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EFFECTS OF ENVIRONMENT ON PRODUCTIVE PERFORMANCE OF HOLSTEIN-FRIESIAN DAIRY COWS IN THREE AGRO-ECOLOGICAL REGIONS OF ZIMBABWE

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Abstract: The level of performance in any livestock production enterprise is a function of genetic and non-genetic factors and their interaction. For the purpose of agricultural production decisions, Zimbabwe was divided into five agro-ecological zones (AEZ) according to rainfall intensity, distribution and length of rainy season. Commercial dairy production, based on specialist dairy breeds such as Holstein, Holstein-Friesian and Jersey, is confined to AEZ I, II, III and IV. The performance of these breeds in contrasting AEZ has not been determined. In this study, farm level data of 7562 Holstein-Friesian cows calving in the period 2003-2011 was used to compare milk yield and milk components across three contrasting AEZ of Zimbabwe. A Generalized Linear Model (GLM) was fitted to investigate the fixed effects of year, season and AEZ and random effects of days in milk (DIM) on milk production and component traits. The factors herd, agro-ecological zone, year and season had significant (P<0.001) effects on all variables tested. The most favourable performance of Holstein-Friesian cows was observed in AEZ II and during the hot-dry season due to higher test-day milk yield, protein, butterfat and total solids, and lower somatic cell counts. However, season and agro-ecological region are not limiting factors for commercial dairy production in Zimbabwe. Where animal performance may be sub-optimal, opportunities do exist for using strategies and technologies that help adapt and cope with climate conditions.

Keywords: Non-genetic factors, Agro-ecological zone, Dairy cattle, Herd, Season

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1. Introduction

Productivity of dairy cattle is determined by interplay between genetic and non-genetic factors and their interactions. Non-genetic factors affecting milk production include herd, year, season, parity, and days in milk, among others. Awareness of non-genetic effects and expected levels of production for particular breeds in specific environments may inform breed choice for those environments and production systems (Valencia et al., 2007). For the scientist, it presents an understanding of whether genetic or environmental factors are the most efficient avenues through which productivity can be improved in specific environments (Hammami et al., 2009). For farmers, knowledge of environmental effects on performance of animals can significantly increase efficiency of genetic improvement programs.

Historically, the Zimbabwe dairy industry has been dual in nature, comprising large scale commercial farms and smallholder multi-enterprise farms that varied with scale of production (Chirinda et al., 2021), Zvinorova et al. (2013) observed that several dairy farming systems co-exist in the country, being shaped by agro-ecological diversity, differences in human settlement patterns and population density, land tenure, resource endowments, and the resultant economic opportunities available for farmers. Consequently, Gororo (2016) proposed a new dairy classification model where farms are demarcated by dairy herd size and defined in terms of business objectives, production levels, labour organization, feeding systems, resource endowments, use of technology and marketing. In that classification scheme, dairy farms are categorized by scale into subsistence (< five dairy animals), small scale (family farms, 5-10 dairy animals); intermediate or medium scale (modest investment in dairy, 10-100 dairy animals); and large scale (corporate or family farms, >100 dairy animals). Medium to large scale dairy operations dominate the industry, producing 95-97% of all formally marketed milk in the country (Chirinda et al., 2021). These farms

are more mechanized, capitalized and employ semi-skilled and skilled labour for all year-round milk production using planted pastures, maize silage, forages and concentrates as feed resources. This diversity means that every farming system is unique, facing distinctive production and marketing challenges and opportunities and recommendation domains for sustainable dairy development for each sector also need to be unique.

Since the mid-1990s, Zimbabwe's dairy production has been declining and the demand-supply gap for milk and milk products in the country has been widening. Milk yield declined from 262 million liters in 1990 to <37 million liters in 2009 followed by a steady but slow increase to 82 million liters in 2021 (Chirinda et al., 2021). Viability and competitiveness challenges, and agrarian reforms led to farm closures, down-sizes, stagnation and shortage of new entrants. Starting in 2017, the country and industry implemented the Dairy Revitalization Program, a multi-pronged approach designed to build local production capacity and reduce the demand-supply gap. The objective of the Dairy Revitalization Program is to increase the national dairy herd and increase farm yields through access to better genetics and breeds for local farmers. Specialist dairy heifers and cows were thus directly imported into the country to replace or complement local non-dairy breeds and dairy cross breeds that predominated small scale dairy farms (Chirinda et al., 2021). The program focused on the importation and distribution of specialist dairy heifers of the Holstein-Friesian breed, as well as small numbers of Jerseys, on diverse farm systems and agro-ecological zones. The Holstein-Friesian was preferred for its higher milk yield potential. However, the breed requires high levels of management, being susceptible to sub-optimal nutrition; parasites, disease and heat stress (Nyamushamba et al., 2012). Jersey and Red Dane breeds on the other hand are reported to be more adaptable and resilient to local production conditions.

For the DRP, there is a challenge of choice of production system- and farmers to target as beneficiaries for imported specialist dairy heifers. Early studies in Holstein-Friesian dairy cows in Zimbabwe focused on milk production potential and the genetic and non-genetic factors influencing milk production, composition and hygienic quality (Missanjo et al., 2010). However, the effect of environment on Holstein-Friesian cows has never been reported in dairy scientific research in Zimbabwe. Awareness of environmental effects and expected levels of production for Holstein-Friesian breed in specific environments may inform breed choice for those environments and production systems. The present study was therefore carried out to assess the effect of agro-ecological zone on the performance of Holstein-Friesian dairy cows in Zimbabwe. The aim was to generate research-based evidence for recommendation domains to use on where and in which system the Holstein-Frisian would perform best in Zimbabwe.

2. Material and Methods

2.1. Study Area

Zimbabwe is a subtropical country located in Southern Africa between latitude 19.0154° S and longitude 29.1549° E. The total land size is 390,759 km^2. Zimbabwe's climate is subtropical, moderated by altitude. The country receives uni-modal rainfall, falling during the hot wet season from November to December followed by a warm wet season (January - March). This season is followed by a cold dry season (April - August) and a hot dry season (September - October).

The country is divided into five agro-ecological regions, depending on the total amount of rainfall received, rainfall distribution and length of rainy season. Rainfall patterns, crop yields and pasture production progressively deteriorate from agro-ecological zones (AEZ) regions I to V. These agro-ecological zones were initially delineated in the 1950s by Vincent et al (1960) and reclassified recently by Mugandani et al., (2012). This study was carried out on farms in AEZ II, III and IV where most of the dairy farms are located (Mhlanga et al., 2018; Chirinda et al., 2021). Agro-ecological zone II (AEZ II) is described as intensive crop and livestock farming region. The region receives moderately high rainfall of 750 – 1000 mm/year in at least 18 wet pentads. Rainfall is confined to summer (November to March) with rare but severe mid-season dry spells. Mean annual temperature is 16-19 ˚C (range: 10-23 ˚C). Vegetation is dominantly *Hyparrhenia* Tall Grass-veld with a grazing capacity of 2.5-3.5 hectares per livestock unit (ha/LU). Agro-ecological zone III is semi-intensive farming region, reserved for mixed crop-livestock systems. Rainfall is uni-modal (November to March), moderately high (650–800 mm/year), and falls in 14–16 wet pentads. Mean annual temperature range is 18-22 ˚C. Natural vegetation is mixed-veld dominated by perennial grasses with a grazing capacity of 5-6 ha/LU. Agro-ecological region IV is a lower potential region, receiving fairly low rainfall of 450 – 650 mm/year in less than 14 wet pentads per year. Rainfall is unreliable, variable with periodic seasonal droughts and severe dry spells. The recommended agriculture system is extensive in nature combined with livestock systems based on drought resistant fodder and forage crops. Natural grazing is a combination of the Eragrostis - other species grass veld (7.5-10 ha/LU) as well as Aristida–other species grass veld (10-16 ha/LU). Mean annual temperatures are higher, averaging 18-24 ˚C.

2.2. Data Collection and Edits

Six commercial dairy farms were selected using stratified random sampling. Only farms practicing intensive dairying, combining grazing, silage and concentrate feeding were considered. From each region, two farms were selected. These farms were on milk recording as part of the Zimbabwe Dairy Herd Improvement (ZDHI) during the period under consideration. Test-day milk production records for 7562 Holstein-Friesian cows calving between 2003 and 2011 were obtained from the

Zimbabwe Dairy Services Agency (ZDSA).

