 30.3% from other supply places (Table 5). This pattern aligns with findings by Katip (2020), who reported that fertilizer dealers are the primary source of supply due to their accessibility and immediate availability. However, reliance on dealers may result in biased product recommendations, as highlighted by Eraslan et al. (2010), who emphasized the importance of agricultural extension services in providing unbiased fertilization recommendations.

**Table 5.** Distribution of Fertilizer Procurement Sources

Fertilizer Supply Locations	Number of Businesses	Percentage
Dealer	229	40.2
Cooperative	168	29.5
Other	173	30.3
Total	570	100

When examining the organizations from which enterprise owners participating in the study obtained information on fertilizer selection, 40.6% (231 enterprise owners) stated “Other”, while 27.7% (158 enterprise owners) mentioned “Fertilizer dealers”. The proportion of those who responded “Agricultural organization staff” was 17.7%, whereas 11.9% of them indicated “Consulting agricultural engineers”. Only 2.1% of the participants responded with “Fertilizer company representatives”. The high percentage of the “Other” category may be due to enterprise owners who do not seek information from any organization when selecting fertilizers (Table 6). The limited reliance on agricultural professionals for fertilizer recommendations is consistent with previous studies. Kızılaslan and Kızılaslan (2005) reported that farmers often prioritize convenience over technical advice. Similarly, Yüzbaşıoğlu (2020) found that while agricultural organizations provide more scientifically grounded advice, their accessibility and engagement with farmers remain limited.

**Table 6.** Distribution of Factors Influencing the Selection of Fertilizer Types

Organizations that provide information on fertilizer selection	Number of Businesses	Percentage
Agricultural organization staff	101	17.7
Fertilizer company representatives	12	2.1
Fertilizer dealers	158	27.7
Consulting agricultural engineers	68	11.9
Other (relatives, friends, those who do not receive information)	231	40.6
Total	570	100

When the criteria that the business owners who participated in the research pay attention to when choosing fertilizer are examined; 42.4% , that is, 242 business owners, said “I buy the most effective fertilizer regardless of the price”, while 23.3%, that is, 144 business owners, said “I buy the fertilizer that an acquaintance uses and recommends”. The rate of those who answered “I buy the fertilizer recommended by the dealer” was 18.4% , while those who said “I buy the fertilizer recommended by agricultural engineers working in agricultural organizations” was 10.4%, and those who answered “I buy the one with the cheapest price” was 3.5%. The majority of business owners prefer fertilizers that they have used before and whose effectiveness (Table 7). These findings align with the study by Kaplan and Gözükar (2021), which emphasized the role of perceived effectiveness in farmers' purchasing decisions. Matsumoto and Yamano (2011) also highlighted that farmers' fertilizer choices are largely influenced by word-of-mouth recommendations rather than scientific evaluation.

**Table 7.** Distribution of Criteria for Fertilizer Selection in Surveyed Enterprises

Criteria for Fertilizer Selection	Number of Businesses	Percentage
I buy the cheapest one	20	3.5
I buy the fertilizer that a friend of mine recommends.	144	25.3
I buy the fertilizer recommended by the dealer	105	18.4
I buy the most effective fertilizer no matter what the price is	242	42.4
I buy the fertilizer recommended by agricultural engineers working in agricultural organizations.	59	10.4
Total	570	100

The majority of the business owners who participated in the survey stated that they did not have soil analysis done. While 92.5% of the business owners , or 527 of them, did not have soil analysis done, 7.5%, or 43 of them, said that they had soil analysis done (Table 8). This result is consistent with findings by Mengel et al. (2006), who reported that soil testing rates remain low in many agricultural regions due to a lack of knowledge, accessibility, and perceived necessity. Similarly, Ilgar (2020) emphasized that increasing farmer awareness about soil analysis through targeted extension programs is essential for improving fertilizer efficiency and reducing environmental impact.

**Table 8.** Soil Testing Status of Surveyed Enterprises

Soil Analysis Status	Number of Businesses	Percentage
It was done	43	7.5
Not made	527	92.5
Total	570	100

According to the research findings, the production area is mostly allocated to forage crops. Since animal husbandry is developed in the region, the production of forage crops is high. Forage crops are followed by wheat, barley, rye and sugar beet, respectively. It is also seen that 19.4% of the agricultural land is not cultivated. Production amounts are also parallel to the production area, the percentage distribution of production amounts is; forage crops 37.4% , wheat 10.6%, barley 4.7%, rye 0.6% and sugar beet 10.0% (Table 9). Studies by Eraslan et al. (2010) and Kızılaslan & Kızılaslan (2005) confirm that regions with developed animal husbandry tend to allocate a higher percentage of their cultivated land to forage crops, ensuring a sustainable feed supply. Additionally, the presence of significant uncultivated land suggests the potential for agricultural expansion or policy-driven land-use optimization.

**Table 9.** Distribution of Products Produced by Surveyed Enterprises

Product Produced	Area (Decare)	Area %	Production (Kg)	Production %
Fodder Plant	34 273	42.4	12 804 310	37.4
Wheat	16 462	20.4	3 630 481	10.6
Barley	6 801	8.4	1 615 302	4.7
Rye	1 246	1.5	194 986	0.6
Sugar beet	827	1.0	3 413 511	10.0
Other	5 527	6.8	15 097 466	36.8
Uncultivated Land	15 661	19.4	-	-
Total	80 877	100.0	34 249 470	100.0

When analyzing the chemical fertilizer usage of the enterprises, nitrogenous fertilizers were found to be the most used, accounting for 50.9% of the total amount. These were followed by phosphate fertilizers (27.1%), compound fertilizers (21.9%), and potassium fertilizers (0.1%). The distribution of land (in decares) where these fertilizers were applied showed a similar pattern. However, when examining the expenditures on chemical fertilizers, although the ranking remained the same, significant differences were observed in percentage distributions. The primary reason for this is the price variations among different fertilizers. For instance, since phosphate fertilizers are more expensive per unit compared to nitrogenous fertilizers, while they were used on 27.1% of the land, they accounted for 33.6% of the total fertilizer expenditures. (Table 10). According to Mengel et al. (2006), nitrogen-based fertilizers play a crucial role in increasing yield, yet excessive use can lead to environmental issues such as nitrate leaching.

**Table 10.** Chemical Fertilizer Usage Statistics of Surveyed Enterprises

Use of Chemical Fertilizers	Area (Decare)	Area %	Quantity (Kg)	Amount %	Value ( TL)	Value %
Nitrogenous	28 258	50.0	511 724	50.9	6 333 600	45.9
Phosphate	16 178	28.6	271 882	27.1	4 628 510	33.6
Potassium	68	0.1	550	0.1	10 900	0.1
Composite	12 012	21.3	220 350	21.9	2 815 600	20.4
Total	56 515	100	1 004 506	100	13 788 610	100

In the Multinomial Probit Model, the dependent variable is how farmers determine their fertilizer needs. Here, the farmers choose one of seven alternative approaches, including: conducting soil analysis, consulting family elders or neighboring enterprises, asking the staff in the agricultural organization, based on my experience, consulting an agricultural engineer advisor, a combination of consulting family elders and relying on personal experience, a combination of relying on personal experience and consulting an agricultural engineer advisor.

In the Multinomial Probit Model, interpretations are based on the criterion of conducting soil analysis. Farmers with higher education levels tend to determine their fertilizer needs by consulting technical staff in agricultural organizations and agricultural engineer advisors (Table 11). More educated farmers are less likely to rely on their personal experience and instead base their decisions on soil analysis. As the experience of working in the sector increases, instead of soil analysis, they determine the need for fertilizer by asking the technical staff in the agricultural organization. Producers who own tractors determine their fertilizer needs by having their soil analyzed. It was also found to be statistically significant.

**Table 11.** Results of the Multinomial Probit Model Analysis of Factors Influencing Farmers ' Decisions on Fertilizer Needs

Variables	To family elders or to neighboring businesses by asking (2)	Asking family elders or neighboring businesses - I decide based on my experiences (2-4)	By asking the technical staff in the Agricultural Organization (3)	I decide based on my experience (4)	I decide based on my experience - By asking my Agricultural Engineer advisor (4-5)	By asking my Agricultural Engineer advisor (5)
Constant	1.14555 1.00998	0.34389 1.24441	-3.14044 1.50854 *	4.57609 *** 0.83203	1.10985 2.70118	-3.74845 ** 1.44988
Education	-0.05600 0.20897	-0.00276 0.24901	0.49399 0.28606	-0.60247 ** 0.17499	-0.47675 0.58838	0.63119 ** 0.26762
Agricultural Experience	-0.01416 0.01782	-0.01193 0.02263	0.04635 0.02464	-0.00812 0.01459	-0.01816 0.05556	0.03249 0.02327
Land size	0.00044 0.00131	-0.00085 0.00259	-0.00149 0.00357	0.00109 0.00109	0.00132 0.00127	0.00132 0.00109
Tractor presence	-0.81883 ** 0.39417	-0.77082 0.50252	-1.08682 0.61746	-0.61975 * 0.33011	-9.87272 0.00001	-0.35889 0.50644
<b>Log likelihood</b>					-267.05232	
<b>Wald chi2(4)</b>					67.37	

\*, \*\*, \*\*\* indicate statistical significance of 0.01, 0.05 and 0.10, respectively.

Table 12, where marginal effects are given, is examined, statistically significant results were found for education level and tractor presence. Those with a higher education level are 2.90% more likely to decide on fertilizer needs by having a soil analysis done compared to other criteria. Similarly, they are 4.10% more likely to decide by asking their elders or neighboring businesses and 1.69% more likely to decide by asking their agricultural engineer consultant. Those with a tractor are 4.56% more likely to determine fertilizer needs by having a soil analysis done compared to those without one.

**Table 12.** Marginal Effects of Factors Influencing Farmers' Decisions on Fertilizer Needs in the Multinomial Probit Model

Variables	By having a soil analysis done	By asking family elders or neighboring businesses	In the Agricultural Organization technical by asking the staff	I decide based on my experience	By asking my Agricultural Engineer advisor
Variables	Coefficient	Coefficient	Coefficient	Coefficient	Coefficient
Education	0.02898 ***	0.04097 ***	0.00865	-0.10883 ***	0.01689 **
Agricultural Experience	0.00048	-0.00083	0.00053	-0.00063	0.00065
Land size	-0.00006	-0.00005	-0.00003	0.00017	0.00001
Tractor presence	0.04565 **	-0.02345	-0.00459	-0.01109	0.00413

\*, \*\*, \*\*\* indicate statistical significance of 0.01, 0.05 and 0.10, respectively.

## CONCLUSION

This study was conducted to examine in detail the agricultural fertilizer use and fertilization costs of agricultural enterprises operating in the TRA1 region, both on a product and enterprise basis, and to analyze the knowledge, attitudes and behaviors of farmers regarding fertilizer use. For this purpose, the findings obtained from the face-to-face survey conducted with agricultural enterprise owners operating in the TRA1 region are summarized.

In order to meet the increasing food demand parallel to the increase in the world population, it is very important to increase the efficiency in agricultural production. Fertilizer use has an important place in the sustainability of efficiency. Therefore, the right time and amount of fertilizer used by the producers as required by the soil affects the efficiency and therefore the continuity of the business.

The study results indicate that the vast majority of businesses determine the type and amount of fertilizer they use based on their own experience or by consulting older family members, while only a very small proportion conduct soil analysis and apply fertilizers according to the results. Only farmers with a high level of education have their soil analyzed and apply fertilizer by taking into account the existing plant nutrients in the soil and the needs of the plant to be planted.

The application of fertilizers without prior soil analysis poses a significant risk of either overuse or underuse relative to the actual needs of the crop. Excessive fertilizer application not only increases production costs but also leads to adverse environmental consequences. Conversely, insufficient fertilizer application may result in inadequate nutrient supply to the crop, thereby causing a decline in yield. The findings of the study suggest that farmers in the region do not place sufficient emphasis on soil analysis when selecting fertilizers and determining application rates. Addressing this deficiency will require comprehensive farmer training programs to enhance awareness and adoption of soil-based fertilization practices.

## Declaration of interests

The authors of the article declare that they do not have any conflict of interest

## Author Contributions

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## Determination of Some Fruit Quality Characteristics of Azman Banana Cultivar at Different Ripening Stages

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### ABSTRACT

Banana (*Musa cavendishii* L.), a member of the Musaceae family, it is a tropical climate plant and an important fruit with high nutritional value that can only grow in the subtropical climate zone. Banana, widely consumed in the countries where it is cultivated, has recently been extensively grown in the coastal regions of our country as well. Due to its richness in bioactive phenols, antioxidants, and potassium, it is recognized as an excellent source of nutrition. This study investigated the effects of different ripening stages on the bioactive compounds in bananas of the Azman cultivar. The results revealed that bioactive compounds varied depending on the stages of ripening. The study determined that L\*, a\*, and b\* color values increased proportionally with the ripening duration. It was found that green bananas had lower total phenolic and antioxidant contents compared to ripe fruits. Furthermore, the soluble sugar content (sucrose, glucose, and fructose) in Azman bananas increased during the ripening process. While the levels of citric, malic, and succinic acids increased with ripening, the content of oxalic acid decreased.

**Key words:** Banana, Dpph scavenging activity, HPLC, Organic acids, Sugars, Total phenol content.

### Azman Muz Çeşidinin Farklı Olgunlaşma Aşamalarında Bazı Meyve Kalite Özelliklerinin Belirlenmesi

### ÖZ

Musaceae familyasının bir üyesi olan muz (*Musa cavendishii* L.), tropik iklim bitkisi olup, ancak subtropik iklim kuşağında yetişebilen besin değeri yüksek önemli bir meyvedir. Yetiştirildiği ülkelerde geniş bir tüketim alanına sahip olan muz bitkisi, son yıllarda ülkemizde de kıyı kuşağında yaygın olarak yetiştirilmeye başlanmıştır. Biyoaktif fenoller, antioksidanlar ve potasyum açısından zengin olması nedeniyle iyi bir besin kaynağı olarak kabul edilmektedir. Bu çalışmada, Azman muz çeşidine ait muz meyvelerinde olgunlaşmanın farklı aşamalarının biyoaktif bileşikler üzerindeki etkisi araştırılmıştır. Sonuçlar, biyoaktif bileşiklerin farklı olgunlaşma aşamalarına göre değiştiğini göstermiştir. Çalışmada, L\*, a\* ve b\*, renk değerlerinin olgunlaşma süresi ile orantılı olarak arttığı belirlenmiştir. Yeşil muzların olgun meyvelere göre daha düşük toplam fenolik ve antioksidan içeriğine sahip olduğu tespit edilmiştir. 'Azman' muz meyvelerinde çözünabilir şekerlerin (sakkaroz, glikoz ve fruktoz) içeriği meyve olgunlaşmasıyla birlikte artmıştır. Sitrik, malik ve süksinik asit içeriği meyve olgunlaşmasıyla birlikte artarken, oksalik asit azalmıştır.

**Anahtar kelimeler:** : Dpph Süpürme Aktivitesi, HPLC, Muz, Organik asitler, Şekerler, Toplam fenol içeriği.

## INTRODUCTION

Banana (*Musa cavendishii* L.) is a fruit in the family Musaceae of Scitamineae. The Musaceae family has two main genera, Musa and Ensete. The genus Musa includes the edible cultivated species and the wild species Ensete, found in the forests of East Africa. The banana plant is native to the islands between South China, India, and Australia. Of the bananas cultivated in the world, 41% are “Cavendish”, 14% are “Gros Michel”, 21% are “Plantain” and 24% are cooking bananas.

Banana fruit is considered a good source of nutrients as it is rich in bioactive phenols, antioxidants, and potassium (Williams, 1995). Banana fruit is also rich in vitamins. It is important for human health and nutrition and has a high energy value. Thanks to its simple digestion and flavor, it is consumed by all age groups in our country. According to Food and Agriculture Organization (FAO) statistics, Asia is the largest banana producer, accounting for 54.4 percent of world banana production. According to FAO 2023 data, India ranks first with 36.614.000 tonnes, China second with 12.062.222 tonnes, Indonesia third with 9.335.232 tonnes, and Brazil fourth with 6.855.724 tonnes. In 2023, world banana production was 139.277.894 tonnes (Anonymous, 2025a). Although the latitudes and longitudes of our country are outside the banana growing areas, it is noteworthy that bananas have increased significantly in our country in recent years. According to TUIK data, banana production in Turkey for 2024 was realized as 875.000 tonnes (Anonymous, 2025b).

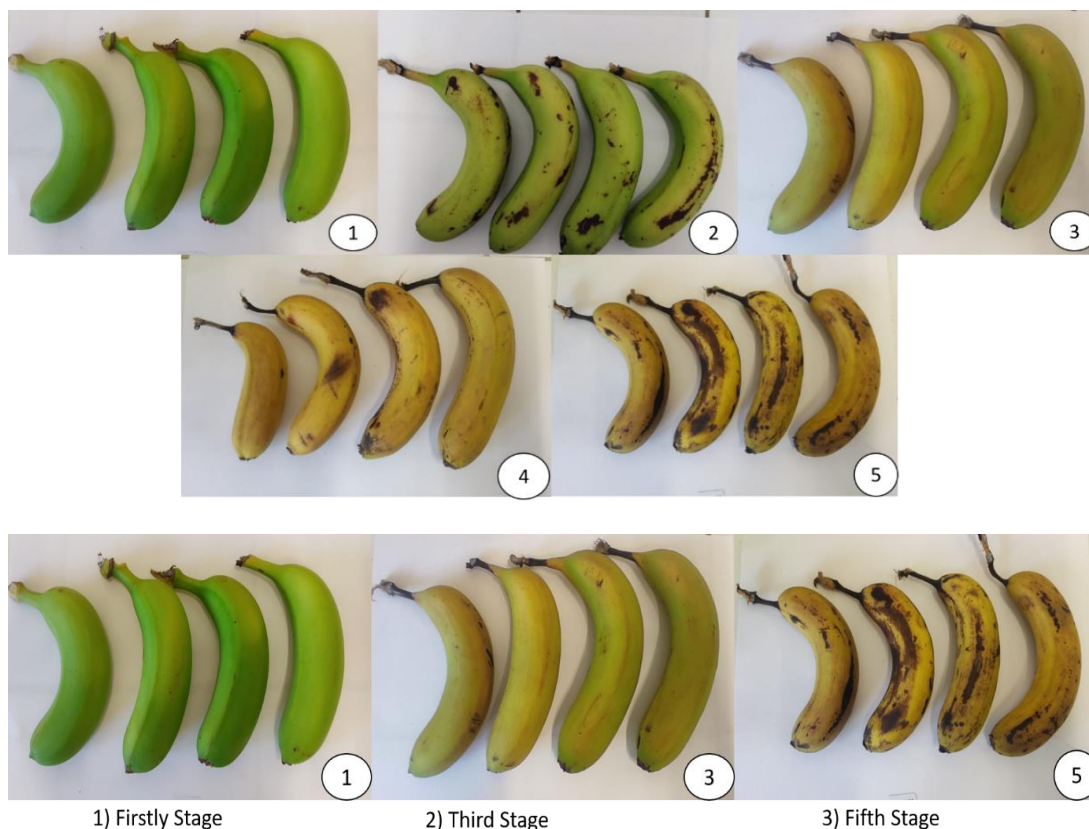
Several studies on banana pulp have examined its properties, ranging from its use as a food supplement to the extraction and recovery of starch, cellulose, and bioactive phytochemicals (Singh et al., 2016). Usually, only ripe pulp is consumed due to its high sugar content and sensory aspects. However, green fruit is also consumed in some regional dishes due to its high starch content. In addition, the inclusion of green fruit flour in some products such as biscuits, fiber-rich bread, and edible films is increasing. Consumption of green bananas (peel and pulp) is beneficial for human health due to their high content of resistant starch, which acts as food fiber in the body (Rodríguezambriz et al., 2008; Oliveira et al., 2015). In addition, banana flour can be an important source of polyphenols, compounds that are considered natural antioxidants (Vergara-Valencia et al., 2007). Banana is a tropical plant that can protect from the oxidative stress caused by high intensity of sunlight and elevated temperature by raising its antioxidant ability (Kanazawa and Sakakibara, 2000). Banana fruits contain many antioxidant compounds such as vitamin E,  $\beta$ -carotene, vitamin C, and flavonoids in both pulp and peel tissues. Macheix et al. (1990) reported that banana pulp contains high levels of total phenolics and tannins. In addition, some enzymes play an important role in increasing the antioxidant capacity of banana pulp (Someya et al., 2002).

Fruit ripening is a highly coordinated, genetically programmed, and irreversible process involving several physiological, biochemical and organoleptic changes such as chlorophyll degradation, increased respiration, ethylene production, biosynthesis of anthocyanins, essential oils, carotenoids, flavor and aroma compounds and activation of cell wall degradation (Prasanna et al., 2007). Banana fruit is a climacteric fruit and respire after harvest, resulting in ripening and browning of the skin color and a decrease in fruit flesh firmness due to ethylene production (Seymour, 2012). Banana fruit has a maximum shelf life of 6 to 8 days after starting to ripen during post-harvest storage (Huang et al., 2014). With the increase in banana production and consumption, knowledge in post-harvest handling is required. A range of pre-harvest and post-harvest factors influences physico-chemical properties, nutritional value, and antioxidant components in fruits. (Xie et al., 2016). Understanding the physico-chemical properties and nutritional characteristics of fruit is important for the design of cost-effective and efficient post-harvest handling equipment, and optimization of bioprocesses in functional food production and pharmaceuticals. Currently, there is a lack of scientific knowledge on the key nutritional changes that occur during the ripeness of banana (*Musa cavendishii* L.) cultivars grown in Türkiye. These fruits do not reach maturity at the same time, green/yellow to ripe fruits coexist on the same tree. Typically, during harvesting, all fruits regardless of their ripening are often picked together. The study aimed to determine rind color, total phenol content, total antioxidant capacity, sugars, and organic acid in different maturity stages across Azman banana cultivar grown in Adana province of Türkiye.

## MATERIALS AND METHODS

### Plant Material

Plants of 'Azman' banana cultivar grown under greenhouse conditions in Sarıçam district of Adana province, Turkey were used as plant material. Fruits were harvested as shown in Figure 1 and analyzed in three stages: first stage, third stage, and fifth stage.



**Figure 1:** Different ripening stages of banana fruit.

## Method

The fruits harvested at different periods in the greenhouse were immediately brought to the Instrumental Analysis Laboratory of the Department of Horticulture, Faculty of Agriculture, Çukurova University. The fruits of Azman banana cultivar were peeled and the pulp was homogenized. It was then stored at  $-20^{\circ}\text{C}$  until analysis. Peel color, total phenolic matter, antioxidant activity, organic acids, and individual sugars were analyzed by spectrophotometer and HPLC (High Performance Liquid Chromatography) with 3 replicates, respectively.

## Color Measurement

The fruit color of different maturity stages Azman banana cultivar measured as  $L$ ,  $a^*$ ,  $b^*$  using Minolta chromometer (Minolta 86 Co model CR-400, Tokyo, Japan) and calculated by a formula and given also as  $C$  and hue ( $h^{\circ}$ ) values of the outer color of the The device was calibrated with a standard white calibration plate before the measurements. Croma ( $C^*$ ), which determines the saturation and vividness of the color, and Hue angle ( $hue^*$ ), which determines the basic components of color (red, yellow, blue, and green), were calculated from the  $a^*$  and  $b^*$  values obtained fruits (Kılıç et al., 2021).

$$C^* = (a^{*2} + b^{*2})^{1/2}, \text{ hue}^* = \tan^{-1} (b^*/a^*)$$

## Determination of total phenol

After homogenizing and weighing banana fruit samples at different stages of ripeness, the total phenolic content of the juice was determined colorimetrically using Folin Ciocalteu reagent (Spanos, 1990). The absorbance value at 760 nm wavelength in the spectrophotometer (MultiskanTM GO microplate spectrophotometer) and the calibration curve created with gallic acid were used to calculate the metric values. The results are given in milligrams of gallic acid equivalent per 100 grams of weight (mg/GAE 100 g).

## Determination of total antioxidant capacity

Banana fruits were weighed and homogenized after harvesting and the total amount of antioxidants in the juice was determined using the DPPH (1,1-diphenyl 2 picrylhydrazyl) method proposed by Brand Williams (1995) with slight modifications. DPPH was prepared fresh at  $0.06 \mu\text{M}$ . The mixture was blended for 1 minute

before being kept in the dark for 30 minutes at room temperature. Then, 1950  $\mu\text{L}$  DPPH- was added to 50  $\mu\text{L}$  banana sample. Absorbance of mixture was measured at 515 nm. Radical scavenging activity %DPPH inhibition was calculated using the following equation:

$$\% \text{Inhibition} = 100 \times [(\text{Abs blank (t = 30)}) - (\text{Abs sample})] / [(\text{Abs blank (t = 30)})]$$

### Liquid chromatographic analysis of sugars

Glucose, fructose, sucrose, and total sugar contents at different ripening stages of banana fruit samples were determined using the HPLC technique, following the method developed by Crisosto et al. (1997).

### Determination of organic acids

Organic acids in different ripening stages of banana fruits were determined by the HPLC analysis (Bozan et al., 1997). The malic, oxalic citric and succinic acid contents in banana fruit samples were determined.

### Statistical analysis

The experiment was performed with a completely randomised experimental block design in three replications for one cultivar and three stages (Green, Medium Ripe, Over Ripe) in each replicate. Data were processed with the SPSS package program version 23.0 (SPSS Inc., Chicago, IL, USA). All data were presented as the mean  $\pm$  standard error (SE) and analyzed by one-way analysis of variance (ANOVA). Differences were considered significant at  $p < 0.05$  (Steel et al., 1997).

## RESULTS AND DISCUSSION

Results of fruit skin color of banana cultivars are presented in Table 1. In the study, there were significant differences in skin color ( $L^*$ ,  $a^*$ ,  $b^*$ , Croma\* and Hue\*) during the ripening period. Fruit skin color ( $L^*$ ,  $a^*$ ,  $b^*$ , Croma\* and Hue\*) was found to be 55.67, -4.20, 35.87, 36.10, 83.39 in green banana (first stage), 63.30, 0.23, 43.20, 43.20, 89.72 in medium ripe (third stage) banana and 64.53, 4.13, 44.30, 44.53, 95.44 (fifth stage) in overripe banana. Color change in the fruit is due to the breakdown of chlorophyll and color change increases with chlorophyll degradation as ripening progresses (Knee, 1972). In the study, significant changes occurred in color values during storage. It was determined that color values increased in proportion to ripening time. The  $L^*$ ,  $a^*$ ,  $b^*$  values we found in this study were similar to Alkarkhi et al. (2011) and Watharkar et al. (2020).

**Table 1.** At different stages of ripeness Color Measurement values of banana samples

Ripeness stages	$L^*$	$a^*$	$b^*$	Croma*	Hue*
First Stage (Green)	55.67 $\pm$ 1.67b	-4.20 $\pm$ 1.61c	35.87 $\pm$ 1.19b	36.10 $\pm$ 1.03b	83.39 $\pm$ 2.21c
Third Stage (Medium Ripe)	63.30 $\pm$ 0.84a	0.23 $\pm$ 1.11b	43.20 $\pm$ 1.54a	43.20 $\pm$ 1.53a	89.72 $\pm$ 1.51b
Fifth Stage (Over Ripe)	64.53 $\pm$ 1.59a	4.13 $\pm$ 0.42a	44.30 $\pm$ 0.93a	44.53 $\pm$ 0.89a	95.44 $\pm$ 0.79a
Sig	0.02	0.02	0.01	0.01	0.01

The difference between means denoted by the same letter is not statistically important ( $p < 0.05$ ).

As a result of this study, it was found total antioxidant and phenolic content of banana samples have changed in different ripening periods. In addition, the statistical differences in the results of TPC and DPPH radical scavenging activities ( $P < 0.05$ ) between banana samples were found to be significant. Total phenol was found to be 27.66 mg GAE /100g in green banana (first stage), 45.02 mg GAE /100g in medium ripe (third stage) banana, and 90.12 mg GAE /100g (fifth stage) in overripe banana. When antioxidant contents were compared, the highest %DPPH radical scavenging value was found in the fruit of the ripe banana (80.95%), while the lowest %DPPH radical scavenging value was found in the fruit of the medium ripe banana (38.80%) (Table 2). We determined that green bananas have lower total phenolic content than overripe fruits. Bilgin et al. (2022) found that the total phenolic and antioxidant content of 'Grand Naine' banana fruits at different ripening stages increased at green (first stage) and extreme ripeness (seventh stage) and decreased at medium ripeness (fourth stage). Fatemeh et al. (2012) also showed that green bananas have lower total phenolic content than ripened fruits. The extracts' radical scavenging abilities (DPPH inhibition) ranged from 26.55 to 52.66 percent (first stage to seventh stage). González-Montelongo et al. (2010) compared different solvents for DPPH scavenging activities. They found that acetone:water extracts had the highest antioxidant activity compared to the other solvents studied, with a factor of 1.3-1.9 (methanol) and 25-35 (acetone) for the DPPH assay and a factor of 2-4 (methanol) and about 10-35 (ethanol, acetone and water in "Grande Naine" banana cultivar and ethanol, acetone and water in "Gruesa") for



the ABTS+ assay. Total antioxidant activity increased with ripening and decreased rapidly with senescence in the banana cultivars studied. In the study, an increase in total phenol and antioxidant value was observed as the ripening time of the fruit increased. Ngoh Newilah et al. (2008) obtained similar results in hybrid bananas where total phenolic content increased with ripening and decreased with time at the full ripening stage. The findings of this study are similar to the literature.

**Table 2.** At different stages of ripeness, total phenolic content (mg/ GAE 100g) and DPPH (%) radical scavenging values of banana samples

Ripeness stages	DPPH radical scavenging%	Total Phenol
First Stage (Green)	38.80±2.84c	27.66±0.85c
Third Stage (Medium Ripe)	65.98±1.43b	45.02±1.54b
Fifth Stage (Over Ripe)	80.95±1.46a	90.12±7.70a
Sig	0.02	0.01

The difference between means denoted by the same letter is not statistically important ( $p < 0.05$ ).

The HPLC sugar profiles show that sucrose is the major sugar, followed by fructose and glucose in all banana samples (Table 2). Especially fructose and glucose contribute both as energy sources and in terms of taste and flavor (Çalışkan and Bayazit, 2012). Ripe bananas contain about 20-25% starch by fresh weight (Do Nascimento et al., 2006). During ripening in banana fruit, one of the main mechanisms causing fruit softening is starch breakdown, (Prasanna et al., 2007), which also provides carbon for sucrose synthesis (Saraiva et al., 2013). The content of soluble sugars (sucrose, glucose and fructose) in the 'Azman' banana increased with fruit ripening (Table 3). In the study, there were significant differences in sugars (sucrose, fructose, and glucose) during the ripening period. Sugar content color (sucrose, glucose fructose, and total sugar) was found to be 262.26, 68.58, 0.00, 330.85 mg/100g in green banana (first stage), 636.07, 113.72, 102.49, 852.28 mg/100g in medium ripe (third stage) banana and 894.58, 293.27, 206.07, 1393.92 mg/100g (fifth stage) in overripe banana. Azman showed increasing sugar content as ripening progressed, being highest when the fruit was fully ripe. Bilgin et al., (2022) examined the sugar content of 'Grand Naine' banana fruits at different ripening stages and found that glucose and fructose values gradually increased in green (first stage), medium ripeness (fourth stage), and extreme ripeness (seventh stage), while sucrose value increased in green (first stage) and extreme ripeness (seventh stage) but decreased in medium ripeness (fourth stage). Fernando et al. (2014) found that the sugar content of Khai banana cultivar increased as ripening progressed and was highest after 8 days of storage. Again, in the study on Hom Thong banana cultivar, they observed that the increases in sugar content occurred during the first 4 days of storage. During ripening, Hom Thong banana cultivar showed the same characteristics as Azman cultivar during ripening. As the banana ripens, starch is converted into sugar and therefore an increase in sugar content occurs, which is a typical characteristic of banana fruit (Valerio-Traya et al., 2002). Cordenunsi and Lajolo (1995) also noticed a significant decrease in starch content accompanying the increase in sugar content. In the study, fructose concentration was lowest and sucrose content was highest in the green and mid-ripening stages, indicating that sucrose dominates glucose and fructose as the rind matures. (Soares et al., 2011). On the other hand, the differences between glucose and sucrose contents are very small at the full maturity stage. The findings of this study are similar to the literature.

**Table 3.** Shows the results of free sugars in banana samples (mg /100 g fresh weight)

Ripeness stages	Sucrose	Glucose	Fructose	Total sugar
First Stage (Green)	262.26±0.80c	68.58±0.75c	0.00±0.00c	330.85±1.36c
Third Stage (Medium Ripe)	636.07±8.43b	113.72±1.03b	102.49±2.48b	852.28±6.87b
Fifth Stage (Over Ripe)	894.58±1.63a	293.27±1.48a	206.07±1.78a	1393.92±1.31a
Sig	0.00	0.00	0.00	0.00

The difference between means denoted by the same letter is not statistically important ( $p < 0.05$ ).

Organic acids are known to affect flavor formation and many physiological processes in fruits depending on the cultivar. Sugar-acid balance and their contents play an important role in determining the taste characteristics of fruits. In this study, oxalic, citric, malic, and succinic acid contents of Azman banana cultivar were analyzed and the results are given in Table 4. According to the results, malic acid is the major organic acid in fruits of banana cultivar. The content of citric, malic, and succinic acids among organic acids in Azman banana increased with fruit ripening while Oxalic acid decreased. Organic acids (citric, malic succinic and oxalic) were found to be 340, 90, 90 mg/100g in green banana (first stage), 370, 340, 90, 40 mg/100g in medium ripe (third

stage) banana and 450, 790, 160, 0 mg/100g (fifth stage) in overripe banana. Malic acid content increased during ripening from 280 mg/100 g in green bananas (first stage) to a maximum in ripe bananas (fifth stage). This behavior of malic acid is in good agreement with Wyman and Palmer (1964) and Agravante et al. (1991).

Maduwanthi et al. (2019) found that citric acid was the most abundant organic acid in the green stage (stage 1), while tartaric acid and oxalic acid were found in low amounts of  $16.1 \pm 2.3$  and  $30.8 \pm 1.6$  mg/100 g, respectively, as a result of Ethephon and Acetylene treatments to Ambul banana cultivar. They also reported that citric acid reached the highest level on day 6 (stage 4), while the amount of malic acid was  $200.83 \pm 2.08$  mg/100 g in stage 1 and reached the maximum ripening level on day 8 (stage 6). Kheng et al. (2012) observed that malic, citric, and succinic acids changed during ripening in Rastali banana cultivar at 11 and 12 weeks. Citric and succinic acid levels decreased as bananas harvested at the 11th and 12th weeks ripened and reached the lowest value on day 5. They also found that malic acid levels increased as ripening progressed from day 0 to day 5 for both bananas harvested at two different stages of ripeness. The findings of this study are similar to the literature.

**Table 3.** Shows the results of organic acids in banana samples (mg / 100 g fresh weight)

Ripeness stages	Citric Acid	Malic Acid	Succinic Acid	Oxalic Acid
First Stage (Green)	340±0.01b	280±0.00c	90±0.00b	90±0.00a
Third Stage (Medium Ripe)	370±0.00b	340±0.00b	90±0.00b	40±0.00b
Fifth Stage (Over Ripe)	450±0.01a	790±0.00a	160±0.00a	0±0.00c
Sig	0.01	0.00	0.00	0.00

The difference between means denoted by the same letter is not statistically important ( $p < 0.05$ ).

## CONCLUSION

Bananas are of great importance in human nutrition due to their rich content of bioactive compounds such as phenolics, carotenoids, and biogenic amines. Many of these compounds possess antioxidant properties and are effective in protecting the human body against certain oxidative stress conditions. In this study, the "Azman" banana cultivar was used as plant material. This variety holds a high market value due to its aromatic and flavorful nature as well as its durability during transportation. Moreover, the Azman banana variety is more resistant to low temperatures compared to other banana varieties, and its plants are robust and productive. In this study, the rind color, total phenols, antioxidants, sugars, and biochemical contents of the Azman banana cultivar grown in a greenhouse in the Sarıçam district of Adana province in the Mediterranean region were analyzed at different ripening stages using the HPLC technique. The results showed that the  $L^*$ ,  $a^*$ , and  $b^*$  color values increased proportionally with the ripening process. Total phenolic content and DPPH radical scavenging activity were found to increase as the fruit ripened. Among the organic acids in Azman bananas, citric, malic, and succinic acid levels increased with ripening, while oxalic acid levels decreased. Additionally, the content of soluble sugars (glucose, sucrose, and fructose) increased significantly during ripening. As ripening progressed, glucose, fructose, sucrose, and total sugar levels showed a sharp increase. The findings indicated that fruits at the fifth ripening stage were richer in biochemical properties and were in their ideal consumption phase. The HPLC technique provided detailed insights into the composition of sugars and organic acids, serving as an effective tool for evaluating the impact of technological processes. This study, which examines the effects of different ripening stages on the bioactive content of the Azman banana cultivar, serves as an important reference source for future research.

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## Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

## Author Contributions

**1<sup>st</sup> Yakup POLAT:** Conceptualization; data curation; formal analysis; funding acquisition

**2<sup>st</sup> Esra EKŞİ :** Investigation; methodology; project administration

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## Tarımda Dayanıklılık ve Sürdürülebilirlik: Kavramsal Çerçeve ve Yeni Yaklaşımlar

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### ABSTRACT

Up to date, concepts of resilience and sustainability have been used without a clear distinction in terms of meaning and purpose in previous studies. The lack of information on the similarities and differences between sustainability and resilience has led to problems in both academic and applied studies. Therefore, the objectives of the study are (i) to reveal the revolution of the concepts of sustainability and resilience, (ii) to examine the working frameworks based on different type of relationship between sustainability and resilience, (iii) to reveal the advantages and disadvantages of existing working frameworks in different applications, and (iv) to determine the historical development and future research trends of studies on sustainability and resilience. Critical literature review, bibliometric analysis, thematic analysis and meta-analysis were used to explore the development, general characteristics and trends of the existing literature focusing on sustainability and resilience. The research findings showed that the concept of sustainability focused more on protection and output, while focus of the concept of resilience were adaptation/improvement and process. The research findings also showed that the competition and complementarity relationships between the concepts of sustainability and resilience were not adequately addressed in previous studies, resulting in encountering major problems in previous academic studies and practice. It was determined that robustness and resilience were used in the same sense in previous studies conducted in Turkey. In addition, it was clear based on the research findings that future studies in the context of resilience and sustainability will focus on climate (resilience, vulnerability, climate change, sustainability, adaptation, vulnerability, governance, climate change adaptation and transformation) and social-ecological systems (ecosystem services, governance and resilient cities). Considering the competitive and complementary relationships between resilience and sustainability and selecting the appropriate working framework can increase the effectiveness of academic and applied studies.