2.3. Statistical Analyses

A complex design of mixed and nesting effects was followed where by the random and fixed factors affecting milk yield and component traits included animal (days in lactation, test day milk yield) and environmental (year, season, agro-ecological zone) factors. There were four seasons: cool dry (April-August), hot-dry (September – October), hot-wet (November - December) and warm-wet (January - March. Test day milk yield (TDMY), milk component traits and somatic cell count (SCC) traits were the response variables. Since herds were only located in specific agro-ecological zones, the factor herd was nested within an agro-ecological zone. SCC data was normalized through log transformation before analyses. Data analysis was done using Generalized Linear Model (GLM) procedure of Minitab 18.1 (Minitab, LLC (2017). Fischer's Least Significant Difference (LSD) was used as the post-hoc test to separate means according to fixed factors, at the 5% significance level. Data was statistically analyzed according to the model given in Equation 1:

$$Y = \mu+Yr+S+F(AEZ)+\beta_1X_1+\beta_2X_2+e \quad (1)$$

where;

Y = butterfat, protein, lactose, total solids, TDMY, SCC

μ = overall mean

Yr = fixed effect of the year (2003 to 2011)

S = fixed effect of the season (cold dry, hot dry, hot wet, warm wet)

F(AEZ) = fixed effect of the farm (F) nested within an agro-ecological zone (AEZ)

β_1 and β_2 = regression coefficients relating to covariates

X_1 and X_2 = random effect of covariates (test day milk yield and days in milk)

e = random residual error (which follows a normal distribution).

3. Results

3.1. Effect of the Year

The annual phenotypic trends for TDMY, butterfat, protein, lactose, total solids and SCC are given in Figure 1, 2 and 3. The year of measurement had a significant effect on all variables tested (P<0.001). TDMY showed an upward trend to a peak of 24.0±0.35 kg/d in 2005. This was followed by a decline to a low yield level of 16.0±0.15 kg/d in the year 2008. Thereafter, TDMY started to rise again to a peak of 24.9±0.15 kg/d by 2011. Total solids did not change much across the years. However, there was a downward trend between 2005 and 2009. An initial upward trend to 2005, followed by a downward trend to 2008 and an upward trend thereafter was observed for the parameter, lactose content. Protein and butterfat content started with a downward trend from 3.60±0.036% and 3.90±0.072% in the year 2003. This downward trend ended in 2008 for milk protein (3.18±0.011%) and 2009 for butterfat (3.42±0.063%). Both protein and butterfat content reached a peak in 2010 at 3.67±0.024% and 4.15±0.048%, respectively. SCC showed a general upward trend over the study period and averaged 590x10^3 cells /ml. The level of SCC in milk spiked to 845x10^3 cells/ml in 2005 from a low of 426x10^3 cells/ml in 2004 and started to decline to 478x10^3 cells/ml in 2007. The other years (2008-2011) had higher, but similar levels of SCC.

3.2. Effect of the Season

Seasonal differences were significant (P<0.001) for all variables evaluated (Table 1). Differences in TDMY were small but significant across seasons, with the highest TDMY observed during the hot dry season. This period was also characterised by significantly lower SCC levels and higher lactose and milk protein content relative to other seasons.

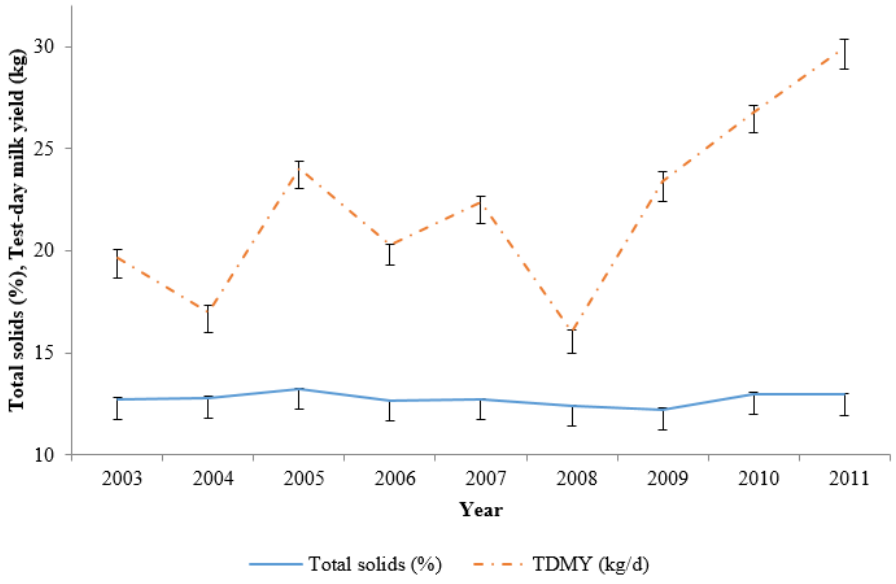

Figure 1. Phenotypic trend of test-day milk yield (TDMY) and Total Solids in Holstein-Friesian cows in Zimbabwe between 2003 and 2011. Error bars indicate SE of mean.

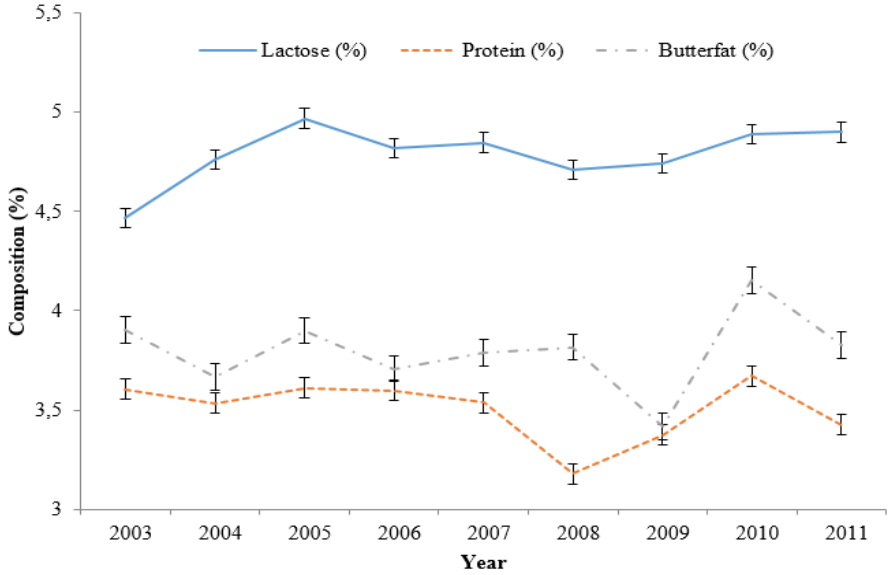

Figure 2. Phenotypic trends for milk components in Holstein-Friesian cows of Zimbabwe between 2003 and 2011. Error bars indicate SE of mean.

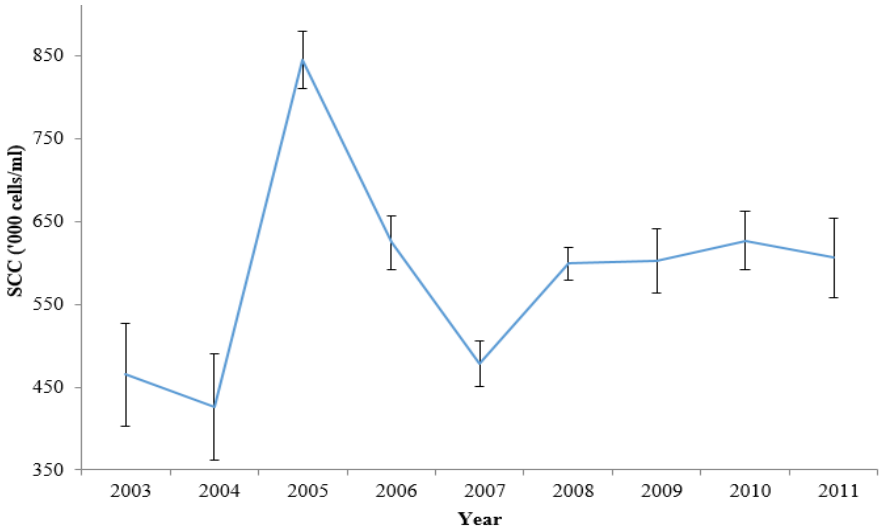

Figure 3. Phenotypic trend for somatic cell counts (SCC) in Holstein-Friesian cows of Zimbabwe between 2003 and 2011. Error bars indicate SE of mean.

The highest SCC was recorded during the cool dry season (604x10^3 cells/ml). The hot wet and warm wet seasons did not differ in this parameter (P>0.05). Butterfat content was higher during the warm-wet and cool-dry seasons (3.87%) and did not differ (P>0.05) between these two seasons. Butterfat content was however, lowest during the hot-dry season (3.67%). Lactose content was significantly higher during the dry seasons compared to the wet seasons. The four seasons differed (P<0.001) in total milk solids with the warm-wet season having the highest (12.92±0.033) and hot wet season having the lowest (12.56±0.033) value for this parameter.

3.3. Effect of the Agro-Ecological Zone

Agro-ecological zone had a significant (P<0.001) effect on all milk yield and composition traits analysed (Table 2). Test-day milk yield was significantly higher in AEZ II (26.8 kg/d) compared to AEZ IV (22.1 kg/d) and AEZ III (17.6 kg/d). AEZ II also had significantly higher milk composition traits (butterfat, milk protein, lactose and total solids) relative to other regions. However, no significant difference was observed in butterfat content for farms in AEZ II and those in AEZ IV. The variable SCC was lower in AEZ II (P<0.001), and similar between AEZ III and IV.

3.4. Effect of the Farm Measurement

Within regions, farm differences were observed in some variables (Table 3). In Region II, the two farms differed (P<0.05) in lactose (4.95% vs. 4.92%) and Log-SCC. Farms in Region III significantly differed from each other in protein (3.17% vs. 3.37%), total solids (12.16% vs. 12.43%), SCC (1,022 vs. 152 × 103 cells/ml) and Log-SCC. In Region IV farms differed from each other in butterfat (3.82% vs. 4.11%), lactose (4.66% vs. 4.71%), total solids (12.63% vs. 12.93%), SCC (668 vs. 481 × 103 cells/ml) and Log-SCC.

Table 1. Effect of season (Mean ±SE) on test-day milk yield (TDMY), milk composition and somatic cell counts (SCC) in Holstein-Friesian cows in Zimbabwe

Variable	Season				P-value
	Cold Dry	Hot Dry	Hot Wet	Warm Wet	
TDMY (kg/d)	21.71±20.435^{bc}	23.65±20.021^{a}	22.03±19.815^{b}	21.28±20.077^{c}	<0.001
Butterfat (%)	3.87±0.027^{a}	3.67±0.029^{c}	3.79±0.025^{b}	3.87±0.029^{a}	<0.001
Protein (%)	3.48±0.013^{b}	3.58±0.014^{a}	3.40±0.012^{c}	3.56±0.012^{a}	<0.001
Lactose (%)	4.86±0.009^{a}	4.85±0.010^{a}	4.71±0.009^{b}	4.73±0.009^{b}	<0.001
Total Solids (%)	12.82±0.033^{b}	12.70±0.038^{c}	12.56±0.033^{d}	12.92±0.033^{a}	<0.001
SCC ('000/ml)	604.4±22.30^{a}	495.6±24.70^{b}	520.8±21.40^{b}	501.0±20.30^{b}	<0.001
Log-SCC	2.40±0.018^{a}	2.25±0.020^{c}	2.35±0.018^{b}	2.34±0.017^{b}	<0.001

^{a,b,c} The same row, means that do not share a letter are significantly different (P<0.001). Seasons were: cold-dry (April-August), hot-dry (September – October), hot-wet (November - December) and warm-wet (January – March). TDMY= test-day milk yield (kg/d)), SCC= somatic cell counts, Log SCC= log somatic cell counts.

Table 2. Least square means (±SE) for effect of agro-ecological zone on milk yield, components and SCC in Holstein-Friesian cows in Zimbabwe.

Variable	AEZ II	AEZ III	AEZ IV
TDMY (kg/d)	26.88±0.159^{a}	17.57±0.201^{c}	22.05±0.161^{b}
Butterfat (%)	3.88±0.041^{a}	3.55±0.020^{b}	3.96±0.022^{a}
Protein (%)	3.76±0.020^{a}	3.27±0.009^{c}	3.49±0.011^{b}
Lactose (%)	4.94±0.014^{a}	4.74±0.007^{b}	4.68±0.008^{c}
Total Solids (%)	13.18±0.053^{a}	12.30±0.026^{c}	12.78±0.029^{b}
SCC ('000/ml)	428.5±34.90^{b}	587.7±16.20^{a}	575.1±18.30^{a}
Log-SCC	2.348±0.0286^{b}	2.201±0.0132^{c}	2.459±0.0150^{a}

^{a,b,c} The means in the same row do not share a letter are statistically different (P<0.001), SCC= somatic cell counts, Log SCC= log somatic cell counts, AEZ= agro-ecological zone.

Table 3. Least square means of effect of farm on milk components and SCC

F(AEZ)	TDMY	Butterfat	Protein	Lactose	Total solids	SCC
1(II)	26.88±0.150^{a}	3.85±0.043^{b}	3.82±0.021^{b}	4.95±0.015^{c}	13.20±0.056^{a}	461.9±36.80^{cd}
2(II)	26.66±0.149^{a}	3.92±0.048^{b}	3.69±0.024^{b}	4.93±0.017^{a}	13.15±0.063^{a}	395.0±41.20^{d}
3(III)	17.57±0.200^{c}	3.52±0.036^{c}	3.17±0.018^{e}	4.74±0.012^{bc}	12.16±0.047^{e}	1,022.9±30.80^{a}
4(III)	16.57±0.250^{c}	3.57±0.039^{c}	3.37±0.019^{d}	4.759±0.014^{b}	12.43±0.051^{d}	152.3±31.50^{e}
5(IV)	22.05±0.160^{b}	3.82±0.031^{b}	3.50±0.015^{c}	4.66±0.011^{d}	12.63±0.040^{c}	668.7±26.20^{b}
6(IV)	22.25±0.130^{b}	4.11±0.030^{a}	3.47±0.015^{c}	4.71±0.010^{c}	12.93±0.039^{b}	481.4±24.60^{c}

^{a,b,c} The means in the same column do not share a letter are statistically different (P<0.001), F(R)= farm nested within agro-ecological zone, SCC= somatic cell counts.

Table 4. Correlation coefficients of test-day milk yield and days in lactation on milk components and SCC in Holstein-Friesian cows of Zimbabwe between 2003 and 2011*

Variable	Days in Lactation		Test Day Milk Yield	
	r	P-value	r	P-value
Test Day Milk Yield	-0.225	0.000	-	-
Butterfat	0.130	0.000	-0.210	0.000
Protein	0.250	0.000	-0.176	0.000
Lactose	-0.190	0.000	0.420	0.000
Total Solids	0.147	0.000	-0.125	0.000
SCC	0.164	0.000	-0.150	0.000
Log-SCC	0.207	0.000	-0.140	0.000

*All correlation coefficients were significant at P<0.001, SCC= somatic cell counts, Log SCC= log somatic cell counts.

3.4. Effect of Animal on Milk Components and SCC

There was significant correlation (P<0.001) between the animal factors, test-day milk yield and days in lactation with all variables tested (Table 4). Test-day milk yield was found to have a moderate and negative correlation with butterfat (0.210), protein (0.180), total solids (0.130) and SCC (0.150), and a high positive correlation with lactose content (0.42). Significant correlations were

also observed for days in lactation with TDMY (-0.225) and lactose (-0.190). Butterfat content, protein, total solids and SCC were moderately and positively correlated to days in lactation.