**Key words:** Resilience, Sustainability, Framework, Bibliometric analysis, Meta-analysis.

## Tarımda Dayanıklılık ve Sürdürülebilirlik: Kavramsal Çerçeve ve Yeni Yaklaşımlar

### ÖZ

Günümüze kadar yapılan birçok çalışmada bu iki kavram anlam ve amaç açısından net bir ayrım yapılmaksızın kullanılmıştır. Sürdürülebilirlik ve dayanıklılık arasındaki benzerlikler ve farklılıklar konusunda var olan bilgi eksikliği hem akademik çalışmalarda hem de uygulamalı çalışmalarda sorunlara yol açmıştır. Bu çerçevede bu çalışmanın amaçları (i) sürdürülebilirlik ve dayanıklılık kavramlarının tarihsel gelişimlerinin ortaya konulması, (ii) sürdürülebilirlik ve dayanıklılığı ilişkilendiren çalışma çerçevelerinin incelenmesi, (iii) mevcut çalışma çerçevelerinin farklı uygulamalardaki avantajlarını ve dezavantajlarının ortaya konulması ve (iv) sürdürülebilirlik ve dayanıklılık konularındaki çalışmaların tarihsel gelişiminin ve gelecek araştırma eğilimlerinin belirlenmesidir. Sürdürülebilirlik ve dayanıklılık konularına odaklanan mevcut literatürün gelişimi, genel nitelikleri

ve eğilimleri kritik literatür incelemesi, bibliyometrik analiz, tematik analiz ve meta analiz yöntemleri yardımıyla ortaya konulmuştur. Araştırma bulguları, sürdürülebilirlik kavramının daha çok korumaya ve çıktıya, dayanıklılık kavramının ise uyum/iyileştirme ve sürece odaklandığını göstermiştir. Araştırma bulguları ayrıca günümüze kadar yapılan çalışmalarda sürdürülebilirlik ve dayanıklılık kavramları arasındaki rekabet ve tamamlayıcılık ilişkilerinin yeterli düzeyde ele alınamadığını ve bu yüzden akademik çalışmalarda ve uygulamada büyük sorunlarla karşılaşıldığını göstermiştir. Türkiye’de gerçekleştirilen akademik çalışmalarda sağlamlık kapasitesi kastedilerek sağlamlık ile dayanıklılık kavramlarının aynı anlamda kullanıldığı belirlenmiştir. Ayrıca, yakın gelecekte dayanıklılık ve sürdürülebilirlik bağlamında çalışmaların iklim (dayanıklılık, kırılabilirlik, iklim değişikliği, sürdürülebilirlik, adaptasyon, güvenlik açığı, yönetim, iklim değişikliği adaptasyonu ve dönüşüm) ve sosyal-ekolojik sistemler (ekosistem hizmetleri, yönetim ve dayanıklı şehirler) odağında ilerleyeceği tespit edilmiştir. Dayanıklılık ve sürdürülebilirlik arasındaki rekabet ve tamamlayıcılık ilişkilerinin dikkate alınması ve uygun çalışma çerçevesinin seçilmesi yapılacak akademik ve uygulamalı çalışmaların etkinliğini artırabilecektir.

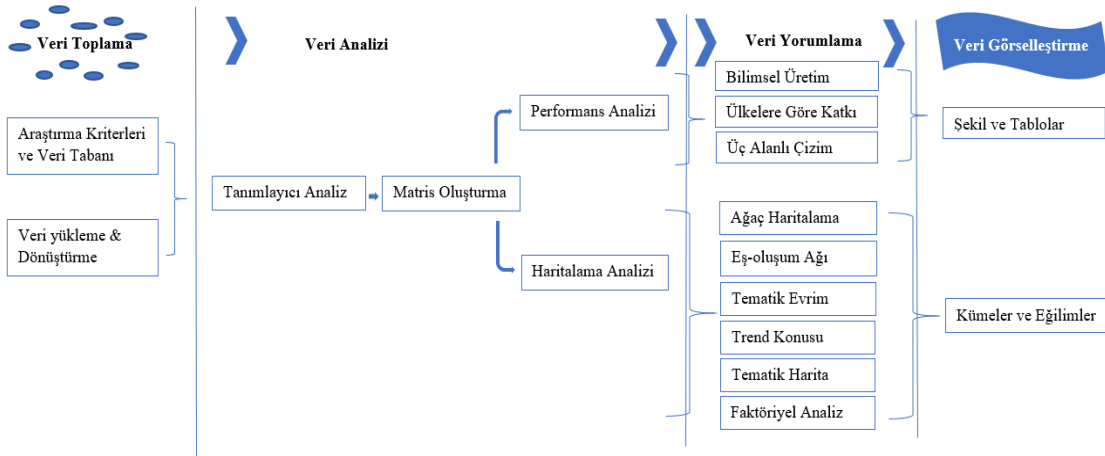
**Anahtar kelimeler:** Dayanıklılık, Sürdürülebilirlik, Çalışma Çerçevesi, Bibliyometrik analiz, Meta-analiz

## GİRİŞ

Dünya nüfusunun hızla artması ve çevresel sorunların derinleşmesi nedeniyle sekiz milyara ulaşan dünya nüfusunun gıda ihtiyacını karşılamak, tarımsal üretim sistemlerini sürdürülebilir ve dayanıklı hale getirmeyi zorunlu kılmaktadır. İklim değişikliği, kuraklık, fırtınalar ve gıda güvenliği gibi artan çevresel risk faktörleri tarım sektörünü tehdit etmektedir. Bu tehdit faktörlerine tedbir almak amacıyla acil müdahale kurumları, sivil toplum kuruluşları (STK) politika geliştirenler ve bu amacı benimseyenler, meydana gelebilecek riskleri kontrol edebilmek ve geleceği güvence altında tutabilmek için sürekli bir mücadelenin içinde bulunmaktadır (Dynes ve Quarantelli, 1975; Ward vd., 2017). Bu mücadeleler sürdürülebilirlik ve dayanıklılık kavramlarına olan ilginin giderek artmasına yol açmıştır (Redman, 2014). Ancak zaman içinde bu iki kavramın tanımları ve kullanımları birçok araştırmacı tarafından farklı şekilde yorumlanmıştır. Günümüze kadar yapılan birçok çalışmada bu iki kavram anlam ve amaç açısından net bir ayırım yapılmaksızın kullanılmıştır. Sürdürülebilirlik ve dayanıklılık kavramları sıkça kullanılan terimler olmasına rağmen, tanımları hakkında hala bazı belirsizlikler ve farklı görüşler bulunmaktadır ve tanımları kullanıldığı durum ve koşullara bağlı olarak değişiklik göstermektedir. Aralarında net sınırların olmaması nedeniyle bu iki kavramın ayırt edilmesi zaman içinde zorlaşmış ve kavram karmaşasına neden olmuştur. Sürdürülebilirlik ve dayanıklılık arasındaki benzerlikler ve farklılıklar konusunda var olan bilgi eksikliği hem akademik çalışmalarda hem de uygulamalı çalışmalarda sorunlara yol açmıştır. Karar vericiler sinerjilerden yeterli düzeyde yararlanamamış ve sürdürülebilirlik ile dayanıklılığın birbirleriyle rekabet eden hedeflerini hesaba katmada başarısız olmuşlardır. Günümüze kadar bu uygulama sorunları fazlaca deneyimlenmiştir. Birçok iklim değişikliği azaltma ve uyum stratejisinde (Lizarralde vd., 2015), kentsel yoğunlaştırma çabalarında (Landauer vd., 2015) ve afet müdahale çabalarında (Asprone ve Manfredi, 2015) sürdürülebilirlik ve dayanıklılık arasındaki benzerlikler ve farklılıklar konusunda bilgisizlik sebebiyle önemli sorunlar yaşanmıştır. Akademik çalışmalarda ise sürdürülebilirlik ve dayanıklılık kavramları kullanılırken kavramsal çerçeveye yeterli özen gösterilmeyip kavramlar yüzeysel olarak ele alınmış, kavramlar eksik veya yanlış kullanılmış ve kavramlar arası karmaşalar oluşmuştur. Bu durum, araştırma bulgularının yaygın etkisini olumsuz etkilemiş ve bulguların karşılaştırılmasını güçleştirmiştir. İki kavramın birbirine karıştırılma potansiyeli nedeniyle, günümüzde sürdürülebilirlik ve dayanıklılık kavramlarının kullanımında karmaşayı giderecek ve bu iki kavramın birbirleriyle etkin bir şekilde ilişkilendirilmesini sağlayacak çalışma çerçevelerin oluşturulmasına duyulan ihtiyaç literatürde yaygın olarak dile getirilmiştir (Anderies vd., 2013; Bocchini vd., 2014; Lizarralde vd., 2015; Saunders ve Becker, 2015; Xu vd., 2015). Sürdürülebilirlik ve dayanıklılık kavramlarının akademik çalışmalarda ve uygulama çalışmalarında birbirleriyle ilişkilendirilerek uygulanması için çalışma çerçevelerinin kullanılması ihtiyacı giderek artmaktadır. Sürdürülebilirlik ve dayanıklılık kavramları büyük, karmaşık ve önemli sistemik sorunları temsil ettiğinden ve benzerlikleri ile farklılıklarının eksik anlaşılması riski olduğundan bu kavramları kullanarak yapılan akademik araştırmalarda ve uygulamalı çalışmalarda kavramsal çerçevenin dikkatlice değerlendirilmesi önem taşımaktadır. Bu sebeple bu çalışmada sürdürülebilirlik ve dayanıklılık kavramlarının gelişimine ve aralarındaki rekabet ve tamamlayıcılık ilişkilerinin ortaya konulmasına odaklanılmıştır. Bu çerçevede bu çalışmanın amaçları (i) sürdürülebilirlik ve dayanıklılık kavramlarının tarihsel gelişimlerinin ortaya konulması, (ii) sürdürülebilirlik ve dayanıklılığı ilişkilendiren çalışma çerçevelerinin incelenmesi, (iii) mevcut çalışma çerçevelerinin farklı uygulamalardaki avantajlarını ve dezavantajlarının ortaya konulması ve (iv) sürdürülebilirlik ve dayanıklılık konularındaki çalışmaların tarihsel gelişiminin ve gelecek araştırma eğilimlerinin belirlenmesidir. Bu çalışmanın, dayanıklılık ve sürdürülebilirliğin etkili bir şekilde ilişkilendirilmesi, sürdürülebilirlik ve dayanıklılık çabalarının mevcut organizasyonlar ve proje çerçeveleri içinde tamamlayıcı bir şekilde çalışmasını sağlamaya yardımcı olacağı düşünülmektedir.

## MATERYAL VE METOD

Sürdürülebilirlik ve dayanıklılık kavramlarının zaman içindeki gelişimi kritik literatür incelemesiyle ve tanımların epistemolojik köküne inilerek ortaya konulmuştur. Sürdürülebilirlik ve dayanıklılık kavramlarına odaklanan daha önce yapılmış çalışmaları sistematik olarak gözden geçirmek için bibliyometrik analiz, tematik analiz ve meta-analiz olmak üzere üç yaklaşım kullanılmıştır. Kavramsal yapıyı görselleştirmek için bilimsel yayınların kapsamlı bir şekilde incelenmesi için titiz ve tekrarlanabilir bir metodoloji oluşturan bibliyometrik analizden yararlanılmıştır. Bibliyometrik analiz, nicel ölçütler kullanarak tanımlanmış bir zaman diliminde çalışmaların zamansal eğilimlerini ve kalıplarını tanımlamaya yardımcı olmaktadır (Garfield ve Sher, 1963; Broadus, 1987; Aria ve Cuccurullo 2017, Aria ve Cuccurullo 2023). Bibliyometrik analiz yapmak için R'deki Bibliometrix paket kullanılmıştır. Bibliyometrik ve tematik analizlerden çıkarılan temaları tasarlamak için meta-analiz kullanılmıştır (Borenstein vd. 2009). Ortaya çıkan temaları incelemek ve meta-analizi desteklemek için tematik analiz yapılmıştır. Çalışmada benimsenen metodoloji veri toplama, veri analizi, veri yorumlama ve veri görselleştirmenin de dahil olduğu dört farklı aşamadan oluşmaktadır (Pyle, 2003) (Şekil 1).



**Şekil 1.** Çalışmada benimsenen metodolojinin genel çerçevesi (Pyle, (2003)'den uyarlanmıştır)

Bibliyometrik analizde uygun araştırmaları belirlemek amacıyla, sistematik incelemeler ve meta-analizler için tercih edilen raporlama öğeleri olan PRISMA çerçevesi kullanılmıştır. PRISMA metodolojisi, tanımlama, tarama, uygunluğun değerlendirilmesi ve dahil etme sürecini tamamlamak üzere birbirini takip eden dört aşamadan oluşmaktadır (Liberati vd., 2009). Yayınlarla ilişkin bibliyografik veriler Web of Science veri tabanından 2004-2024 yıllarını kapsayacak şekilde elde edilmiş ve daha sonra BibTeX formatına dönüştürülmüştür. Veri toplama sürecinde, arama kriterleri olarak makale belge türü, başlıklar, yayın yılı, özetler ve anahtar kelimeler kullanılmıştır. Veri toplama sürecinde arama terimleri olarak "sustainability" ve "resilience" gibi spesifik anahtar kelimeler kullanılmıştır. Çalışmanın incelenmesi yayın yılı (2004-2024), belge türleri = (yayınlanmış makale) ve dil (İngilizce) ile sınırlandırılmış ve veri setine diğer çalışmalar olarak referans verilen çalışmalar da dâhil edilmiştir. Bu bağlamda yapılan kapsamlı bir değerlendirmenin ardından, bibliyometrik analiz için toplam 1263 çalışma incelenmiştir.

## BULGULAR VE TARTIŞMA

Araştırma bulguları sürdürülebilirlik ve dayanıklılık kavramlarının zaman içinde gelişim gösterdiğini ve farklı bilim alanlarının farklı tanımlamalar üzerinde durduğunu göstermiştir. Benzer şekilde, ulusal ve uluslararası düzeydeki literatürde aynı bilim alanında bile sürdürülebilirlik ve dayanıklılık kavramlarının tanımlamalarında ve kapsamında farklılaşmaların ve dağınıklığın olduğu tespit edilmiştir. Diğer taraftan, literatürde sürdürülebilirlik ve dayanıklılık kavramları arasındaki ilişki ihmal edilerek, bu iki kavramın araştırmalarda farklı yaklaşım ve anlayışlarla bir araya getirildiği görülmüştür. Araştırmada elde edilen bulgular sürdürülebilirlik ve dayanıklılık kavramları için ayrı ayrı olacak şekilde aşağıda belirtilmiştir.

### Sürdürülebilirlik Kavramı ve Gelişimi

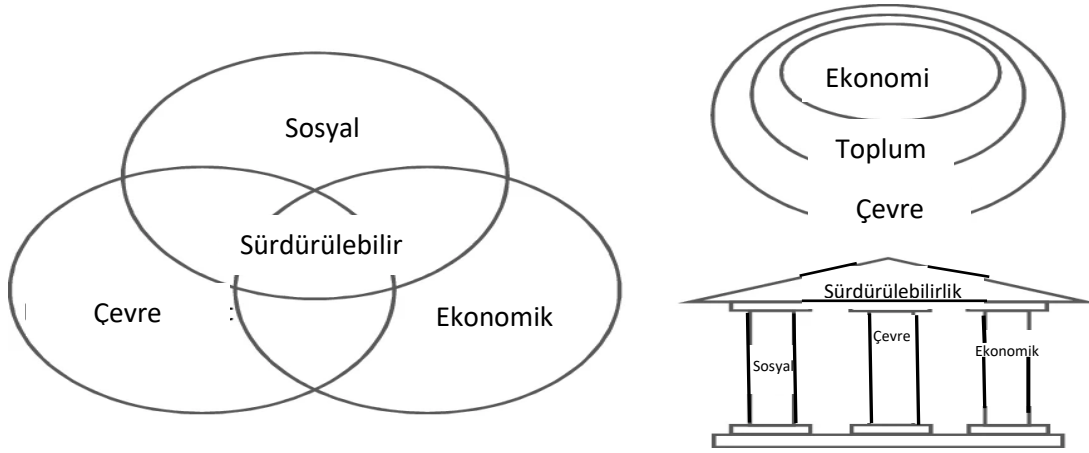
Sürdürülebilirlik Latince "*sustinere*" kelimesinden türemiş bir terimdir ve sürdürmek, desteklemek, ayakta tutmak veya dayanmak anlamında kullanılabilmektedir (Onions, 1964). Mevcut literatür, sürdürülebilirliğin standart bir tanımının yapılmasının mümkün olmayabileceği konusunda hemfikirdir. Sürdürülebilirlik, sayısız yorum ve bağlama özgü anlayışa sahip açık bir kavram olmaya halen devam etmektedir.

Çocuklarımız ve onlara bırakacağımız dünyayla ilgili olan sürdürülebilirlik, geçmişten günümüze kadar farklı kurumlar ve bilim insanları tarafından farklı şekillerde kullanılmış ve ifade edilmiştir. Sürdürülebilirlik kavramının ilk kullanımı 1700'li yıllara dayanmaktadır. Ormancılık alanında çalışan Evelyn ve Carlowits sürdürülebilir verim kavramını ilk kullanan araştırmacılar (Warde, 2011; Grober ve Cunningham, 2012). Ormancılıkta doğal bir kaynağın uzun vadeli ve sorumlu bir şekilde kullanılması anlamında kullanan Hans Carl von Carlowitz sürdürülebilirlik kavramı kullanımının öncülerinden olmuştur (Thory, 2019; Frederich ve Symons, 2022). Bin dokuz yüz altmışlı yıllarda sürdürülebilirlik daha çok çevresel sürdürülebilirliğe atıfta bulunmakta ve doğal kaynakların dikkatli kullanımına dikkat çekmektedir. Sürdürülebilirlik kavramının küresel anlamda modern bir görünüme kavuşması 1970'li yıllarda Meadows vd. (1972) tarafından ileri sürülen "Büyüme Sınırları" ve Ekoloji editörleri (1972) tarafından dile getirilen "sürdürülebilir bir dünya sistemi" isimli yayınlanmamış eserlerle başlamıştır (Grober, 2012). Sürdürülebilirlik kavramının hızla yaygınlaşması 1970'lerin başında olmuştur. Bin dokuz yüz yetmiş dört yılında "İnsanın ve Toplumun Geleceği" komisyonu, büyüme sınırlarının kullanılması yerine "sürdürülebilir bir toplum" kavramının kullanımını önermiştir (Grober 2012). Ekoloji Partisi (bugünkü İngiliz Yeşil Partisi) 1975 yılında "Sürdürülebilir Bir Toplum İçin Manifestoları" deklare etmiştir. Takiben sürdürülebilirlik kavramını öne çıkaran bir dizi kitap yayınlanmıştır (Stivers 1976; Buxton ve Meadows 1977; Pirages 1977; Cleveland 1979; Coomer 1979). Günümüzde en sık kullanılan sürdürülebilirlik tanımı Birleşmiş Milletler (BM) Dünya Çevre ve Kalkınma Komisyonuna (DÇKK) aittir. BMDÇKK sürdürülebilirliği "*gelecek nesillerin kendi ihtiyaçlarını karşılama yeteneğini tehlikeye atmadan bugünün ihtiyaçlarını karşılamak*" olarak tanımlamıştır (BM, 1987). UCLA ise sürdürülebilirliği sistem yaklaşımına vurgu yaparak "*bu nesil ve gelecek nesiller için gelişen, sağlıklı, çeşitli ve dayanıklı toplumlar yaratmak amacıyla çevresel sağlık, sosyal eşitlik ve ekonomik canlılığın bütünleştirilmesi*" şeklinde ifade etmiştir. Oxford İngilizce Sözlüğü sürdürülebilirliği "*çevresel olarak sürdürülebilir olma özelliği; bir sürecin veya girişimin doğal kaynakların uzun vadede tükenmesini önleyerek sürdürülebilirlik veya devam ettirilebilirlik derecesi*" olarak tanımlamıştır (Halliday, 2016). İyileştirme ve sürdürme kapasitesi yaklaşımını benimseyen Harrington (2016) ise sürdürülebilirliği uzun vadede arzu edilen malzemelerin veya koşulların durumunu ve bulunabilirliğini sürdürme veya iyileştirme kapasitesi olarak ifade etmiştir. Berg (2020), sürdürülebilirliği bir nevi kuşaklar arası etik biçimi olarak düşünmüş ve bir topluluğun sosyal kurumlar kümesinin veya toplumsal uygulamanın uzun vadeli uygulanabilirliği olarak tanımlamıştır.

Sürdürülebilirliğin iç içe geçmiş üç daire ile betimlenmesi ilk olarak Edward Barbier tarafından 1987 yılında yapılmıştır. Günümüze kadar farklı araştırmacılar sürdürülebilirliği farklı terimleri kullanarak açıklamışlardır. Bazı araştırmacılar üç sütun (pillars) paradigmasını kullanmayı tercih etmişlerdir (Basiago 1999; Pope vd., 2004; Gibson 2006; Waas vd., 2011; Moldan vd., 2012; Schoolman vd. 2012; Boyer vd. 2016). Bazı araştırmacılar ise boyut (dimension) terimini kullanmışlardır (Stirling 1999; Lehtonen 2004; Carter ve Moir 2012; Mori ve Christodoulou 2012). Diğer bazı araştırmacılar ise sürdürülebilirliği bileşen (components) (Du Pisani 2006; Zijp vd., 2015), tabure bacakları (stool legs) (Dawe ve Ryan 2003; Vos 2007), yön (aspects) (Goodland 1995; Lozano 2008; Tanguay vd. 2010) ve perspektifler (perspectives) (Brown vd., 1987; Arushanyan vd., 2017) terimlerini kullanarak açıklamışlardır. Sürdürülebilirlik kavramının üç sütun olarak ifadesinin literatürde baskın bir yorum olduğunu söylemek mümkündür (Purvis vd., 219). Gonzalez (2017) ise sürdürülebilirlik etrafındaki söylemlerin çoğunun ilk olarak Barbier (1987) tarafından kullanılan üç dairesel ölçüt etrafında düzenlendiğini belirtmiştir (Şekil 2). Ancak, Purvis vd. (2019) ise üç boyut paradigmasının teorik temelini zayıf olduğunu belirtmişlerdir. Basitlikleri nedeniyle çekici olsalar da bu diyagramların ilettiği anlam ve daha geniş 'sütun' kavramının kendisinin belirsiz olduğunu ve tutarlı bir şekilde işlevselleştirilme yeteneğini engellediğini iddia etmişlerdir. Ayrıca, söz konusu üç boyutun kavramsallaşmasının çok daha öncelere dayandığını düşünmektedirler.

O'Riordan (1985) ekolojik ve sosyokültürel olmak üzere iki ana sürdürülebilir kullanım türü önermiştir. Brown vd. (1987) ise literatür incelemelerine dayanarak sosyal perspektif, ekolojik ve ekonomik olmak üzere üç perspektif veya bağlam tanımlamışlardır. Sosyal perspektif, bireylerin temel insan ihtiyaçlarının sürekli tatmini ile ilgilidir. Ekolojik perspektif ekosistemlerin sürekli üretkenliği ve işleyişi ile genetik kaynakların korunması ve biyolojik çeşitliliğin muhafazası üzerine odaklanmaktadır. Ekonomik perspektif, sürdürülebilir bir toplumun ekonomik büyümeye koyması gereken sınırlamalar ile ilgilidir. Aynı yıl, Barbier (1987) kalkınma sürecini üç sistem (biyolojik sistem, ekonomik sistem ve sosyal sistem) arasındaki etkileşim olarak ifade etmiş ve kesişen daireler diyagramını ortaya koymuştur.





**Şekil 2.** Sürdürülebilirliği İfade Etmede En Sık Kullanılan Gösterimler

Her sisteme genetik çeşitlilik, dayanıklılık, biyolojik üretkenlik, temel ihtiyaçları karşılama (yoksulluğu azaltma), eşitliği artırma, faydalı mal ve hizmetleri artırma, kültürel çeşitlilik, kurumsal sürdürülebilirlik, sosyal adalet, katılım vb. gibi hedefler atfetmişlerdir. Sürdürülebilir kalkınmanın amacını, tüm bu sistemlerdeki hedefleri, bir uzlaşma süreciyle uyarlamalı bir şekilde maksimize etmek olarak belirlemişlerdir. Cocklin (1989), Barbier (1987)'den yararlanarak sürdürülebilirliği sosyal, ekonomik ve çevresel alt sistemlerle ilgili bir dizi hedef açısından kavramsallaştırmıştır.

Üç sütun paradigmasında yer alan sütunların kavramsallaştırılmasında, bazı araştırmacılar bireysel sütunları ayrı, ancak etkileşimli sistemler olarak düşünüp Barbier'in yaklaşımını benimsemişlerdir (Cocklin, 1989; Hancock, 1993; Basiago, 1995). Barbier (1987) ve Cocklin (1989) sistemlerin bütünleştirilmesinin ve aralarındaki takasların (trade off) yönetiminin önemini vurgulamışlardır. Sistem yorumuna alternatif olarak, "üç ayağı" sürdürülebilirliğin farklı perspektifleri olarak ele alan araştırmacılar da vardır. Bu araştırmacılar, sütunların bireysel olarak sahip olduğu hedeflerin bütünleştirilmesine ve dengelenmesine yani uzlaştırmaya vurgu yapmışlardır (Le'le', 1991; Campbell, 1996; Munasinghe, 1993; Altieri, 1995; Milne, 1996; Goodland ve Daly, 1996; Custance ve Hillier, 1998). Seçimlerin sonuçlarını bilmeden uzlaşmalar hakkında karar vermek zor olduğundan uygun bir fayda maksimizasyonu yaklaşımı sunulamamaktadır. Teori ile uygulama arasındaki bu eksiklik, Barbier ve Markandya (2013) ve Barbier ve Burgess (2017) tarafından daha sonraki çalışmalarda sorun olarak incelenmiştir. Ancak, bir yandan yetersiz teorileştirme ile diğer yandan uygulamaya olanak verecek gerekli politik değer yargılarında bulunma hususlarında var olan bilgi boşluğu günümüzde halen devam etmektedir.

Sürdürülebilirlik tanımlarında en güçlü ortak nokta, mevcut neslin ihtiyaçlarını karşılamak ile gelecek nesillerin ihtiyaçlarını tehlikeye atmadan kaynakları kullanma vurgusudur. Dengeli gelişim ve etkileşim, uzun vadeli perspektif ve toplumsal sorumluluk ve adalet diğer ortak noktalar. Bu ortak noktalar, sürdürülebilirlikte temel amacın her zaman geleceğin ihtiyaçlarını düşünerek bugünü yaşamak olduğuna işaret etmektedir. Yani, sürdürülebilirlik sadece bir çevre koruma meselesi değil, bir yaşam tarzıdır. Tarihsel süreçte yapılan tanımlamalardaki ortak noktalara dayanarak sürdürülebilirliği mevcut nesillerin ihtiyaçlarını karşılayarak çevresel, sosyal ve ekonomik dengeyi korurken, gelecek nesillerin ihtiyaçlarını tehlikeye atmadan kaynakları verimli bir şekilde kullanma ve uzun vadeli iyileşme sağlamayı amaçlayan bir yaklaşım olarak tanımlamak mümkündür.

Genellikle aynı şeyi ifade etmek için kullanılan "sürdürülebilirlik" ve "sürdürülebilir kalkınma" kavramları da birbirleriyle yakından ilişkilidir. Ancak, sürdürülebilirlik genel bir kavram iken, sürdürülebilir kalkınma bir politika veya düzenleme ilkesi olarak ele alınmaktadır. Bilim insanları sürdürülebilirliğin daha geniş bir kavram olduğunu, çünkü sürdürülebilir kalkınmanın esas olarak insan refahına odaklandığını söylemektedirler (Harrington, 2016). Sürdürülebilir kalkınma kavramı BM Brundtland komisyonu tarafından 1987 yılında yayınlanan rapor sonrasında uluslararası politika söylemine girmiş ve çevre hareketinin baskın paradigması haline gelmiştir. Sürdürülebilir kalkınmayı çevresel ve sosyal sorunları birbirine bağlayan küresel bir kavram olarak tanımlayan bu rapor, sürdürülebilir kalkınma kavramının popülarleşmesine ve yaygınlaşmasına sebep olmuştur (Basiago 1999; Castro 2004; Johnston vd., 2007; Pope vd., 2004; Redclift 2005). BM (1987) çevresel bozulmanın yoksulluğun azaltılmasıyla azaltılabileceğini savunmakta ve yoksulluğu azaltmak için geliştirmekte olan ülkelerin daha serbestleşmiş piyasalar gerektiren ekonomik büyümeyi teşvik etmektedir (Castro 2004). Ancak, BM'nin benimsediği sürdürülebilirlik yaklaşımının daha önce yapılmış eleştirel büyüme çalışmalarıyla çeliştiğini söyleyen araştırmacılar vardır. Tulloch (2013), Brundtland Raporunun sürdürülebilirliği ana akım yapan küresel çabalarına eleştirel yaklaşmış ve marjinal bir hareketi küreselleşmiş neoliberal politikayı meşrulaştırmak ve gizlemek için bir



platforma dönüştürmekten sorumlu olduğunu savunmuştur. Nitekim Dryzek (2005) çevresel söylemleri kategorize ederken, sürdürülebilirliği sınırlar söylemi gibi sistemik değişimi savunan “radikal” söylemlerin aksine “reformist” olarak tanımlamaktadır.

### Dayanıklılık Kavramı ve Gelişimi

“Resilience” kavramı farklı disiplinlerde farklı Türkçe kelime ile ifade edilmiştir. Kaya (1994) “sürdürerek aşma” terimini kullanmayı tercih etmiştir. İşletme biliminde İngilizce kavramın Türkçe okunuşu olan “rezilyans” olarak kullanılmıştır (Kırbaşlar ve Yılmaz Börekçi, 2014). Bazı araştırmacılar, “yine/yenilenme” terimini kullanmayı önermişlerdir (Gerçek ve Yılmaz Börekçi, 2014; Gerçek ve Yılmaz Börekçi, 2019). Ancak bütün disiplinler için geçerli olacak ortak bir terim olmadığından bu çalışmada günümüze kadar en çok kullanılan “dayanıklılık” teriminin kullanımına devam edilmiştir. Dayanıklılık kavramı, yirminci yüzyılın başlarında bilimsel yayınların başlıklarında yer almaya başlamıştır. Dayanıklılık kelimesi Latince kökenli ‘resilio’ kelimesinden gelmektedir ve “geri sıçramak” veya “geri dönmek” anlamına gelmektedir. Genellikle, bir şeyin eski haline veya başlangıç noktasına geri dönme eylemini ifade eden dayanıklılık kavramı, başlangıçta malzeme bilimi ve özellikle tekstil araştırmalarında ön plana çıkmıştır. Söz konusu alanlarda dayanıklılık, malzemelerin fiziksel şoklara karşı direncini ifade etmek için kullanılmış ve bir şeyin bir tür bozulmadan sonra bir referans durumuna gerilebilme yeteneği olarak düşünülmektedir (Winson, 1932; Hoffman 1948). Bin dokuz yüz yetmişli yıllardan günümüze kadar dayanıklılık kavramının en fazla kullanıldığı alanlar psikoloji ve ekolojidir. Bu dönemde her iki disiplinde de dayanıklılık kavramı uzun tartışmaların konusu olmuştur. Psikolojide dayanıklılık zorluklara rağmen gelişimsel hedeflere ulaşmak, stres altında sürdürülebilir yeterlilik ve travma sonrasında iyileşme yeteneği olarak kullanılmıştır (Murphy, 1974) Psikoloji alanında dayanıklılık kavramının kullanılmasında amaç genellikle bireylerin dayanıklılığını açıklayan koruyucu faktörleri veya koruyucu mekanizmaları bulmak olmuştur (Rutter 1987). Ekoloji alanında ise dayanıklılık, çeşitlilik ve istikrarın birlikte hareket ettiği fikri üzerine tartışma bağlamında anlaşılmış ve 1950'ler ve 1960'lar boyunca ekoloji alanında kabul görmüştür (MacArthur, 1955; Elton, 1958). Bu dönemde dayanıklılık yaygın kullanılan bir terim olmuştur ve dayanıklılık kavramı direnç (resistance), kalıcılık (persistence), sabitlik (constancy), histerezis (hysteresis) ve elastikiyet (elasticity) gibi terimlerle birlikte kullanılmıştır. Yirminci yüzyılın sonlarında Grimm ve Wissel (1997) istikrar ile ilgili 70 farklı terim ve 163 tanım listelemiş ve özel öneme sahip iki dayanıklılık kavramı ortaya koymuştur. Bunlardan birincisi, geçici bir bozulmadan sonra referans durumuna (veya dinamiğe) geri dönme şeklindedir. Schrader-Franchette ve McCoy (1993) buna “dinamik denge” adını verirken, Holling (1973) önce “istikrar” demeyi tercih etmiş, daha sonraki çalışmalarında “mühendislik dayanıklılığı” adını vermiştir (Holling 1996; Holling ve Gunderson 2002). İkinci dayanıklılık tanımı Holling (1973) tarafından yapılan tanımdır. Başlığında dayanıklılık kelimesi bulunan ve en çok atıf alan makalesinde Holling (1973) istikrar ve dayanıklılık kavramları arasında bir ayrım yapmıştır. Dayanıklılığın bir sistem içindeki ilişkilerin devamlılığını belirlediğini, bu sistemlerin durum değişkenlerindeki, sürücü değişkenlerindeki ve parametrelerdeki değişiklikleri absorbe etme ve yine de devamlılık gösterme yeteneğinin bir ölçüsü olduğunu ifade etmiştir. Bu tanımlamada dayanıklılık sistemin bir özelliğidir ve devamlılık veya yok olma olasılığı da bunun sonucudur (Holling, 1973). Bu anlayışa göre dayanıklılık referans durumuna geri dönmekle ilgili değildir, daha ziyade sistem içinde bir tür tampondur. Sistemin rahatsız edildiğinde belirli yapısal özelliklerini korumasına izin veren bir marjdir. Holling (1973) için dayanıklılık, bir sistemin kendi dinamikleri aracılığıyla yok olacağı bir yörüngeye itilmeden ne kadar büyük bir rahatsızlığa dayanabileceğini bize söyleyen ve ideal olarak ölçülebilen bir büyüklüktür. Orians (1975) bir sistemin dışsal bozulmalara direnme yeteneği için atalet (inertia) terimini kullanmıştır. Schrader-Franchette ve McCoy (1993), bu amaçla “kalıcılık” terimini kullanmayı tercih ederken, Grimm ve Wissel (1997) Holling’in (1973) dayanıklılık tanımını “çekim alanı” terimiyle ilişkilendirmiştir.

Çok gündemde olmasına rağmen, herkesin hemfikir olduğu bir dayanıklılık tanımı yoktur. Dayanıklılık kavramı farklı kurum ve araştırmacılar tarafından farklı şekillerde tanımlanmaktadır. Ele alınan sistemler farklı formlar alabildiğinden dayanıklılık kavramı farklılaşmaktadır (Kumar vd., 2015). Keating vd. (2014) dayanıklılık kavramının ekoloji, mühendislik ve psikoloji gibi çeşitli alanlarda farklı şekilde yorumlandığını ve uygulandığını belirtmiştir. Dayanıklılık kavramının ilk kullanıldığı alanlardan biri olan ekolojik bilimlerde bile, önemli ölçüde farklı dayanıklılık tanımları mevcuttur (Brand ve Jax 2007). Ekoloji alanında çalışan bazı araştırmacılar dayanıklılığı, bir sistemin bir bozulmadan sonra denge durumuna ne kadar hızlı döndüğünün bir ölçüsü olarak kabul ederken, Holling (1973) dayanıklılığı, bir sistemin farklı bir rejime geçmeden nasıl bozulabileceğinin bir ölçüsü olarak tanımlamıştır. Walker vd. (2002), dayanıklılığı, bir sistemin belirli bir yapıda kalma ve bozulma kaynaklı değişimin ardından geri bildirimleri, işlevleri ve yeniden organize olma yeteneğini sürdürme potansiyeli olarak tanımlamaktadır. Bir sistemin, esasen aynı işlevi, yapıyı, geri bildirimi ve dolayısıyla kimliğini korurken şokları deneyimleme kapasitesidir (Walker vd. 2006). Brand ve Jax (2007), panarşi (panarchy) terimini ortaya atarak sistemik-sezgisel tanımı diğer dayanıklılık tanımlarını arasına eklemiştir. Panarşinin temel odağı, değişim ve kalıcılık, öngörülebilir ve öngörülemez arasındaki etkileşimi uygun bir şekilde dikkate almaktır. Holling vd. (2002),

deneyleri sürdüren, sonuçlarını test eden ve uyarlanabilir evrime izin veren yapıları temsil etmek için gömülü ölçekler arasındaki etki hiyerarşileri kavramından, yani panarşiden yararlanmışlardır. Farklı ekolojik dayanıklılık tanımları, sistemin rahatsızlıkları emme veya tamponlama ve yine de temel niteliklerini koruma yeteneği, sistemin kendi kendini organize etme yeteneği ve değişim bağlamında öğrenme ve uyum sağlama kapasitesi olmak üzere üç temel özelliğine dayanmaktadır (Berkas vd., 2003). Brand and Jax (2007) dayanıklılığı, birbirine bağlı sosyo-ekolojik sistemlerin, temel yapıları, süreçleri ve geri bildirimleri korumak için kasırgalar veya seller gibi tekrarlayan rahatsızlıkları absorbe etme kapasitesi olarak tanımlanmaktadır. Adger vd. (2005) dayanıklılık, karmaşık bir uyarlanabilir sistemin kendi kendini organize etme kabiliyetinin derecesini ve sistemin öğrenme ve adaptasyon kapasitesini inşa edebilme derecesini yansıttığını ifade etmişlerdir. Benzer şekilde Folke vd. (2002) sosyal-ekolojik sistemler için dayanıklılığın sistemin emebileceği ve belirli bir durumda kalabileceği şokun büyüklüğü, sistemin kendi kendini organize edebilme yeteneğinin derecesi ve sistemin öğrenme ve adaptasyon kapasitesini geliştirebilme derecesi ile ilgili olduğunu belirtmişlerdir. Brian Walker vd. (2004) ise dayanıklılığı, "bir sistemin değişime uğrarken rahatsızlığı absorbe etme ve yeniden organize olma kapasitesi, böylece temelde aynı işlevi, yapıyı, kimliği ve geri bildirimleri koruyabilme" olarak tanımlamıştır. Dayanıklılık kavramını "dayanıklılık düşüncesi" veya "dayanıklılık teorisinin" ötesinde çok daha geniş kapsamlı tanımlayan araştırmacılarda vardır (Folke vd, 2010; Strunz, 2012). Strunz (2012) dayanıklılığı bilimsel söyleme ve akademik kurumlara bağlı olmayan bir kaynak yönetimi yaklaşımı ve dünya görüşü olarak ifade etmiştir. Carpenter vd. (2001) dayanıklılık kavramının ekosistem-sosyal sistem metaforu olmadığını ancak metaforik bir aktarımın parçası olduğunu, karmaşık sosyal varlıkların/sistemlerin ekosistemler olarak görülmesi olduğunu belirtmiştir. Brand ve Jax (2007), dayanıklılığın bir tür sınır nesnesi olarak işlediğini ileri sürmüşlerdir. Bu tür nesnelerin esneklik, yerel ihtiyaçlara uyum sağlama ve katılık olmak üzere üç özelliği bulunmaktadır (Star ve Griesemer 1989; Collins, Evans ve Gorman 2007).