4. Discussion

Significant fluctuations in test-day milk yield and compositional traits were observed in this study (P<0.001). These temporal variations may be related to socio-economic and environmental factors experienced each year (Fontaneli et al., 2005). Kunaka and Makuza (2005) reported that the amount and distribution of rainfall has a positive effect on forage and feed resource availability and disease challenges experienced each year. Serious drought conditions were experienced in the years 2007-2009. In addition, this coincided with the period of economic recession and hyper-inflation, probably reducing the managerial capacity of the farms. This may partly explain lower milk yields and poor compositional quality during the period. These annual fluctuations in milk yield and quality have been reported before for Holstein-Friesian (Nyamushamba et al., 2012) and Jersey cows (Missanjo et al., 2010) in Zimbabwe.

Seasonal differences were observed for test-day milk yield and composition in this study. The year was divided into four seasons and data analysed by season. It was observed that the hot-dry season had the highest test-day milk yield, lactose and milk protein, and the lowest butterfat content. In addition, this was the season with significantly lower SCC relative to the other seasons. A higher milk yield and lower butterfat content observed this season can partly be explained by negative genetic correlation between these two traits. It could also be explained by the absence of green forage, necessary for producing richer, deep yellowish milk.

However, the observation of peak milk yield in the hot-dry season was not expected, since it is the period of forage scarcity and heat stress. Earlier studies (Muchenje et al., 1997; Makuza and McDaniel, 1997; Kunaka and Makuza, 2005) reported peaks for milk yield and compositional traits in the cooler months between May and August. Theoretically, milk production in cows decreases with increasing heat load (Rodriguez et al., 1985). In that case, the cows were expected to partition more and more metabolic energy to homeostasis at the expense of productive functions such as milk production. This study did not interpolate bioclimatic data to check whether heat stress could have been at play. The Temperature Humidity Index (THI) is commonly used to measure the degree at which environmental conditions affect performance of dairy cattle. THI measures the risk of heat stress in dairy cows by combining relative humidity and air temperature. However, Kunaka and Makuza (2005) computed monthly THI for dairy areas using data from the period 1979-1998 and observed that they ranged from 55.95 in the month of July to 67.78 in the hottest month of January. None of these monthly THI values exceeded the limits (~72) for heat load in the Holstein-Friesian breed (Collier et al., 2011). Beyond THI 72, an area is deemed unsuitable for dairy farming as the lactating cow would have to channel significant amounts of metabolic energy to maintenance of body temperature (Mhlanga et al., 2018). Therefore, environmental variations in humidity and temperature may not have had any influence on milk composition and yield. Mhlanga et al. (2018) used three different change scenarios to model and predict future suitability of Zimbabwean landscape for dairy production. Mugandani et al. (2012) reclassified Zimbabwe's agro-ecological zones and found that AEZ II had significantly decreased. These studies show that the naturally suitable areas for dairy are decreasing in extent. Opportunities however exist for using strategies and technologies that help adapt and cope with current and future scenarios (Gwatibaya, 2012; Mhlanga et al., 2018).

Matekenya (2016) posits that better all year-round performance of commercial dairies is partially due to access to extensive grazing areas, conserved forages and financial resources for purchasing supplementary feeds during the dry period. It could be speculated that the higher yields could be related to better management given to the cows during this season as farmers sought to reduce heat stress, maximize production from conserved forages and take advantage of seasonally higher milk prices. Due to availability of conserved forages such as maize silage and hay, season is not a limiting factor for commercial dairy production in Zimbabwe.

Zimbabwe is divided into five agro-ecological regions or zones (AEZ) based mostly on rainfall amount and season quality. When milk yield and composition data for the period 2003-2011 was disaggregated by AEZ, it was observed that AEZ II had the highest milk yield and composition and the lowest SCC. In addition, herd differences were observed for most of the variables investigated. Similar to present findings, Nyamushamba et al. (2012) reported a decrease in lactation yield from AEZ I to AEZ V. In that study, the lowest milk yield was observed in AEZ IIb, whereas AEZ IIa and AEZ III had similar milk yields. Observed regional differences could be related to environmental conditions characterizing each of the respective AEZs.

Herd differences in milk yield and composition are widely reported in Zimbabwe (Makuza and McDaniel, 1997; Kunaka and Makuza, 2005; Nyamushamba et al., 2012). These differences can be ascribed to variations in herd effects of management and nutrition on the various farms. Available feed resources and strategies for delivering them to the farm usually differ from one herd to another. Dairying in Zimbabwe is pasture based with concentrates provided as supplementary feed. During the wet seasons, cows are grazed on natural, planted or reinforced natural pasture up to about March when the grazing starts to lose its feeding value. During the dry non-growing period, roughage is supplied in the form of conserved forage - native or improved grass hay and maize silage. In both seasons, concentrates are given to

achieve nutritional requirements for milk production and other physiological needs. Farms differ in their systems of concentrate delivery to the cows, and in the quality and quantity of concentrates and forages delivered. Farms may elect to use conventional (feed to yield), flat rate, budget, lead feeding or combinations of these strategies. General nutritional management of dairy cows in Zimbabwe is detailed in the Dairy Farmers Handbook (Oliver, 1987). Thus, feeding strategy and system are partly responsible for significant variations in performance of farms, even those within the same agro-ecological region. Commercial dairy production involves harvesting and conservation of excess forage during periods of plenty for feeding to cows during periods of scarcity and deficits. Therefore, the non-genetic factors - herd, season and agro-ecological region - may not be serious limiting factors for dairying in Zimbabwe.

In this study, negative correlations were observed between milk yield and milk components, except lactose content, which had a high significant correlation with milk yield. Correlations were in the range of those generally reported in literature, falling between −0.20 and −0.56 (Missanjo et al., 2010; Wongpom et al., 2017). In addition, higher milk yields are associated with less SCC. The negative correlation of milk yield and lactose content with days in lactation is consistent with the standard lactation curve of cows. In early lactation milk yield is rising until it reaches a peak eight weeks post calving and it starts to decline until the cow is dried off. As lactation days advance, milk yield decreases and milk components decrease. SCC was positively correlated to days in lactation. Generally, SCC is higher immediately after calving but drops rapidly during the first week of lactation. The high cell counts the first days of lactation is due to the high immunoglobulin content in the colostrum. It has generally been observed that SCC increases with advancing stage of lactation as drying-off approaches (Hagnestam-Nielsen et al., 2009). For cows with subclinical mastitis, SCC increase significantly towards end of lactation.

5. Conclusion

This study revealed that environment affects performance of Holstein-Friesian cows across contrasting agro-ecological zones of Zimbabwe. It was observed that season and agro-ecological region are not limiting factors for commercial dairy production in Zimbabwe. However, the most favourable performance of cows was observed in AEZ II and during the hot-dry season. It is during these when test-day milk yield and composition traits were higher and SCC lower. AEZ II offers the best conditions for dairy production based on the Holstein-Friesian breed. However, with good fodder and forage planning and management of heat stress, commercial dairy production in Zimbabwe is not limited by season and agro-ecological region.

Author Contributions

O.T. (50%) and T.K.M. (50%) initiated the research, collected data, analyzed and interpreted the data. T.K.M. (50%) and E.G. (50%) wrote the manuscript. E.G. (50%) and O.T. (50%) supervised the research, suggested the research methods, structured the paper and edited the manuscript. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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IN VITRO MICROPROPAGATION OF MAINTAINER WHITE HEAD CABBAGE LINES USING COTYLEDON AND HYPOCOTYL EXPLANTS

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Abstract: *Brassica* species are the most widely cultivated vegetable crops and improvement programs started in the last thirty years in Türkiye. Hybrid seed production is very difficult for *Brassica* vegetable species. Because the production of a new F1 hybrid cultivar needs a male sterile line (A), maintainer line (B), and also a male line (C). Biotechnological methods provide an excellent opportunity for new F1 hybrid cultivar improvement via *in vitro* maintenance of the breeding lines. Thus, *in vitro* propagation possibilities of Matsunami F1 cultivar and 3 white head cabbage maintainer inbred lines were examined using hypocotyl and cotyledon explants. The most successful results were obtained from MS + 2 mg/l and 4 mg/l BAP combinations. Matsunami F1 was prominent in terms of proliferation rate. Root formation was found to be considerably high in all genotypes. The use of in vitro propagation techniques is expected to provide significant benefits in head cabbage breeding programs.