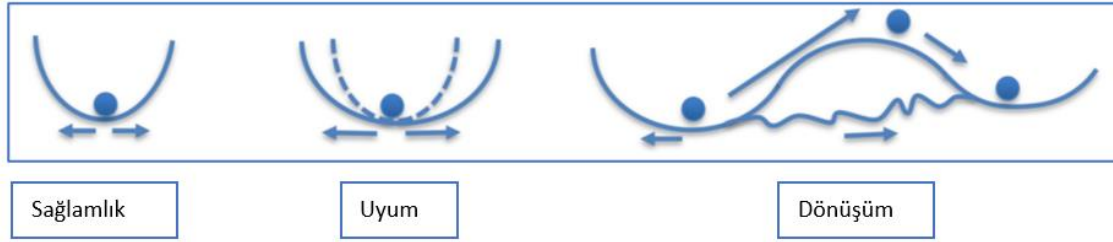
Dayanıklılık kavramı politika süreçlerinde çok kullanılmaya başladığından dayanıklılık tanımını farklı kurumlar tarafından da tanımlanmıştır. Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) dayanıklılığı, *"bireylerin, hanelerin, toplulukların, şehirlerin, kurumların, sistemlerin ve toplumların, çok çeşitli risklerle karşı karşıya kaldıklarında, kabul edilebilir bir işleyiş seviyesini korurken ve sürdürülebilir kalkınma, barış ve güvenlik, insan hakları ve herkes için refah için uzun vadeli beklentileri tehlikeye atmadan, olumlu, verimli ve etkili bir şekilde önleme, direnme, absorbe etme, uyarılma, yanıt verme ve kurtarma yeteneği"* şeklinde tanımlamaktadır (FAO vd., 2018). Hükümetler Arası İklim Değişikliği Paneli (IPCC) ise dayanıklılığı, *"sosyal, ekonomik ve çevresel sistemlerin tehlikeli bir olay, eğilim veya rahatsızlıkla başa çıkma, temel işlevlerini, kimliklerini ve yapılarını korurken aynı zamanda uyum, öğrenme ve dönüşüm kapasitelerini de koruyarak yanıt verme veya yeniden düzenleme kapasitesi"* olarak ifade etmiştir (IPCC, 2014). OECD dayanıklılığı *"olumsuzluğa veya koşullardaki bir değişikliğe direnme, bunları absorbe etme, bunlardan kurtulma veya bunlara başarılı bir şekilde uyum sağlama yeteneği"* şeklinde tanımlamıştır (OECD, 2014).

Farklı disiplinlerde farklı tanımlar olmakla birlikte dayanıklılık tanımlarını yerel ve küresel dayanıklılık olmak üzere iki grupta özetlemek mümkündür (Brand ve Jax, 2007). Yerel dayanıklılık, belirli bir sistemin tek bir çekim alanını ilgilendirdiği için bu şekilde anılmaktadır ve rahatsızlık/şoktan sonra geri dönme yeteneği olarak karakterize edilmektedir. Yerel dayanıklılık ile ilgilenen araştırmacılar sistemin geri dönüş hızıyla (Holling (1973) bunu istikrar olarak tanımlamaktadır) veya rahatsızlık/şok öncesi ve sonrası durum arasındaki farkla ilgilenmektedirler. Küresel dayanıklılık ise bir sistemin bir referans durumuna geri dönmesiyle değil, bir sistem tarafından bir rahatsızlığın emilmesiyle ilgilidir. Küresel teriminin seçimi, genellikle bir sistemin bir özelliği olarak kabul edilmesinden daha ziyade, bir sisteme ait bir özellik olarak kabul edilmesinden kaynaklanmaktadır. Küresel dayanıklılık belli bir rahatsızlığı absorbe (emme) ve koruma yeteneği olarak tanımlanmaktadır. Küresel dayanıklılık, sistem rahatsız edildiğinde sistemin bazı özelliklerinin sabit tutulmasını içermektedir, yani rahatsızlık sırasında bazı özelliklerin korunması anlamında kullanılmaktadır. Carpenter ve Gunderson (2001), küresel dayanıklılığın sistemlerin içsel bir özelliği olmadığını, ancak bir sistem ile belirli bir (tür) rahatsızlık arasındaki bir ilişki olduğunu ve belirli bir sistemin farklı rahatsızlıklara karşı farklı derecelerde dayanıklı olabileceğini belirtmişlerdir.

Birçok araştırmacı Holling (1973)'in dayanıklılık tanımının yalnızca ekosistemlerin değil, aynı zamanda sosyo-ekolojik, ekonomik ve sosyal dahil olmak üzere diğer alanların da dinamik özelliğini yakalayabileceğini düşünmektedirler (Gunderson ve Holling 2002; Adger vd. 2005; Folke vd. 2010). Brand ve Jax (2007) ve Strunz (2012) dâhil olmak üzere birkaç araştırmacı, dayanıklılık kavramının heterojen olduğunu belirtmiş ve bu heterojenliğin çeşitli şekillerde farklı disiplinleri birbirine bağlamada yardımcı olabileceğini savunmuştur. Dayanıklılık durumunda soyutlamanın, disiplinleri birbirine bağlamanın göz ardı edilen ve önemli bir başka yolu olduğu öne sürmüşlerdir. Günümüze kadar dayanıklılık kavramı ekseninde yapılan çalışmaların alanlar itibarıyla dağılımı, dayanıklılığın farklı alanlarda kullanılabileceği yaklaşımını doğrular niteliktedir. Son yıllarda dayanıklılık kavramı literatürde oldukça görünür hale gelmiş ve tarım politikası oluşturma sürecinde giderek daha fazla gündemde olan bir kavrama dönüşmüştür. Holling (1973) tarafından tanıtıldıktan günümüze kadar geçen sürede

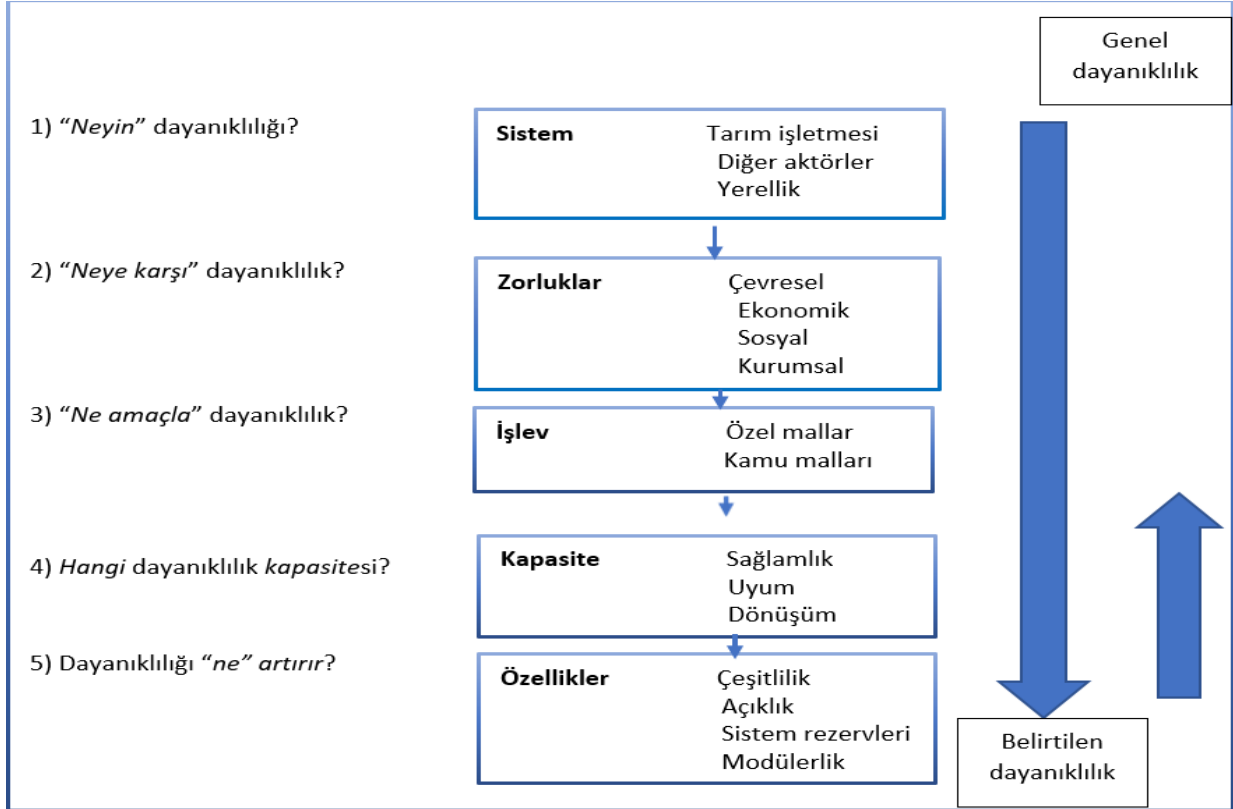
dayanıklılık kavramı, ekolojik sistemlerin yapısı ve işlevindeki değişim modellerini tanımlamak için kullanılan kavramsal bir çerçeveye dönüşmüştür. Ekolojik bilimlerde temellendirilmiş olmasına rağmen, dayanıklılık kavramı, doğa ve sosyal bilimciler arasında da popülerlik kazanmıştır. Bazı araştırmacılar sosyal-ekolojik sistemler arasındaki bağlantıları incelemiştir (Berkas ve Folke 1998; Davidson-Hunt vd., 2003; Armitage ve Johnson 2006; Walker vd., 2006). Diğer bazı araştırmacılar ise kurumsal ve örgütsel düzenlemeler arasındaki bağlantılara odaklanmıştır (Gunderson ve Holling 2002; Anderies vd., 2004). Holling (1973)'in tanımladığı dayanıklılık kavramı arkeoloji (Weiberg 2012), sosyoloji (Davidson 2010), toplum psikolojisi (Norris vd. 2008), toplumsal tıp (Almedom ve Glandon 2007), tarım (Ge vd., 2016; Diogo vd., 2022; Feindt vd., 2018; Meuwissen vd., 2019) gibi diğer alanlardaki araştırmacılar tarafından kullanılmıştır.

Farklılık gösteren dayanıklılık tanımları sistemlerin risk ve bozulmalar karşısında sağlamlığına (robustness), uyum kapasitesine (adaptability) ve dönüşme yeteneğine (transformability) vurgu yapmaktadır. Dayanıklılık konusunda yapılmış önceki çalışmalar tarım sektöründe ve gıda sistemlerinde dayanıklılık için absorbe etme (sağlamlık), uyum sağlama ve dönüştürme olmak üzere üç kapasitenin önemli olduğuna işaret etmektedir (Walker vd., 2004; Folke vd., 2010; Béné vd., 2012; Mitchell vd., 2013; Anderies vd., 2013; Douchamps vd., 2017; Moore-O'Leary vd., 2017; Termeer vd., 2017; FAO vd., 2018; Meuwissen vd., 2019). Sağlamlık, çiftçilik sisteminin streslere ve beklenmeyen şoklara dayanma kapasitesidir. Uyum sağlama yeteneği, sistemin yapılarını ve geri bildirim mekanizmalarını değiştirmeden şoklara ve streslere yanıt olarak girdilerin, üretimin, pazarlamanın ve risk yönetiminin bileşimini değiştirme kapasitesidir. Dönüştürülebilirlik, iş yapmayı imkânsız kılan şiddetli şoklara veya kalıcı strese yanıt olarak sistemin iç yapısını ve geri bildirim mekanizmalarını önemli ölçüde değiştirme kapasitesidir ve sistemin işlevlerinde değişiklikler gerektirebilmektedir. Dönüşüm, dönüm noktalarından ve çöküşten sonra meydana gelebileceği gibi, küçük ve artımlı değişikliklerin bir dizisinden de kaynaklanabilmektedir. Kısa vadeye atıfta bulunan sağlamlık ve uyum kapasitelerinden farklı olarak, dönüşüm kapasitesi uzun döneme ait bir kavramdır (Şekil 3).



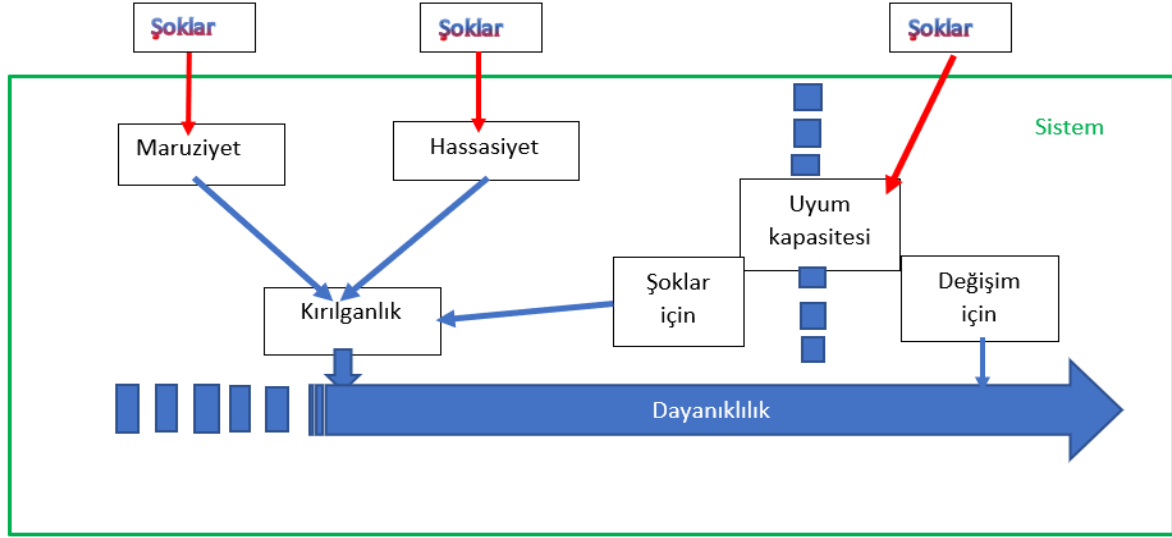
**Şekil 3.** Dayanıklılık kapasiteleri (Meuwissen vd. (2019)'dan uyarlanmıştır)

Daha önce yapılan tanımların ortak özelliklerden hareketle dayanıklılığı "*olumsuz olaylara karşı sağlam olma ve risk ve bozulmalara yanıt olarak daha başarılı bir şekilde uyum sağlama ve dönüşme yeteneği*" olarak tanımlamak mümkündür. Bütün olası olumsuz olayları kapsadığından, çok boyutlu kapasiteleri vurguladığından ve uzun vadede bir sistemin devam edebilmek için değişebilmesi gerektiğini kabul ettiğinden bu tanımın, tarım sektörü ve gıda sistemleri için yapılacak çalışmalarda oldukça uygun olduğu düşünülmektedir. Dayanıklılık kavramı kullanılırken, kimin için dayanıklılık (hedef ölçek veya analiz birimi), neye karşı dayanıklılık (hedef risk kaynağı) dayanıklılık özellikleri konusunda ortak bir anlayış oluşturulmalıdır. Tarımda dayanıklılık çalışmalarında ölçek parsel, tarım işletmesi, bölge, ülke veya hatta küresel gıda sistemi olabilir (Bullock vd., 2017). Dayanıklılık, sellere karşı dayanıklılık veya fiyat oynaklığına karşı dayanıklılık gibi tek belli zorluklara karşı dayanıklılık (belirtilen dayanıklılık) veya sistemin bilinmeyenle, belirsizlikle ve sürprizle başa çıkma kapasitesi (genel dayanıklılık) olarak ele alınabilmektedir (Anderies vd., 2013). Bir sistemin dayanıklılığı değerlendirilirken belirtilen dayanıklılık ve genel dayanıklılık kavramları ayırımına dayalı dayanıklılık çerçevesi kullanılmaktadır. Dayanıklılık çerçevesi 5 sorunun cevabına bağlı adımları içermektedir (Carpenter vd., 2001; Quinlan vd., 2016; Alvarado-Herrera, 2017; Meuwissen vd., 2019) (Şekil 4). Dayanıklılığı artırmak, aktörlerin hem şokların sonuçlarını yönetmelerini hem de bunların meydana gelmesini öngörmelerini ve hazırlanmalarını gerektirir. Bu bağlamda maruziyet (exposure), kırılganlık (vulnerability) ve uyarlanabilir kapasite (adaptive capacity) kavramları önem taşımaktadır (IPCC, 2012). Benzer şekilde, kırılganlık kavramı ile dayanıklılık kavramları arasındaki bağlantının anlaşılması da dayanıklılık bağlamında yapılacak çalışmalarda büyük önem taşımaktadır.



Şekil 4. Tarım Sistemleri için Dayanıklılık Çerçevesi (Meuwissen vd. (2019)'dan uyarlanmıştır)

Dayanıklılık ve kırılganlık, sistemlerin ve aktörlerin değişime, şoklara ve sürprizlere, ayrıca yavaş yavaş ortaya çıkan değişimlere verdiği tepkileri anlamak için birbirleriyle ilişkili ancak farklı iki yaklaşımı temsil etmektedirler. Kırılganlık, yaralanma anlamına gelen Latince *vulnus* kelimesinden türemiştir ve bir sistemin zayıflığına atıfta bulunmaktadır. Kırılganlık, bozulmanın/şokun meydana gelmesi esnasında bir bütün olarak sistemin veya sistemin bir parçasının zarar görme olarak tanımlanmıştır. Kırılganlık, sistemin ortaya çıkan olayla başa çıkma yeteneğinden kaynaklanan fiziksel, sosyal ve ekonomik yönler ve çıkarımlarla ilişkili bir risk ölçüsünü ifade etmektedir (Lanlan vd., 2024). Urruty vd. (2016), kırılganlığı tarımsal sistemlerin bozulma/şoklar nedeniyle zarar görme olasılığı olarak ifade etmiştir. Kırılganlık fiziksel kırılganlık, sosyal kırılganlık ve ekonomik kırılganlık olmak üzere üç grupta incelenebilmektedir. Fiziksel kırılganlık binalar, altyapı vb. gibi fiziksel varlıklarla ilgilidir. Geçim için gerekli olan mahsul ve diğer altyapıların potansiyel kaybını da içermektedir. Sosyal kırılganlık kadınlar, zihinsel ve fiziksel engelli kişiler, çocuklar ve yaşlılar, yoksullar, mülteciler vb. gibi dezavantajlı gruplarla ilgilidir. Ekonomik kırılganlık ise, ekonomik varlıklara ve süreçlere yönelik tehlike yaratan kayıp riskini kapsamaktadır. Ekonomik kırılganlık fiziksel ve sosyal altyapının hasar görmesi veya yok olması ve onarım veya değiştirme maliyeti ile mahsul hasarı gibi doğrudan olabileceği gibi, üretim, istihdam, hayati hizmetler, gelir eşitsizlikleri kaybı gibi dolaylı da olabilmektedir (Proag, 2014). Kırılganlık kavramı, 1970’li yıllarda coğrafya ve diğer sosyal bilim alanlarında risk yönetimi konularında depremler, gıda değişim krizleri gibi çevresel veya sosyo-ekonomik risklerle karşı karşıya kalan ülkelerin kırılganlığını tanımlamak için kullanılmaya başlanmıştır (Blaikie vd., 1999; Watts ve Bohle 1993). Hükümetler Arası İklim Değişikliği Paneli’nin (IPCC) küresel ısınmanın bölgesel ve küresel düzeylerdeki potansiyel etkilerini değerlendirmek için kırılganlık kavramını benimsemesiyle bu kavramın kullanımı hızlı bir şekilde artmıştır (Kates vd., 2001; Downing vd., 2015; Adger, 2006; Luers vd., 2003; Paavola, 2008; Turner vd., 2003). Bu yaklaşım kullanılarak tarımda da kırılganlık bağlamında bazı araştırmalar yapılmıştır (Jalan ve Ravallion, 1999; Luers vd., 2003; Reidsma ve Ewert, 2008; Simelton vd., 2009). Kırılganlığın (i) maruziyet (exposure) (incelenen sistemi etkileyen bozulmaların sıklığı, yoğunluğu ve süresi), (ii) hassasiyet (sensitivity) (incelenen sistemin bozulmalara maruz kalmaktan etkilenme derecesi), (iii) başa çıkma kapasitesi ve (iv) uyum kapasitesi (adaptive capacity) (incelenen sistemin başa çıkabileceği değişkenlik düzeyini artırma yeteneği) olmak üzere 4 boyutu/unsuru bulunmaktadır (Urruty vd., 2016). Sağlamlık kapasitesi bağlamında dayanıklılık genellikle kırılganlığın tersi olarak ele alınmaktadır (Lanlan vd., 2024). Başa çıkma ve uyum kapasiteleri sayesinde kırılganlıklar giderilerek dayanıklılığı artırmak mümkündür (Şekil 5).



**Şekil 5.** Kırılganlık ve Dayanıklılık ilişkisi (Gitz and Meybeck (2012)’den uyarlanmıştır)

#### Sürdürülebilirlik ve Dayanıklılık Kavramları Arasındaki Benzerlik ve Farklılıklar

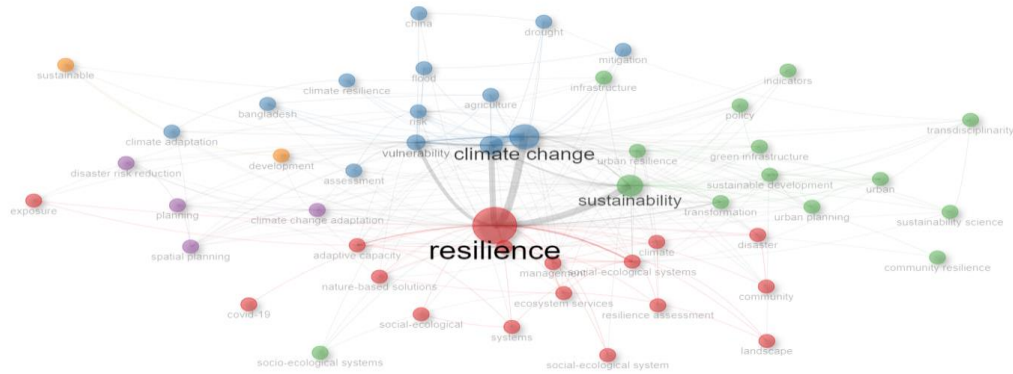
Sürdürülebilirlik kavramı, genel olarak hem şimdiki hem de gelecek nesiller için çevresel, sosyal ve ekonomik hususlar açısından yaşam kalitesini artırmaya odaklanmaktadır (Collier vd., 2013). Dayanıklılığın odağı ise sistemlerin hem aşırı rahatsızlıklara hem de sürekli strese verdiği tepkidir (Ulusal Araştırma Konseyi, 2012; Folke, 2016). Bir sistemi tanımlamak için kullanıldıklarından, sürdürülebilirlik ve dayanıklılık kavramları arasında benzerlikler vardır (Carpenter vd., 2001). İki kavramın ilk benzerliği, her ikisinin de bir sistemin veya özelliğın zaman içindeki durumunu ifade etmesi ve normal çalışma koşulları altında ve bozulmalara yanıt olarak bu sistemin devamlılığına odaklanmalarıdır (Fiksel vd., 2014). Ele alınan sistemin hayatta kalmasına yönelik bu ortak odaklanma nedeniyle, sürdürülebilirlik ve dayanıklılık kavramları sıklıkla yaşam döngüsü analizi, yapısal analiz ve sosyo-ekonomik analiz metodolojileri ile birlikte kullanılmaktadırlar (Bocchini vd., 2014). Küresel politik eğilimlerle bağlantılı olmaları dayanıklılık ve sürdürülebilirlik kavramlarının ikinci ortak özelliğidir (Folke vd., 2002). Sürdürülebilirlik ve dayanıklılığın hedeflerinin örtüşmesi ve ortak uygulama alanlarına sahip olmaları ulusal veya uluslararası düzeyde birçok kurum/kuruluşta birleşik sürdürülebilirlik ve dayanıklılık bölümlerinin kurulmasına yol açmıştır.

Benzerliklerinin yanında, sürdürülebilirlik ve dayanıklılık kavramları arasında önemli bazı farklılıklar bulunmaktadır. Mekânsal ve zamansal ölçek farklılıkları sürdürülebilirlik ve dayanıklılık kavramları arasındaki ilk temel farklılıktır. Sürdürülebilirlik çalışmaları genellikle dayanıklılıktan daha büyük mekânı kapsamakta ve daha uzun zamanı ele almaktadır (Redman, 2014; Meacham vd., 2016). Sürdürülebilirliğin daha çok “*korumaya*”, dayanıklılığın ise “*uyum ve iyileştirmeye*” odaklanması aralarındaki ikinci temel farktır. Toplum geliştirme bağlamında, sürdürülebilirlik girişimleri genellikle geleneksel kaynak kullanım yöntemlerini, geçim kaynaklarını, çevresel bilgiyi ve çevresel kaynakları korumaya odaklanma eğilimindedir. Buna karşılık, dayanıklılık girişimleri genellikle yeni koşullara uyum sağlamaya, geleneksel bilginin yenilikçi kullanımlarını oluşturmaya, yeni çevresel bilgi yaratmaya ve yaşam koşullarını ve istihdamı iyileştirmeye odaklanma eğilimindedir (Lew vd., 2016). İki kavram arasındaki üçüncü fark süreç veya çıktıya odaklanma bağlamındadır. Dayanıklılık sistemlerin veya özelliklerin süreçlerine öncelik verme eğilimindeyken, sürdürülebilirlik sistemin sonuçlarına öncelik vermektedir (Lee vd., 2013; Saunders ve Becker, 2015). Dayanıklılık bir yaklaşımın alternatif sonuçlar arasında seçim yapmayı değil, istenen işlevsel bir süreci seçmeyi içermektedir. Sürdürülebilirliğin hedefi ise süreçleri zenginleştiren gelişim yoluyla toplum refahını yükseltmektir. Dayanıklılık zorlukların/şokların oluşturduğu kesintiye bir yanıt olarak uygulanmaktadır ve hazırlıklı olmaya dayanmaktadır (Chelleri vd., 2015; Linkov ve Palma Oliveira, 2017).

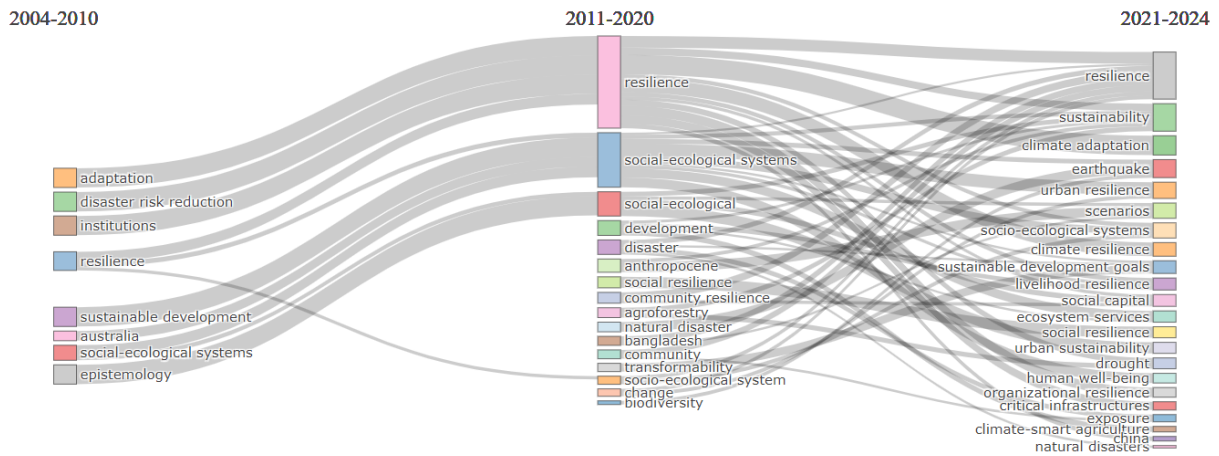
#### Sürdürülebilirlik ve Dayanıklılık Üzerine Yapılan Çalışmaların Bibliyografik Analizi

Dayanıklılık ve sürdürülebilirlik konularında yapılan bilimsel araştırmaların sayısı son on yılda artış eğilimindedir. 2014-2024 yıllarını kapsayan son on yıllık dönemde 1210 makale yayınlanmışken, 2014 yılından önceki dönemde yalnızca 53 makale yayınlanmıştır. Dayanıklılık ve sürdürülebilirlik konularında en fazla araştırma yayınlayan ülkeler sırasıyla Amerika Birleşik Devletleri, Çin, Birleşik Krallık, İtalya ve Almanya’dır. Günümüze kadar, tarımda

sürdürülebilirlik ve dayanıklılık konularında en yoğun iş birliği ABD ile İsveç, Kanada ve Birleşik Krallık arasında gerçekleşmiştir. Çin, ABD, İtalya, Almanya, Birleşik Krallık, İsveç, Avustralya, Güney Afrika, Kanada ve Hollanda'daki yazarların/araştırmacıların sınır ötesinde en çok iş birliği yapan ülkelerdir. Sürdürülebilirlik ve dayanıklılık bağlamındaki araştırmalara en fazla katkı sağlayan kurumlar Stockholm Üniversitesi, Arizona Devlet Üniversitesi, Stellenbosch Üniversitesi, Wageningen Üniversitesi ve Michigan Devlet Üniversitesidir. Yingqi Liu, Jian Liu, Yuhuan Li, Per Olsson, Carl Folke ve Luca Salvati sürdürülebilirlik ve dayanıklılık kavramlarına dayanarak yapılan araştırmalar bağlamında en üretken yazarlardır. Folke, 2006'dan 2018'e kadar uzanan yayın akışı ile ufuk açıcı bir araştırmacı olarak ön plana çıkmaktadır. En fazla atıf alan araştırmacılar Folke, Olsson ve Biggs'tir. En çok küresel atıf yapılan çalışma Walker'ın (2004) "Sosyal-ekolojik sistemlerde dayanıklılık, uyarlanabilirlik ve dönüştürülebilirlik" başlıklı çalışmasıdır. Sürdürülebilirlik ve dayanıklılık konularında en çok kullanılan anahtar kelimeler dayanıklılık, iklim değişikliği, sürdürülebilirlik, sosyal ekolojik sistem, adaptasyon, güvenlik açığı, ekosistem hizmetleri ve dönüşümdür. Günümüze kadar sürdürülebilirlik ve dayanıklılık bağlamında yapılan bilimsel çalışmaları dayanıklılık (kırmızı), sürdürülebilirlik (yeşil), iklim değişikliği (mavi) ve iklim değişikliğine uyum (mor) olmak üzere 4 kümede toplamak mümkündür (Şekil 6). Sürdürülebilirlik ve dayanıklılık araştırmalarının üç boyut dönemi arasındaki tematik kaymalar incelendiğinde, 2010 yılına kadar uyum ve afet riskinin azaltılması ön plana çıkmaktayken, 2011-2020 yılları arasında ise dayanıklılık ve sosyal-ekolojik sistemler ön sıralara yükselmiştir. Son yıllarda, dayanıklılık, sürdürülebilirlik, iklim uyumu, deprem ve kentsel dayanıklılık konuları gündemin ön sıralarındadır (Şekil 7).



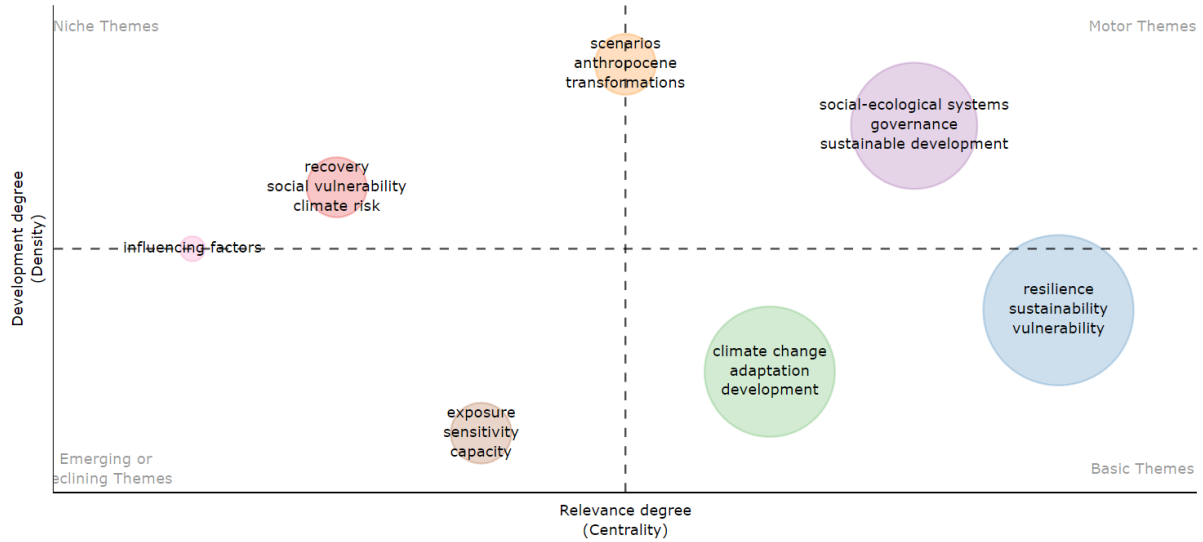
Şekil 6. Eş Oluşum Ağı- Yazarın Anahtar Kelimeleri- Kümeleme Algoritması: Louvain



Şekil 7. Sürdürülebilirlik ve Dayanıklılık Bağlamında Konuların Tematik Evrimi

Trend analizi sonuçları yakın gelecekte çalışmaların iklim ve sosyal-ekolojik sistemler odağında ilerleyeceğini göstermiştir. İklim odağında dayanıklılık, kırılganlık, iklim değişikliği, sürdürülebilirlik, adaptasyon, güvenlik açığı, yönetim, iklim değişikliği adaptasyonu ve dönüşüm konularında yoğunlaşma olacağı tahmin edilmektedir. Sosyal-ekolojik sistemler odağında ise ekosistem hizmetleri, yönetim ve dayanıklı şehirler ile ilgili konular ön plana çıkacaktır (Şekil 8).





Şekil 8. Sürdürülebilirlik ve Dayanıklılık Kavramlarının Tematik Gelişimi

### Sürdürülebilirlik ve Dayanıklılık Çerçeveleri

Günümüze kadar sürdürülebilirlik ve dayanıklılık temalı çok sayıda araştırma yapılmış ve uygulama çalışması gerçekleştirilmiştir. Sürdürülebilirlik ve dayanıklılık arasındaki benzerlikler ve farklılıkların yeterli düzeyde dikkate alınmaması, kavramların epistemolojik köküne inilmemesi, iki kavram arasındaki etkileşimin ihmal edilmesi sebepleriyle hem AR-GE çalışmalarında hem de uygulamalı çalışmalarda sorunlar yaşanmıştır. Günümüze kadar uygulanan birçok iklim değişikliği azaltma ve uyum stratejisinde, kentsel yoğunlaştırma çabalarında ve afet müdahale çabalarında sürdürülebilirlik ve dayanıklılık arasındaki benzerlikler ve farklılıklar konusunda bilgisizlik sebebiyle önemli sorunlar yaşanmıştır (Lizarralde vd., 2015; Landauer vd., 2015; Asprone ve Manfredi, 2015). Akademik çalışmalarda ise sürdürülebilirlik ve dayanıklılık kavramları kullanılırken kavramsal çerçeveye yeterli özen gösterilmeyip kavramlar yüzeysel olarak ele alınmış, kavramlar eksik veya yanlış kullanılmış ve kavramlar arası karmaşalar oluşmuştur. Bu durum, araştırma bulgularının yaygın etkisini olumsuz etkilemiş ve bulguların karşılaştırılmasını güçleştirmiştir. Sürdürülebilirlik ve dayanıklılık kavramları çoğunlukla birbirinden bağımsız bir şekilde ele alınmıştır. Ancak, her iki kavramın ilişkilendirilerek kullanıldığı çalışmalarda bulunmaktadır. Sürdürülebilirlik ve dayanıklılık kavramları için tanım, metodoloji, araçlar ve uygulama alanlarındaki farklılıklar göz önüne alındığında, tüm paydaşların ihtiyaçlarını karşılayacak tek bir ortak çalışma çerçevesi önerilmesi mümkün değildir. Dayanıklılık ve sürdürülebilirlik için bütün disiplinleri kuşatacak ortak çalışma çerçevesi geliştirilmesi için önemli çabalar sarf edilmiştir. Ahern (2013) ve Bocchini vd. (2014) sürdürülebilirlik ve dayanıklılık hedeflerine öncelik verilmesi gerektiğini ifade etmişlerdir. Redman (2014) ve Ulanowicz vd. (2009) ise sinerjilerden yararlanılmasını önermiştir. Bu görüşe göre, dayanıklılık farklı disiplinlerden gelen bilgileri birbirine bağlama ve koordine etme fırsatı sunarak sürdürülebilirlik biliminde önemli bir araç olarak kullanılabilir (Kates vd. 2001; Jerneck vd. 2011). Bazı araştırmacılar ise sürdürülebilirlik ile dayanıklılık kavramları arasındaki çatışmaların olumsuz etkilerinin hafifletilmesine odaklanılması gerektiğini vurgulamışlardır (Derissen vd., 2011; Gasparini ve Manfredi, 2014).

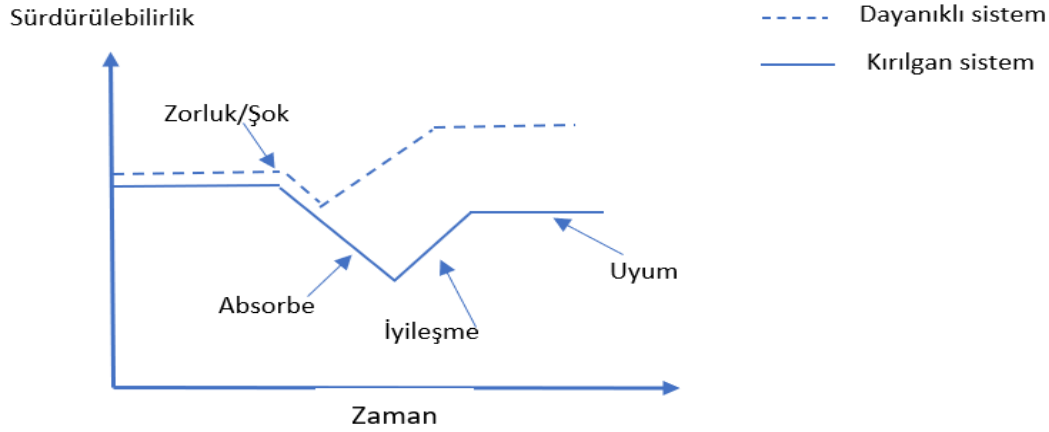
Günümüze kadar yapılan sürdürülebilirlik ve dayanıklılık araştırmaları ve uygulamalı çalışmaların, sürdürülebilirlik ve dayanıklılığın hedeflerini yakalayan üç genelleştirilmiş çalışma çerçevesi altında sınıflandırılabilirliğini göstermiştir. Bu çalışma çerçevelerinden birincisi, dayanıklılığı sürdürülebilirliğin bir bileşeni olarak ele almaktır. Sürdürülebilirliği dayanıklılığın bir bileşeni olarak ele almak ikinci çalışma çerçevesidir. Son çalışma çerçevesi dayanıklılık ve sürdürülebilirliği ayrı hedefler olarak ele almaktır (Marchese vd., 2018).

### Sürdürülebilirliğin Bileşeni Olarak Dayanıklılık

Bu çalışma çerçevesinde sürdürülebilirlik, dayanıklılıktan daha geniş bir kavram olarak ele alınmaktadır ve dayanıklılığı sürdürülebilirliğin bir bileşeni olarak değerlendirmektedir. Birincil hedef sürdürülebilirliktir ve bir sistemin dayanıklılığının sağlanması o sistemi daha sürdürülebilir kılmamanın bir aşamasıdır (Marchese vd., 2018). Bu çalışma çerçevesinde dayanıklılık kavramı sürdürülebilirlik bağlamında bir sistemin hedeflerine ulaşmak olarak kullanılmaktadır (Anderies vd., 2013). Bu çalışma çerçevesi benimsenerek yapılan çalışmalarda, bir sistemin sürdürülebilirliğinin sağlanmasının, o sistemi her zaman daha dayanıklı kılmayacağını akılda tutmak gerekmektedir. Blackmore ve Plant (2008), bir sistemin sürdürülebilir olması için planlama sürecinde o sistemin bozulmalara karşı kırılganlıklarının mutlaka dikkate alınması gerektiğini belirtmiştir. Benzer şekilde, Ahern (2013)



dayanıklılık dikkate alınmadan yapılacak sürdürülebilirlik çalışmaları sonucunda bir sistemin yalnızca kırılgan sürdürülebilirliğe ulaşabileceği konusunda uyarıda bulunmuştur. Bir sistemin dayanıklılığının o sistemin sürdürülebilirliğini nasıl etkileyebileceği ve dayanıklı bir sistemin dayanıklılığın uyarlanabilir bileşeni aracılığıyla bir zorluktan kurtulduktan sonra nasıl sürdürülebilir hale gelebileceği Şekil 9’da gösterilmiştir. Kırılgan sistemlerde şokla karşılaşıldığında absorbe süreci daha uzun, iyileşme süreci daha kısa ve denge mevcut durumdan daha düşük düzeyde olmaktadır. Dayanıklı sistem şokla karşılaştığında ise absorbe süreci daha kısa ancak iyileşme süreci daha uzundur. Doğal olarak yeni denge mevcut durumdan daha yüksek bir düzeyde oluşmaktadır



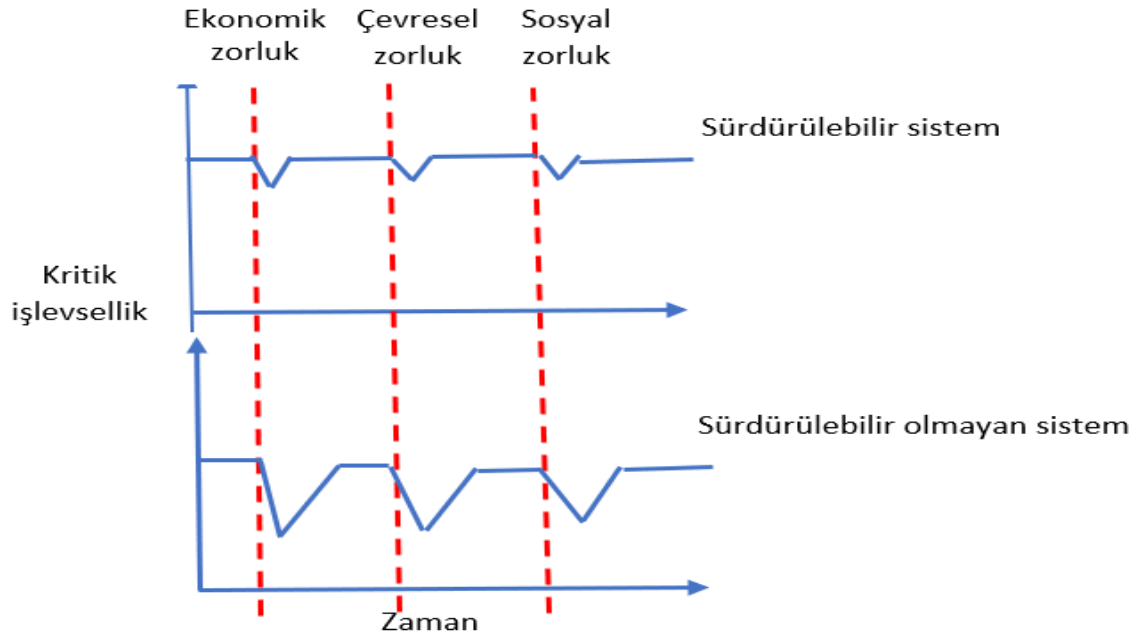
**Şekil 9.** Sürdürülebilirliğin Bileşeni Olarak Dayanıklılık (Marchese vd. (2018)’den uyarlanmıştır)

Günümüze kadar bu çalışma çerçevesini kullanarak toplum dayanıklılığı, doğal kaynaklar yönetimi, kamu politikası, işletme yönetimi, sürdürülebilirlik bilimi ve şehir planlama alanlarında çeşitli çalışmalar gerçekleştirilmiştir. Yapılan bu çalışmalarda genellikle sürdürülebilirliğin bazı boyutları ile dayanıklılığın bazı kapasiteleri farklı kombinasyonlarda kullanılmıştır. Bu çalışmaların bazılarında sürdürülebilirlik boyutları (sosyal, çevresel, ekonomik) ve dayanıklılık kapasitelerinin (absorbe etme, uyum, dönüşüm) tamamı bir bütün olarak tanımlamalarda veya çalışma çerçevesinin temel bileşeni olarak ele alınmıştır (Seager, 2008; Milman ve Short, 2008; Ahern, 2011; Ahern, 2013; Park vd., 2013; Saunders ve Becker, 2015; Xu vd., 2015). Günümüze kadar sadece Park vd. (2013) sürdürülebilirliğin ekonomik ve çevresel boyutlarını, dayanıklılığın bütün kapasiteleri ile birlikte çalışma çerçevesinin temel bileşeni olarak ele alarak bu çerçeveyi uygulamaya aktarmıştır. FAO (2014)’ün yayınladığı “Gıda ve Tarım Sistemlerinin Sürdürülebilirlik Değerlendirmesi Kılavuzunda (SAFA)” bu çerçeve benimsenmiştir. Türkiye’de yapılan akademik çalışmalarda ise sürdürülebilirlik kavramı genellikle dayanıklılıktan bağımsız olarak ele alınmış olup, dayanıklılığı sürdürülebilirliğin bir alt bileşeni olarak kabul edildiği bir çalışmaya rastlanmamıştır. İncelenen çalışmaların bazılarında işletmelerin sürdürülebilirlik raporlarındaki çevresel, ekonomik ve sosyal göstergelerin önem düzeyleri ve risk faktörlerinin sürdürülebilirlik düzeyindeki önemi araştırılmıştır (Korga ve Aslanoğlu, 2024; Şen vd., 2018). Tarım sektörü özelinde ise daha önce bu çerçeveyi kullanan bir çalışmaya rastlanmamıştır.

#### Dayanıklılığın Bileşeni Olarak Sürdürülebilirlik

Bu çalışma çerçevesinde dayanıklılık sistemin ana hedefidir, sürdürülebilirlik ise dayanıklılığa katkıda bulunan bir bileşen olarak ele alınmaktadır. Temel olarak bir sistemin sürdürülebilirliğini artırmanın, o sistemi daha dayanıklı hale getirdiğine odaklanılmaktadır. Kuşkusuz, bir sistemin dayanıklılığını geliştirmenin o sistemi mutlaka daha sürdürülebilir hale getirmeyeceği hususu sürekli hatırlanmalıdır. Bu bağlamda dayanıklılığın odak noktası, rahatsızlıklar sırasında ve sonrasında bazı temel hedefleri veya kritik işlevleri sürdürmektir. Bir sistemin sürdürülebilirliğinin o sistemin dayanıklılığını nasıl etkileyebileceği ve sürdürülebilirliğin sağlanması yoluyla nasıl dayanıklı hale gelinebileceği Şekil 10’da gösterilmiştir. Günümüze kadar bu çalışma çerçevesini kullanarak kamu politikası (Chapin vd., 2009; Saxena vd., 2016), işletme yönetimi (Avery ve Bergsteiner, 2011) ve değer zinciri yönetimi (McEvoy vd., 2006; Ahi ve Searcy, 2013; Closs vd., 2011; Bansal ve DesJardine, 2014) alanlarında çeşitli çalışmalar gerçekleştirilmiştir. Yapılan bu çalışmalarda genellikle sürdürülebilirliğin bazı boyutları ile dayanıklılığın absorbe etme (sağlamlık) ve iyileşme kapasitelerinin farklı kombinasyonları üzerinde durulmuştur. Türkiye’de ve

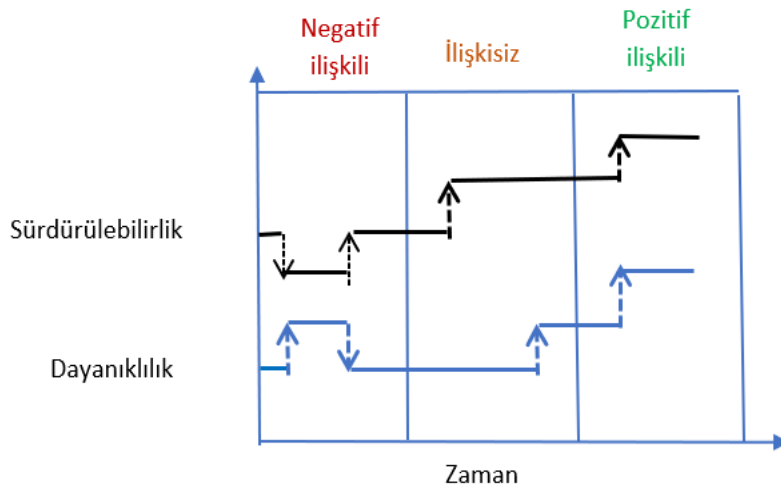
özellikle de tarım sektörü özelinde dayanıklılığın bir alt bileşeni olarak sürdürülebilirliğin ele alındığı çalışmaya rastlanmamıştır.



**Şekil 10.** Dayanıklılığın Bileşeni Olarak Sürdürülebilirlik (Marchese vd. (2018)'den uyarlanmıştır)

#### Ayrı Hedefler Olarak Sürdürülebilirlik ve Dayanıklılık

Bu çalışma çerçevesinde, sürdürülebilirlik ve dayanıklılık birbirleriyle rekabet edebilen veya tamamlayıcı olan ama ayrı hedefleri olan kavramlar olarak değerlendirilmektedir. Dayanıklılık ve sürdürülebilirlik bazen negatif veya pozitif yönlü ilişkili, bazen de ilişkisiz olabilmektedir. Günlük hayatta uygulanan proje ve politikaların dayanıklılığı, sürdürülebilirliği veya her ikisini birden etkileyebileceği öne sürülmektedir (Bocchini vd., 2014; Derissen vd., 2011; Fiksel vd., 2014; Lew vd., 2016; Lizarralde vd., 2015; Meacham, 2016). Bir sistemde sürdürülebilirlik ve dayanıklılığın rekabet halinde veya tamamlayıcı olma durumu Şekil 11'de gösterilmiştir.



**Şekil 11.** Ayrı Hedefler Olarak Sürdürülebilirlik ve Dayanıklılık (Marchese vd. (2018)'den uyarlanmıştır)

Günümüze kadar bu çalışma çerçeve kullanılarak ekonomi (Derissen vd., 2011), toplumsal dayanıklılık (Lew vd., 2016), kentsel planlama (Fiksel vd., 2014; Ulanowicz vd., 2009), sivil altyapı (Bocchini vd., 2014; Manyena vd., 2008; Meacham, 2016; Ning vd., 2013) ve kamu politikası (Lizarralde vd., 2015; Redman, 2014) alanlarında çalışmalar yapılmıştır. Bocchini vd. (2014) dayanıklılığı düşük olasılıklı, yüksek etkili olaylara verilen yanıt olarak, sürdürülebilirliği ise etkilerin yaşam döngüsünün alt yapısına yayıldığı, yüksek olasılıklı olaylara verilen yanıt olarak tanımlamışlar ve yaşam döngüsü analizini kullanarak değerlendirme yapmışlardır. Türkiye'de

yapılan akademik çalışmalarda ise sürdürülebilirlik kavramı dayanıklılık kavramından bağımsız olarak ele alınmıştır. Yapılan çalışmalarda genellikle sürdürülebilirlik kavramı ekonomik, sosyal ve çevresel boyutuyla (Eryılmaz vd., 2019; Başer vd., 2017; Güneş ve Karakaş, 2022; Yılmaz, 2021; Gürsoy, 2023; Akkuş, 2023; Keskinliç, 2019) ele alınmıştır. Diğer bazı araştırmalarda ise ekonomik, sosyal ve çevresel boyuta ek olarak sürdürülebilirliğin biyo-fiziksel (Ceyhan, 2010), kültür (Ayyıldız, 2018), biyolojik ve politik (Kaya, 2023) boyutlarına da değinilmiştir. Sürdürülebilirliği bağımsız olarak teknik ve ekonomik sürdürülebilirlik şekline ele alan araştırmacılar da olmuştur. Turhan (2005) sürdürülebilirliği üreticinin uzun dönemdeki geliri, doğal kaynaklar, çevre, yönetsel özellikler ve sosyo-ekonomik etkiler boyutuyla ele almıştır. Yıldız (2017) sürdürülebilirliği, Çiftlik Sürdürülebilir Endeksi'ni kullanarak çiftçi ve bölge koşullarına uygun olan on dört değişkenle ölçerek değerlendirmiştir. Sürdürülebilirliğin aksine, Türkiye'de tarım sektörü özelinde dayanıklılık çalışmalarına rastlanmamıştır.

## SONUÇLAR

Bu çalışmada sürdürülebilirlik ve dayanıklılık kavramlarının tarihsel gelişimlerinin ortaya konulması, sürdürülebilirlik ve dayanıklılığı ilişkilendiren çalışma çerçevelerinin incelenmesi, mevcut çalışma çerçevelerinin farklı uygulamalardaki avantajlarını ve dezavantajlarının ortaya konulması ve sürdürülebilirlik ile dayanıklılık konularındaki çalışmaların tarihsel gelişiminin ve gelecek araştırma eğilimlerinin belirlenmesi amaçlanmıştır. Araştırma bulguları, sürdürülebilirlik ve dayanıklılık arasındaki benzerlikler ve farklılıkların yeterli düzeyde dikkate alınmadığını, kavramların epistemolojik köküne inilmediğini, iki kavram arasındaki etkileşimin ihmal edildiğini ortaya koymuştur. Türkiye özelinde bu sorunun daha da derinleşmiş olduğu tespit edilmiştir. Türkiye'de günümüze kadar yapılan çalışmalarda sürdürülebilirlik ve dayanıklılık arasındaki rekabet ve tamamlayıcılık ilişkileri yeterli düzeyde ele alınmadığından akademik çalışmalarda sorunlarla karşılaşmaktadır. Türkiye'de sürdürülebilirlik ve dayanıklılık kavramları çoğunlukla birbirinden bağımsız bir şekilde ele alınmaktadır. Sürdürülebilirlik ve dayanıklılık kavramları kullanılırken genellikle kavramsal çerçeveye yeterli özen gösterilmeyip kavramlar yüzeysel olarak ele alınmakta ve zaman zaman kavramlar eksik veya yanlış kullanılarak kavramlar arası karmaşalar oluşmaktadır. Dayanıklılık kavramının üç kapasitesi olan absorbe etme (sağlamlık), uyum ve dönüşüm kapasitelerini içerecek şekilde kullanılmadığı, dayanıklılık kavramının daha çok sağlamlık kapasitesi kastedilerek sınırlı bir şekilde kullanıldığı (sağlamlık ile dayanıklılık aynı anlamda) tespit edilmiştir. Yurt dışında yapılan çalışmaların büyük bir çoğunluğunda sürdürülebilirlik ve dayanıklılık kavramları net olarak tanımlanmakta ve çalışma çerçevesi netleştirilmektedir. Türkiye'de yapılan çalışmaların büyük bir çoğunluğunda bunun karşılığının olmadığı görülmüştür. Dolayısıyla kavramsal olarak bir çatışma ve belirsizlik görülmektedir. Bu durum, akademik çalışmaların etkinliğini ve yaygın etkisini olumsuz etkilemiş ve bulguların karşılaştırılmasını güçleştirmiştir. Dayanıklılık ve sürdürülebilirlik arasındaki rekabet ve tamamlayıcılık ilişkilerinin dikkate alınması ve uygun çalışma çerçevesinin seçilmesi yapılacak akademik ve uygulamalı çalışmaların etkinliğini artıracaktır. Yapılacak akademik ve uygulama çalışmaları ile politika oluşturma süreçlerinde dayanıklılık kavramının sürdürülebilirlik, uyarlanabilir kapasite ve kırılganlık kavramları ile bağlantısının dikkate alınması dayanıklı ve sürdürülebilir tarım sektörü oluşturma gayretlerinden daha iyi sonuçlar elde edilmesini sağlayabilecektir. Sürdürülebilirliğin dayanıklılık ve ekonomik verimlilik gibi diğer yönetim hedefleriyle ilişkisinin hem ulusal düzeyde hem de uluslararası düzeyde uzlaşmalar yoluyla kurulması sürdürülebilir ve dayanıklı bir tarım sektörünün oluşturulmasına olumlu katkıları olabilecektir.

Yakın gelecekte dayanıklılık ve sürdürülebilirlik bağlamında çalışmaların iklim (dayanıklılık, kırılganlık, iklim değişikliği, sürdürülebilirlik, adaptasyon, güvenlik açığı, yönetim, iklim değişikliği adaptasyonu ve dönüşüm) ve sosyal-ekolojik sistemler (ekosistem hizmetleri, yönetim ve dayanıklı şehirler) odağında ilerleyeceği tahmin edilmektedir. Dayanıklılık ve sürdürülebilirlik bağlamında gelecekte yapılacak çalışmalarda bu yönelimin dikkate alınması dayanıklı ve sürdürülebilir bir tarım sektörü oluşturulması çalışmalarının etkinleştirmesine katkıda bulunabilecektir.

## Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.


## Araştırmacıların Katkı Oranı Beyan Özeti


**Cansu KADAKOĞLU** :Veri düzenleme; biçimsel analiz; yazma; inceleme; düzenleme.


**Vedat CEYHAN** :Kavramsallaştırma; yazma; inceleme; düzenleme.

**Orhan GÜNDÜZ** :Metodoloji; yazma; inceleme; düzenleme.

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## Impact of Elevated Ammonia Concentration on *Scenedesmus dimorphus* Growth in a Flat-Plate Photobioreactor

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### ABSTRACT

Animal feeding operations (AFOs) are significant sources of airborne pollutants, particularly ammonia (NH<sub>3</sub>), which pose considerable environmental and health risks. In response to these challenges, photobioreactor (PBR) systems utilizing microalgae have emerged as a promising solution. These systems can effectively absorb and metabolize pollutants such as NH<sub>3</sub> and carbon dioxide (CO<sub>2</sub>), thereby improving air quality while simultaneously producing valuable biomass. The present study specifically investigated the effects of elevated NH<sub>3</sub> concentrations on algal growth within PBRs. Ammonium chloride (NH<sub>4</sub>Cl) was employed to simulate NH<sub>3</sub> concentrations typical of animal housing, specifically at a loading rate of 50 ppm (78 mg L<sup>-1</sup> d<sup>-1</sup> NH<sub>4</sub>Cl). Over a 21-day experimental period, control tanks containing standard Bold's Basal Medium (BBM) were compared against those with NH<sub>3</sub> exposure. Results indicated that while normalized cell concentrations were highest in control tanks (1.79±0.09, p<0.01), the dry biomass was significantly greater in tanks subjected to the 50 ppm NH<sub>3</sub> loading rate (1.34±0.02, p<0.01). These findings suggest that microalgae possess a remarkable capacity to adapt to high NH<sub>3</sub> levels, highlighting their potential role in emission mitigation and sustainable biofuel production. The integration of PBR systems utilizing microalgae represents a viable strategy for addressing the environmental and health challenges posed by AFOs. By effectively utilizing pollutants such as NH<sub>3</sub>, these systems not only enhance air quality but also contribute to the development of sustainable biofuels, thus supporting broader environmental sustainability goals.

**Key words:** Microalgae, mitigation, animal barns, emission

### Yüksek Amonyak Konsantrasyonunun Düz Panel Fotobiyoreaktörde *Scenedesmus dimorphus* Gelişimi Üzerindeki Etkisi

### ÖZ

Yoğun hayvancılık faaliyetleri, amonyak (NH<sub>3</sub>) da dahil olmak üzere havayla taşınan önemli kirleticiler üreterek çevre ve sağlık riskleri oluşturmaktadır. Fotobiyoreaktör (PBR) sistemleri, NH<sub>3</sub> ve CO<sub>2</sub> gibi kirleticileri absorbe etmek ve metabolize etmek, hava kalitesini artırmak ve değerli biyokütle üretmek için mikroalgleri kullanan umut verici bir çözüm olarak ortaya çıkmıştır. Bu çalışma, hayvan barınaklarında tipik olarak görülen yüksek amonyak konsantrasyonlarını azaltmak için yüksek NH<sub>3</sub> konsantrasyonlarının PBR'lerde alg büyümesi üzerindeki etkisini araştırmaktadır. Amonyum klorür (NH<sub>4</sub>Cl), NH<sub>3</sub> yerine kullanılmış ve nitrat içermeyen BBM ortamı hayvan barınaklarında tipik olarak görülen 50 ppm (78 mg L<sup>-1</sup> d<sup>-1</sup> NH<sub>4</sub>Cl) NH<sub>3</sub> konsantrasyonunu simüle etmiştir. Her deney 21 gün boyunca normal BBM (0,25 g L<sup>-1</sup> NaNO<sub>3</sub>) içeren kontrol tanklarında gerçekleştirilmiştir. Normalize edilmiş hücre konsantrasyonları NH<sub>3</sub> içermeyen PBR tanklarında en yüksek (1.79±0.09, p<0.01), ancak kuru biyokütle 50 ppm NH<sub>3</sub> yükleme oranına sahip tanklarda daha yüksekti (1.34±0.02, p<0.01). Hücre konsantrasyonları 50 ppm NH<sub>3</sub> ile azalırken, kuru biyokütle NH<sub>3</sub> içermeyen tanklara kıyasla artmıştır. Sonuçlar, mikroalglerin emisyonları azaltma, yetiştirme stratejilerini optimize etme ve sürdürülebilir biyoyakıt üretimini destekleme potansiyelini ortaya koymakta ve PBR'lerin çevresel etkileri azaltma ve atık kaynakları geri dönüştürmedeki rolünü vurgulamaktadır.

**Anahtar kelimeler:** Mikroalg, azaltım, hayvan barınakları, emisyon

## INTRODUCTION

The increasing concentration of air pollutants in agricultural operations, particularly in animal houses, poses significant environmental and health challenges. Intensive animal husbandry operations generate substantial amounts of airborne pollutants, including ammonia (NH<sub>3</sub>), particulate matter (PM), and greenhouse gases, which can adversely affect air quality and contribute to climate change (Guo et al., 2022; Tschöfen et al., 2019). Among these pollutants, ammonia (NH<sub>3</sub>) is particularly concerned due to its ability to penetrate deep into the respiratory system, posing health risks not only to animals but also to surrounding communities (Xi et al., 2022). As such, innovative strategies are required to mitigate these emissions effectively.

Photobioreactor systems (PBRs) have emerged as a promising technology for addressing air pollution in animal housing environments. These systems utilize microalgae, which can absorb and metabolize various pollutants, including ammonia and carbon dioxide, thereby improving air quality (Uguz et al., 2022; Julianto, 2024). The integration of photobioreactors into animal housing can facilitate the biological treatment of waste gases, converting harmful emissions into biomass that can be further utilized for biofuel production or as animal feed (Uguz and Sozcu, 2024; Dumont, 2018). Research has shown that microalgae such as *Scenedesmus* species can thrive in controlled environments, effectively utilizing nutrients from waste streams while simultaneously reducing airborne pollutants (Uguz et al., 2022; Sun et al., 2014; Zhang et al., 2014). Moreover, the design and operational parameters of photobioreactors can be optimized to enhance their pollutant removal efficiency. Factors such as light intensity, nutrient concentration, and airflow rates play critical roles in maximizing algal growth and pollutant uptake (Abdel-Baset, 2024; Thành et al., 2016). This innovative approach not only contributes to cleaner air but also promotes sustainable agricultural practices by recycling waste products into valuable resources (Tschöfen et al., 2019).

The flat-plate photobioreactors has garnered gained attention due to its potential applications in biofuel production and environmental sustainability. The growth of *S. dimorphus* is influenced by various factors, including nutrient availability, light intensity, and environmental conditions. Among these factors, ammonia concentration, which is often prevalent in animal housing environments, poses a unique challenge and opportunity for optimizing algal growth. Ammonia can serve as a nitrogen source, which is essential for algal growth, but excessive concentrations can lead to toxicity and inhibit growth (Zhang et al., 2014). Flat-plate photobioreactors are particularly advantageous for cultivating microalgae due to their efficient light utilization and ease of temperature control (Zhang et al., 2012). These systems allow for the precise manipulation of growth conditions, enabling researchers to explore the effects of varying ammonia concentrations on the growth kinetics of *S. dimorphus*. Previous studies have demonstrated that the growth rates of microalgae can be significantly affected by the concentration of nitrogen sources, including ammonia, highlighting the need for careful optimization in photobioreactor designs. Moreover, the interaction between light intensity and nutrient availability, including ammonia, is critical in maximizing biomass productivity and lipid accumulation, which are vital for biofuel applications (Gris et al., 2013; Koller et al., 2016). Research into the effects of ammonia on *S. dimorphus* growth is essential for understanding how to leverage wastewater and agricultural runoff as nutrient sources in algal cultivation. Such studies not only contribute to the development of sustainable biofuel production methods but also address environmental concerns related to nutrient pollution (Zhang et al., 2014).

This study aimed to investigate the mitigation potential of microalgae grown in a flat-plate photobioreactor to reduce high ammonia concentrations from livestock houses. By examining the impact of high ammonia levels typical of animal houses on the growth of *Scenedesmus dimorphus* (*S. dimorphus*) in flat-plate photobioreactors, this research seeks to provide insights that could enhance algal cultivation strategies and promote the use of waste resources for pollutant reduction in animal houses.

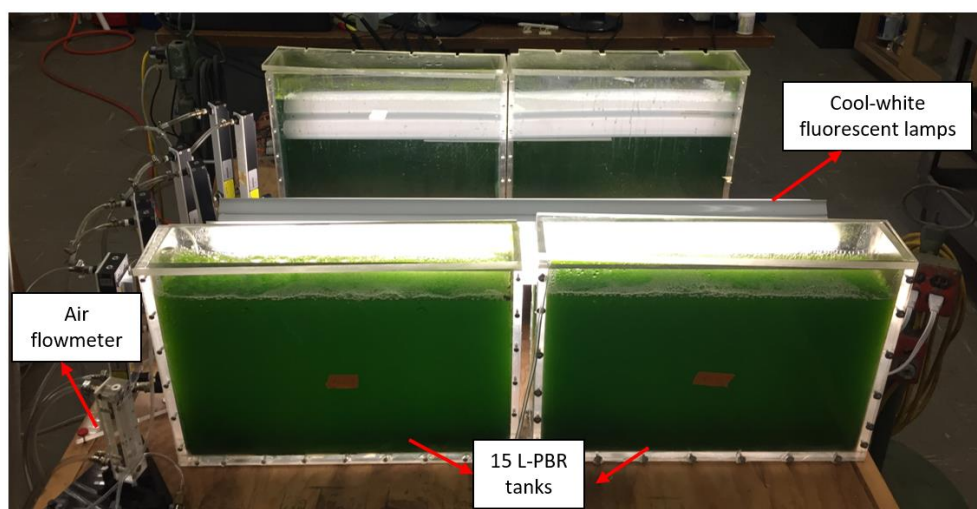
## MATERIALS AND METHODS

### Algal Cultivation

The microalgae strain (*S. dimorphus*-UTEX 1237) obtained from the UTEX Culture Collection of Algae (Texas University, USA) was used due to its high growth rate and effective NH<sub>3</sub> removal capabilities (Uguz et al., 2022; Kang et al., 2014). *S. dimorphus* was cultivated in a 1 L Erlenmeyer flasks containing 200 mL of Bold's basal medium (BBM), with the pH adjusted to 6.8–7.0 using 0.5M HCl or NaOH. The composition of the BBM can be found in Uguz et al. (2022). The prepared BBM was sterilized at 121°C for 20 minutes. Algae were initially grown in 1-L Erlenmeyer flasks containing 100 mL BBM and doubled weekly, then transferred to 15 L flat plate PBRs (constructed as given by Uguz et al. (2022), Figure 1). During the experiments, the PBR tanks were continuously aerated with air (~350 ppm CO<sub>2</sub>) at 0.5 L min<sup>-1</sup> per PBR volume. Air flowmeters (Cole-Parmer, USA) adjusted the volumetric flow rate.

### Flat-Plate Photobioreactor Setup

PBR tanks were constructed from clear acrylic sheets, with the dimensions of 35 cm in height, 50 cm in length, and 10 cm in width. CO<sub>2</sub>-enriched air was supplied through a difusser at the bottom of each PBR. Flow meters with needle valves controlled the airflow, adjusted to meet specific air requirements. Each PBR received 7.5 L min<sup>-1</sup> (0.5 vvm) of aerated air. Illumination was provided by two cool white fluorescent lamps (40 W, 122 cm long, 2100 Lumens) providing 60–70 μmol m<sup>-2</sup> s<sup>-1</sup>. Environmental conditions, including pH, temperature, water level (maintained with deionized water), aeration, and light intensity, were monitored throughout the experiments. The pH was adjusted daily to remain between 6.8–7.0 using 0.5 M HCl or 0.5 M NaOH. The room temperature was kept between 23–25°C during the 21-day experimental period. The temperature and pH were measured with a digital pH meter (Hanna Instrument, HI98128). Algae were cultivated under these conditions for 21 days, with daily cell counts measured using hemocytometers under an optical microscope. Figure 1 shows the flat-plate PBR tanks running in the laboratory.



**Figure 1.** Control and treatment PBRs running in the experiment

### Experimental Prodecure

Ammonium chloride (NH<sub>4</sub>Cl) was used as a substitute for NH<sub>3</sub> gas found in animal barn air due to NH<sub>3</sub>'s high solubility and tendency to adsorb onto surfaces, making precise control challenging (Kang et al., 2014; Uguz et al., 2022). This approach aligns with established methods for studying algal responses to ammonia stress under reproducible laboratory conditions. The nitrate-free BBM medium, making NH<sub>4</sub>Cl the sole nitrogen source, was added daily to simulate NH<sub>3</sub> concentration of 50 ppm (78 mg L<sup>-1</sup>d<sup>-1</sup> NH<sub>4</sub>Cl mass equivalent on daily bases). This concentration (50 ppm) represents maximum NH<sub>3</sub> levels in typical animal houses. Ammonia (NH<sub>3</sub>) concentrations in animal housing facilities can vary widely depending on livestock type, housing system, and environmental conditions. Typically, levels range from 0 to 40 ppm, but poor ventilation can cause concentrations to exceed 60 ppm (Kim et al., 2021; Tang et al., 2019). Each experiments were run for 21-days with three replicates each, with controls set up for comparison. Control tanks were cultivated with regular BBM (containing 0.25 gL<sup>-1</sup> NaNO<sub>3</sub>).

### Determination of Growth Parameters

Cell concentrations were determinated using a hemocytometer and an optical microscope (Olympus, Olympus Corp, Tokyo, Japan). The specific growth rate (μ, d<sup>-1</sup>) was calculated using the Eq. 1:

$$\mu = \frac{\ln N_t - \ln N_0}{t - t_0} \quad (1)$$

where N<sub>0</sub> and N<sub>t</sub> are the algae cell concentrations (cell mL<sup>-1</sup>) at times t<sub>0</sub> and t, respectively (Jiang et al., 2013; Toledo-Cervantes et al., 2013). The dry biomass concentration was determined by vacuum-filtering 25 mL of an algal sample and weighing the filter after drying it in a vacuum oven at 80 °C for 3 hours, following established protocols for dry biomass measurement (g L<sup>-1</sup>) (Uguz et al., 2022). The algal dry cell weight was calculated by dividing the dry biomass concentration by the cell count of the same sample, then normalizing it to the initial dry cell weight. The biomass weight was determined using the formula provided in Eq. 2 (Uguz et al., 2024).

$$\text{Dry biomass conc. (g L}^{-1}\text{)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Sample volume (25 mL)}} \times 1000 \text{ (mL/L)} \quad (2)$$

NH<sub>4</sub>Cl mass equivalent on a daily basis (mg L<sup>-1</sup>d<sup>-1</sup>) was calculated as:

$$M_{eq}(\text{NH}_4\text{Cl}) = M_{\text{NH}_3} \times (M_{m(\text{NH}_4\text{Cl})}/M_{m(\text{NH}_3)}) \quad (3)$$

Where  $M_{eq}(\text{NH}_4\text{Cl})$  is the NH<sub>4</sub>Cl mass equivalent (g L<sup>-1</sup>d<sup>-1</sup>),  $M_{m(\text{NH}_4\text{Cl})}$  is the molecular weight of NH<sub>4</sub>Cl (53.5 g mol<sup>-1</sup>),  $M_{m(\text{NH}_3)}$  is the molecular weight of NH<sub>3</sub> (17 g mol<sup>-1</sup>),  $M_{\text{NH}_3}$  is the NH<sub>3</sub> loading on daily basis in the influent gas (g L<sup>-1</sup>d<sup>-1</sup>) and calculation as:

$$M_{\text{NH}_3} = C_{gas} \times \rho_{gas} \times Q_{PBR} \times \left( \frac{M_{m(\text{NH}_3)}}{M_{m(\text{air})}} \right) \quad (4)$$

where  $C_{gas}$  is NH<sub>3</sub> concentration in the influent air (ppm),  $\rho_{gas}$  is the gas density at 25° and 1 atm (0.6943 kg m<sup>-3</sup>),  $Q_{PBR}$  is the airflow rate at each PBR (m<sup>3</sup> L<sup>-1</sup> d<sup>-1</sup>), and  $M_{m(\text{air})}$  is the molecular weight of air (29 g mol<sup>-1</sup>).

### Statistical Analysis

The results were presented as mean ± standard deviation. Analysis of Variance (ANOVA) and t-tests were conducted to compare the cell and dry biomass concentrations under different test conditions. 95% confidence level was chosen to assess significance. Software package JMP (version 13.0) was used for the statistical analysis.

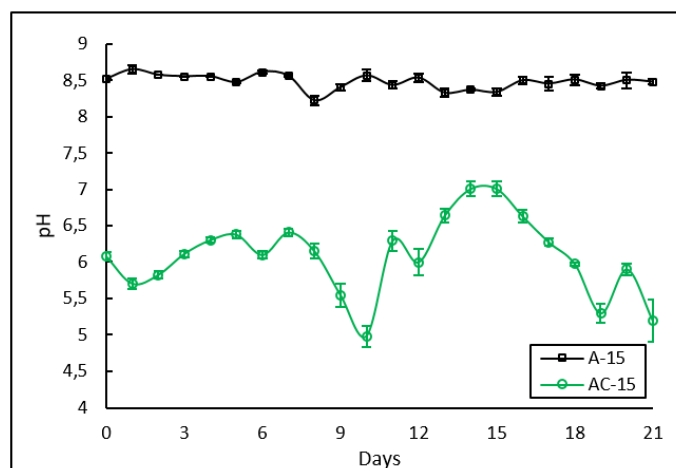
## RESULTS AND DISCUSSION

### PH in the PBR Tanks

The pH of algal culture media is a critical factor influencing algal growth, nutrient absorption, and overall biomass productivity. Optimal pH levels are essential for maximizing photosynthesis and metabolic processes in microalgae. Research indicates that most algal species thrive within a pH range of approximately 6 to 8 (Chaudhuri, 2020). Deviations from this range can lead to significant physiological stress, inhibiting enzyme activity and photosynthetic efficiency, ultimately resulting in reduced biomass production (Ambat et al., 2019; Rana, 2024). The relationship between pH and nutrient availability is particularly noteworthy. For instance, the solubility of essential nutrients such as nitrates and phosphates is pH-dependent, which directly affects algal growth (Rana, 2024; James et al., 2013). At higher pH levels, the availability of carbon dioxide decreases, which can limit photosynthesis and growth rates (Singh, 2017). Conversely, low pH levels can lead to the inhibition of enzyme activity critical for nutrient uptake and metabolic processes (Ambat et al., 2019; Rana, 2024). Therefore, maintaining an optimal pH is vital for ensuring that microalgae can efficiently utilize available nutrients.

The pH of the each PBR tank was monitored daily and adjusted to 7 using 0.5M HCl or 0.5M NaOH. The pH was influenced by dissolved CO<sub>2</sub> in the air and NH<sub>3</sub> provided by NH<sub>4</sub>Cl, as well as their uptake by algal cells. An initial pH increase in the control PBRs on Day 1 was due to the photosynthetic uptake of CO<sub>2</sub> by algae. Daily adjustments maintained a stable pH in the control group. In experimental groups receiving NH<sub>4</sub>Cl, pH ranged from 5.1 to 7.1 throughout the experiment, with significant drops observed after Day 15 due to high NH<sub>4</sub>Cl concentrations. Subsequent algal uptake and daily adjustments helped stabilize the pH. Figure 2 shows the pH change throughout of the 21-day experiment.