Keywords: Cabbage breeding, Explant, *in vitro* regeneration, Maintainer lines

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1. Introduction

Vegetables and oilseeds have the highest economic value in *Brassica* species. *Brassica* vegetables are grown extensively all over the world and mainly belong to the family of *B. oleracea* (Ravanfar et al., 2014). Hybrid seed production is very difficult for *Brassica* species. Obtaining the commercial hybrids of *Brassica oleracea* L. is a highly laborious effort because they are strictly cross-pollinated plants and the maintaining of parental lines used in hybrid combinations involves a lot of time and financial support (Balkaya et al., 2005; Cristea et al., 2009; Karaagac and Kar, 2016; Dogru et al., 2017). The low price of seeds has limited the opportunities for mass micropropagation of vegetables and thereby restricted the use of clonal multiplication for breeding purposes (Kieffer et al., 2001). Plant tissue culture techniques provide an excellent opportunity for new cultivar development. Vegetative populations are of interest because the self-incompatibility and biennial life cycle of many *Brassica oleracea* crops can make the recovery of sufficient seeds for large-scale studies slow and difficult (Kumar and Srivastava, 2015). This technology allows the regeneration of a large number of plants, independently of the season, in controlled conditions. The genetic material thus obtained is authentic, homogeneous, healthy, high quality, and productive, avoiding phytosanitary risks and early biological cycles (Cristea et al., 2009). However, the development of *in vitro* regeneration techniques is useful to produce uniform and true-to-type plants of the cultivar and is also essential for the future recovery of transgenic plants (Ravanfar et al., 2014; Kurtar et al., 2020). For genetic improvement of cabbage using genetic engineering, efficient and reproducible *in vitro* regeneration protocols are required. In *Brassica*, remarkable results regarding the regeneration of new plants using different explants such as cotyledon and hypocotyl were obtained through direct organogenesis and embryogenesis (Cristea et al., 2009; Gambhir et al., 2017).

Brassica vegetable improvement programs started in the 1990s in Türkiye. The new cultivars produced until now are a result of traditional breeding procedures. However, most of the hybrids used in winter vegetable growing in Türkiye are imported cultivars. This is because hybrid seed production is very difficult for Brassica vegetable species and some problems occur during this process. Very few in vitro propagation studies have been conducted in Türkiye to overcome problems in the cabbage breeding process.

The main objective of the present study was to investigate the shoot induction and plantlet regeneration from hypocotyl and cotyledon explants of promising

maintainer white head cabbage breeding lines accelerate to the production of local F1 white head cabbage cultivars with desirable traits.

2. Material and Methods

2.1. Materials

The experiment was carried out in the field and laboratory of the Black Sea Agricultural Research Institute in 2019. Three promising maintainer white head cabbage inbred lines (P62-1, 4, 145) including our gene pool and Matsunami F1 cultivar were used as donors.

2.2. Explant Preparation

Hypocotyl and cotyledon explants used in the study were obtained by *in vitro* seed sowing. Firstly, one-year-old seeds of donors were rinsed in 50% (v/v) ethanol for 5 minutes and following this soaked in sterile water. Then, they were surface-sterilized in 50% (v/v) commercial bleach solution for five minutes and subsequently rinsed in distilled sterile water three times and were placed on sterilized filter paper to desiccate excessive surface water. The sterilized seeds were germinated in 60x15 mm sterile Petri dishes (six seeds per dish) on MS basal medium containing 1% sucrose and 0.8% agar (w/v), pH 5.8 without plant growth regulators (Gerszberg et al., 2015). The seeds were germinated in a growth chamber at 23±1 °C, illuminated with white fluorescent 32 W lamps (3000 lx) under a 16/8 h photoperiod.

2.3. Shoot Induction

Cotyledon and hypocotyl explants were aseptically excised from 10-d-old seedlings and cultured on 6 different solid MS shoot-regenerating media supplemented with 1% sucrose, 0.8% agar, and with different concentrations and combinations of plant growth regulators (M1: MS Basal medium; M2: MS + 2 mg/l BAP; M3: MS + 4mg/l BAP; M4: MS + 0.1 mg/l NAA; M5: MS + 2 mg/l BAP + 0.1 mg/l NAA; M6: MS + 4 mg/l BAP + 0.1 mg/l NAA). The cotyledons containing 1 - 2 mm petioles and the 5 - 10 mm long hypocotyl segments excised from seedlings were placed on the shoot regeneration medium (Dai et al., 2009). All cultures were maintained in a growth chamber under an 8-h dark/16-h light photoperiod (~3000 lx) at 23±1 °C. Explants were regularly subcultured at four-week intervals. The frequency of shoot regeneration and the number of shoots per explant were counted after every four weeks of culture (Gerszberg et al., 2015).

2.4. Root Induction

After the culture on shoot-regenerating media, regenerated shoots were transferred to rooting media. At the stage of shoot regeneration; explants were cultured for four weeks in four different MS media supplemented with 1% sucrose and 0.7% agar to determine the effect of IBA content and ½ MS media on rooting (M1: MS Basal medium; M2: MS + 0.5 mg/l IBA; M3: ½ MS; M4: ½ MS + 0.5 mg/l IBA). Then, explants that were divided into small pieces on sterile paper were placed in 300 ml jars containing 100 ml medium. Explants were maintained in a growth chamber under an 8-h dark/16-h light photoperiod (~3000 lx) at 25±1 °C.

Rooting rate (%), rooted plant length (mm), and stem diameter (mm) were measured. The average number of roots and the number of leaves were counted, and the number of branched roots and average root diameter (mm) were determined in rooting plantlets.

2.5. Acclimatization

Rooted and elongated plants removed from the culture chamber were washed in tap water and purified from the medium. Afterward, the plants were treated with 1% fungicide solution (containing the Benlacide active ingredient) for two min. The plants were planted in the containers prepared with a 1:1 peat-perlite mixture, subsequently. Planted samples were covered with an air-permeable cover to provide moisture control. At this stage, the survival rate (%) was determined according to Basak et al. (2012).

2.6. Statistical Analysis

The study was carried out according to a randomized plot factorial experimental design. The analyses were performed in three replicates, with six Petri dishes in each replicate at the seedling stage, and three replicates with 20 plants per replicate at the stage of shoot reproduction and rooting. All the obtained data were subjected to variance analysis in JMP-SAS 5.01 statistical software. However, arcsin √x transformation was also applied for %-valued parameters such as rooting rate. The criteria that were found statistically significant as a result of the analyses were grouped by Duncan's multiple comparison test (Genç and Soysal, 2018).

3. Results and Discussion

3.1. Multiple Shoot Induction from Cotyledon and Hypocotyl Explants

In this study, ten-day-old seedlings grown *in vitro* were used as a source of hypocotyl and cotyledon explants for shoot regeneration (Figure 1a). As presented in Table 1, in cotyledon explants cultured for four weeks in shoot propagation media shoot formation rate (%) varied between 24.44% (line 145 + 04) and 97.78% (Matsunami F1 + M2) in cotyledon explants (Table 1). Otherwise, hypocotyl explants did not produce any shoot in M1 and M4 medium in all genotypes. In this respect, the highest shoot formation rate was obtained from Matsunami F1 in the M2 medium with 28.89%. It was determined that in some of the hypocotyl explants cultured in shoot regeneration media, calli formations occurred together with shoot formations. Since calli and shoot formation rates obtained from hypocotyl explants were inadequate, the next step was continued only with shoots from cotyledon explants. In terms of regeneration ability, hypocotyl explants were prominent in some of the *in vitro* propagation studies conducted in *Brassica* species, while cotyledon explants were prominent in other studies. Ertaş and Tuncer (2016) found that hypocotyl explants were more successful than cotyledon explants in terms of shoot regeneration. In a different study carried

out using cotyledon explants in broccoli, it was reported that an average of 10 shots can be obtained from each cotyledon explant (Ravanfar et al., 2014). It is thought that these differences that the studies observed in the explant type are due to the genotype effect. However, cotyledon, hypocotyl, epicotyl, and root segments could be used for shoot formation in white cabbage, but different combinations of hormones should be applied for high regeneration rates in these explants (Sretenovic-Rajicic et al., 2002). Pal et al. (2007) stated that *in vitro* induction of organogenesis depends on the endogenous concentration of plant growth regulator or interaction with an exogenously supplied growth regulator.

In cotyledon explants cultured in the shoot propagation medium, the new shoots formed from a shoot were counted, and the multiplication index (MI), which theoretically represents the total number of plants that can be obtained from a shoot, were calculated. The data regarding the obtained MI are presented in Table 2.

Among the cabbage genotypes, the highest MI was determined in the Matsunami F1 with an average of 7.11 shoots/explant. As a result of the research, when the media is evaluated; the highest MI was obtained from the medium used cytokinin in plant growth regulators (Figure 1b). The highest MI was obtained from the M2 + 2 mg/l BAP with an average of 7.75 shoots/explants (Figure 1c). This was followed by the M3 + 2 mg/l BAP with an average of 6.33 shoots/explants. The results obtained in different studies have shown that cytokinins added to the media have a positive effect on shoot formation and multiplication (Ahmad and Anis, 2005; Ravanfar et al., 2014). While IAA, IBA, and NAA-like auxins are effective in root formation; BAP and kinetin-like cytokinins are effective in shoot formation (Pant and Manandhar, 2007). The auxin group of plant growth regulators promotes cell division and callus formation. Therefore, shoot formation did not occur in the media that used NAA alone (Ravanfar et al., 2014). On the other hand, Pavlovic et al. (2010) determined the MI as 8.7 for broccoli and 13.4 for savoy sprouts. Besides, it has been determined that the effect of the genotype factor is prominent in the differences between the media in terms of shoot reproduction.

3.2. Rooting Rate

The formation of a healthy and strong root structure is one of the most important factors that increase the survival rate of plants during their acclimatization process. Rooting rate values obtained at the end of the culture period from the explants cultured for four weeks in rooting media are presented in Table 3.

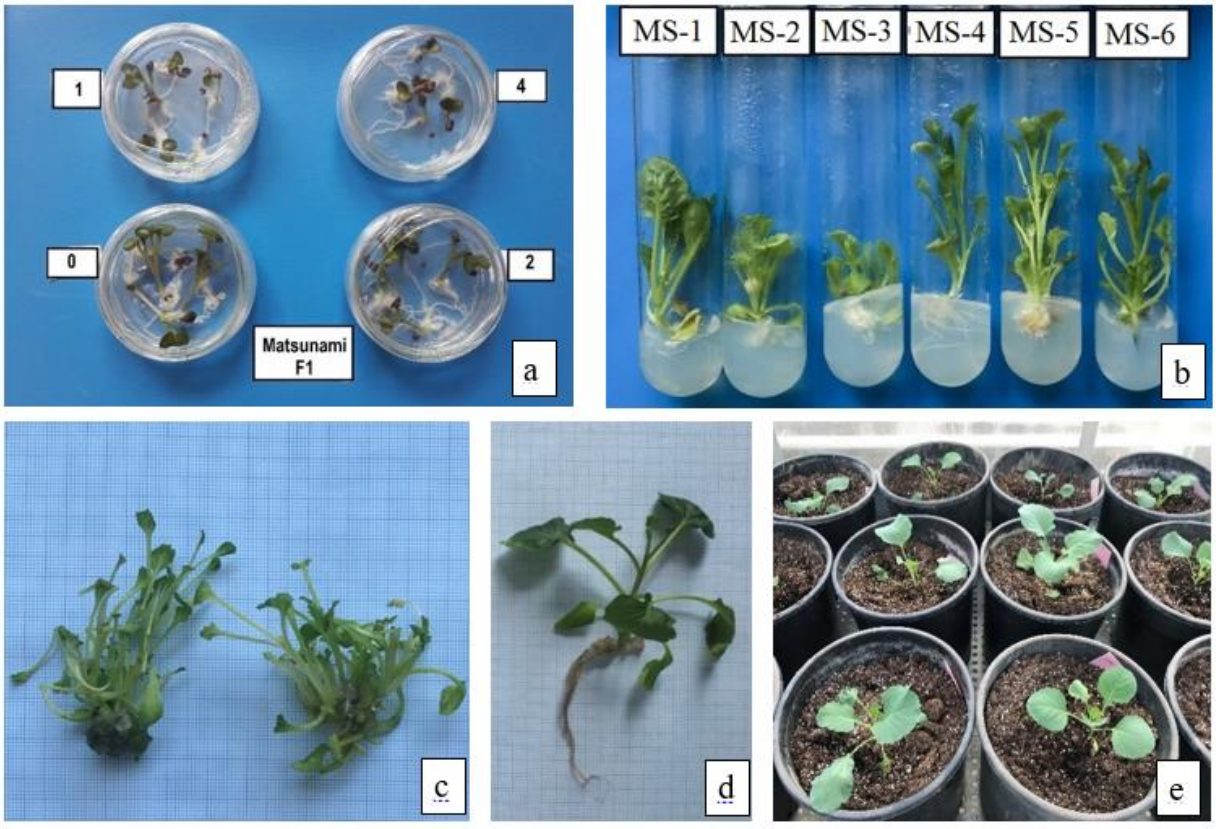

Figure 1. Efficient plant regeneration from cotyledon and hypocotyl explants of white cabbage (*Brassica oleracea* L. var. c*apitata*). a. Aseptically germinated 10-day-old seedlings of cabbage cv. Matsunami F1 on different sucrose concentrations. b. Callus formation and shoot initiation from cotyledon explants on different media. c. Shoot regeneration from cotyledon explants on MS basal media supplemented with 2 mg/l BAP. d. Fully developed plantlets of cabbage taken out of media. e. Plantlets transferred to the potting mixture.

Table 1. The effect of media containing different plant growth regulators on average shoot induction (%), callogenesis (%) and rooting (%)

Genotype	Media	Shoot Induction (%)		Callogenesis (%)		Rooting (%)	
		C*	H*	C*	H*	C*	H*
P62-1	M1	64.44	0.00	0.00	0.00	97.78	0.00
	M2	86.67	4.44	95.56	0.00	0.00	0.00
	M3	91.11	6.67	97.78	6.67	0.00	0.00
	M4	31.11	0.00	0.00	0.00	93.33	13.33
	M5	88.89	15.56	0.00	0.00	91.11	0.00
	M6	93.33	15.56	84.44	4.44	8.89	0.00
	Average	75.93	7.04	46.30	1.85	48.52	2.22
145	M1	55.56	0.00	4.44	0.00	91.11	0.00
	M2	93.33	6.67	97.78	0.00	0.00	0.00
	M3	93.33	8.89	95.56	0.00	0.00	0.00
	M4	24.44	0.00	0.00	0.00	95.56	0.00
	M5	93.33	24.44	93.33	15.56	0.00	26.67
	M6	88.89	20.00	60.00	0.00	26.67	13.33
	Average	74.81	10.00	58.52	2.59	35.56	6.67
4	M1	53.33	0.00	0.00	0.00	91.11	0.00
	M2	71.11	6.67	46.67	0.00	0.00	0.00
	M3	80.00	15.56	53.33	0.00	0.00	0.00
	M4	55.56	0.00	0.00	0.00	91.11	0.00
	M5	93.33	2.22	86.67	11.11	4.44	0.00
	M6	88.89	17.78	84.44	6.67	0.00	0.00
	Average	73.70	7.04	45.19	2.96	31.11	0.00
Matsunami F_1	M1	68.89	0.00	0.00	0.00	93.33	0.00
	M2	97.78	28.89	93.33	0.00	0.00	15.56
	M3	95.56	24.44	95.56	0.00	0.00	13.33
	M4	64.44	0.00	2.22	6.67	91.11	0.00
	M5	93.33	2.22	91.11	22.22	4.44	17.78
	M6	93.33	4.44	88.89	26.67	2.22	0.00
	Average	85.56	10.00	61.85	9.26	31.85	7.78
	Overall	76.81	8.52	52.96	4.17	36.76	4.17

*C= cotyledon, H= hypocotyl.