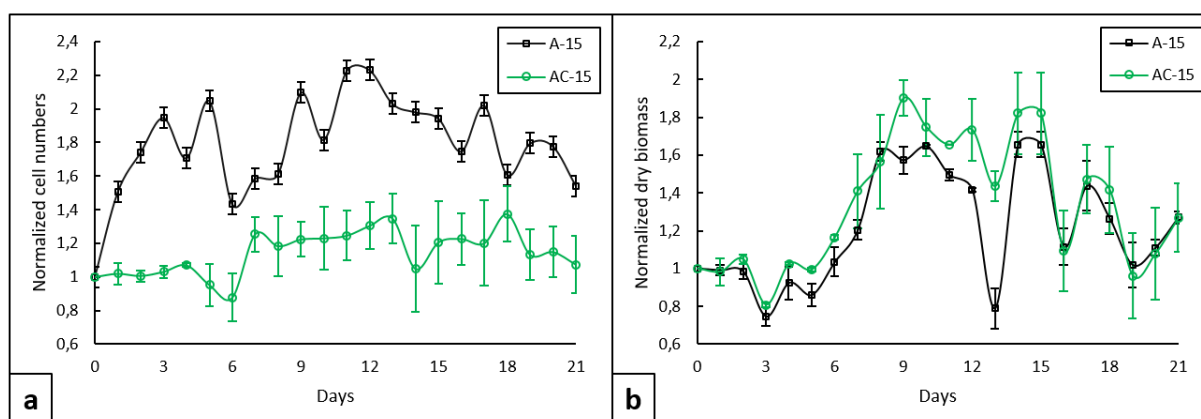
The dynamics of pH changes in algal cultures supplemented with NH<sub>4</sub>Cl are influenced by several factors, including the initial concentration of ammonium, the specific algal species, and the overall metabolic activity of the culture. For instance, studies indicate that as algal cells grow and fix carbon dioxide, they can initially increase the pH of the medium through the consumption of CO<sub>2</sub>, which is a weak acid (Roopnarain et al., 2014). However, when ammonium is added, the subsequent uptake can lead to a net decrease in pH, particularly if the concentration of ammonium is high (Pahl et al., 2012). This interplay between ammonium uptake and pH dynamics is crucial for optimizing algal cultivation conditions, as excessively low pH levels can lead to detrimental effects such as reduced enzyme activity and impaired nutrient absorption (Zhao et al., 2015; Ayre et al., 2017). The addition of ammonium chloride to algal culture media has a profound effect on pH, which in turn influences algal growth and productivity. The complex interactions between ammonium uptake, pH dynamics, and environmental factors necessitate careful monitoring and management to optimize algal cultivation conditions.



**Figure 2.** PH changes during the 21-day experiment (AC-15: PBR tanks fed with  $\text{NH}_4\text{Cl}$  and A-15 control tanks)

### Cell and Dry Biomass Concentration

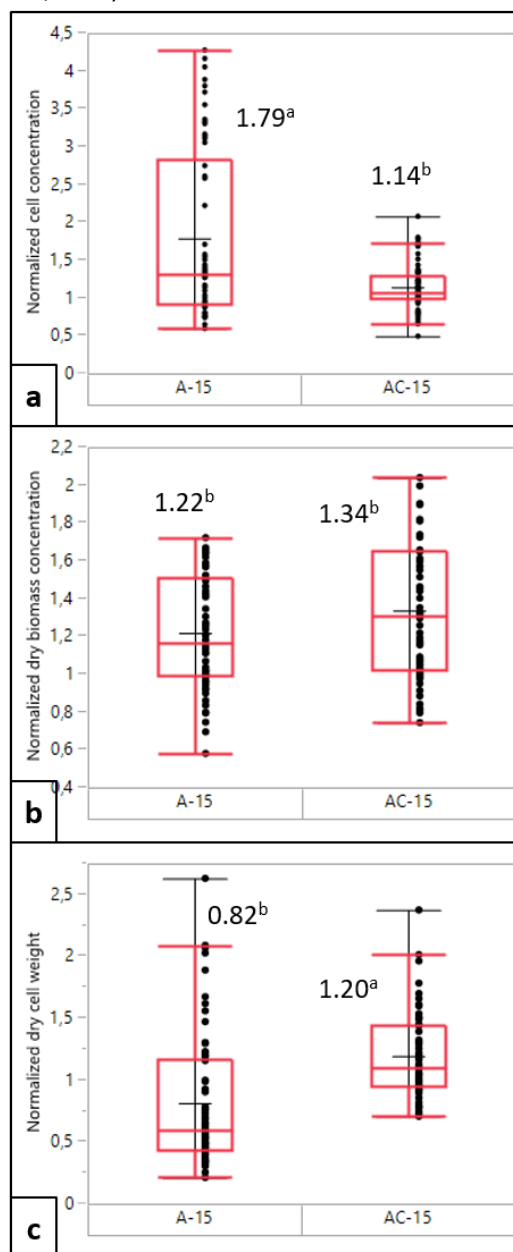
Ammonia ( $\text{NH}_3$ ) concentrations in animal housing can reach significant levels, varying by species and housing conditions. In animal production facilities, ammonia concentrations typically range from 0 to 40 ppm, with reports of levels exceeding 60 ppm under poor ventilation (Kim et al., 2021; Tang et al., 2019). Specifically, ammonia can reach up to  $35 \text{ mg/m}^3$  (about 25 ppm) in winter due to reduced ventilation (Tang et al., 2019). In poultry houses, particularly in broiler and layer systems, ammonia levels often range between 25 and 30 ppm, sometimes exceeding 50 ppm in extreme cases (Yi et al., 2016; Wei et al., 2015). For years, our team has worked on integrating photobioreactor systems into animal production to reduce ammonia and carbon dioxide. Given that ammonia levels in production can reach 50-60 ppm, this study investigates the impact of 50 ppm ammonia (worst-case scenario) on microalgae growth in photobioreactors. In this study, to investigate the effect of high  $\text{NH}_3$  concentrations on algal growth in PBRs, microalgae in A-15 PBR tanks were grown with standard BBM, while AC-15 PBR tanks were fed daily with  $78 \text{ mg L}^{-1} \text{ d}^{-1} \text{ NH}_4\text{Cl}$ , equivalent to a 50 ppm  $\text{NH}_3$  loading rate. Figure 2 shows the normalized cell and dry biomass concentration, and normalized dry cell weight of *S.dimorphus* grown under different culture conditions. Normalized algal cell number concentrations was the highest ( $1.79 \pm 0.09$ ,  $p < 0.01$ ) in the PBR tanks without  $\text{NH}_3$ . However, the dry biomass concentration was higher ( $1.34 \pm 0.02$ ,  $p < 0.01$ ) in the PBR tanks fed with 50 ppm  $\text{NH}_3$  gas loading rate. Figure 3 shows the normalized cell and dry biomass growth curves of *S.dimorphus* for 21-day experiment



**Figure 3.** Normalized cell and dry biomass numbers of *S.dimorphus* for 21-day experiment

Experiments showed a decrease in algal cell concentrations towards 50 ppm  $\text{NH}_3$ , but dry biomass concentration was increased at 50 ppm  $\text{NH}_3$  loading rate compared to  $\text{NH}_3$ -free PBR tanks (Figure 4). Ammonium ions ( $\text{NH}_4^+$ ) are a preferred nitrogen source for many algal species, as they can be assimilated more readily than other nitrogen forms, such as nitrates or urea. However, the effects of varying  $\text{NH}_3$  concentrations on algal growth are complex and can lead to both beneficial and detrimental outcomes. At optimal concentrations, ammonium can significantly enhance algal growth by providing essential nitrogen for the synthesis of proteins, nucleic acids, and chlorophyll, which are vital for cellular functions and photosynthesis (Vasileva et al., 2020). For

instance, studies have shown that moderate levels of ammonium can promote the growth of species like *Chlorella vulgaris* and *Chlamydomonas reinhardtii*, leading to increased biomass and lipid production (Liu et al., 2017; Ayre et al., 2017). The presence of free ammonia ( $\text{NH}_3$ ), which can form at higher pH levels, poses additional risks, as it is more toxic to algal cells than ammonium ions (Qian et al., 2023). The relationship between ammonia concentration and algal growth is also influenced by environmental factors such as pH, temperature, and the presence of other nutrients. For instance, maintaining a balanced pH is essential, as a drop in pH due to high ammonium levels can further exacerbate growth inhibition (Scherholz & Curtis, 2013). Additionally, the interaction between ammonium and other nitrogen sources can affect overall growth dynamics; some algal species may exhibit preferences for certain nitrogen forms, which can influence their growth rates under varying ammonia conditions (Vasileva et al., 2020).



**Figure 4.** Normalized cell and dry biomass concentration, and normalized dry cell weight of *S. dimorphus* grown under different culture conditions

The  $\text{NH}_3$  concentration in algal culture media significantly influences the dry biomass concentration of algal species, with varying effects depending on the ammonia levels present. Ammonium ions ( $\text{NH}_4^+$ ) are a preferred nitrogen source for many microalgae, promoting growth and biomass accumulation when supplied in optimal concentrations. However, the relationship between ammonia concentration and algal biomass is complex, as both low and high concentrations can lead to distinct outcomes in biomass production. At moderate



ammonia concentrations, algal growth is generally enhanced due to the efficient assimilation of nitrogen, which is crucial for protein synthesis and cellular metabolism. For instance, studies have shown that algal species such as *Chlorella protothecoides* exhibit increased biomass production when supplied with adequate ammonium levels, as nitrogen is a key component of *chlorophyll* and other cellular structures (Shabana et al., 2019; Ambat et al., 2019).

The ability of algae to uptake  $\text{NH}_3$  efficiently can lead to significant increases in dry biomass concentrations, particularly in nutrient-rich environments (Wrede et al., 2018). Conversely, excessive  $\text{NH}_3$  concentrations can have detrimental effects on algal growth and biomass accumulation. High levels of ammonium can lead to toxicity, primarily due to the formation of free ammonia ( $\text{NH}_3$ ) at elevated pH levels, which is harmful to algal cells (Zhu et al., 2020; Palkar et al., 2020). Research indicates that when  $\text{NH}_3$  concentrations exceed certain thresholds, algal growth can be inhibited, resulting in lower dry biomass concentrations. This toxicity can manifest as decreased photosynthetic efficiency, impaired metabolic functions, and ultimately reduced biomass accumulation (Wu et al., 2020).

The influence of ammonia concentration on algal biomass is also mediated by environmental factors such as temperature, light intensity, and the presence of other nutrients. For instance, Wrede et al. (2018) observed that seasonal variations in ammonia concentrations correlated with changes in algal biomass, with higher  $\text{NH}_3$  levels in winter associated with lower biomass production (Wrede et al., 2018). This suggests that while  $\text{NH}_3$  is essential for algal growth, its effectiveness as a nutrient source is contingent upon optimal environmental conditions.

## CONCLUSION

The findings in this study indicate that microalgae, specifically *Scenedesmus dimorphus*, can efficiently utilize elevated levels of ammonia typical of animal housing environments, converting these pollutants into valuable biomass. Optimal growth conditions, including controlled pH, temperature, and nutrient levels, are essential for maximizing algal growth and pollutant uptake. The research highlights that flat-plate PBRs are particularly advantageous due to their efficient light utilization and ease of temperature control, enabling the precise manipulation of growth conditions to enhance pollutant removal efficiency. The study also emphasizes the importance of optimizing PBR operational parameters, such as light intensity, nutrient concentration, and airflow rates, to maximize biomass productivity. The ability of microalgae to thrive under varying ammonia concentrations makes them a sustainable solution for reducing environmental pollutants in agricultural settings. Moreover, the generated biomass can be further utilized for biofuel production or as an alternative protein source in animal feed, contributing to a circular economy. Overall, this research underscores the potential of PBR systems as a sustainable technology for improving air quality in animal housing environments while supporting bioresource recovery. Future research should focus on scaling up these systems, refining operational strategies, and exploring the long-term impacts of using microalgae-based feed supplements to enhance the economic feasibility and environmental benefits of PBR integration in animal agriculture.

For Türkiye's livestock sector, where intensive poultry, dairy, and cattle operations are prevalent, integrating PBR technology could address critical environmental and economic challenges. Türkiye's livestock houses often face elevated ammonia levels (frequently exceeding 30 ppm), particularly in densely populated regions like the Aegean and Central Anatolia, where ventilation is limited during winter months. The high ammonia emissions not only pose health risks to animals and workers but also contribute to soil and water eutrophication. Microalgae-based PBRs could be deployed in these settings to:

- **Reduce Ammonia Emissions:** By absorbing  $\text{NH}_3$  directly from barn air, PBRs could complement existing ventilation systems, especially in confined poultry and dairy facilities where ammonia concentrations peak.
- **Utilize Local Resources:** Türkiye's abundant sunlight and moderate climate favor outdoor or greenhouse-integrated PBRs, reducing energy costs for algal cultivation.
- **Generate Co-Products:** The harvested biomass could supplement animal feed (e.g., for poultry or aquaculture), addressing Türkiye's reliance on imported feed ingredients while valorizing waste.
- **Comply with Regulations:** As Türkiye strengthens environmental policies (e.g., alignment with EU IPPC directives), PBRs could help farms meet emission limits sustainably.

However, challenges such as initial investment costs, maintenance expertise, and seasonal temperature fluctuations must be addressed through pilot projects and government incentives. Future research should focus on optimizing PBR designs for Türkiye's regional climates (e.g., insulating systems for eastern Anatolia's harsh winters) and quantifying long-term economic benefits for farmers. By aligning PBR technology with Türkiye's livestock characteristics—such as small-to-medium farm sizes and high ammonia-producing poultry operations—this approach could advance both environmental sustainability and circular bioeconomy goals.



Main significant results of the study should be presented in a clear and concise way and should be numbered.

### Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

### Author Contributions

**1<sup>st</sup> Seyit Uguz** :Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; software; writing— original draft; writing—review and editing.

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## Phosphorus And Nitrogen Removal Performance Of Zeolite And Rice Husk From Wastewaters

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### ABSTRACT

Nitrogen and phosphorus-rich wastewater effluents result in the eutrophication of water resources and threaten aquatic life. Therefore, aquatic life can be supported by wastewater treatment. In this study, filter column tests assessed the phosphorus and nitrogen removal performance of both zeolites, abundantly available in Türkiye, and rice husk, abundantly available waste material, from synthetic wastewaters. Materials were used alone and in mixtures (25:75, 50:50, and 75:25). Phosphorus and nitrogen solutions at different concentrations (0, 10, 25, and 50 mg L<sup>-1</sup>) pass through filter media at a flow rate of 5.0 mL/min. Effluent samples taken at the end of the 3rd, 6th, 12th, 24th, and 48th hours were subjected to pH, electrical conductivity (EC), total phosphorus (TP), and total nitrogen (TN) analyses. The highest nitrogen removal efficiency (75.1%) was achieved with 75:25 zeolite: rice husk mixture at the end of the 3rd hour from 10 mg L<sup>-1</sup> influent concentration and the lowest with the same mixture at the end of the 48th hour from 50 mg L<sup>-1</sup> influent concentration. The highest phosphorus removal efficiency (19.4%) was achieved with pure zeolite at the end of the 3rd hour from 10 mg L<sup>-1</sup> influent concentration and the lowest (0.14%) with 25:75 zeolite: rice husk mixture at the end of the 48th hour from 50 mg L<sup>-1</sup> influent concentration. Based on the present findings, it was concluded that rice husk further improved the nitrogen removal efficiency of zeolite.

**Keywords:** Column studies, nitrogen, phosphorus, rice husk, zeolite

## Atık Sulardan Zeolit Ve Pirinç Kabuğunun Fosfor ve Azot Giderim Performansının Belirlenmesi

### Öz

Azot ve fosfor açısından zengin atıksu deşarjları, su kaynaklarında ötrofikasyona yol açarak sucul yaşamı tehdit etmektedir. Bu nedenle, atıksuların arıtılması ile sucul yaşam desteklenebilir. Bu çalışmada, Türkiye’de bolca bulunan zeolit ve atık malzeme olarak bolca bulunan pirinç kabuğunun, sentetik atıksulardan fosfor ve azot giderme performansı, filtre kolon testleriyle değerlendirilmiştir. Malzemeler hem tek başlarına hem de karışımlar halinde (25:75, 50:50 ve 75:25) kullanılmıştır. Farklı konsantrasyonlardaki fosfor ve azot çözeltileri (0, 10, 25 ve 50 mg L<sup>-1</sup>) filtre ortamından 5.0 mL/dakika debiyle geçirilmiştir. 3. saat, 6. saat, 12. saat, 24. saat ve 48. saat sonunda alınan effluent örnekleri pH, elektriksel iletkenlik (EC), toplam fosfor (TP) ve toplam azot (TN) analizlerine tabi tutulmuştur. En yüksek azot giderme verimliliği (%75.1), 10 mg L<sup>-1</sup> giriş konsantrasyonundan 3. saatin sonunda, 75:25 zeolit:pirinç kabuğu karışımıyla elde edilmiştir; en düşük verimlilik ise aynı karışımla 48. saatin sonunda 50 mg L<sup>-1</sup> giriş konsantrasyonunda elde edilmiştir. En yüksek fosfor giderme verimliliği (%19.4), 10 mg L<sup>-1</sup> giriş konsantrasyonundan 3. saatin sonunda saf zeolit ile elde edilmiştir; en düşük verimlilik ise (%0.14) 50 mg L<sup>-1</sup> giriş konsantrasyonundan 48. saatin sonunda 25:75 zeolit:pirinç kabuğu karışımıyla elde edilmiştir. Elde edilen bulgulara dayanarak, pirinç kabuğunun zeolit azot giderme verimliliğini artırdığı sonucuna varılmıştır.

**Anahtar kelimeler:** Azot, Fosfor, Kolon çalışmaları, Pirinç Kabuğu, Zeolit

## INTRODUCTION

By 2015 and 2030, the world's population will grow to approximately 7.2 and 8.3 billion, respectively. Significant improvements in food production are necessary in such a situation. There are several ways to increase food production: farming more land, raising crop yields, planting two or three crops annually on the same plot of acreage, or combining these methods. They all have different benefits and drawbacks, however. Some other options call for more labor-intensive production techniques. For example, use mechanization, high-yield cultivars, irrigation, fertilizers, and pesticides (Özkay et al., 2014).

Water is an indispensable component of life on Earth. Although 75% of the world is covered with water, it does not necessarily mean water is abundant. Only 0.75% of water resources are drinkable. The world population, approximately 1 billion at the end of the 18th century when the Industrial Revolution began, has reached 7.92 billion. With a rapidly increasing population, domestic, industrial, and agricultural water needs have also increased. Besides these increasing needs, global warming and climate change seriously pressure available water resources. Developing industries and technology are insufficient for treating wastewater (Vo et al., 2014).

According to long-term research of the International Water Management Institute (IWMI), countries with an available water resource of <1700 m<sup>3</sup> per capita are classified as “water-stressed” countries. On the other hand, countries with an available water resource of < 1,000 m<sup>3</sup> are classified as “water-poor” countries (IWMI, 2023).

Türkiye has an annual total available water resources of 112 billion m<sup>3</sup> and a population of 85 million. Therefore, the annual amount of available water resources per capita is approximately 1315 m<sup>3</sup> (112 billion / 85 million). With that value, Türkiye is classified among the water-stressed countries (DSI, 2022). Given the nation's limited water supplies, reusing low-quality or treated water is crucial for agricultural irrigation. However, pollutants such as heavy metals and trace elements found in treated waters can cause several issues for plants and soils (Özkay et al., 2014).

Therefore, reuse of water resources has become a prominent issue. Wastewater primarily contains nitrogen, phosphorus, pathogenic organisms, heavy metals, and trace organic pollutants. Nitrogen and phosphorus pollution mainly result in the eutrophication of water resources, thus adversely affecting water quality, and these waters cannot be used as drinking water. Such pollution threatens aquatic life (USEPA, 1988; Eugenia Valsami, 2004; EEA, 2006).

Nitrogen may generate dangerous ground water pollution and pose serious environmental and human health risks. Spalding and Exner (1993) indicated nitrate as groundwaters' most common chemical pollutant. Nitrate-contaminated waters can reach surface waters through groundwater flows, disrupt nitrogen-phosphorus balance, and result in algae formation and eutrophication of these environments (Bolger & Stevens, 1999). Agrochemicals constitute the most significant source of nitrate in groundwaters.

Intensive use of cleaning chemicals, such as detergents, soaps, and shampoos, generates phosphorus pollution of water. Various methods, such as activated carbon, reverse osmosis, and electrodialysis, are used for phosphorus removal from wastewater (Yeoman et al., 1988). Recent research primarily focused on phosphorus removal using adsorbent solid media.

Water resources that are already used are considered wastewater. Wastewaters have to pass through treatment processes not to alter the physical, chemical, and ecological characteristics of the environments into which they are discharged (SKKY, 2004). Various treatment methods are used to reuse water resources. These methods include physical processes (aeration, sieves, and filters), biological processes (anaerobic treatment and oxidation), chemical processes (neutralization, disinfection, and flocculation), and membrane processes (filtration and osmosis) (Rezai and Allahkarami, 2021).

In cases where the population density is not high and conventional treatment plants cannot be built by making high-cost investments, natural treatment systems are constructed with low investment costs and without negative impacts on the environment. These systems generally serve small communities. Natural treatment systems called constructed wetlands, are similar to natural wetlands and imitate the ongoing processes of natural wetlands (Gökalp and Tas, 2018). Artificial wetlands are used as low-cost and sustainable wastewater treatment systems, especially in rural areas. For the removal of phosphorus, which is the primary source of pollution in domestic wastewater, the adsorption capacity of the filler material plays a critical role (Akçakoca and Gökalp, 2020).

Constructed wetlands contain plants and soil (filter material) to treat wastewater (EPA, 1993). They are commonly used for the treatment of industrial, agricultural, and domestic wastewater (Dombush, 1989; Du Bowry and Reaves, 1994; Riviera et al., 1997; Trautmann et al., 1989; Cooper et al., 1997; Schreijer et al., 1997).

Zeolites are hydrated aluminum silicates in crystalline structures originating from volcanic rocks. They contain discontinuous micropores and are widely used as adsorbents and catalysts (Chmielewska, 2014; Anonymous, 2022). Zeolites are used as soil stabilizers, fertilizer additives, water retainers, and soil aerators in

agriculture, as a growing medium in plant production, in landscaping and gardening, as feed additive and animal bedding material in livestock operations, as mechanical and chemical filters in treatment, in holding toxic wastes, as filter material in pools and spas, in aquariums, as cat litter, in cleaning materials, textile, paper and energy industries, in health sector, as a catalyst in aquaculture and chemistry. They also have a wide range of uses in waste and utility water treatment (Çeğin and İmamoğlu, 2005; MTA, 2021).

Paddy farming is practiced by over 1.3 million decare in Türkiye, and annual production is about 1 million tons. While 600 thousand tons of this production is rice, the remaining 400 thousand tons emerge as husk, a waste material (TUIK, 2022). Rice husk is a kind of bio-waste mainly used as an adsorbent. Rice husk creates significant environmental problems in managing and disposing of agricultural wastes (Kalderis et al., 2008; Asadi et al., 2008).

Current research on constructed wetlands mainly focuses on better and more efficient filter or substrate materials for phosphorus and nitrogen removal from wastewater. Different materials have been tested, and porous materials are more successful in removing phosphorus and nitrogen from wastewater. In this study, filter column tests assessed the phosphorus and nitrogen removal performance of both zeolites, abundantly available in Türkiye, and rice husk, abundantly available waste material, from synthetic wastewaters.

## MATERIALS AND METHODS

Zeolite and rice husk were used as adsorbent materials in column tests. Gördes Zeolite Co. supplied zeolite, and a local farmer supplied rice husk. The material size was selected as between 0.5 - 1.0 mm. Physico-chemical characteristics of zeolite and rice husk are provided in Table 1. Besides pure zeolite and rice husk, different mixtures (25:75, 50:50, and 75:25) of these materials were also used in the present experiments (Table 2).

**Table 1.** Physico-chemical characteristics of rice husk and zeolite

Rice Husk Property	Value	Zeolite Property	Value
SiO <sub>2</sub>	90.89%	SiO <sub>2</sub>	71.6%
Al <sub>2</sub> O <sub>3</sub>	0.93%	Al <sub>2</sub> O <sub>3</sub>	11.3%
CaO	1.25%	CaO	2.27%
Fe <sub>2</sub> O <sub>3</sub>	0.47%	Fe <sub>2</sub> O <sub>3</sub>	1.39%
K <sub>2</sub> O <sub>3</sub>	2.34%	K <sub>2</sub> O	3.67%
MgO	0.81%	MgO	0.86%
Na <sub>2</sub> O	-	Na <sub>2</sub> O	0.86%
pH	6.5-7.5	pH	6.5–7.5
Moisture Content	8–12%	Moisture Content	8%
Water Retention Cap.	(105). %	Water Retention Cap.	75%

**Table 2.** Experimental treatments (mixture ratios, %)

Treatments	Rice Husk (%)	Zeolite (%)
100R	100	-
100Z	-	100
75R25Z	75	25
50R50Z	50	50
25R75Z	25	75

Column tests were conducted in glass pipes with a length of 60 cm and an internal diameter of 30 mm. Initially, substrate materials were placed into filter columns (100 cm<sup>3</sup>). Substrate columns were saturated with phosphorus solutions (mono potassium phosphate) and nitrogen solutions (potassium nitrate) at different concentrations (0, 10, 25, and 50 mg L<sup>-1</sup>). The synthetic solution of each concentration was passed through the column at flow rates of 5.0 mL min<sup>-1</sup>. A peristaltic pump (Longer Pump brand) was used to adjust flow rates. Substrate columns were subjected to 3, 6, 12, 24, and 48 hours of hydraulic retention times. Effluent samples were taken at the end of each hydraulic retention time. Samples were then analyzed for pH, EC, phosphorus, and nitrogen concentration. Sample pH was measured using a Hanna-brand pH meter with a glass electrode. Sample EC was measured using an EC-meter (Hanna brand). Total phosphorus (TP) was measured using the ammonium molybdate blue color method in a spectrophotometer at 882 nm. Total nitrogen (TN) was measured using a nitrate-selective electrode (Hanna brand).

## RESULTS AND DISCUSSION

Solutions containing different phosphorus and nitrogen concentrations (10, 25, and 50 mg L<sup>-1</sup>) prepared under laboratory conditions were applied to the columns containing filter materials at a flow rate of 5 mL min<sup>-1</sup>. Effluent samples were taken from column ends at 3rd, 6th, 12th, 24th and 48th hours. The samples obtained were subjected to pH, EC, total nitrogen (TN), and total phosphorus (TP) analyses.

### pH Values

The pH values of effluent samples are given in Table 3. Initial pH levels of the solutions prepared at different concentrations (10, 25, and 50 mg L<sup>-1</sup>) were determined as 5.70, 5.41, and 5.03, respectively. Effluent pH values varied between 6.20 (50 mg L<sup>-1</sup>, 50Z50R) and 7.37 (10 mg L<sup>-1</sup>, 25Z75R) at the end of the 3rd hour, between 6.13 (50 mg L<sup>-1</sup>, 50Z50R) and 7.28 (10 mg L<sup>-1</sup>, 100Z) at the end of the 6th hour, between 6.06 (50 mg L<sup>-1</sup>, 100Z) and 6.88 (10 mg L<sup>-1</sup>, 100R) at the end of the 12th hours, between 6.07 (50 mg L<sup>-1</sup>, 75Z25R) and 6.96 (10 mg L<sup>-1</sup>, 25Z75R) at the end of the 24th hour and between 6.01 (50 mg L<sup>-1</sup>, 25Z75R) and 7.56 (10 mg L<sup>-1</sup>, 50Z50R) at the end of 48th hour. According to the Water Pollution Control Regulation of the Ministry of Environment, Urbanization and Climate Change of the Republic of Türkiye, pH values of wastewater effluents should be between 6-10 (SKKY, 2004). Present pH values all comply with discharge criteria.

**Table 3.** Effects of mixture ratios on filtrate pH

Mixtures	Hydraulic retention times / Solution concentrations (mg L <sup>-1</sup> )														
	3 h			6 h			12 h			24 h			48 h		
	10	25	50	10	25	50	10	25	50	10	25	50	10	25	50
Zeolite	7.32	6.71	6.4	7.28	6.55	6.14	6.76	6.33	6.06	6.73	6.31	6.11	6.64	6.35	6.15
Rice Husk	6.92	6.48	6.35	6.9	6.43	6.33	6.88	6.42	6.27	6.72	6.38	6.24	6.68	6.25	6.22
75Z - 25R	7.05	6.84	6.49	7.02	6.57	6.35	6.82	6.44	6.13	6.68	6.25	6.07	6.63	6.24	6.06
50Z - 50R	6.88	6.52	6.2	6.8	6.44	6.13	6.77	6.41	6.11	6.87	6.36	6.09	7.56	6.33	6.08
25Z - 75R	7.37	6.54	6.23	6.8	6.42	6.16	6.82	6.45	6.12	6.96	6.48	6.1	6.9	6.49	6.01

### EC (μS/cm) Values

The EC values of effluent samples are given in Table 4. Initial EC values of the solutions prepared at different concentrations (10, 25, and 50 mg L<sup>-1</sup>) were determined as 116.5, 227.58, and 442.89 μS/cm, respectively. Effluent EC values varied between 139.20 (10 mg L<sup>-1</sup>, 50Z50R) and 618.0 μS/cm (50 mg L<sup>-1</sup>, 100R) at the end of 3rd hour, between 136.70 (10 mg L<sup>-1</sup>, 75Z25R) and 579.90 μS/cm (50 mg L<sup>-1</sup>, 100R) at the end of 6th hour, between 132.60 (10 mg L<sup>-1</sup>, 75Z25R) and 580.60 μS/cm (50 mg L<sup>-1</sup>, 100R) at the end of 12th hour, between 128.30 (10 mg L<sup>-1</sup>, 100Z) and 602.60 μS/cm (50 mg L<sup>-1</sup>, 100R) at the end of 24th hour and between 125.70 (10 mg L<sup>-1</sup>, 75Z25R) and 559.30 μS/cm (50 mg L<sup>-1</sup>, 100R) at the end of 48th hour. According to the Water Pollution Control Regulation of the Ministry of Environment, Urbanization and Climate Change of the Republic of Turkey, EC values of wastewater effluents should be <2000 μS/cm. Present EC values all comply with discharge criteria.

**Table 4.** Effects of mixture ratios on filtrate EC (mS/cm)

Hydraulic retention times / Solution concentrations (mg L <sup>-1</sup> )	Mixtures	Zeolite	Rice Husk	75Z - 25R	50Z - 50R	25Z - 75R
3 h	10	144.6	153.4	147.8	139.2	210.7
	25	259.1	294.8	280.6	244.7	283.6
	50	455.6	618	486.5	490.4	535.4
6 h	10	142.4	140.3	136.7	145.5	176.9
	25	252.8	282.4	261.1	249.2	280.9
	50	467.8	579.9	499	493.1	542.2
12 h	10	146	172.8	132.6	165.2	154.6
	25	259.7	278.2	259.2	270.4	278.9
	50	498.6	580.6	512.2	510.2	544.9
24 h	10	128.3	195.9	129.6	168.5	156.8
	25	272.6	288	272.7	277	283.1
	50	513.8	602.6	516.8	519.3	551.5
48 h	10	213.2	132.5	125.7	173.6	216.5
	25	281.3	285.1	280.1	284.1	285.7
	50	546.9	559.3	524.1	525.7	555.8



Synthetic wastewaters were prepared using potassium nitrate (KNO<sub>3</sub>) and potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>). Increasing phosphate and nitrate salt concentrations are expected to increase EC values. Therefore, it is important to carry out studies to increase the exchange capacity of materials to achieve more successful results.

#### TN (mg L<sup>-1</sup>) Values

The TN values of effluent samples are given in Table 5. Effluent TN values varied between 2.49 (10 mg L<sup>-1</sup>, 75Z25R) and 40.33 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 50Z50R) at the end of 3rd hour, between 3.78 (10 mg L<sup>-1</sup>, 75Z25R) and 45.73 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 50Z50R) at the end of 6th hour, between 5.36 (10 mg L<sup>-1</sup>, 75Z25R) and 48.36 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 50Z50R) at the end of 12th hour, between 6.93 (10 mg L<sup>-1</sup>, 75Z25R) and 48.90 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 50Z50R) at the end of 24th hour and between 8.45 (10 mg L<sup>-1</sup>, 75Z25R) and 49.65 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 75Z25R) at the end of 48th hour. According to the Water Pollution Control Regulation of the Ministry of Environment, Urbanization and Climate Change of the Republic of Turkey, TN values of wastewater effluents should be < 100 mg L<sup>-1</sup> (SKKY, 2004). Present TN values were all comply with discharge criteria.

Montalvo et al. (2011) applied an ammonium solution at a concentration of 100 ppm to zeolite and limestone and achieved 95% success. Lin et al. (2014) applied ammonium solutions at a concentration of 200 ppm to 300 g of zeolite material at a flow rate of 10 ml/min and achieved a success of up to 100%. Kumak (2022) applied nitrogen solutions at 10, 20, and 40 ppm concentrations to zeolite and pumice materials for 96 hours and achieved 58% removal efficiency in 50-50% mixtures of both materials. Karapınar (2009) applied ammonium solution at 10, 20, and 40 ppm concentrations to zeolite material for 90 minutes and achieved about 40% removal efficiency.

**Table 5.** Effects of mixture ratios on filtrate TN (mg L<sup>-1</sup>)

	Mixtures	Hydraulic retention times / Solution concentrations (mg L <sup>-1</sup> )				
		Zeolite	Rice Husk	75Z - 25R	50Z - 50R	25Z - 75R
3 h	10	2.57	4.48	2.49	3.09	3.05
	25	14.80	12.8	12.39	13.10	14.56
	50	35.46	37.33	31.50	14.56	38.10
6 h	10	4.71	6.79	3.78	5.57	5.68
	25	19.23	14.86	14.92	14.90	18.3
	50	39.66	41.36	39.43	45.73	40.9
12 h	10	6.13	7.78	5.36	8.35	6.60
	25	22.93	18.36	16.63	15.36	22.83
	50	43.46	45.26	42.46	48.36	43.13
24 h	10	8.25	8.44	6.93	8.90	7.22
	25	23.03	22.36	19.71	16.73	23.43
	50	47.26	46.40	47.31	48.90	46.43
48 h	10	9.20	9.26	8.45	9.35	8.98
	25	24.76	23.63	22.53	19.53	24.23
	50	49.53	48.53	49.65	49.50	48.26

#### TN Removal Performance (%)

Nitrogen removal performance (%) of different filter materials from wastewater was calculated. The nitrogen removal performance of the materials varied between 19.34% (50 mg L<sup>-1</sup>, 50Z50R) and 75.10% (10 mg L<sup>-1</sup>, 25Z75R) at the end of the 3rd hour, between 8.54% (50 mg L<sup>-1</sup>, 50Z50R) and 62.20% (75Z25R) at the end of the 6th hour, 3.28% (50 mg L<sup>-1</sup>, 50Z50R) to 46.40% (10 mg L<sup>-1</sup>, 75Z25R) at the end of the 12th hour, between 2.20% (50 mg L<sup>-1</sup>, 50Z50R) and 30.70% (10 mg L<sup>-1</sup>, 75Z25R) at the end of the 24th hour and between 0.70% (50 mg L<sup>-1</sup>, 75Z25R) and 15.50% (10 mg L<sup>-1</sup>, 75Z25R) at the end of the 48th hour (Table 6). The 25Z75R mixture showed the highest nitrogen removal performance among the materials used in this study.

Present findings on nitrogen removal efficiencies comply with the findings of Lin et al. (2014), Karapınar (2009), Montalvo et al. (2011), and Kumak (2022).

**Table 6.** Nitrogen removal performance of substrate materials (%)

Hydraulic retention times / Solution concentrations (mg L <sup>-1</sup> )	Mixtures	Zeolite	Rice Husk	75Z - 25R	50Z - 50R	25Z - 75R
	10	74.3	55.20	69.50	69.10	75.10
3 h	25	40.80	48.68	41.76	47.60	50.44
	50	29.08	25.34	23.80	19.34	37.00
	10	52.90	32.10	62.20	44.30	43.20
6 h	25	23.08	40.56	40.32	40.40	26.80
	50	20.68	17.28	21.14	8.54	18.20
	10	38.70	22.20	46.40	16.50	34.00
12 h	25	8.28	26.56	33.48	38.56	8.68
	50	13.08	9.48	15.08	3.28	13.74
	10	17.50	15.60	30.70	11.00	27.80
24 h	25	7.88	10.56	21.16	33.08	6.28
	50	5.48	7.20	5.38	2.20	7.14
	10	8.00	7.40	15.50	6.50	10.20
48 h	25	0.96	5.48	9.88	21.88	3.08
	50	0.94	2.94	0.70	1.00	3.48

### TP Values

The TP values of effluent samples are given in Table 7. Effluent TP values varied between 8.06 (10 mg L<sup>-1</sup>, 100Z) and 49.20 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 100Z) at the end of the 3rd hour, between 8.64 (10 mg L<sup>-1</sup>, 100Z) and 49.65 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 25Z75R) at the end of the 6th hour, between 8.90 (10 mg L<sup>-1</sup>, 75Z25R) and 49.77 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 25Z75R) at the end of the 12th hour, between 8.44 (10 mg L<sup>-1</sup>, 100Z) and 49.90 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 25Z75R) at the end of the 24th hour and between 8.86 (10 mg L<sup>-1</sup>, 100Z) and 49.93 mg L<sup>-1</sup> (50 mg L<sup>-1</sup>, 25Z75R) at the end of the 48th hour. According to the Water Pollution Control Regulation of the Ministry of Environment, Urbanization and Climate Change of the Republic of Turkey, TP values of wastewater effluents should be < 10 mg L<sup>-1</sup> (SKKY, 2004). In this study, only 10 mg L<sup>-1</sup> influent concentration met this criterion, and the other influent concentrations (25 and 50 mg L<sup>-1</sup>) did not meet the discharge criterion.