Table 2. The effect of MS media containing plant growth regulators at different concentrations on the multiplication index (MI) in shoot propagation media

Genotype	Media[x]						Average
	M1	M2	M3	M4	M5	M6	
P62-1	1.00 ^{j}	4.33 ^{efg}	3.66 ^{fgh}	1.00 ^{j}	4.33 ^{efg}	5.33 ^{de}	3.27 ^{c}
145	1.33 ^{j}	7.66 ^{bc}	7.33 ^{bc}	1.00 ^{j}	4.66 ^{ef}	5.33 ^{de}	4.55 ^{b}
4	1.33 ^{j}	6.33 ^{cd}	3.00 ^{ghi}	1.66 ^{ij}	3.66 ^{fgh}	3.33 ^{fgh}	3.22 ^{c}
Matsunami F_1	2.33 ^{hij}	12.66 ^{a}	11.33 ^{a}	2.33 ^{hij}	8.33 ^{b}	5.66 ^{de}	7.11 ^{a}
Average	1.50 ^{d}	7.75 ^{a}	6.33 ^{b}	1.50 ^{d}	5.25 ^{c}	4.91 ^{c}	
Genotype ** ; Media ** ; Genotype x Media **							

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 19.38.

When Table 3 is examined, it can be seen that root formation was successful in all cabbage genotypes (Figure 1d). The effect of genotype and genotype x media interaction on the rooting rate was found as statistically significant. There was no significant differences among medium. When the rooting rate is examined in terms of genotypes, rooting occurred in 145 (94.17%), Matsunami F1 (96.25%), and 4 (100.00%) genotypes with a high rate. The lowest rooting rate was determined in the P62-1 breeding line with 79.17%.

The auxin group plant growth regulators used in the media have a positive effect on rooting in in vitro propagation studies. However, it has been reported in the literature that Brassica group species have a high regeneration capacity in terms of root formation in tissue culture (Gerszberg et al., 2015).

Table 3. The effect of different media used in the rooting stage on the rooting rate (%)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	77.78 bc	83.33 b	66.67 c	88.89 ab	79.17 b
145	100.00 a	76.67 bc	100.00 a	100.00 a	94.17 a
4	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
Matsunami F_1	100.00 a	100.00 a	100.00 a	85.00 b	96.25 a
Average	94.44	90.00	91.66	93.47	
Genotype ** ; Media NS ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 1.92.

Genotypes with sufficient internal auxin levels in cabbage and cauliflower do not need auxin supplementation for rooting traits (Qamar et al., 2014). The rooting rate varies from 66.6% to 100.0% in the local Erciş white head cabbage genotype depending on the IBA concentration in the media. Also, it was determined that higher rooting rates were obtained from lower auxin levels (Ertaş and Tuncer, 2016).

3.3. Average Root Number

The plant obtained in the *in vitro* propagation studies should have a healthy and strong root structure for the successful acclimatization process. Therefore, it is required that the number of plants transferred to the rooting medium is high.

When the root numbers obtained in the rooting medium were examined at the genotype level, the highest number of roots was obtained from genotype 4 among all cabbage genotypes (Table 4). In terms of genotype x media interaction, the highest root formation was determined in genotype 4 using ½ MS media. The auxin group plant growth regulators used in the *in vitro* propagation studies promote a healthy and strong root formation. Indole Butyric Acid (IBA) is the most appropriate auxin for increasing the number of roots (Munshi et al., 2007; Gerszberg et al., 2015). IBA at a concentration of 0.5 mg/l used in the rooting media in white cabbage had a positive effect on the rooting rate and root number (Munshi et al., 2007). The differences observed in the rooting stage were due to the genotype effect (Murata and Orton, 1987). In this study, the highest root formation was obtained in MS media without IBA in 4 inbred line. These results showed that the genotype effect was highly effective on root formation in white head cabbage genotypes.

3.4. Number of Branching Roots

In *in vitro* propagation studies, root branching must be formed for a strong root system as well as the number of roots for successful acclimation of the plants must be determined. The effect of different media used in the rooting stage on the number of branching roots is shown in Table 5.

When Table 5 is examined, it has been shown that genotype, media, and genotype x media interactions have a significant effect on the number of branching roots. When the cabbage genotypes were compared, the highest branching root number was obtained from the Matsunami F1. When the results were examined in terms of genotype x media interaction, the highest value was determined in Matsunami F1, which was cultured using 0.5 mg/l IBA in the media. The auxin group plant growth regulators used in white cabbage in the media do not affect the rooting rate but have positive effects on healthy and strong root development (Gerszberg et al., 2015). On the other hand, root formation was weaker in cauliflower plants without auxin in the rooting media (Bhatia et al., 2015).

Table 4. The effect of different nutrient media used in the rooting stage on the average root number (unit)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	15.67 c-f	18.67 a-e	16.00 c-f	18.67 a-e	17.25 ab
145	23.00 abc	15.33 a-e	16.00 c-f	10.33 f	17.00 b
4	11.67 ef	21.00 a-d	29.00 a	27.67 ab	22.33 a
Matsunami F_1	14.67 def	22.00 a-d	17.33 b-e	16.33 c-f	17.58 ab
Average	16.25	20.08	19.58	18.25	
Genotype ** ; Media NS ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 9.67.

Table 5. The effect of different media used in the rooting stage on the number of branching roots (unit).

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	2.66 d	3.66 cd	2.66 d	4.00 cd	3.25 c
145	4.66 c	6.33 b	3.33 cd	3.66 cd	4.50 b
4	4.33 c	4.33 c	2.66 d	6.66 b	4.50 b
Matsunami F_1	6.66 b	7.33 b	6.33 b	10.00 a	7.58 a
Average	4.58 c	5.41 b	4.58 c	6.08 a	
Genotype ** ; Media ** ; Genotype x Media **					

[x]Aaccording to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 18.58.

3.5. Average Root Diameter

Average root diameter is another parameter that shows a healthy and strong root structure. The roots should be thicker and stronger in plants transferred from the rooting media to the external environment. The effect of different media on average root diameter in cabbage genotypes during the rooting stage is summarized in Table 6.

In the study, the genotype effect on the mean root diameter was found to be statistically significant at the level of 5%. Genotype x medium interaction effect was determined as very important at the 1% level. Besides, the effect of the media on average root diameter was found to be statistically insignificant (Table 6). When cabbage genotypes were compared, the thickest root diameter was measured with 0.56 mm in the Matsunami F1. The difference between the root diameters of genotypes P62-1, 145, and 4 were statistically insignificant. In terms of genotype x media interactions, the highest root diameter value was determined in Matsunami F1 cultured in a rooting medium using 0.5 mg/l IBA. The IBA used in the media had a positive effect on the average root diameter in all cabbage genotypes. These findings are similar to the results obtained from *in vitro* propagation studies carried out on *Brassica* species (Munshi et al., 2007; Bhatia et al., 2015).

3.6. The Length of the Rooted Plant

The results obtained in terms of the effect of different media used in the rooting stage on the length of the rooted plant are given in Table 7. When the data obtained in terms of the length of the rooted plant were analyzed, it was found that the effects of genotype and genotype x media interaction were statistically significant. The effect of the media was found to be statistically insignificant. The highest plant height was measured in genotype 4 with an average of 14.06 cm. Also, the highest rooted plant length in terms of genotype x media was measured in genotype 4 cultured in M4 rooting media that used 0.5 mg/l IBA. When the cabbage genotypes were compared, it was determined that the plants belonging to genotype 4 were long but their plant structure was weak. A strong plant structure is required for *in vitro* propagation studies. For this reason, it is undesirable to have a very high plant height.

Table 6. The effect of different nutrient media on average root diameter (mm) during the rooting stage

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	0.36 e	0.33 e	0.34 e	0.35 e	0.35 b
145	0.37 de	0.31 e	0.40 cde	0.35 e	0.36 b
4	0.30 e	0.54 abc	0.36 e	0.33 e	0.38 b
Matsunami F_1	0.50 bcd	0.55 ab	0.54 ab	0.65 a	0.56 a
Average	0.38	0.43	0.41	0.42	
Genotype ** ; Media NS ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 19.21.

Table 7. The effect of different media used in rooting stage on rooted plant length (cm)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	12.03 cde	12.73 b-e	10.63 ef	11.83 cde	11.80 b
145	17.67 a	14.63 a-d	11.56 de	12.26 cde	14.03 a
4	7.63 fg	15.70 ab	15.06 abc	17.87 a	14.06 a
Matsunami F_1	12.00 cde	11.03 e	16.70 a	6.56 f	11.57 b
Average	12.33	12.52	13.49	12.13	
Genotype ** ; Media NS ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 15.69.