**Table 7.** Effects of mixture ratios on filtrate TP (mg L<sup>-1</sup>)

Hydraulic retention times / Solution concentrations (mg L <sup>-1</sup> )	Mixtures	Zeolite	Rice Husk	75Z - 25R	50Z - 50R	25Z - 75R
	10	8.06	8.70	8.36	9.27	8.91
3 h	25	23.59	23.88	23.07	23.44	23.61
	50	49.20	48.29	43.42	47.24	48.73
	10	8.64	9.35	8.80	9.35	9.35
6 h	25	23.52	24.07	22.72	24.43	24.30
	50	49.31	48.76	45.74	48.99	49.65
	10	8.99	9.43	8.90	9.42	9.55
12 h	25	23.13	24.33	22.24	24.60	24.67
	50	48.01	49.44	47.67	49.13	49.77
	10	8.44	9.68	8.97	9.50	9.63
24 h	25	23.66	24.67	22.09	24.65	24.69
	50	47.68	49.38	48.59	49.64	49.90
	10	8.86	9.92	9.37	9.62	9.85
48 h	25	24.17	24.80	21.88	24.96	24.89
	50	47.44	49.82	49.05	49.87	49.93

Ping et al. (2008) applied phosphorus at a concentration of 1.5 ppm to zeolite material for 24 hours and achieved 95% removal efficiency. Montalvo et al. (2011) applied phosphorus solution at 40 ppm concentration to zeolite and limestone and achieved 67% success in phosphorus removal. Uzun et al. (2021) applied phosphorus solutions at 10, 20, and 40 ppm concentrations to sand, pumice, and zeolite materials for 96 hours and achieved an average of 90% success. Vera et al. (2014) applied a phosphorus solution at a concentration of 100 ppm to a 12-liter volume of zeolite material and determined a removal efficiency of up to 50% after one year. Lin et al. (2014) applied phosphorus solutions at a concentration of 400 ppm to 300 g of zeolite material at a flow rate of 10 ml/min and achieved a success of up to 100%. Jiang et al. (2013) applied phosphorus solution at two ppm concentrations to zeolite and iron sponges for 48 hours, achieving an average of 90% success. Karapınar (2009) applied a phosphorus solution at 10, 20, and 40 ppm concentrations to zeolite material for 90 minutes and determined approximately 40% removal efficiency. Shi et al. (2019) applied a solution containing 10 ppm phosphorus to zeolite material at a flow rate of 22.5 ml/min for 360 minutes and determined 70% removal efficiency.

### TP Removal Performance (%)

Phosphorus removal performance (%) of different filter materials from wastewater was calculated. The phosphorus removal performance of the materials varied between 1.60% (50 mg L<sup>-1</sup>, 100Z) and 19.40% (10 mg L<sup>-1</sup>, 100Z) at the end of 3<sup>rd</sup> hour, between 0.70% (50 mg L<sup>-1</sup>, 24Z75R) and 13.60% (10 mg L<sup>-1</sup>, 100Z) at the end of the 6<sup>th</sup> hour, between 0.46% (50 mg L<sup>-1</sup>, 25Z75R) and 11.04% (25 mg L<sup>-1</sup>, 75Z25R) at the end of the 12<sup>th</sup> hour, between 0.20% (50 mg L<sup>-1</sup>, 25Z75R) and 15.60% (10 mg L<sup>-1</sup>, 100Z) at the end of the 24<sup>th</sup> hour and between 0.14% (50 mg L<sup>-1</sup>, 25Z75R) and 12.48% (25 mg L<sup>-1</sup>, 75Z25R) at the end of the 48<sup>th</sup> hour (Table 8). In this study, while 100Z yielded quite a high removal efficiency at an influent concentration of 10 mg L<sup>-1</sup>, a 25% rice husk mixture increased the effect of material at higher concentrations.

**Table 8.** Phosphorus removal performance of substrate materials (%)

Hydraulic retention times / Solution concentrations (mg L <sup>-1</sup> )	Mixtures		Zeolite	Rice Husk	75Z - 25R	50Z - 50R	25Z - 75R
	3 h	10	19.40	13.00	16.40	7.30	10.90
		25	5.64	4.48	7.72	6.24	5.56
		50	1.60	3.42	13.16	5.52	2.54
	6 h	10	13.60	6.50	12.00	6.50	6.50
		25	5.92	3.72	9.12	2.28	2.80
		50	1.38	2.48	8.52	2.02	0.70
	12 h	10	10.10	5.70	11.00	5.80	4.50
		25	7.48	2.68	11.04	1.60	1.32
		50	3.98	1.12	4.68	1.74	0.46
24 h	10	15.6	3.20	10.30	5.00	3.70	
	25	5.36	1.32	11.64	1.40	1.24	
	50	4.46	1.24	2.82	0.72	0.20	
48 h	10	11.40	0.80	6.30	3.80	1.50	
	25	3.32	0.80	12.48	0.16	0.44	
	50	5.12	0.36	1.90	0.26	0.14	

Present findings on phosphorus removal efficiencies comply with the findings of previous studies. It was determined that the findings obtained from the study showed similar results to previous studies (Lin et al., 2014); Karapinar, 2009; Montalvo et al., 2011; Kumak, 2022). Differences were mainly attributed to influent concentrations, filter materials, and sampling times.

### CONCLUSION

In present column tests, different concentrations (10, 25, and 50 mg L<sup>-1</sup>) of phosphorus and nitrogen-containing synthetic wastewaters were applied to zeolite, rice husk, and mixtures of these materials (25% - 75%, 50% - 50%, and 75% - 25%) for 48 hours and effluent samples were taken at the end of 3rd, 6th, 12th, 24th and 48th hours. Present findings revealed that effluent pH, EC, and TN values were at desired levels after treatment and met the discharge criteria specified in Water Pollution Control Regulations. However, TP values met relevant criteria only at 10 mg L<sup>-1</sup> influent concentration and did not meet the criteria at higher concentrations. Present findings revealed that zeolite had quite a high nitrogen removal efficiency, and rice husk further improved the nitrogen removal efficiency of zeolite. Zeolite was also highly efficient in phosphorus removal from wastewater, especially at low concentrations and flow rates.

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### Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Author Contributions

**1<sup>st</sup> Yusuf Can Aşık:** formal analysis; investigation; writing— original draft; writing—review and editing.

**2<sup>st</sup> Hasan Ali İrik :**Conceptualization; data curation; funding acquisition; methodology; project administration; software; writing— original draft; writing—review and editing.

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Original Article

## Determinants of Environmental Pollution Awareness Among University Students: A Case Study of Atatürk University

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### ABSTRACT

Environmental pollution has adverse effects on living organisms and contributes to a wide range of health issues in individuals. Given this reality, enhancing public awareness and knowledge regarding environmental issues is of utmost importance. Accordingly, this study aims to identify the factors influencing environmental pollution awareness levels among university students. The primary objective is to determine which components of air, radiation, and noise pollution students are most aware of and concerned about. Achieving this objective required conducting a survey through face-to-face interviews with 400 students enrolled in various departments at Atatürk University in Erzurum, Turkey. The survey was designed to collect data for an academic study and to examine the extent to which students' understanding of environmental pollution is influenced by various sociodemographic and behavioral factors, including gender, household education level, mother's employment status, regular breakfast and exercise habits, interest in social sciences, and daily television consumption. The findings indicate that these factors significantly shape students' awareness of environmental pollution. Results from logistic regression analysis reveal that each of these variables exerts a statistically significant impact when considered simultaneously. Based on the study's conclusions, it is recommended that family members serve as the primary initiators of environmental education. Furthermore, local governments should implement systematic and consistent educational programs addressing environmental pollution. Additionally, the media holds significant potential for disseminating awareness and information effectively, thereby playing a crucial role in fostering students' environmental consciousness..

**Key words:** Environment, awareness, logit, university students, Turkey.

## Üniversite Öğrencilerinde Çevre Kirliliği Farkındalığını Etkileyen Faktörler: Atatürk Üniversitesi Örneği

### ÖZ

Çevre kirliliği canlılar üzerinde olumsuz etkilere sahiptir ve bireyler üzerinde çok çeşitli rahatsızlıklara neden olabilmektedir. Bu durum göz önüne alındığında insanların çevre sorunları konusunda farkındalığını ve bilgi düzeyini artırmak son derece önemlidir. Bu nedenle bu çalışma üniversite öğrencileri arasında çevre kirliliği farkındalık düzeyini etkileyen unsurları belirlemek için yapılmıştır. Öğrencilerin hava, radyasyon ve gürültü kirliliğinin hangi bileşenlerinin en çok farkında olduklarını ve endişe duyduklarını belirlemek çalışmanın temel amacını oluşturmuştur. Bu amacı gerçekleştirmek için Erzurum ilinde bulunan Atatürk Üniversitesi'nin çeşitli bölümlerine kayıtlı dört yüz öğrenciyle fakültelerde yüz-yüze görüşülerek anket soruları cevaplandırılmıştır. Bu anketin amacı, akademik çalışma için bilgi toplamak olup öğrencilerin çevre kirliliği anlayışının; cinsiyet, hane halkının eğitim düzeyi, annenin çalışma durumu, düzenli kahvaltı ve spor yapma alışkanlığı, sosyal bilimlere ilgisi

ve günlük televizyon izleme süresi gibi çeşitli faktörlerden ne derece etkilendiğini araştırmaktır. Araştırma sonucunda, bu faktörlerin tamamının öğrencilerin çevre kirliliği konusundaki farkındalığını önemli ölçüde etkilediği belirlenmiştir. Lojistik regresyon analizi sonuçları, bu unsurların her birinin aynı anda önemli bir etki uyguladığını göstermiştir. Çalışmanın sonuçlarına göre çevre eğitiminin öncelikli başlatıcısının aile bireyleri olması gerektiği, yerel yönetimlerin çevre kirliliği konusunda tutarlı ve metodik eğitim programları düzenlemesi gerektiği, medyanın da farkındalık ve bilgilendirmeyi başarılı bir şekilde yaygınlaştırma potansiyeli ile öğrencilerin çevre bilinci konusunda eğitilebileceği sonucuna varılmıştır.

**Anahtar kelimeler:** Çevre, farkındalık, logit, üniversite öğrencileri, Türkiye.

## INTRODUCTION

Global environmental pollution poses a major challenge to the planet's long-term habitability. The environment is essential for sustaining life on Earth; however, increasing global pollution has become a major concern in recent years (Awewomom et al., 2024). Harmful substances and pollutants released into the air, soil, and water cause significant damage to the environment and human health, leading to environmental contamination and pollution (Li et al., 2019). Environmental contamination has received significant scrutiny as a critical issue over the past century, posing a serious threat to all forms of life unless appropriate measures are taken. Globally, nations strive to enhance their citizens' quality of life through development; however, this often leads to environmental challenges such as pollution and degradation. Rapid economic growth frequently results in environmental destruction, particularly in developing countries. The transformative effects of economic activities and expansion put pressure on the environment, leading to the redefinition of green spaces, the depletion of natural resources, and the pollution of land, water bodies, and air (Long et al. 2023). Environmental pollution can occur in different forms, such as air, water, soil, radioactive, noise, and light pollution (Michalski and Ficek, 2016).

Urbanization and industrialization have resulted in an increase in air pollution, which has detrimental effects on human health, plant life, and agricultural practices. Wildfires, livestock emission, and volcanoes are the natural sources of air pollution. Anthropogenic sources include agriculture, deforestation, denitrification, transportation, urbanization, and industry. These processes lead to the formation of contaminants and suspended particles in the atmosphere, which have adverse effects on human health, plant growth, and agricultural activities (Rafie-Rad et al., 2024). In 2019, the global mortality toll from environmental air pollution reached 7 million, with 4.1 million fatalities attributed to ambient pollution and 2.3 million to indoor pollution. Air pollution causes 9–12 million fatalities per year, primarily affecting impoverished populations with low and middle incomes. According to the World Health Organization, more than 80% of individuals residing in cities with air quality monitoring systems inhale polluted air that surpasses the levels recommended by the WHO (2024) (Henning, 2024).

Health concerns frequently arise due to repeated violations of the World Health Organization's recommended standards. Urban areas contribute to water pollution, public health issues, and river contamination. Climate change and urbanization are major contributors to environmental pollution (Noor et al., 2023). Nations use extensive pesticides and chemicals in agriculture to boost per capita income, ensure economic growth, and meet nutritional demands, resulting in soil and water contamination. Additionally, the escalating population pressure makes it challenging to obtain less than 1% of high-quality freshwater (Zahoor and Mushtaq, 2023). Industrialization and disorganized urbanization cause soil pollution, which poses a serious threat to the earth's soil ecosystem and the health of trillions of living organisms. Plants can absorb contaminants in soil, which then move up the food chain and impact human health. The concentration of pollutants in soils varies according to anthropogenic emissions, production, urbanization, and the competitive environment. Growing pollutants in agricultural soils are considered minimal contaminants, but they have a significant negative effect on individuals and the environment (Gautam et al., 2023).

The impact of urban expansion on biological variety is a significant problem, as it disrupts the natural environment through the introduction of artificial sound and light, which affects the life forms of living animals (Morelli et al., 2023). Pollution is currently the primary environmental factor responsible for disease and premature mortality on a global scale. Air transportation noise, often associated with air pollution in metropolitan areas, is an increasingly recognized environmental issue. Europe has an estimated 12,000 early deaths and 48,000 new cases of ischemic heart disease each year as a result of prolonged exposure to environmental noise (Münzel et al., 2021). Human activities have caused a significant increase in light pollution, which has had a major impact on the Earth's ecosystems. This increase in light intensity and illuminated surfaces is primarily due to socioeconomic factors and large-scale cultural activities. Furthermore, we expect this trend to



continue and even accelerate owing to lower production and usage costs and advancements in technology (Ramírez et al., 2023).

According to Cankurt et al. (2016), the most efficient approach to addressing this issue is to enhance individuals' consciousness regarding the preservation of the natural environment and amplify their understanding of environmental challenges. In this context, the study aimed to determine the elements that university students find most important in terms of environmental pollution and the factors that have an effect on the environmental pollution elements which the students identify as significant. Therefore, this study aims to serve as a resource for research focused on raising awareness and consciousness about environmental pollution, which has increasingly become a global threat to human health in recent years.

## MATERIALS AND METHODS

### Materials

Face-to-face interviews with students at the central campus of Atatürk University in Erzurum provided data for this study on environmental pollution. The sample size was determined using a proportional sampling method with a 95% confidence interval and 5% margin of error. The formula used to calculate the sample size was as follows, as stated by Uzundumlu and Sezgin (2017) and Akan et al. (2021):

$$n = \frac{N \cdot p \cdot (1-p)}{(N-1) \cdot (Q_{px})^2 + p \cdot (1-p)} \quad (\text{Eq.1})$$

where n: size of the sampling,

N: the number of active students in graduate programs in the central county of Atatürk University (29,600),

P: the proportion of environmentally conscious students (50%),

Q<sub>px</sub>: variance (0.000650771).

r: mean deviation (5%),

Z<sub>α/2</sub>: z or t table value=1.96,

variance = (r/t table value)<sup>2</sup>=0.000650771

The data for this study on environmental pollution was obtained through face-to-face interviews conducted with students at the central campus of Atatürk University in Erzurum. The sample size was determined using a proportionate sampling procedure with a 95% confidence level and a 5% margin of error. The formula utilized for determining the sample size was as follows:

$$n_i = \frac{N_i}{N} * n \quad (\text{Eq.2})$$

n<sub>i</sub>= Number of students sampled in faculty of i,

N<sub>i</sub>= Number of students in faculty of i,

n= Total number of samples for the population

The formula (2) was used to determine the number of students to be involved in the study from different departments and classes.

In addition, a pre-survey was carried out within the scope of the study with 100 students from different departments. The students were given air, radiation, water, soil, noise, and light pollution choices as environmental pollution elements, and the three most disturbing elements were determined (Table 1). These elements were identified as air, noise, and radiation respectively. The items in the questionnaire were designed based on these three elements.

**Table 1.** The distribution of pollution elements rated by the extent of students' disturbance

Pollution elements	Percentage
Air	28
Noise	25
Radiation	19
Water	12
Soil	9
Light	7
Total	100.00

## Methods

The logistic regression method was employed in the analysis of data obtained from the questionnaires. In econometric studies, the categorical models whose dependent or explained variables are responded as yes-no or successful-unsuccessful and coded as 0 and 1 are called bipolar limited dependent variable models. This type of model is estimated using three different methods. These are Linear Probability Model, Logit Model, and Probit Model. Probit model is used as an alternative to the Logit model (Kalayci, 2018; Miran, 2018). However, Logit model is generally used instead of Probit model. The three environmental pollution (air, noise, and radiation) elements that the students were disturbed by were presented to the students as a dependent variable, and the factors causing disturbance were identified. The following is the functional form of the regression model used to estimate the effect of the factors having an influence on the issue.

$Y = f(X_1, X_2, X_3, X_4, X_5, \dots, X_{12})$  where

Y1= the state of disturbance or getting influenced by air pollution (yes=1, no=0)

Y2= the state of disturbance or getting influenced by the radiation in the environment (yes=1, no=0)

Y3= the state of disturbance or getting influenced by noise pollution (yes=1, no=0)

X1: age of the student (year),

X2: gender of the student (male:1, and female:0),

X3: education level of the householder (year),

X4: employment status of the mother

X5: residence in the city center

X6: the student's education period so far (year),

X7: studying science

X8: daily regular sport participation (hour/day)

X9: sleeping time of student (hour/day),

X10: regular daily breakfast consumption (hour/day)

X11: television viewing time of the student (hour/day)

X12: total time spent surfing the Internet (hour/day)

## RESULTS AND DISCUSSION

The study conducted a survey of 400 students from various departments. The average age of the students participating in the study was 20.7 years, and 38% of them were male (Table 2). Uzundumlu et al. (2019) found that the average student age was 21.5 years, with 40% of the participants being male. However, in this study, the majority of participants were male. The average length of education of the head of the household was approximately nine years, while Uzundumlu et al. (2019) reported an average of 9.30 years. Furthermore, the data indicated that 20% of the students had employed mothers, and 71% lived in rural areas. The study participants had an average educational attainment of 14 years, with approximately 64% pursuing science-related subjects. Uzundumlu et al. (2019) reported an average educational attainment of 14.98 years among students. Students engaged in sports for less than 30 minutes on average. Pavlovic et al. (2023) found that 44.6% of university students did not participate in any sports activities. The mean duration of sleep was 7.4 hours per day, and 58% of the students consistently had breakfast. Özdişli & Yildiz (2021) found that university students slept an average of 7.1 hours per day, while Yamamoto et al. (2021) reported that 81.0% of students did not skip breakfast. On average, students spent 1.14 hours watching television and 4.68 hours using the internet in this study. Similarly, Benaich et al. (2021) observed that students spent an average of 1.11 hours watching television, while Chowdhury et al. (2020) found that more than half of the students spent 2 to 4 hours on the internet daily, with an average internet usage of 3.7 hours.

**Table 2.** Statistical summary and description of the variables

Variables	Description	Mean	St. Dev.
Age	Continuous variable	20.7	2.43
Gender	Male: 1, Female: 0	0.38	0.48
Education level of the householder	Continuous variable (year)	9.04	4.24
Employment status of the mother	Yes: 1, No: 0	0.20	0.40
Residence in the city center	Center: 1, Rural: 0	0.71	0.45
The student's education period	Years	14.43	1.65
Studying science	Yes: 1, No: 0	0.64	0.48
Sport participation (hour/day)	None:1, 0-0.5:2 0.5-1: 3, More than 1 hour: 4	1.76	1.10
Sleeping time (hour/day)	Continuous variable	7.44	1.35
Regular breakfast	Yes: 1, No: 0	0.58	0.49
Total time spent watching television (hour/day)	Continuous variable	1.14	1.61
Total time spent surfing the Internet (hour/day)	Continuous variable	4.68	2.84

According to the table, female students reported 10.4% more air pollution disturbances than male students (Table 3). Bozoglu et al. (2016) said that gender had very high impacts on the environmental awareness and behaviors of the students in question. Cankurt et al. (2016) also determined that women were more sensitive to air pollution. There are other studies coming up with similar findings, too (Chen & Chen 2021; Liao et al. 2021). On the contrary Carneiro et al (2021) found that boys were more sensitive to air pollution than girls. The likelihood of experiencing air pollution increases by 1% for each additional year of education of the household head. Yadama et al. (2012) found that the likelihood of a family having extra ventilation improves by 14% for every additional year of schooling that the head of the household receives. Those who regularly exercise for an hour more are 4.9% more sensitive to air pollution. Tainio et al. (2021) concluded that children who exercised in areas with high air pollution had significantly lower maximum oxygen available to them during exercise than children who exercised in areas with low air pollution.

**Table 3.** The results of the logistic regression analysis relating to the state of getting disturbed from air pollution

Variables	Coefficient	St. Dev.	P-value	Marginal effect
Constant	0.218	1.142	0.849	
Age	-0.015	0.056	0.789	-0.004
Gender	-0.449**	0.212	0.035	-0.104
Education level of the householder	0.047**	0.024	0.046	0.011
Residence in the city center	-0.057	0.216	0.791	-0.013
The student's education period	-0.020	0.082	0.804	-0.005
Studying science	0.119	0.206	0.564	0.027
Sport participation	0.218**	0.093	0.019	0.049
Regular sleep	0.018	0.071	0.797	0.005
Regular breakfast	0.076	0.196	0.697	0.017

\*  $\alpha=0.10$  \*\*  $\alpha=0.05$  \*\*\*  $\alpha=0.01$

The results indicate that male students were 8.2% more disturbed by noise pollution than female students. This could be attributed to the hypothesis that male students are exposed to louder surroundings, possess higher vocal frequencies, and experience greater levels of congestion in dorms, among other factors

(Table 4). But Okimiji et al. (2023) determined that women are more affected by noise pollution than men. Due to their heightened sensitivity, women may be more susceptible to noise, particularly in shared spaces. Also, Kocaman (2021) did not found significant difference observed between the independent variable of gender and the impression of noise pollution.

**Table 4.** The results of the logistic regression analysis relating to the state of getting disturbed from noise pollution

Variables	Coefficient	St. Dev.	P-value	Marginal effect
Constant	- 0.358	0.926	0.699	
Age	0.031	0.041	0.448	0.007
Gender	0.346*	0.230	0.099	0.082
Employment of the mother	0.624***	0.237	0.009	0.150
Education level of the householder	0.044*	0.023	0.055	0.011
Studying science	- 0.193	0.199	0.331	- 0.046
Daily time spent watching TV	- 0.126*	0.066	0.056	- 0.030
Daily time spent surfing the Internet	0.013	0.034	0.709	0.003
Sport participation	- 0.042	0.088	0.631	- 0.010
Regular breakfast	- 0.062	0.191	0.744	- 0.015

\*  $\alpha=0.10$  \*\*  $\alpha=0.05$  \*\*\*  $\alpha=0.01$

Our findings indicate that mothers' employment is associated with a 15% higher risk of noise pollution. Aryee and Ageziire's (2022) study found that children raised by employed mothers exhibit higher levels of environmental consciousness than children from households where the mother does not work. It is typical for mothers employed in professional sectors to have a significant level of education and hire private tutors to educate their children, thereby augmenting their knowledge.

When the head of the household's education year increases by one year, the child's susceptibility to noise pollution rises by 1.1%. This finding can be attributed to high level of awareness in the family. Şenyurt et al. (2011) and Özden (2016) found similar findings in their studies. They also reported in their study that children who grew up in families with educated parents had a higher level of environmental awareness.

Students who watched television every day for an extra hour had a 3% lower sensitivity to noise pollution. According to Iqbal et al. (2023), 76% of Pakistani university students acknowledge noise pollution as a significant environmental hazard that negatively impacts both health and the environment. Additionally, 72% of the participants report experiencing traffic noise, indicating that mass media, particularly television, fails to adequately address this issue. This perception is prevalent among university students.

When the house leader's education level increases by one year, their susceptibility to radiation pollution rises by 1.6%. The rising degree of education of the person responsible for the household logically influences the environmentally sensitive and knowledgeable children in the household (Table 5). Ahmar et al. (2022) said that the education level of the household head is positively effective in the use of clean and modern energy sources.

**Table 5.** The results of the logistic regression analysis relating to the state of getting disturbed from radiation

Variables	Coefficient	St. Dev.	P-value	Marginal effect
Constant	- 0.548	1.064	0.632	
Age	0.084	0.057	0.145	0.019
Gender	- 0.210	0.214	0.326	-0.048
Education level of the householder	0.070***	0.023	0.003	0.016
Residence in the city center	- 0.162	0.214	0.451	-0.038
The student's education period	- 0.147*	0.082	0.072	-0.034
Studying science	- 0.572***	0.205	0.005	-0.134
Sport participation	0.109	0.089	0.221	0.025
Regular sleep	- 0.031	0.071	0.660	-0.007
Regular breakfast	0.390**	0.194	0.044	0.090

\*  $\alpha=0.10$  \*\*  $\alpha=0.05$  \*\*\*  $\alpha=0.01$

When a university student's educational level rises by one year, their susceptibility to radiation pollution falls by 3.4%. Research has revealed that students' concern for the environmental pollution resulting from radioactive emissions from technological vehicles is insufficient. Technological advancements, which have made their lives more efficient and pleasurable, have contributed to this lack of concern. Hanin et al. (2019) stated that the damage caused by radioactive substances is caused by dangerous ionizing radiations such as alpha, beta and gamma, and that cancer is the most common disease caused by radioactive pollution.

The sensitivity to radiation pollution increases by 9% in people who eat regular breakfast compared to those who do not eat breakfast. Islam and Hasan (2023) stated that students who live in a polluted environment and are exposed to inorganic compounds, heavy metals, or radionuclides need a good diet along with a good breakfast. In this sense, students who know of the effects of radiological contamination in their living spaces do not skip breakfast.

## CONCLUSION

Environmental pollution poses a substantial risk to living organisms. Consequently, the global community has undertaken comprehensive investigations and dedicated significant resources to addressing and reducing environmental pollution. Nevertheless, the primary objective is to enhance knowledge and understanding of environmental pollution and foster a mindful and careful attitude towards the environment. It is critical to determine individuals' perceptions, sensitivities, and awareness of environmental contamination, as well as the elements that influence them. The findings of this study indicate that to raise awareness about environmental contamination, it is advisable to initially focus on reaching families. Local authorities should systematically and continuously organize educational efforts that specifically target environmental pollution. In order to promote awareness among the general population about healthy lifestyles, it is crucial to create educational programs that emphasize the influence of having a regular breakfast and engaging in physical activity on environmental consciousness. Furthermore, effective mass media utilization will help to raise public awareness about environmental pollution. Also, the importance of maintaining clean and efficient energy conversion is increasing. Consequently, it is imperative to establish a training program to educate individuals about various sources of pollution. Universities should incorporate interdisciplinary departments focused on climate change, technological advancements, and preventive measures against pollution into their educational offerings.

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## Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Author Contributions

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## A new Stereoid Basidiomycete Record for Türkiye; *Xylobolus illudens* (Berk.) Boidin

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### ABSTRACT

Russulales, one of the 12 orders in Homobasidiomycetes, classified by DNA-based advanced molecular techniques and morphological characteristics, also includes Stereoid fungi, an artificial group of mostly resupinate, stipitate basidiomata, flat hymenophores and hyaline spores. This study has focuses on the micro and macro characters of the Turkish specimen of *Xylobolus illudens*, which was transferred from the genus *Stereum* to the genus *Xylobolus* by Kew Mycology in 2015, found on a branch fragment near the dam lake in Bayramiç district, Çanakkale, in January 2025. During the field studies, the macroscopic data of the specimen were meticulously examined and recorded. Then the microscopic characteristics of the specimen, which was turned into fungarium material, were examined in the laboratory. As a result of macroscopic and microscopic examinations, the specimen was identified as *Xylobolus illudens*. It was determined that it is a new record for the mycobiota of the country. Microscopic characters of the macrofungus (basidia, spores, cystid structures, etc.) were drawn using the CorelDRAW drawing program to make the micromorphological features more understandable. *Xylobolus* has 11 species worldwide, and there is only one species in Türkiye. With this study, the number of species of the genus in the country has been increased to two, thus contributing to the mycobiota of Türkiye.

**Key words:** Russulales, Homobasidiomycete, New record, Çanakkale.

## Türkiye İçin Yeni Bir Stereoid Basidiomycete Kaydı; *Xylobolus illudens* (Berk.) Boidin

### ÖZ

Homobasidiomycetes familyasındaki 12 takımdan biri olan, DNA tabanlı ileri moleküler teknikler ve morfolojik özelliklerle sınıflandırılan Russulales, çoğunlukla resupinat, stipitat basidiomata, düz himenofor ve renksiz sporlu, yapay bir grup olan Stereoid mantarları da içermektedir. Bu çalışma, 2015 yılında Kew Mycology tarafından *Stereum* cinsinden *Xylobolus* cinsine nakledilen *Xylobolus illudens*'in Ocak 2025 tarihinde Çanakkale, Bayramiç ilçesi, baraj gölü yakınlarında bir dal parçası üzerinde tespit edilen Türkiye örneğinin mikro ve makro karakterlerine odaklanmıştır. Saha çalışmaları esnasında örneğin makroskobik verileri titizlikle incelenerek kaydedilmiş, daha sonrasında fungaryum materyali haline getirilen örneğin mikroskobik özellikleri laboratuvarında incelenmiştir. Makroskobik ve mikroskobik incelemeler sonucunda örnek *Xylobolus illudens* olarak tanımlanmış ve ülke mikobiyotası için yeni bir kayıt olduğu tespit edilmiştir. Makrofungusun mikroskobik karakterlerinin (basidia, spores, sistit yapıları vb.) daha anlaşılır olması açısından CorelDRAW çizim programı kullanılarak mikromorfolojik özellikler çizilmiştir. Dünyada 11 türü bulunan *Xylobolus*'un Türkiye'de sadece bir türü bulunmaktadır. Bu çalışma ile cinsin ülkedeki tür sayısı iki olmuştur ve böylece Türkiye mikobiyotasına katkı sağlanmıştır.

**Anahtar kelimeler:** Russulales, Homobasidiomycete, Yeni kayıt, Çanakkale.

## INTRODUCTION

Friesian concepts emphasize the importance of appearance in macrofungi, and this view dominated classification systems in homobasidiomycetes until the mid to late 20<sup>th</sup> century. Before then, agaric, resupinate, gasteromycete, and coralloid fungi were considered more closely related within each sporophore type, and it was unthinkable that taxa with diverse sporophore morphologies could be classified together (Miller et al., 2006). Donk (1971), suggested that the family *Hericiaceae* may be related to other basidiomycetes with different sporophore types. The common characteristic of these fungi was that they contained gloeopler hyphae, and their spores had amyloid ornaments. According to Donk, 'Each taxon may retain its status as a separate family, but they can also be considered interconnected links of a single large system.' He states, 'They can be considered as members of a single large order, but in this case, a group with a fairly large number of reduced species or genera appears, in which the characters originally selected as defining features are lost.'

In a similar period, Singer and Smith (1960) questioned the necessity of making a sharp distinction between agaric forms and sequestrate or gasteroid taxa in the order Hymenogasterales. Later, Oberwinkler at 1977 proposed that the order *Russulales* is a tightly-knit group of fungi containing all known sporocarp types within the homobasidiomycetes. This view was widely accepted and formed the basis for the classification system in sources such as the Dictionary of Fungi by Kirk et al. (2001) (Miller et al., 2006).

*Russulales* is positioned as one of the 12 major lineages in the *Homobasidiomycetes* recently described by molecular sequence data. One of the most morphologically diverse orders, it also includes the Stereoid fungi, an artificial group with mostly effused-reflexed, resupinate, stipitate basidiomata, flat hymenophores, and hyaline spores (Miller et al., 2006; Welden, 2010; Xavier de Lima, 2020). This artificial group is also traditionally considered among the corticioid fungi under a single genus, *Stereum* Hill (Xavier de Lima, 2020; Burt, 1920; Bernicchia and Gorjón, 2010). The genus was subsequently divided based on morphological and phylogenetic analyses (Hibbett et al., 2014). In Welden's monograph on neotropical stereoid fungi, 13 genera were described. *Xylobolus* was not included among these genera. However, the genus *Stereum* was included in the study 'Synopsis Fungorum 32' by Leif Ryvarden at Kew Mycology (UK) in 2015. As a result of morphological and phylogenetic analyses, *Stereum illudens* was reclassified *Xylobolus illudens*.

When Sesli et al. (2020) and some recent studies (Sesli, 2022; Acar and Dizkırı, 2023; Sesli, 2023; Acar and Karabıyık, 2024a,b; Akata et al., 2024a,b,c; Allı et al., 2024; Aslan et al., 2024; Karaduman et al., 2024; Kesici et al., 2024; Uzun et al., 2024) *Xylobolus illudens* (Berk.) Boidin is a new record for *Xylobolus* of Türkiye.

## MATERIALS AND METHODS

The material of this study consists of basidiomata specimens of *Xylobolus illudens* collected on a branch fragment in Bayramiç (Çanakkale) district on 25.01.2025. The study material was photographed and documented in its natural habitat from different angles. In addition, the field notebook meticulously recorded all macroscopic data points for the specimen, including the place and date of collection, and the substrate on which the specimen was found. The collected specimens were then transported to the laboratory. The specimens were boxed, placed in polyethylene bags, labelled, and placed in the fungarium for further examination. At the diagnostic stage, microscopic examinations were conducted under a Leica DM500 (Germany) light microscope. After the micromorphological characters such as basidia, spores, and cystitis were clarified under the microscope, different pictures were taken for each character. At least 20 measurements were made using Leica Application Suite (version 3.4.0) software to ensure that the photographed microcharacters' most significant and minor sizes fell within reliable ranges. *X. illudens*, the second record of *Xylobolus* in the mycobiota of Türkiye, was identified following the methods described in the literature (Cooke, 1879; Eicker and Louw, 1998; Salcedo et al., 2006; Anonymous, 2025a). For clarity, the micromorphological characters of *X. illudens* were drawn using CorelDRAW (64-bit) (Canada) software. After the species identification process was completed, *X. illudens* was transformed into fungarium material and preserved under appropriate conditions in the Fungarium of Van Yüzüncü Yıl University, Faculty of Science, Department of Biology (VANF).

## RESULTS AND DISCUSSION

**Basidiomycota** R.T. Moore

**Agaricomycetes** Doweld

**Russulales** Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David

**Stereaceae** Pilát

**Xylobolus** P. Karst.

**Xylobolus illudens** (Berk.) Boidin

(Figure 1,2)



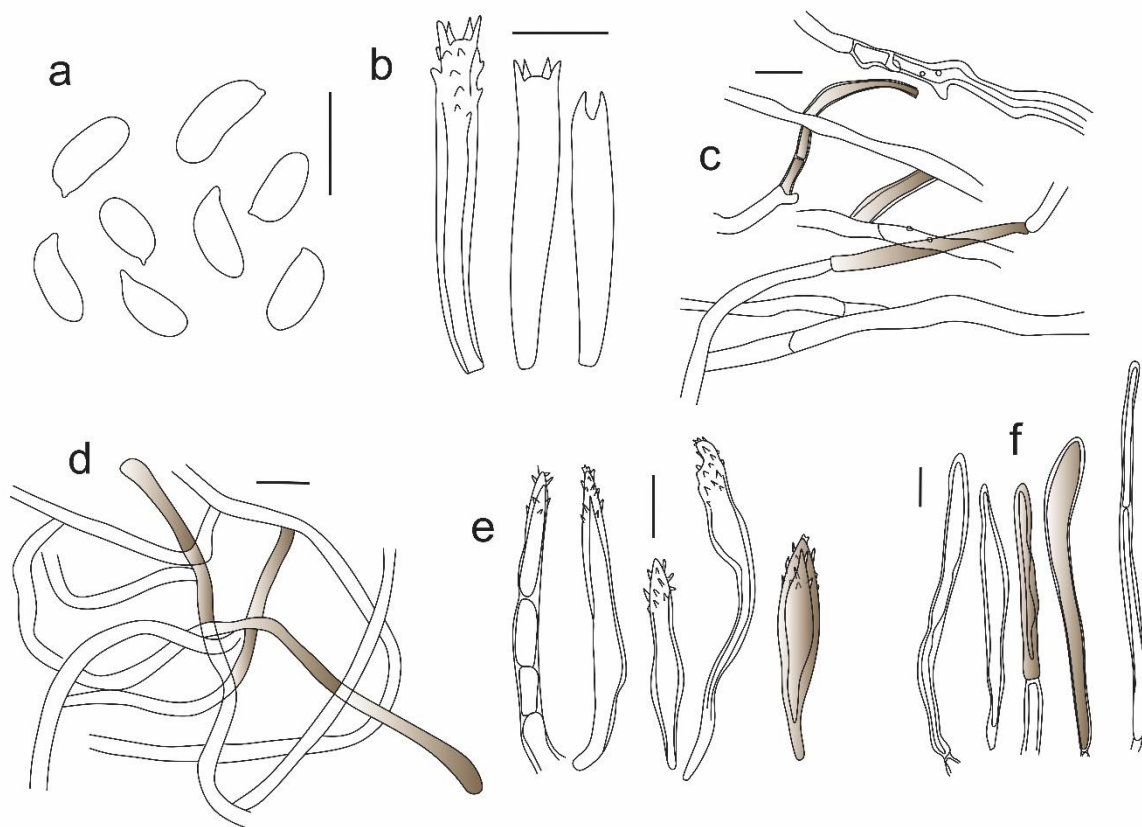
**Basidiomata** sessile, leathery, flexible when young, stiff, and brittle when dry, stratified, and circular, may coalesce to form a continuous basidioma, sometimes up to 70 cm long. **Pileus** 10– 30 × 10–15 mm, effused-reflexed, concentrically zoned, flabelliform, umbonate or resupinate, surface dark brown with chestnut or greyish base, with hairy surface, grouped, consisting of brown and shiny hairs, zoned, consisting of parallel, long, spiral, septate hyphae. **Hymenium** smooth or slightly zoned, pale reddish brown at the edges and reddish violet in the center, dark brown when mature, with whitish margins and slightly fibrillose. **Subhymenium** 2.7–4.1  $\mu\text{m}$ , hyaline, thin or slightly thick-walled, without fibula. **Hyphal system** dimitic, generative hyphae subiculum thick-walled, yellowish or pale brown, needleless, 2.5–4.5  $\mu\text{m}$  in diam., no clamp connections. Skeletal hyphae 3.5–6  $\mu\text{m}$  wide, walls 1  $\mu\text{m}$  thick, yellow-brown in color, darker with mature. **Basidia** 22–35 × 4–6  $\mu\text{m}$ , subclavate, sometimes apex with protrusions, without the basal fibula, with (2)4 sterigmata. **Basidiospores** 6.5–9.2 × 2.8–4  $\mu\text{m}$ , cylindrical to ellipsoidal, subballantoid, smooth, hyaline and amyloid. **Acanthocystidia** 30–50 × 3.5–6.5  $\mu\text{m}$ , abundance, thick-walled, slightly protruding, fusiform or cylindrical, 7–15 finger-like protrusions of 0.5–3  $\mu\text{m}$  length in the apical region, at first transparent and finally brown. **Pseudocystidia** 50–120 × 5.8–11.3  $\mu\text{m}$ , brownish, corresponding to the tips of the skeletal hyphae, with rounded ends, moniliform or slightly fusiform, oily content.

**Specimens examined:** Türkiye, Çanakkale, around Bayramiç Dam Lake, 39° 48'47"N, 26° 39'56"E, 188 m, on a branch fragment, 25.01.2025, Acar 2055.



Figure 1. *Xylobolus illudens* a–d. Basidiomata

Scale bar: 10 mm for (a)



**Figure 2.** *Xylobolus illudens* a. basidiospores, b. basidia, c. hairs, d. generative hyphae, e. acanthocystidia, f. pseudocystidia **Scale bar:** 10 µm

*Xylobolus illudens* is a species with records from South America, Spain, Tasmania, New Zealand, Papua New Guinea, and mainly Australia (Salcedo et al., 2006; Anonymous, 2025b). It colonizes the wood of different angiosperms, and, as mentioned by Cunningham (1963), it is more common in members of the *Myrtaceae* family, such as *Eucalyptus*. In the forested area, where there are *Pinus* and *Quercus* sp. and other tree species, it was impossible to identify precisely which tree branch the specimen found on a branch fragment on the ground belonged to. In the field these species can be confused with *Chondrostereum purpureum* (Fr.) Pouz, *Hydnoporia tabacina* (Sowerby) Spirin, Miettinen and K.H. Larss., sometimes with some specimens of *Stereum ostrea* (Blume and T. Nees) Fr. and *S. sanguinolentum* (Alb. and Schwein.) Fr. (Breitenbach and Kränzlin, 1986; Salcedo et al., 2006; Anonymous, 2025b). However, it can be easily distinguished from similar species by the striking coloration of the hymenium, pale reddish or reddish-purple with white margins and dark brown pubescence on the surface, as well as by its peculiar micromorphological characters (presence of acanthocystidia and basidia with a spiny apical region) (Cooke, 1879; Salcedo et al., 2006; Anonymous, 2025b).

**Table 1.** Comparison of microscopic characters of *X. illudens* with previous studies [25,27].

<i>X. illudens</i>	Cooke (1879)	Salcedo et al. (2006)	Current study
<b>Basidia</b>	24–30 × 5–6 µm, 4-spored	20–35 × 4–5.5 µm, 2 or 4-spored	22–35 × 4–6 µm, 2 or 4-spored
<b>Basidiospores</b>	7–9 × 2.5–4 µm	7–9.2 × 3–4 µm	6.5–9.2 × 2.8–4 µm
<b>Acanthocystidia</b>	aspect ratio not specified	30–45 × (3.5–)4–6 µm	30–50 × 3.5–6.5 µm
<b>Pseudocystidia</b>	aspect ratio not specified	60–100(–130) × 6–11 µm	50–120 × 5.8–11.3 µm

When Table 1 is analyzed, the microscopic characters reported by Cooke (1879) and Salcedo et al. (2006) overlap with those of our specimens. numbered.

## CONCLUSION

As stated in Sesli et al. (2020) and Lohweg (1957;1964) *X. frustulatus* (Pers.) P. Karst. is the only record of the *Xylobolus* genus in the Çatalca-Kocaeli Department, Western, and Eastern Black Sea Departments in our



country. This study contributed to the distribution of the genus *Xylobolus* in the country and increased the number of species from one to two, thus contributing to the mycobiota of Türkiye.

#### Declaration of Interests

The authors declare that there is no conflict of interest between them.

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## Determination of Phenolic Content and Antioxidant Capacity of Olives in Fethiye Region

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### ABSTRACT

In this study, the phenolic compound content and antioxidant activity of olive fruits collected from olive groves in the Fethiye region of Türkiye from different geographical directions (North, South, East and West) were investigated. Olive samples were extracted using different solvents, and the total phenolic content of the extracts was determined by spectrophotometric method. Individual phenolic compound analysis HPLC and antioxidant activities were determined by free radical cleaning analyses. The results showed that olive fruit has a wide range of phenolic compounds, such as catechin, ellagic, epicatechin, gallic acid, protocatechuic, caffeoyl, caffeic, sinapic, vanillic, p-coumaric, ferulic, rosmarinic, and rutin. In addition, significant differences have been determined between breeding zones in terms of phenolic composition and antioxidant capacity due to genetic and environmental factors. These results reveal that olives in the Fethiye region can be considered as important functional food components in terms of health and contribute to the regional economy.

**Key words:** Olive, phenolic compound, antioxidant activity, DPPH, Fethiye

### Fethiye Bölgesindeki Zeytinlerin Fenolik İçeriği ve Antioksidan Kapasitelerinin Belirlenmesi

### ÖZ

Bu çalışmada, Türkiye'nin Fethiye bölgesindeki zeytin bahçelerinden farklı coğrafi yönler (kuzey, güney, doğu ve batı) göre toplanan zeytin meyvelerinin fenolik bileşik içerikleri ve antioksidan aktiviteleri incelenmiştir. Zeytin örnekleri, farklı çözücüler kullanılarak ekstrakte edilmiş ve elde edilen özütlerin toplam fenolik içeriği spektrofotometrik yöntemle, fenolik madde profil analizi HPLC ile, antioksidan aktiviteleri ise serbest radikal süpürme analizleriyle belirlenmiştir. Sonuçlar, zeytin meyvesinin catechin, ellagic, epicatechin, gallic asit, protocatechuic, caffeoyl, caffeic, sinapic, vanillic, p-coumaric, ferulic, rosmarinic, rutin gibi geniş bir fenolik bileşikler yelpazesine sahip olduğunu göstermiştir. Ayrıca, yetiştirme bölgeleri arasında genetik ve çevresel faktörlere bağlı olarak fenolik kompozisyon ve antioksidan kapasite açısından önemli farklılıklar tespit edilmiştir. Bu sonuçlar, Fethiye bölgesindeki zeytinlerin sağlık açısından önemli fonksiyonel gıda bileşenleri olarak değerlendirilebileceğini ve bölgesel ekonomiye katkı sağlayabileceğini ortaya koymaktadır.

**Anahtar kelimeler:** Zeytin, Fenolik Bileşik, Antioksidan Aktivite, DPPH, Fethiye

### INTRODUCTION

The olive tree (*Olea europaea* L.), indigenous to the Mediterranean region, has long been a significant agricultural and economic asset. Its cultivation can be traced back to 3000 BC, with ancient societies such as Crete and Egypt utilising it for farming and trade purposes (Rocha et al., 2020; Haddad et al., 2020). Today, the Mediterranean region remains a crucial hub for olive and olive oil production, accounting for 98% of global olive output (Haddad et al., 2020; Loureiro et al., 2006). Olive fruits are known for their distinctive bitter taste and high oil content. Olive oil is obtained by processing olive fruit and is one of the basic components of the



Mediterranean diet (Sánchez-Romero, 2021; Marcelino et al., 2019). Olive oil is rich in health-promoting phenolic compounds and unsaturated fatty acids, which are beneficial to heart health (Marcelino et al., 2019; Al-Asmari et al., 2021). In addition, olives and olive oil have historically played an important role in the diets of indigenous peoples in the Mediterranean region (Rocha et al., 2020; Maraulo et al., 2021). Olive trees are long-lived, resistant plants that make them attractive for agricultural production (Sánchez-Romero, 2021; Maesano et al., 2021). Olive cultivation is considered not only an economic activity but also a cultural heritage. Suitable conditions provided by the Mediterranean climate for olive cultivation allow a large part of the worldwide production of this plant to occur in this region (Haddad et al., 2020; Loureiro et al., 2006).

Phenolic compounds are recognised as important antioxidant molecules, owing to their ability to counteract oxidative stress. The hydroxyl (OH) groups in their structure provide the capacity to neutralise free radicals by donating electrons and hydrogen. The redox properties of phenolic compounds make them effective electron donors and free-radical scavengers, which forms the basis of their antioxidant activity (Chandra et al., 2014; Rudrapal et al., 2022). These properties enable the synthesis of phenolic compounds in response to ecological and physiological pressures in plants, thereby becoming an integral part of their defence systems (Khoddami et al., 2013). According to Duh and Yen (1995), the ability of phenolic compounds to inhibit lipid peroxidation is related to their antioxidant properties. It has been observed that the consumption of foods rich in phenolic compounds reduces the risk of cardiovascular diseases and slows down the development of atherosclerosis (Vázquez-Ruiz et al., 2022; Dueñas et al., 2004). In this context, the beneficial effects of phenolic compounds on cellular mechanisms stand out, particularly in protecting mitochondrial function and delaying cellular ageing. Studies have shown that, owing to their strong antioxidant activity, phenolic compounds provide protective effects by reducing cellular stress (Vázquez-Ruiz et al., 2022; Sharifi-Rad et al., 2020).

The phenolic composition of olives varies depending on various factors such as growing conditions, fruit maturity, and environmental stress factors. The techniques used during olive processing can also affect phenolic compound content. For example, it has been determined that different olive varieties and cultivation methods affect the total phenolic content in olive oil. In addition, the quality of olive oil is directly related to the presence of phenolic compounds; olive oils with higher phenolic contents have greater health benefits (Cerretani et al., 2010; Guasch-Ferré et al., 2014; Rohman et al., 2019). In recent years, with the increasing interest in olives and olive oil, scientific studies on the effects of these products on health have also increased. In particular, the positive effects of olive oil on cardiovascular health have been supported by large-scale studies such as the PREDIMED (PREvención con Dieta MEDiterránea) study. These studies show that olive oil, especially that with high phenolic content, supports heart health and reduces the risk of metabolic diseases (Guasch-Ferré et al., 2014; Yubero-Serrano et al., 2018).

The Fethiye region has suitable climate and soil conditions for olive cultivation. Determining the phenolic content and antioxidant capacity of olives in the region will help to understand the potential health benefits of this valuable agricultural product and reveal its contribution to the regional economy. The aim of this study was to evaluate changes in the phenolic compound content and antioxidant activities of olive samples collected from different geographical locations and altitudes in the Fethiye region. In addition, the relationship between these changes and environmental factors such as altitude above sea level and geographical direction was investigated.

## MATERIALS AND METHODS

### Materials and Instruments




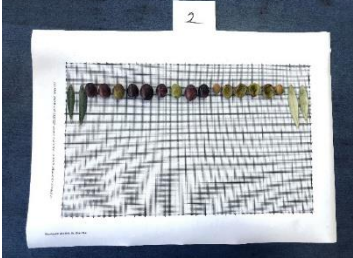

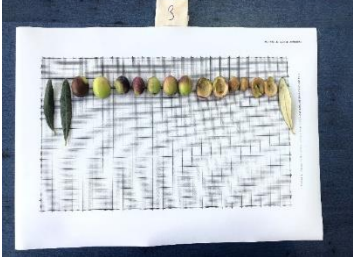

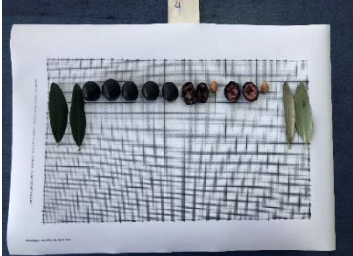

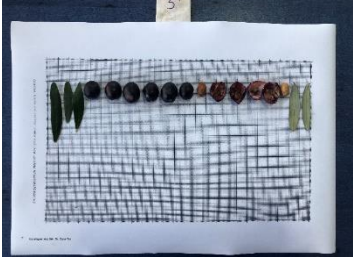


The chemicals used in this study were obtained from different suppliers. Folin reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH•) and sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) were supplied by Sigma-Aldrich; methanol, ethanol, acetone and gallic acid were supplied by Isolab. Catechin, ellagic acid, epicatechin, gallic acid, protocatechuic acid, syringic acid, caffeic acid, cinnamic acid, vanillic acid, p-coumaric acid, ferulic acid and rosmarinic acid used in HPLC analyses were supplied by Merck.

The devices used in the analyses included a PG Instruments T60 model spectrophotometer for absorption measurements, a Kern ACS 220-4 model precision balance for weighing, a Memmert UN 110 model oven for the heating and drying processes, and a Heidolph brand evaporator to remove the solvent after extraction. The HPLC system used for the determination of phenolic substances in olive extracts belongs to the Shimadzu Prominence brand and has a DAD detector, SPD-M20A system controller, SIL-20A automatic sampler, and an LC-20 AT pump system. A Zorbax C18 (220 × 4.60 mm) 5 µm HPLC column was used.


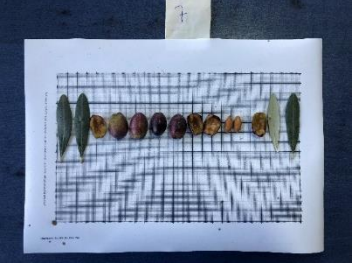

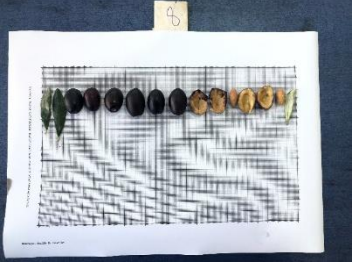

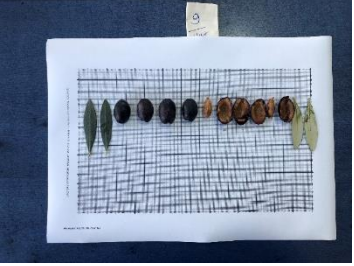

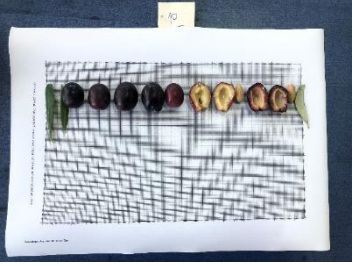

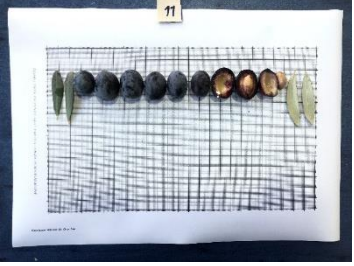

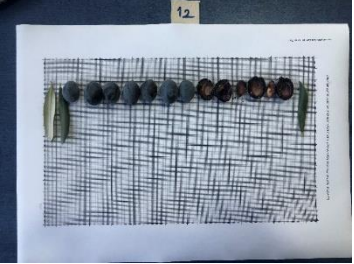
### Collection of Olive Fruits and Preparation of Extracts





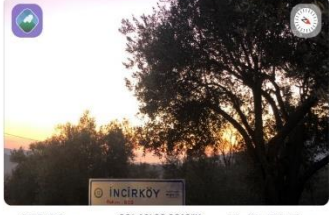
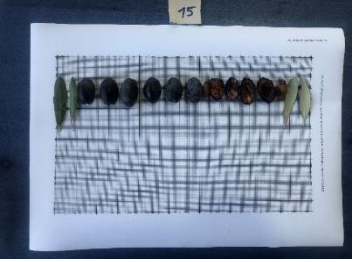



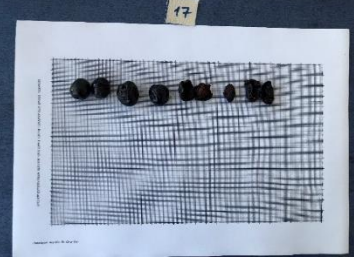


Olive fruits were collected from olive groves in the Fethiye region, taking into account different geographical directions (north, south, east, and west) and elevations above sea level, and stored at -16°C until extraction. The location of the olive samples is presented in Table 1.

**Table 1.** Locational Information of Collected Olive Samples

Address	Direction	Coordinate	Olive Samples
Karagözler neigh.	North	 <p>89.27 m Rakım</p> <p>36° 36' 59.1396"K 29° 6' 37.3842" D Koordinat</p> <p>Muğla/Türkiye Konum</p>	
Kayaköy neigh.	South east	 <p>168.27 m Rakım</p> <p>36° 35' 18.8264"K 29° 5' 44.9359" D Koordinat</p> <p>Muğla/Türkiye Konum</p>	
Kayaköy neigh.	West	 <p>150.80 m Rakım</p> <p>36° 35' 10.1508"K 29° 5' 46.3700" D Koordinat</p> <p>Muğla/Türkiye Konum</p>	
Ölüdeniz neigh.	South	 <p>28.79 m Rakım</p> <p>36° 33' 3.2981"K 29° 7' 20.7996" D Koordinat</p> <p>Muğla/Türkiye Konum</p>	
Ölüdeniz neigh.	North east	 <p>15.09 m Rakım</p> <p>36° 31' 58.1260"K 29° 7' 34.3226" D Koordinat</p> <p>Muğla/Türkiye Konum</p>	
Kelebek Valley	South	 <p>361.97 m Rakım</p> <p>36° 30' 4.8178"K 29° 7' 38.4199" D Koordinat</p> <p>Muğla/Türkiye Konum</p>	



Faralya neigh.	West	 <p>286.65 m      36° 29' 32.6660"K      Muğla/Türkiye Rakım      Koordinat      Konum</p>	
Kabak Cove	South	 <p>81.53 m      36° 27' 51.9167"K      Muğla/Türkiye Rakım      Koordinat      Konum</p>	
Ovacık neigh.	South west	 <p>333.83 m      36° 34' 14.3364"K      Muğla/Türkiye Rakım      Koordinat      Konum</p>	
Taşyaka neigh.	North	 <p>17.88 m      36° 37' 22.3386"K      Muğla/Türkiye Rakım      Koordinat      Konum</p>	
Günlükbaşı neigh.	North	 <p>9.78 m      36° 38' 44.0104"K      Muğla/Türkiye Rakım      Koordinat      Konum</p>	
Yeşilüzümlü neigh.	North	 <p>504.72 m      36° 44' 50.7658"K      Muğla/Türkiye Rakım      Koordinat      Konum</p>	

Yeşilüzümlü neigh.	North	 <p>499.54 m    36° 44' 50.7708"K    Muğla/Türkiye Rakım    Koordinat    Konum</p>	
Yeşilüzümlü neigh.	North	 <p>632.69 m    36° 46' 21.7411"K    Muğla/Türkiye Rakım    Koordinat    Konum</p>	
İncir Village	South	 <p>652.96 m    36° 46' 33.9318"K    Muğla/Türkiye Rakım    Koordinat    Konum</p>	
Karaçulha neigh.	South west	 <p>96.64 m    36° 38' 53.9388"K    Muğla/Türkiye Rakım    Koordinat    Konum</p>	
Karagedik neigh.	South	 <p>41.24 m    36° 41' 2.5263"K    Muğla/Türkiye Rakım    Koordinat    Konum</p>	
Çiftlik neigh.	North	 <p>40.85 m    36° 42' 4.9921"K    Muğla/Türkiye Rakım    Koordinat    Konum</p>	



Yanıklar	East	 <p>16.04 m      36° 42' 5.4239"K      Muğla/Türkiye Rakım      Koordinat      Konum</p>	
Göcek	South	 <p>5.65 m      36° 45' 26.5765"K      Muğla/Türkiye Rakım      Koordinat      Konum</p>	

In this study, four different solvents with different polarities (ethanol, methanol, acetone, and water) were used in the extraction process, and the bioactive performance of the obtained liquid extracts was compared. The extraction processes performed with mixtures of solvents with the highest bioactive activity prepared with different water ratios were also evaluated, and the most suitable solvent-water mixture was determined. The solvent-water mixture obtained as a result of this optimization was also applied to other olive samples. The extraction process for the preparation of olive extracts was performed in a double-necked flask. In this process, 3 g of a fresh olive sample was mixed with 30 mL of the selected solvent and extracted at 30°C for 45 min. After the extraction was complete, the filtrate was passed through a blue band filter paper to separate the supernatant and evaporated under vacuum at 40°C to remove the solvent.

#### Determination of Total Phenolic Substance and Antioxidant Activity (DPPH Radical Scavenging Test)

Total phenolic substance amounts in olive samples was determined according to the method reported by Singleton and Rossi (1965). For this purpose, 5 mL of 0.2 N Folin Cioceltau reagent was added to 100 µL of olive extract and the mixture was kept in the dark for 5 min. Then, 4 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> and 900 µL of distilled water were added to the mixture, which was tightly closed, vortexed, and kept in the dark for 2 h at room temperature (20-25 °C). At the end of two hours, the absorbance values of the samples were determined using a UV-vis spectrometer at a wavelength of 765 nm. For the gallic acid calibration curve, methanol solutions of gallic acid were prepared at 5 different concentrations (0.5-5 mg/L). The aforementioned procedures were also applied to gallic acid solutions of different concentrations prepared for the calibration curve. Total phenolic substance amounts were calculated as mg GAE/kg using a calibration chart prepared with gallic acid standard. Each measurement was repeated thrice.

The antioxidant capacity of olive extracts was determined based on the DPPH radical scavenging activity (Mishra et al., 2012). Different volumes of the extraction product (25, 50, 100, 200, 400, and 600 µL) were placed in glass tubes and 600 µL of 1 mM DPPH radical solution was added. The final volume was adjusted to 6 mL with ethyl alcohol. The mixtures were then incubated in the dark for 15 min at room temperature. The absorbance was measured at a wavelength of 517 nm against the prepared control solution (pure ethyl alcohol and DPPH radicals). The results are expressed as % inhibition and IC<sub>50</sub> values. The individual inhibition values for each olive sample were calculated using the following equation:

$$\% \text{ inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the reaction mixture.

### HPLC Analysis

For the HPLC analysis of phenolic compounds in plant extracts, calibration graphs of 15 different phenolic compound standards were drawn separately. Two different solvents were used as the mobile phases in the analyses (A: 3% formic acid and B: Methanol). The gradient program was as follows: 93% A + 7% B for three minutes, 72% A + 28% B for 28 min; 67% A + 33% B for 60 min; 58% A + 42% B for 62 min; 50% A + 50% B for 70 min; 30% A + 70% B for 75 min; and 93% A + 7% B for 90 min (Gomes vd.,1999). The results obtained from the HPLC analysis are given in micrograms/gram with a 95% confidence interval.

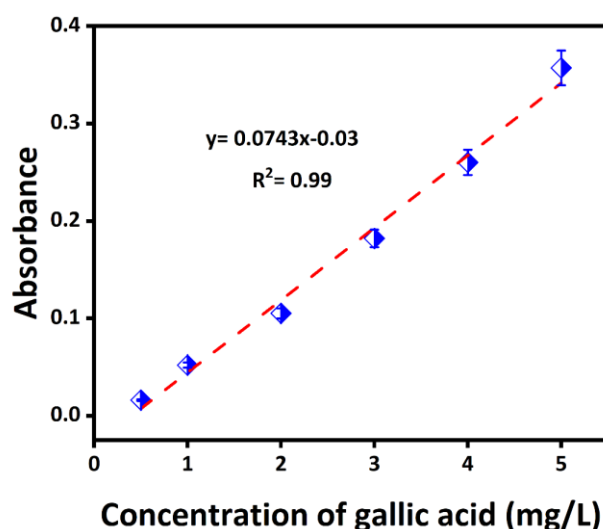
### Statistical Analysis

The total phenolic content of olive fruits collected from olive groves in the Fetiye region considering geographical directions (north, south, east, and west) and altitudes above sea level were applied by both One-Way ANOVA and Kruskal-Wallis tests according to which group the samples belonged to (e.g., altitude groups, direction groups, etc.). These analyses allowed us to determine which factors (altitude, direction, etc.) created statistically significant differences in the composition of olives.

## RESULTS AND DISCUSSION

### Determination of Total Phenolic Substance Quantity

The total phenolic content of olive extracts was determined using the Folin-Ciocalteu method. The gallic acid solutions used as standards were prepared in ethanol at five different concentrations. Absorbance readings were recorded at a wavelength of 765 nm. A gallic acid calibration graph is shown in Figure 1.



**Figure 1.** Calibration graph of gallic acid

Various phenolic compounds with varying polarity properties exist in the olive fruit. Depending on their structure, these compounds show better solubility in solvents with different polarities. For example, polar solvents such as water are effective in the extraction of more polar phenolic compounds (such as hydroxytyrosol), whereas less polar solvents such as ethanol, methanol, or acetone provide better extraction of less polar phenolic substances (such as oleuropein). The purpose of the extraction process using solvents of different polarities is to obtain the widest possible range of phenolic compounds in the olive fruit. Thus, the substance content and antioxidant capacity of olive oil or other olive products can be determined more comprehensively. In addition, extracts obtained with different solvent polarities can be compared in terms of their phenolic profiles and bioactivity. This allowed for the determination of the most effective antioxidant compounds in olive fruit suits. For this purpose, firstly, the solvent type with the highest phenolic composition was determined by using extracts obtained using four different solvents (water, methanol, ethanol, and acetone) for an olive fruit sample. The results obtained are listed in Table 2.



**Table 2.** Phenolic substance amounts of extracts obtained with different solvents

Solvents	Total Phenolic Substance Amount (mg GA/g)
Water	0.74± 0.04
Ethanol	0.68± 0.03
Methanol	1.68± 0.06
Acetone	0.75± 0.04

As shown in Table 2, the highest gallic acid content was obtained using methanol as the solvent, yielding  $1.193 \pm 0.059$  GA mg/g, whereas the lowest content was recorded with ethanol, at  $0.684 \pm 0.034$  GA mg/g. According to the obtained results, the methanol extract was richer in terms of phenolic substances than the other solvent extracts. Some compounds may have higher solubilities in mixed solvent systems than in pure solvents. The polarity of the solvent system was optimized by changing the methanol/water ratio. This can lead to a solvent system with a polarity that matches well with the target compounds and more efficient extraction (Zuorro et al., 2014). For this purpose, the most efficient solvent system was determined for the extraction of olive fruits using different methanol-water fractions (75%, 50%, and 25% v/v). The obtained data are presented in Table 3.

**Table 3.** Phenolic substance amounts in extracts obtained in different methanol-water fractions

Solvent ratio	Total Phenolic Substance Amount (mg GA/g)
Methanol (100%)	1.75± 0.09
Methanol-water (75% v/v)	4.19± 0.21
Methanol-water (50% v/v)	3.88± 0.19
Methanol-water (25% v/v)	2.39± 0.12

When Table 3 is examined, it is seen that the highest value in terms of phenolic substance was obtained with 75% methanol-water ratio. In line with the obtained results, extracts of other olive fruits were obtained using a solvent system with a 75% methanol-water ratio, and the results are given in Table 4.

Table 4 presents the TPSA of olive samples collected from various locations within the Fethiye region, expressed as mg GA/g. The results demonstrated significant variation in phenolic content based on geographical location and altitude. Notably, samples collected from higher altitudes such as Yeşilüzümlü (505 m, 8.70 mg GA/g), İncir (653 m, 5.21 mg GA/g), and Karagedik (42 m, 6.82 mg GA/g) exhibited higher phenolic contents compared to those from lower altitudes like Kelebek Valley (362 m, 1.99 mg GA/g) and Faralya (287 m, 1.91 mg GA/g). This suggests that environmental stressors commonly associated with higher altitudes, such as increased exposure to ultraviolet (UV) radiation, temperature fluctuations, and reduced oxygen levels, may enhance phenolic biosynthesis as part of the plant's natural defense mechanisms. Additionally, the orientation of olive cultivation sites plays a critical role in the phenolic content, as regions exposed to more sunlight generally show higher TPSA values. For instance, samples from southern-oriented locations, such as Yeşilüzümlü and Karaçulha, demonstrated higher TPSA values than those from shaded or less exposed areas. Furthermore, regional differences may be attributed to variations in soil composition, microclimate conditions, and other environmental factors specific to each area. This variability highlights the complexity of phenolic compound production in olives and underscores the importance of environmental factors in determining phenolic profiles. In addition, one-way ANOVA and Kruskal-Wallis tests were applied to the results obtained for a more detailed examination. The results are presented in Table 5.

When the data in Table 5 were examined, there was a statistically significant difference between the altitude groups for both analysis models ( $p < 0.05$ ), while there was no statistically significant difference between the direction groups. As a result, the total phenolic content of plant samples in the Fethiye region was closely related to the altitude of the samples but was not affected by geographical orientation. Such regional differences are important in terms of showing the extent to which the secondary metabolite content of plants is affected by environmental factors.

**Table 4.** Total Phenolic Substance Amounts (TPSA) of Olive Fruits According to Their Locations

Number	Address	Height Above Sea Level (m)	Direction	Coordinate	TPSA (mg GA/g)
1	Karagözler neigh.	~90	North	36°36'59.1396"N 29°6'37.3842"E	3.62±0.18
2	Kayaköy neigh.	~169	South east	36°35'18.8264"N 29°5'44.9359"E	4.04±0.21
3	Kayaköy neigh.	~151	West	36°35'10.1508"N 29°5'46.3700"E	3.66±0.18
4	Ölüdeniz neigh.	~29	South	36°33'3.2981"N 29°7'20.7996"E	2.89±0.14
5	Ölüdeniz neigh.	~15	North east	36°31'58.1260"N 29°7'34.3226"E	2.23±0.11
6	Kelebek Valley	~362	South	36°30'4.8178"N 29°7'38.4199"E	1.99±0.09
7	Faralya neigh.	~287	West	36°29'32.6660"N 29°7'38.3764"E	1.91±0.08
8	Kabak Cove	~82	South	36°27'51.9167"N 29°7'44.3890"E	2.81±0.14
9	Ovacık neigh.	~334	South west	36°24'14.3364"N 29°8'38.3174"E	3.87±0.19
10	Taşyaka neigh.	~18	North	36°37'22.3386"N 29°8'48.7133"E	2.91±0.14
11	Günlükbaşı neigh.	~10	North	36°38'44.0104"N 29°8'6.1419"E	4.61±0.23
12	Yeşilüzümlü neigh.	~505	North	36°44'50.7658"N 29°14'15.1012"E	8.70±0.43
13	Yeşilüzümlü neigh.	~500	North	36°44'50.7708"N 29°14'14.0436"E	3.19±0.16
14	Yeşilüzümlü neigh.	~633	North	36°46'21.7411"N 29°13'4.3288"E	4.71±0.23
15	İncir Village	~653	South	36°46'33.9318"N 29°13'3.5350"E	5.21±0.26
16	Karaçulha neigh.	~97	South west	36°38'53.9388"N 29°12'41.6915"E	4.31±0.22
17	Karagedik neigh.	~42	South	36°41'2.5263"N 29°7'24.2712"E	6.82±0.34
18	Çiftlik neigh.	~40	North	36°42'4.9921"N 29°5'29.0978"E	4.63±0.23
19	Yanıklar	~16	East	36°42'5.4239"N 29°4'11.5638"E	6.44±0.33
20	Göcek	~5	South	36°45'26.5765"N 28°56'18.4578"E	3.44±0.17

**Table 5.** Statistical analysis of olive samples according to TPSA altitude and directions

Variables	ANOVA		Kruskal-Wallis	
	F- statistic	p-value	H- statistic	p-value
Height Above Sea Level	4.805	0.039	7.211	0.027
Direction	2.375	0.122	4.745	0.191

#### DPPH• (1,1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Effect

The DPPH• radical-scavenging assay is a frequently used method to evaluate antioxidant activity. DPPH• is a chemical compound with free-radical properties that causes its color to change by reacting with an

antioxidant substance. The basic principle of this method is based on the scavenging of DPPH• radicals using antioxidant compounds. An antioxidant substance donates a hydrogen atom or electron to the DPPH• radical and converts it into a stable compound. As a result, the purple color of DPPH• becomes lighter and its absorbance decreases (Mishra et al., 2012). Olive samples of different concentrations (25, 50, 100, 200, 400, and 600 µL) were mixed with DPPH• solution and incubated for a certain period. After incubation, the decrease in absorbance of DPPH• was measured spectrophotometrically. The results obtained were evaluated by creating dose-response curves, and the parameters representing antioxidant activity were calculated as EC<sub>50</sub> (Effective Concentration 50). The results are presented in Table 6.

**Table 6.** EC<sub>50</sub> values of olive fruits according to their location

Number	Address	Height Above Sea Level (m)	Direction	Coordinate	EC <sub>50</sub> (g)
1	Karagözler neigh.	~90	North	36°36'59.1396"N 29°6'37.3842"E	0.36±0.02
2	Kayaköy neigh.	~169	South east	36°35'18.8264"N 29°5'44.9359"E	0.59±0.03
3	Kayaköy neigh.	~151	West	36°35'10.1508"N 29°5'46.3700"E	0.91±0.04
4	Ölüdeniz neigh.	~29	South	36°33'3.2981"N 29°7'20.7996"E	0.13±0.01
5	Ölüdeniz neigh.	~15	North east	36°31'58.1260"N 29°7'34.3226"E	0.33±0.02
6	Kelebek Valley	~362	South	36°30'4.8178"N 29°7'38.4199"E	0.70±0.04
7	Faralya neigh.	~287	West	36°29'32.6660"N 29°7'38.3764"E	0.63±0.03
8	Kabak Cove	~82	South	36°27'51.9167"N 29°7'44.3890"E	0.22±0.01
9	Ovacık neigh.	~334	South west	36°24'14.3364"N 29°8'38.3174"E	0.18±0.01
10	Taşyaka neigh.	~18	North	36°37'22.3386"N 29°8'48.7133"E	0.41±0.02
11	Günlükbaşı neigh.	~10	North	36°38'44.0104"N 29°8'6.1419"E	0.28±0.01
12	Yeşilüzümlü neigh.	~505	North	36°44'50.7658"N 29°14'15.1012"E	0.06±0.01
13	Yeşilüzümlü neigh.	~500	North	36°44'50.7708"N 29°14'14.0436"E	0.17±0.01
14	Yeşilüzümlü neigh.	~633	North	36°46'21.7411"N 29°13'4.3288"E	0.16±0.01
15	İncir Village	~653	South	36°46'33.9318"N 29°13'3.5350"E	0.14±0.01
16	Karaçulha neigh.	~97	South west	36°38'53.9388"N 29°12'41.6915"E	0.11±0.01
17	Karagedik neigh.	~42	South	36°41'2.5263"N 29°7'24.2712"E	0.12±0.01
18	Çiftlik neigh.	~40	North	36°42'4.9921"N 29°5'29.0978"E	0.27±0.01
19	Yanıklar	~16	East	36°42'5.4239"N 29°4'11.5638"E	0.09±0.01
20	Göcek	~5	South	36°45'26.5765"N 28°56'18.4578"E	0.24±0.01

The analysis of Table 6 reveals that EC<sub>50</sub> values range from 0.06 g to 0.91 g, with lower EC<sub>50</sub> values indicating greater antioxidant activity. The most potent antioxidant activity was observed in samples from the Yeşilüzümlü neighbourhood (EC<sub>50</sub>=0.06 g) and Karaçulha neighbourhood (EC<sub>50</sub>=0.11 g). Overall, specimens

collected at higher elevations demonstrated enhanced antioxidant properties, which can be attributed to the tendency of high-altitude environments to stimulate the production of secondary metabolites in plants.

The comparison of data presented in Table 4 (Total Phenolic Substance Amounts, TPSA) and Table 6 (DPPH Radical Scavenging Activity - EC<sub>50</sub>) reveals a noticeable relationship between the total phenolic content of olive samples and their antioxidant activity. Generally, lower EC<sub>50</sub> values indicate better antioxidant activity, whereas higher TPSA values suggest a richer phenolic profile. In most cases, the samples exhibiting high TPSA values also demonstrated strong antioxidant activity, as reflected by the low EC<sub>50</sub> values. For instance, the samples collected from Yeşilüzümlü (505 m) with a TPSA of 8.70 mg GA/g displayed the most potent antioxidant activity, with an EC<sub>50</sub> value of 0.06 g. Similarly, samples from Karaçulha (97 m) with a TPSA of 4.31 mg GA/g also exhibited strong antioxidant activity, with an EC<sub>50</sub> value of 0.11 g. However, some exceptions are observed. For example, samples from Günlükbaşı (10 m), with a relatively high TPSA value of 4.61 mg GA/g, displayed moderate antioxidant activity with an EC<sub>50</sub> value of 0.28 g. Another noticeable mismatch occurred with samples from Kayaköy (169 m) which had a TPSA of 4.04 mg GA/g, but demonstrated poor antioxidant activity with an EC<sub>50</sub> value of 0.59 g. This indicates that a high total phenolic content does not always correspond to a high antioxidant activity (Shim, 2011; Kosanić et al., 2011; Kamdem et al., 2012; Susanti, 2019). The discrepancy between TPSA and EC<sub>50</sub> values can be explained by several factors. First, not all phenolic compounds contributed equally to antioxidant activity. The DPPH assay primarily measures the hydrogen-donating capacity of phenolics, which may not reflect the overall antioxidant potential if the dominant phenolic compounds in the sample have weak radical scavenging abilities. Additionally, the structural characteristics of phenolic compounds, including their hydroxyl group positioning, conjugation, and glycosylation, significantly influence their effectiveness as antioxidants. Compounds with more hydroxyl groups generally exhibit higher antioxidant activities, whereas glycosylated or esterified forms may display reduced activities. Moreover, the interactions between different phenolic compounds may play a critical role in determining the antioxidant potential of an extract. Some compounds may work synergistically to enhance antioxidant effects, while others may have antagonistic effects that reduce the overall efficacy. Furthermore, the solvent system used for extraction was optimised for total phenolic determination, which may not have selectively extracted phenolic compounds with high antioxidant potential (Solar & Stampar, 2011; Sheik & Chandrashekar, 2014; Makhafola et al., 2016).

In general, the findings suggest that although there is a strong positive correlation between high TPSA and low EC<sub>50</sub> values, this relationship is not absolute. The antioxidant capacity of olive samples is influenced by the type and structural characteristics of the phenolic compounds, their interactions, and the extraction method employed. Further studies involving the identification and quantification of individual phenolic compounds are necessary to better understand the relationship between the phenolic content and antioxidant activity.

#### HPLC Analysis

HPLC analysis of the olive fruit extracts revealed the presence of 13 distinct phenolic compounds. The identified compounds are listed in Table 7. The presence of ellagic acid (EA) in olives indicates its potential as a natural source of this compound. Ellagic acid is predominantly recognized for its antioxidant, anti-inflammatory, and potential anticancer properties, making its identification in olives important for nutritional and therapeutic applications. Previous studies have corroborated the notion that olives and their related products are rich in bioactive phenolic compounds. For instance, research has previously linked ellagic acid with preventive effects against various cancers and oxidative stress-related disorders, emphasizing the functional relevance of olives in disease prevention and health promotion (Hsieh et al., 2016; Mishra & Vinayak, 2015). The identification of high levels of EA aligns with findings in other fruits, where similar beneficial effects of ellagic acid have been documented, thereby illustrating the potential of olives as functional foods (Neveu 2010; Djurić et al., 2014). In addition, the notable concentrations of epicatechin (EC) found in olive samples significantly contribute to their antioxidant potential. Epicatechin, a well-established polyphenolic compound, exhibits strong free-radical scavenging activity and has been associated with various health benefits, including cardiovascular protection and anticancer effects. The predominance of EC in olives compared to other phenolic compounds—such as rosmarinic acid (RA) and rutin (RU)—underscores olives' potential as a functional food ingredient, which can further promote overall health and facilitate disease prevention (Singh et al., 2011; Khanal et al., 2010). The health benefits of epicatechins have been extensively documented in the literature, suggesting their role in mitigating chronic diseases (Khan and Mukhtar, 2018; Gorai et al., 2020). Moreover, the significant amounts of caffeic acid (CA) and cinnamic acid (CI) observed in olive fruits reinforce their role as sources of hydroxycinnamic acids.

**Tablo 7.** Phenolic compound content of olive fruits (mg/kg)

No	CA	EA	EC	GA	PA	SA	CA	CI	VA	Pc	FE	RA	RU
1	4.12	459	6.3	0.07	0.04	1.62	40	62	1.33	0.02	0.01	27	0.09
2	1.03	8.8	0.11	0.12	0.19	0.18	69.81	142.45	13.2	3.24	0.01	0.93	0.09
3	1.24	18.3	6.3	0.11	0.83	0.13	76.80	8.85	0.18	0.79	0.51	2.82	0.11
4	0.23	78.6	16.4	0.13	0.26	0.36	23.77	2.47	0.85	0.13	0.65	0.12	0.08
5	0.56	58.7	2.79	0.04	0.17	0.09	17.22	0.80	3.58	1.04	0.23	0.09	0.05
6	0.20	2.3	0.83	0.03	0.10	0.39	2.47	0.31	0.80	0.05	3.22	0.44	0.04
7	0.21	9.6	1.5	0.10	0.11	0.05	2.15	0.07	0.49	0.13	0.95	1.92	0.05
8	1.18	91.8	29.7	0.16	0.10	0.64	9.43	0.26	1.10	0.02	0.01	1.26	3.67
9	2.46	85.2	18	0.02	0.31	0.06	20.87	1.55	0.35	0.03	3.79	1.84	2.84
10	2.45	215	3.65	0.14	0.62	0.96	59.90	0.46	0.08	0.03	0.73	0.34	0.85
11	1.61	345.6	13.4	0.35	0.90	2.27	49.67	0.003	0.25	0.04	3.97	1.99	1.67
12	1.95	0.67	51.3	0.58	0.11	2.16	79.87	1.73	21.2	0.04	1.12	3.03	1.55
13	0.34	79.4	19.6	0.25	0.15	1.88	18.55	0.27	3.49	0.08	1.69	0.10	4.24
14	0.39	25.4	7.49	0.01	0.05	0.96	1.62	3.61	11.7	0.05	1.64	0.37	0.08
15	0.98	16.3	29.8	0.14	0.16	0.28	4.95	23	5.89	1.21	18.8	0.74	0.11
16	1.92	58.4	5.65	0.34	0.40	2.07	35.11	3.52	2.77	0.84	52.9	0.26	0.30
17	1.64	156	32.8	0.60	0.39	0.22	22.34	1.69	27.4	4.00	1.13	0.15	0.15
18	1.38	250	4.94	0.04	0.27	406	85.38	18.84	3.51	0.01	411	24.72	0.10
19	2.48	27.8	35	0.22	0.16	0.34	61.4	2.31	0.55	0.02	43.5	0.28	0.20
20	0.55	43	4.85	0.01	0.06	0.41	23.21	0.25	0.50	0.30	0.20	2.0	0.12

CA: Catechin, EA: Ellagic, EC: Epicatechin, GA: Gallic, PA: Protocatechuic, SA: Syringic, CA: Caffeic, CI: Cinnamic, VA: Vanillic, Pc: p-Coumaric, FE: Ferulic, RA: Rosmarinic, RU: Rutin

These compounds are known for their potent antioxidant and antimicrobial activities. Numerous studies have highlighted the efficacy of phenolic acids, such as caffeic acid, in preventing oxidative damage and inhibiting microbial growth, thus expanding the potential applications of olives in food preservation and nutraceutical formulations (Giampieri et al., 2015; Redford et al., 2021). The antioxidant capabilities of these phenolic acids have been further validated in various studies, which emphasize their importance in enhancing the preservation of food products and contributing to health benefits (Ingólfsson et al., 2011; Hsieh et al., 2016). In conclusion, the findings related to ellagic acid, epicatechin, caffeic acid, and cinnamic acid concentrations in olives underscore their considerable value as sources of bioactive phenolic compounds. This position is not only nutritionally beneficial but also therapeutically relevant in the realm of functional foods, offering a range of health benefits owing to their rich phytochemical profile.

## CONCLUSION

In this study, the phenolic compound contents and antioxidant capacities of olive fruits collected from different geographical locations and altitudes in the Fethiye region were evaluated. The results showed that the methanol-water (75%) mixture provided the highest phenolic compound extraction, and that the samples collected from high altitudes were richer in phenolic substance content. In particular, the total phenolic content of olives collected from the Yeşilüzümlü Neighborhood was significantly higher than that in other regions. In addition, the low EC<sub>50</sub> values in antioxidant activity assessments revealed that phenolic compounds can effectively neutralize free radicals. This finding supports the hypothesis that high altitude and environmental stress factors increase secondary metabolite production in olives. HPLC analysis revealed the presence of various phenolic compounds, such as catechin, ellagic acid, epicatechin, gallic acid, protocatechuic acid, cinnamic acid, and ferulic acid in olives. In particular, the high concentrations of ellagic acid and epicatechin highlight the potential health benefits of these olives. Previous studies have shown that ellagic acid has antioxidant and anticancer properties and epicatechin has positive effects on cardiovascular health by effectively scavenging free radicals. These results show that olives grown in the Fethiye region not only have regional economic value, but may also be an important source of nutritional and pharmacological terms.

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### Declaration of interests

The authors declare that they have no conflicts of interest.

### Author Contributions

The authors declare that they contributed equally to this work.

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## Effect of Salinity Stress on Plant Growth Parameters and Antioxidant Activity In Some Cucumber Cultivars (*Cucumis sativus* L.) Grown Under *In Vitro* Conditions

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### ABSTRACT

In this study, effects of salinity (NaCl) at different concentrations (0, 50, 100, 150, 200, 250, 300 mM) on the growth of cucumber plants were determined in *in vitro* conditions. The study was carried out in the plant tissue culture laboratory of Agricultural Sciences and Technology Faculty of Sivas Science and Technology University (Sivas, Türkiye). Experiments were conducted with 3 different cucumber varieties. Murashige and Skoog (MS) was used as the basic nutrient medium. During the study, some parameters were evaluated such as germination rate (%), salt tolerance index (%), stem fresh and dry weight (g), root fresh and dry weight (g), actual water content (%), stem and root lengths and visual scale values. Also, antioxidant capacity were determined by DPPH and ABTS. When the effect of different NaCl doses on the varieties was evaluated, the highest antioxidant capacity was obtained from HD medium (173.21  $\mu\text{mol TE g}^{-1} \text{dw}$ ) in the DPPH analysis and from HC medium (251.06  $\mu\text{mol TE g}^{-1} \text{dw}$ ) in the ABTS analysis. In the study, the highest germination rate was obtained in the variety 1 (94.36%) and in the HA medium (96.05%). In the actual water content, the variety average was the highest in the variety 2 (91.16%) and the medium average was the highest in the HA (91.71%). The highest stem length was determined in the variety 1 (26.40 mm) and in the HA medium (24.48 mm). Considering the results of the study, although there were differences among the varieties, the increase in NaCl concentration negatively affected germination and plant growth in cucumber.

**Key words:** *In vitro*, NaCl, Cucumber, Antioxidant

### *In Vitro* Koşullarda Yetiştirilen Bazı Hıyar Çeşitlerinde (*Cucumis sativus* L.) Tuzluluk Stresinin Bitki Büyüme Parametreleri ve Antioksidan Aktivite Üzerine Etkisi

### ÖZ

Bu çalışmada, farklı konsantrasyonlarda (0, 50, 100, 150, 200, 250, 300 mM) tuzun (NaCl) *in vitro* koşullarda hıyar bitkilerinin büyümesi üzerine etkileri tespit edilmiştir. Çalışma, Sivas Bilim ve Teknoloji Üniversitesi Tarım Bilimleri ve Teknoloji Fakültesi bitki doku kültürü laboratuvarında gerçekleştirilmiştir. Denemeler, 3 farklı hıyar çeşidi ile yürütülmüştür. Çalışmada temel besin ortamı olarak Murashige ve Skoog (MS) kullanılmıştır. Çalışma süresince, çimlenme oranı (%), tuz tolerans indeksi (%), gövde yaş ve kuru ağırlığı (g), kök yaş ve kuru ağırlığı (g), gerçek su içeriği (%), gövde ve kök uzunlukları ve görsel skala değerleri değerlendirilmiştir. Ayrıca, antioksidan kapasite DPPH ve ABTS ile belirlenmiştir. Farklı tuz dozlarının çeşitler üzerindeki etkisi değerlendirildiğinde, DPPH analizinde en yüksek antioksidan kapasite değeri HD ortamından (173,21  $\mu\text{mol TE g}^{-1}$  kuru madde), ABTS analizinde ise HC ortamından (251,06  $\mu\text{mol TE g}^{-1}$  kuru madde) elde edilmiştir. Çalışmada, en yüksek çimlenme oranı çeşit 1'de (%94,36), ve HA ortamından (%96,05) elde edilmiştir.

Gerçek su içeriğinde çeşit ortalaması en yüksek 2 numaralı çeşitte (%91,16), orta ortalaması ise en yüksek HA ortamında (%91.71) tespit edilmiştir. En yüksek gövde uzunluğu 1 numaralı çeşitte (26.40 mm) ve HA ortamında (24.48 mm) belirlenmiştir. Çalışma sonuçları dikkate alındığında, çeşitler arasında farklılıklar olmasına rağmen, NaCl konsantrasyonundaki artış hıyarda çimlenmeyi ve bitki büyümesini olumsuz etkilemiştir.

**Anahtar kelimeler:** *In vitro*, NaCl, Hıyar, Antioksidan

## INTRODUCTION

Cucumber (*Cucumis sativus* L.), belongs to the *Cucurbitaceae* family, is native to Asia and has been consumed for more than 3000 years (Pandey and Kujur, 2022; Gelaye, 2023). It has very high water content and commonly consumed in salads and as pickle, and desserts. It is also rich in phosphorus, potassium, and oxalic acid, about 80% of the edible part of the cucumber fruit consists of 95% water, 0.7% protein, 0.1% fat, 3.4% carbohydrate, 0.4% fiber, and 0.4% ash (Onimisi and Ovansa, 2015; Abbey et al., 2017; Gupta et al., 2021). Cucumber is also useful in preventing jaundice and similar diseases and constipation. Since cucumber is a low-calorie vegetable, it helps weight loss by regulating blood sugar (Abbey et al., 2017). Cucumber cultivation is carried out both in open field and greenhouse as table and pickled cucumbers, and the production amount is 94,718,396 tons in the world (FAO, 2022). In Türkiye, cucumber cultivation is mostly carried out in the Mediterranean and Aegean and Marmara Regions with a production of 1,871,712 tons (TÜİK, 2023).

Salinity stress is one of the most common abiotic stresses that cause significant losses in agricultural production, especially in arid and semi-arid regions. According to FAO, approximately 800 million hectares of land worldwide are affected by salinity. Therefore, it is vital to know the mechanisms of salinity tolerance in order to obtain plants that respond better to abiotic stress. At the same time, it is necessary to achieve these goals through sustainable agricultural practices that will ensure more productive crops in the future climate change scenario (Hernández, 2019). The first symptoms of salt stress are manifested through exposure to osmotic stress, which is associated with the accumulation of phytotoxic ions in the roots, occurring from the early hours to several days later (Acosta-Motos et al., 2017). All these responses to salinity contribute to detrimental effects on plants, but there are plants tolerant to NaCl that can implement a series of adaptations to acclimatize to salinity, helping them survive. These adaptation mechanisms include morphological, physiological, biochemical, and molecular changes (Acosta-Motos et al., 2017). High salt levels affect 20% of cultivated land and half of the irrigated area (Chinnusamy et al., 2005; Sarwar et al., 2021).

Antioxidants neutralize free radicals and reactive oxygen species, preventing oxidative damage to cell membranes, proteins, DNA, and lipids, while also playing a protective role against environmental stress factors (temperature fluctuations, salt stress, drought, etc.), allowing plants to grow and develop healthily. Antioxidants protect plant health by enhancing their resistance to environmental stresses and supporting their productivity (Tabassum et al., 2024). In plants under salt stress, the activity of certain enzymes (glutathione peroxidase, superoxide dismutase, peroxidase, catalase, etc.) may change. These enzymes help neutralize free radicals and reactive oxygen species (ROS), preserving the plant's cellular structures (Zia-ur-Rehman et al., 2023). During salt stress, the regulation of potassium and sodium balance can affect the effectiveness of the plant's antioxidant systems. Maintaining this balance can enable better adaptation of the plants (Kanwal et al., 2024). As a result, salt stress can alter the antioxidant capacity in plants. However, this effect may vary depending on the plant species, the severity of the stress, and its duration. Some plants may tolerate this stress better, while others may experience more oxidative damage and lower productivity (Zia-ur-Rehman et al., 2023; Gill et al., 2024).

Plant tissue culture is the process of transferring parts of a plant to artificial nutrient media under sterile conditions. The goal of plant tissue culture were to obtain disease especially virus-free materials, to propagate species that are difficult to reproduce, to protect species at risk of extinction, and to produce a large number of plants in a short period. In this context, the aim of this study was to determine the effects of NaCl at different concentrations (0, 50, 100, 150, 200, 250, 300 mM) on the growth parameters of cucumber under *in vitro* conditions. The study also aimed to determine the antioxidant capacity of cucumber varieties in response to salt stress applied at different doses.

## MATERIALS AND METHODS

This study was carried out in the plant tissue culture and also quality and soil analysis laboratories of Agricultural Sciences and Technology Faculty of Sivas University of Science and Technology (Sivas, Türkiye). Besta F1 (1), MRC 1724 F1 (2) and Tulga F1 (3) varieties obtained from Manier Seed Company (Adana, Türkiye) were used in the study.

### Preparation of Nutrient Media and Seed Sowing

Murashige and Skoog (MS) (1962) was used as the basal nutrient medium. Sucrose and agar were used as the carbon source and solidification, respectively. Different doses of NaCl (0, 50, 100, 150, 200, 250, 300 mM) were tested (Table 1). After adjusting pH of the nutrient media to 5.8, they were sterilized in an autoclave for 15 minutes at 121°C and a pressure of 1.2 atm. After sterilization, the media were poured into petri dishes on a sterile bench. The cucumber seeds used in the experiments were kept in a 25% sodium hypochlorite solution for 20 minutes, then they were rinsed 4-5 times with sterile distilled water. The seeds sterilized were sown into the previously prepared nutrient media in the sterile bench. After sowing, the cucumber seeds were kept in the plant growth room at a temperature of  $25 \pm 2^\circ\text{C}$  with light intensity of 3000 lux for an 8-hours dark and 16-hours light conditions. During the study, germination rate (%) (Kaya et al., 2006), salt tolerance index (%) (Rahman et al., 2008; Khayatnezhad et al., 2011; Güldüren, 2012; Aydın and Atıcı, 2015), real water content (RWC, %), stem fresh weight (g), stem dry weight (g), root fresh weight (g), root dry weight (g), stem length (mm), root length (mm) (Keleş, 2019) and visual scale evaluations in plants after salt stress (Daşgan et al., 2002) were evaluated.

**Table 1.** Cucumber varieties and NaCl doses used in the study

NaCl Dose (mM)	Variety		
	1	2	3
0 (Control)	HA	HA	HA
50	HB	HB	HB
100	HC	HC	HC
150	HD	HD	HD
200	HE	HE	HE
250	HF	HF	HF
300	HG	HG	HG

### Determination of Antioxidant Capacity by DPPH

For DPPH, 1 gram of dried samples was weighed, 25 ml of 80% methanol was added, and the mixture was shaken in a shaking water bath for 2.5 hours at room temperature. The homogenized samples were transferred to a falcon tube and centrifuged at 7,000 rpm for 15 minutes. The supernatant of the centrifuged samples was filtered through blue band filter paper, and the resulting liquid extract was stored at  $+4^\circ\text{C}$ . The radical DPPH scavenging activity of the extract was determined by modifying the method described by Masuda et al. (1999). A 1900  $\mu\text{l}$  DPPH solution was added to 100  $\mu\text{l}$  of the sample and kept in the dark for 30 minutes. The samples were analyzed in a microplate reader at 517 nm, and absorbance values were recorded. Trolox was used as a reference antioxidant for the calculation of antioxidant capacity. Solutions of different concentrations were prepared and analyzed under the same conditions to obtain a calibration curve. The antioxidant capacity of the samples was determined as  $\mu\text{mol TE g}^{-1}$  dry weight (dw) in terms of trolox equivalent using this curve.

### Determination of Antioxidant Capacity by ABTS

A modified spectrophotometric method developed by Re et al. (1999) was used. 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (7 mM) and potassium peroxydisulfate solution (2.45 mM) were dissolved in 50 ml and 50 ml of distilled water, respectively. To prepare the stock solution, 10 ml of ABTS solution was taken, and 10 ml of  $\text{K}_2\text{O}_8\text{S}_2$  (potassium persulfate) solution was added. The mixture was kept in the dark for 16 hours at room temperature to prepare the ABTS radical solution. The working solution was prepared by diluting the stock solution to give an absorbance value of  $0.700 \pm 0.02$  at 734 nm, and the initial absorbance value was determined. Then, 3 ml of the working solution (ABTS + peroxydisulfate) or standard/sample was added to 100  $\mu\text{l}$  of the extract, and after mixing, the reaction was allowed to occur for 10 minutes in a dark environment at room temperature. Absorbance values were then read at 734 nm. Trolox was used as a reference antioxidant for the calculation of antioxidant capacity. Solutions of different concentrations were prepared and analyzed under the same conditions, and a calibration curve was obtained. The antioxidant capacity of the samples was determined as  $\mu\text{mol TE g}^{-1}$  dry weight (dw) in terms of trolox equivalent using this curve.

### Statistical Analysis

This study was carried out according to the Factorial Experimental Design with 4 replications and 5 petri dishes in each replication. Statistical analyses were performed using the JMP program.

### RESULTS AND DISCUSSION

The average germination rate of different cucumber varieties and nutrient media including different doses of NaCl were given in Table 2. Variety mean, medium mean and variety x medium interaction were found to be statistically significant. Variety average was the highest in variety 1 (94.36%) and the lowest in variety 2 (84.99%). In terms of medium average, the highest germination rate was observed in HA (96.05%) and the lowest in HG (84.80%). Singh and Gopal (2019) determined the effects of water and salt stress on germination rate, germination time and seedling growth of onion seeds. They found that increasing stress level caused a gradual and significant decrease in germination rate and seedling growth. Abdel-Farid et al. (2020) determined the effects of different doses of NaCl (25, 50, 100 and 200 mM) on seed germination and seedling growth of *Cucumis sativus* and *Solanum lycopersicum* in *in vitro* and *in vivo* studies. Seed germination rate and time was slightly decreased and delayed and shoot length was significantly reduced under the highest salt concentration (200 mM) in cucumber, while seed germination rate, germination time and seedling growth were significantly affected under all NaCl concentrations in tomato. Öztürk Gökçe et al. (2022) determined the effect of different salt concentrations on onion breeding lines and obtained the highest germination rate in the control medium without salt. Our study results were similar.

**Table 2.** Average germination percentage (%) of different cucumber varieties on different NaCl concentrations

	Variety			
Medium	1	2	3	Medium mean
HA	98.00ab	92.00f	98.16a	96.05A
HB	96.36abc	90.91f	96.12bc	94.46B
HC	95.36cd	85.00hı	96.00cd	92.12C
HD	94.54cd	84.91hı	94.16de	91.20C
HE	92.54ef	83.63ij	92.10f	89.42D
HF	92.03f	81.81j	88.36g	87.40E
HG	91.72f	76.67k	86.00h	84.80F
Variety mean	94.36A	84.99C	92.99B	
LSD <sub>variety</sub> ***:0.74    LSD <sub>medium</sub> ***:1.13    LSD <sub>varietyxmedium</sub> ***:1.95				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) \*\*\*  $p \leq 0.001$ .

The differences in salt tolerance index between different cucumber varieties and nutrient media prepared at different concentrations of NaCl were found statistically significant (Table 3). In terms of salt tolerance index, variety mean was the highest in the variety 1 (96.29%) and the lowest in the variety 2 (92.38%). The medium average was the highest in HA (100.00%) and lowest in HG (88.18%). When the variety x medium interaction was analyzed, the highest salt tolerance index was obtained from HA (100.00%) medium of varieties 1, 2 and 3, and the lowest was found in HG (83.34%) medium of variety 2, which were statistically in the same group. Kara et al. (2024) determined the growth of tomato plants in MS medium including sodium chloride at different concentrations (0, 50, 100, 150 and 200 mM) under *in vitro* conditions. In their study, the lowest salt tolerance index was observed in DE (200 mM) (81.25%) medium.



**Table 3.** Salt tolerance index of cucumber varieties at the different concentrations of NaCl (%)

	Variety			
Medium	1	2	3	Medium mean
HA	100.00a	100.00a	100.00a	100.00A
HB	98.33abc	98.82ab	97.92bc	98.36B
HC	97.31bcd	92.39gh	97.80bcd	95.83C
HD	96.47cd	92.29gh	95.93de	94.90C
HE	94.43ef	90.90hi	93.83fg	93.05D
HF	93.91fg	88.92ij	90.02i	90.95E
HG	93.59fg	83.34k	87.61j	88.18F
Variety mean	96.29A	92.38C	94.73B	
LSD <sub>variety</sub> ***:0.75    LSD <sub>medium</sub> ***:1.15    LSD <sub>varietyxmedium</sub> ***:1.99				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) \*\*\*  $p \leq 0.001$ .

Table 4 shows the actual water contents of different cucumber varieties and nutrient media including NaCl at various concentrations. The variety mean and medium mean were found to be statistically significant in the experiments. The variety mean was the highest in the variety 2 (91.16%) and the lowest in the variety 1 (89.07%). In terms of medium mean, the highest actual water content was found in HA (91.71%), while the lowest was detected in HG (89.44%) and HF (88.74%), which were in the same group.

**Table 4.** Actual water contents of cucumber varieties at the different concentrations of NaCl (%)

	Variety			
Medium	1	2	3	Medium mean
HA	90.82	92.24	92.07	91.71A
HB	90.17	91.01	90.56	90.58B
HC	89.75	90.68	90.24	90.22BC
HD	88.17	91.90	90.34	90.13BC
HE	89.56	91.19	89.91	90.22BC
HF	87.92	90.76	89.65	89.44CD
HG	87.09	90.33	88.80	88.74D
Variety mean	89.07C	91.16A	90.22B	
LSD <sub>variety</sub> ***:0.69    LSD <sub>medium</sub> ***:1.06    LSD <sub>varietyxmedium</sub> : N.S.				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) \*\*\*  $p \leq 0.001$ .

The stem fresh weight of different cucumber varieties and nutrient media including NaCl at different concentrations was given in Table 5. As a result of the analyses, both variety mean and medium mean were found to be statistically significant. According to Table 5, the highest the variety average was obtained from variety 2 (0.401 g), while the lowest was found in the varieties 3 (0.306 g) and 1 (0.302 g), which were statistically in the same group. The medium mean was the highest in HA (0.408 g) and the lowest in HG (0.251 g). Abu-Romman et al. (2012) reported that the shoot fresh and dry weights of cucumber plants decreased with increasing doses of NaCl (0.0, 25, 50, 75, and 100 mM). Kiran et al. (2015) examined the response of some eggplant rootstocks to salinity stress. When the plants reached to 4-5 true leaves stage, a decrease in the fresh weight of the green parts was observed at a concentration of 100 mM. The results obtained from our study have also showed a similar trend.

**Table 5.** Stem fresh weight of different cucumber varieties at the different concentrations of NaCl (g)

	Variety			
Medium	1	2	3	Medium mean
HA	0.396	0.456	0.372	0.408A
HB	0.356	0.455	0.330	0.380AB
HC	0.339	0.450	0.329	0.372AB
HD	0.274	0.447	0.305	0.342BC
HE	0.264	0.383	0.300	0.316CD
HF	0.251	0.310	0.290	0.284DE
HG	0.213	0.304		0.251E
Variety mean	0.302B	0.401A	0.306B	
LSD <sub>variety</sub> ***:0.03    LSD <sub>medium</sub> ***:0.04    LSD <sub>varietyxmedium</sub> : N.S.				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) \*\*\*  $p \leq 0.001$ .

Table 6 shows the stem dry weights of different cucumber varieties and nutrient media including NaCl at various concentrations. Both variety mean and medium mean were found to be statistically significant in the analyses. According to the Table 6, the highest variety mean was observed in the variety 2 (0.034 g), while the lowest was found in the varieties 1 (0.031 g) and 3 (0.031 g), which were statistically in the same group. The medium mean was the highest in HA (0.035 g) and the lowest in HG (0.027 g).

**Table 6.** Stem dry weight of different cucumber varieties at the different concentrations of NaCl (g)

	Variety			
Medium	1	2	3	Medium mean
HA	0.0341	0.0379	0.0341	0.035A
HB	0.0317	0.0382	0.0333	0.034AB
HC	0.0305	0.0383	0.0326	0.034AB
HD	0.0306	0.0341	0.0318	0.032BC
HE	0.0306	0.0332	0.0307	0.032BC
HF	0.0314	0.0304	0.0285	0.030CD
HG	0.0275	0.0289	0.0247	0.027D
Variety mean	0.031B	0.034A	0.031B	
LSD <sub>variety</sub> ***:0.74    LSD <sub>medium</sub> ***:1.13    LSD <sub>varietyxmedium</sub> : N.S.				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) \*\*\*  $p \leq 0.001$ .

The root fresh weight of different cucumber cultivars and nutrient media including NaCl at various concentrations was shown in Table 7. Both variety mean and medium mean were found to be statistically significant in the experiments. According to Table 7, the highest variety mean was found in the variety 2 (0.289 g), while the lowest was found in the variety 1 (0.088 g). The medium mean was the highest in HA (0.220 g) and the lowest in HG (0.100 g). In a study carried out by Afsar et al. (2020), the response of arugula to salt stress was investigated and shoot length, plant height, number of leaves, plant fresh weight, and plant dry weight were significantly reduced in plants under salt stress compared to the control. Baktemur (2023) investigated the growth of zucchini plants in nutrient media containing different concentrations of sodium chloride under *in vitro* conditions. The highest root fresh weight was observed in KD medium (3.78 g), while the lowest root fresh weight was found in the 250 mM in KF medium (2.03 g) including 250 mM NaCl.

**Table 7.** Root fresh weight of different cucumber varieties at the different concentrations of NaCl (g)

Medium	Variety			Medium mean
	1	2	3	
HA	0.130	0.384	0.147	0.220A
HB	0.117	0.341	0.135	0.198AB
HC	0.094	0.295	0.125	0.172BC
HD	0.077	0.294	0.120	0.164CD
HE	0.077	0.266	0.119	0.154CD
HF	0.068	0.247	0.080	0.132DE
HG	0.055	0.195	0.051	0.100E
Variety mean	0.088C	0.289A	0.111	
<i>B</i>				
LSD <sub>variety</sub> ***: 0.02    LSD <sub>medium</sub> ***: 0.13    LSD <sub>varietyxmedium</sub> : N.S.				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) \*\*\*  $p \leq 0.001$ .

Table 8 shows root dry weights of different cucumber varieties and nutrient media including NaCl at various concentrations. The differences in the analyses were not statistically significant.

**Table 8.** Root dry weight of different cucumber varieties at the different concentrations of NaCl (g)

Medium	Variety			Medium mean
	1	2	3	
HA	0.011	0.024	0.011	0.015
HB	0.007	0.019	0.008	0.011
HC	0.007	0.018	0.008	0.011
HD	0.006	0.016	0.007	0.010
HE	0.006	0.014	0.007	0.009
HF	0.005	0.001	0.006	0.008
HG	0.004	0.016	0.004	0.007
Variety mean	0.006	0.016	0.007	
LSD <sub>variety</sub> ***: 0.002    LSD <sub>medium</sub> ***: 0.005    LSD <sub>varietyxmedium</sub> ***: 0.003				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) \*\*\*  $p \leq 0.001$ .

The average stem length of different cucumber varieties and nutrient media including NaCl at various concentrations was presented in Table 9. Both variety mean and medium mean were found to be statistically significant in the study. The highest variety mean was observed in the variety 1 (26.40 mm), while the lowest was found in the variety 3 (21.55 mm). In terms of medium mean, the highest stem length was observed in HA (24.48 mm), and the lowest was in HG (20.23 mm). In a study carried out by Alrahman et al. (2005), growth and physiological characteristics of cucumber under *in vitro* salt stress (0, 50, 75, and 100 mM NaCl) determined and shoot length, fresh shoot weight, dry shoot weight, root length, and number of roots generally decreased in response to increasing salt concentrations. Kara et al. (2024) found that stem length was the highest in the DA medium (57.04 mm) and shortest in the DE medium (39.37 mm) in tomato plant growth subjected to salt stress.

**Table 9.** Stem length of different cucumber varieties at the different concentrations of NaCl (mm)

	Variety			
Medium	1	2	3	Medium mean
HA	30.50	27.08	24.86	24.48A
HB	29.18	27.02	22.61	26.27AB
HC	26.97	26.06	22.43	25.15BC
HD	25.71	24.95	22.44	24.37CD
HE	24.71	24.81	21.21	23.58DE
HF	24.08	24.37	19.09	22.51E
HG	23.66	18.87	18.18	20.23F
Variety mean	26.40A	24.74B	21.55C	
LSD <sub>variety</sub> ***:0.06	LSD <sub>medium</sub> ***:0.13	LSD <sub>varietyxmedium</sub> : N.S.		

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) \*\*\*  $p \leq 0.001$ .

Table 10 shows the average root length of different cucumber varieties and nutrient media including NaCl at various concentrations. Variety mean, medium mean, and variety x medium interaction were found to be statistically significant in the experiments. The highest variety mean was observed in the variety 2 (32.91 mm), while the lowest was found in the variety 1 (22.62 mm). In terms of medium mean, the highest root length was obtained from HA (36.11 mm), and the lowest was in HG (17.36 mm). When the variety x medium interaction was analyzed, the maximum root length was determined in the variety 2-HA medium (41.83 mm), and the minimum root length was found in the variety 1HF medium (14.16 mm), variety 3-HG medium (11.95 mm), and variety 1-HF medium (11.83 mm), which were statistically in the same group. Öztekin and Tüzel (2011) determined that root length decreased with the increase in salt levels in tomato. Kurum et al. (2013) investigated the effects of different salt doses on root length on rootstock squash varieties; the longest root was obtained in the control group (54.3 cm), while the shortest root was recorded at 16 dS m<sup>-1</sup> (30.1 cm). Mahmood and Pirlak (2023) investigated the responses of aronia plants to salt stress in both *in vitro* and *in vivo* conditions. In the experiments, it was observed that plant height, plant dry weight, root length, chlorophyll content, and protein content decreased with increasing salt concentrations. Similar results were obtained in our study.

**Table 10.** Root length of different cucumber varieties at the different concentrations of NaCl (mm)

	Variety			
Medium	1	2	3	Medium mean
HA	35.08b	41.83a	31.42cd	36.11A
HB	27.26efg	35.23b	30.87cd	31.11B
HC	24.36gh	34.29bc	30.66de	29.77BC
HD	24.13gh	31.57cd	30.03def	28.58C
HE	21.51h	29.73def	28.14def	26.46D
HF	14.16i	29.44def	26.91fg	23.50E
HG	11.83i	28.31def	11.95i	17.36F
Variety mean	22.62C	32.91A	27.14B	
LSD <sub>variety</sub> ***:1.32	LSD <sub>medium</sub> ***:2.02	LSD <sub>varietyxmedium</sub> ***:3.49		

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) \*\*\*  $p \leq 0.001$ .

The visual scale evaluations for different cucumber varieties and nutrient media including NaCl at various concentrations was presented in Table 11. Both variety average and medium average were found to be statistically significant in the experiments. The highest variety average was obtained from variety 2 (2.97), while the lowest was found in the variety 1 (1.69). In terms of medium average, the highest damage was observed in HG (3.40) and HF (3.07), while the least damage was observed in HA (1.33) (HA was in the same group with HG

and HF. The importance of visual scale evaluation was reported as an important parameter by Aktaş (2002) in pepper, Daşgan et al. (2002) in tomato, and Kuşvuran (2010) in melon for selection of genotypes. Kuşvuran (2011) examined the responses of genotypes to salt stress in determining salt-tolerant genotypes in okra. It was reported that the genotypes Okr-49, Okr-6, Okr-92, and Okr-36 were the least affected by salt stress with scale values ranging from 2 to 2.50, while the genotypes Okr-93 and Okr-112 were the most damaged by salt stress with scale values of 4. Baktemur (2023) reported increased damage in zucchini plants with increased NaCl concentrations. Similar trends were observed in our study.

**Table 11.** Visual scale values (1-5) of different cucumber varieties at the different concentrations of NaCl

	Variety			
Medium	1	2	3	Medium mean
HA	1.00	1.80	1.20	1.33D
HB	1.60	2.60	2.20	2.13C
HC	1.60	2.60	2.40	2.20BC
HD	1.60	2.80	2.60	2.33BC
HE	1.60	3.40	2.60	2.53B
HF	1.80	3.80	3.60	3.07A
HG	2.60	3.80	3.80	3.40A
Variety mean	1.69C	2.97A	2.63	
<i>B</i>				
LSD <sub>variety</sub> ***:0.08    LSD <sub>medium</sub> ***:0.12    LSD <sub>varietyxmedium</sub> : N.S.				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. N.S. – non significant (2) \*\*\*  $p \leq 0.001$ .

The antioxidant capacity of different cucumber varieties and nutrient media including NaCl at various concentrations was presented according to DPPH method (Table 12). Variety mean, medium mean, and variety x medium interaction were found to be statistically significant. The highest variety mean was obtained from variety 1 (163.57  $\mu\text{mol TE g}^{-1} \text{dw}$ ), while the lowest was found in variety 2 (147.68  $\mu\text{mol TE g}^{-1} \text{dw}$ ). In terms of medium mean, the highest average was observed in HD (173.21  $\mu\text{mol TE g}^{-1} \text{dw}$ ), and the lowest was in HF (138.36  $\mu\text{mol TE g}^{-1} \text{dw}$ ). Nasrin et al. (2015) reported that the scavenging percentage (%) of DPPH free radicals was concentration-dependent. Specifically, the concentration of the extract between 5-100  $\mu\text{g/ml}$  significantly increased the inhibitory activity. The IC<sub>50</sub> value for MCS was 13.06  $\mu\text{g/ml}$ , while it was 13.17  $\mu\text{g/ml}$  for standard ascorbic acid. In a study conducted Shariff et al (2021) by polyphenol content and antioxidant capacity of different parts of cucumber were determined. Results showed that the ethanol peel extract contained the highest levels of phenolics (23.08 mg GAE/g) and flavonoids (14.02 mg QE/g). Furthermore, the ethanol bark extract showed a significantly ( $p < 0.05$ ) higher FRAP value. Pearson correlation analysis revealed positive correlations ( $p < 0.01$ ) between FRAP analysis and TPC (Total Phenolic Content) and TFC (Total Flavonoid Content).

**Table 12.** Antioxidant activity ( $\mu\text{mol TE g}^{-1} \text{dw}$ ) recorded by DPPH analysis in the nutrient media including different concentrations of NaCl

	Variety			
Medium	1	2	3	Medium mean
HA	148.97ef	139.59h	147.22fg	145.26D
HB	162.86d	141.41h	161.27d	155.18C
HC	183.14a	161.71d	168.27c	171.05AB
HD	181.08a	163.77cd	174.78b	173.21A
HE	182.87a	152.36e	175.75b	170.33B
HF	139.56h	131.14i	144.37fgh	138.36E
HG	146.50fg	143.81gh	153.19e	147.83D
Variety mean	163.57A	147.68C	160.69B	
LSD <sub>variety</sub> ***: 2.83    LSD <sub>medium</sub> ***: 1.85    LSD <sub>varietyxmedium</sub> ***: 4.90				

In Table 13, the antioxidant capacity of different cucumber varieties and nutrient media including NaCl at different concentrations was presented according to ABTS method. In the study, the variety average, medium average, and the variety  $\times$  medium interaction were found to be statistically significant. The variety average was the highest in the variety 3 (242.17  $\mu\text{mol TE g}^{-1} \text{ dw}$ ) and the lowest in the variety 2 (210.56  $\mu\text{mol TE g}^{-1} \text{ dw}$ ). Regarding the medium average, the highest values were found in HC (251.06  $\mu\text{mol TE g}^{-1} \text{ dw}$ ) and HE (249.02  $\mu\text{mol TE g}^{-1} \text{ dw}$ ) media, while the lowest value was observed in the HF (208.15  $\mu\text{mol TE g}^{-1} \text{ dw}$ ) medium. Kanwal et al. (2024) conducted a study to alleviate the negative effects of sodium chloride (NaCl) on pea through foliar application of ascorbic acid (AsA). They found that enzymatic antioxidants, such as SOD (22.3%), POD (34.1%), and CAT (39%), were increased in both varieties under stress,. In another study, a salt stress was simulated by irrigating with a 100 mM NaCl solution, and then the leaves were sprayed with 1.0 mM salicylic acid (SA) to determine resistance of cherry rootstocks to salt stress (Xu et al., 2024). The salt treatment increased the antioxidant enzyme (peroxidase, catalase, and superoxide dismutase) activities in cherry rootstocks under salt stress, and this increase was more pronounced under salt stress with SA application (Xu et al., 2024). In plants under salt stress, the activity of certain enzymes (glutathione peroxidase, superoxide dismutase, peroxidase, catalase, etc.) may change. These enzymes help neutralize free radicals and reactive oxygen species (ROS), preserving the plant's cellular structures (Zia-ur-Rehman et al., 2023).

**Table 13.** Antioxidant activity ( $\mu\text{mol TE g}^{-1} \text{ dw}$ ) of different cucumber varieties determined by ABTS analysis in the nutrient media including different concentrations of NaCl

Medium	Variety			Medium mean
	1	2	3	
HA	226.46f	198.54j	234.39e	219.79D
HB	235.08e	201.51ij	251.27c	229.29C
HC	263.42a	233.58e	256.18bc	251.06A
HD	257.49b	210.65h	245.10d	237.75B
HE	255.38bc	226.99f	264.69a	249.02A
HF	218.51g	196.54j	209.40h	208.15E
HG	218.34g	206.13hi	234.19e	219.56D
Variety mean	239.24B	210.56C	242.17A	
LSD <sub>variety</sub> ***: 3.41    LSD <sub>medium</sub> ***: 4.90    LSD <sub>varietyxmedium</sub> ***: 5.91				

(1) Statistical differences between the averages shown in separate letters in the same column were found to be significant. (2) \*\*\*  $p \leq 0.001$ .

## CONCLUSION

This study aimed to determine the effects of of salt applications at different concentrations on the growth of cucumber plants under *in vitro* conditions. The negative effects of salinity, one of the most important abiotic stresses, are increasing globally. As usable agricultural areas have continued to decrease, it is crucial to develop salinity-resistant plant varieties. As seen from the results of this study, plant damage increases with higher salinity concentrations.

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## Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

## Author Contributions

M.D. : Setting up experiments and performing all analyses, statistical analyses & writing; Y.Ç: Antioxidant analyses, E. K. : Data analysis-review, editing & writing, G. B.: Designing of the study, controlling of all analysis and measurements, supervising & writing.



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