3.7. Stem Diameter

For successful acclimatization, plants removed from the rooting medium are desired to have a strong stem. The effect of different media on the average stem diameter during the rooting phase is summarized in Table 8.

Accordingly, it was determined that the genotype effect on the stem diameter has statistically significant differences. The effect of the media and genotype x media interaction was found to be statistically insignificant. The thickest stem diameter was measured in Matsunami F1 with an average of 2.43 mm. It is reported by many researchers that the most important factor in terms of the parameters examined is the genotype effect in *in vitro* propagation studies (Murato and Orton, 1987; Dai et al., 2009; Qamar et al., 2014; Gerszberg et al., 2015). Our findings are similar to the literature mentioned above.

3.8. Average Leaf Number

Plants that are transferred from the rooting media to the external environment must photosynthesize to live healthily. For this reason, a high number of healthy leaves is desirable in terms of increasing the survival rate. The effect of different media used in the rooting stage on the average leaf number is presented in Table 9.

When cabbage genotypes were compared in terms of leaf number, the highest number of leaves was determined in Matsunami F1. When the genotype x media interaction was examined, the highest leaf number was obtained from Matsunami F1 that used 0.5 mg/l IBA in the rooting media. It has been reported that IBA used in the media promotes the formation of new leaves in *in vitro* propagation studies (Akturk, 2009). As a result of the research, although the highest number of leaves was determined in Matsunami F1, in which IBA was used, in general, IBA did not have a significant effect on increasing the number of leaves.

3.9. Survival Rate

One of the most important factors in the commercial use of in vitro propagation studies is the plant survival rate. No matter how high the MI is, it is very difficult to use it in practice if the survival rate of the plants transferred to the external environment is low. The effect of different nutrient media used in the rooting phase on the survival rate is shown in Table 10.

When the data obtained as a result of the research were evaluated in general, it was determined that the survival rate of the plants transferred to the external environment was quite high (Figure 1e). According to Table 10, genotype, media, and genotype x media interactions had statistically significant effects on the survival rate. When the survival rates were evaluated in terms of genotype, the highest survival rate was determined in Matsunami F1 with an average of 95.02%. When the interaction of genotype x media was examined, the highest survival rate was determined in Matsunami F1, which was transferred to the external environment after culturing using 0.5 mg/l IBA in the rooting media. The results obtained in different studies show that the survival rates in Brassica group species are generally at high levels (Munshi et al., 2007; Bhatia et al., 2015; Gerszberg et al., 2015).

Table 8. The effect of different media on the average stem diameter (mm) during the rooting stage

Genotype	Media				Average[x]
	M1	M2	M3	M4	
P62-1	1.77	1.49	1.76	1.94	1.74 ^{b}
145	1.57	1.49	1.40	1.42	1.49 ^{bc}
4	0.90	1.68	1.72	1.38	1.41 ^{c}
Matsunami F_1	2.09	2.25	2.71	2.66	2.43 ^{a}
Average	1.58	1.73	1.90	1.87	
Genotype ** ; Media NS ; Genotype x Media NS					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 17.04.

Table 9. The effect of different media used in the rooting stage on the number of leaves (unit)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	5.33 ^{f}	6.33 ^{def}	10.33 ^{b}	6.33 ^{def}	7.08 ^{b}
145	9.66 ^{bc}	7.33 ^{def}	6.33 ^{def}	5.67 ^{ef}	7.25 ^{b}
4	7.33 ^{def}	5.33 ^{f}	7.66 ^{cde}	5.67 ^{ef}	6.50 ^{b}
Matsunami F_1	9.66 ^{bc}	15.33 ^{a}	10.66 ^{b}	8.0 ^{cd}	10.91 ^{a}
Average	8.00 ^{a}	8.58 ^{a}	8.75 ^{a}	6.42 ^{b}	
Genotype ** ; Media ** ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 16.26.

Table 10. The effect of different media used in the rooting stage on the survival rate (%)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	85.71 f	94.44 bc	91.67 d	83.33 g	88.79 c
145	93.10 cd	83.33 g	93.33 cd	90.00 e	89.94 b
4	93.33 cd	95.83 ab	96.00 ab	94.44 bc	94.90 a
Matsunami F_1	92.00 d	97.50 a	94.44 bc	96.15 ab	95.02 a
Average	91.04 c	92.78 b	93.47 a	90.98 c	
Genotype ** ; Media ** ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 1.92.

4. Conclusion

In vitro plant regeneration studies play a significant role in germplasm conservation and mass multiplication of the vegetable species. In this study, in vitro propagation possibilities in white cabbage genotypes were examined in detail. As a result of this study, plants obtained using cotyledon explants were acclimated successfully. But the capacity of regeneration strongly depends on the genotype and quantity of exogenous hormones that are used in the media. Thus, by using the results obtained *in vitro* propagation studies in breeding programs, genetic materials with the risk of loss due to inbreeding depression in white head cabbage genotypes could be ensured to continue. Besides, in male sterility studies, clonal propagation can be applied to obtain a large number of plants in both male sterile lines and maintainers. By integrating all these gains into various breeding programs, it will be possible to minimize the problems experienced in the classical breeding process of cabbage.

Author Contributions

S.M.D. (34%), A.B. (33%) and E.S.K. (33%) design of study. S.M.D. (34%), A.B. (33%) and E.S.K. (33%) data acquisition and analysis. S.M.D. (34%), A.B. (33%) and E.S.K. (33%) writing up. S.M.D. (34%), A.B. (33%) and E.S.K. (33%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Research Article
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DETERMINATION OF SATURATED AND UNSATURATED FATTY ACIDS IN SECOND CROP SEASON PEANUT CULTIVATION IN THE EASTERN MEDITERRANEAN

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Abstract: In this study; the saturated and unsaturated fatty acid composition of 11 different peanut cultivars, Runner (Georgia Green) Virginia (NC-7, Masal, Halisbey, Wilson, Com, Brantley, Duzici-1) Spanish (Florispan, Nigeria-1), widely grown in the eastern Mediterranean Transition Zone were determined. The research was carried out for two years (2020 to 2021) under second crop season conditions in the trial areas of the Oil Seeds Research Institute. The experiment was set up in a randomized block design with 3 replications. In the research, palmitic acid, stearic acid, arachidonic acid, oleic acid, linoleic acid, oleic/linoleic ratio, iodin value, behenic acid, arachidic acid properties were investigated. The highest oleic acid ratio was obtained from Masal (79.71%), the highest palmitic acid from florispan (11.06%), and the highest linoleic acid (34.08%) from florispan. The behenic acid ratio was found between 2.51% (Wilson) and 3.14% (Georgia Green).

Keywords: *Arachis hypogea* L., Oleic acid, Linoleic acid, Palmitic acid, Behenic acid

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1. Introduction

Peanut (*Arachis hypogaea* L.) is an annual plant from the Leguminosae family, with 2n=40 chromosomes (Hammons et al., 2016). Peanut, originating from South America and the eastern parts of the Andes, has a very large cultivation area due to its good adaptation to the tropical and subtropical regions of the World (Stalker, 2017; Xie et al., 2020).

With the advancing technology and industry, especially with the help of the oil industry, peanut production has increased and will continue to increase day by day. The nutrition requirement of people is a big problem in almost every part of the world. Today, more than 70% of the world's population does not have enough nutrition. The basic principle in the protection and maintenance of human health is adequate and balanced nutrition. Today's people need to provide a balanced diet by supplying 15% of total calories from protein, 25% from fat and 60% from carbohydrates (Tang et al., 2013; Mekdad et al., 2021).

The protein, which peanut seed conteins 20-30% has a very high ratio of exogen ratio essential amino acids and biological value of 49 (Yaşlı et al., 2020; Yılmaz and Çiftçi, 2021). In terms of nutritional value, peanut has higher quality protein compared to other plant proteins. The fact that many of the amino acids that make up the peanut proteins are easily digestible, that increase their nutritional value. Therefore, peanut seeds can be consumed as fresh or dry roasted in large quantities as a snack (Cheng et al., 2018).

Since peanut oil very stable in high temperatures, it is widely used as frying oil. Due to its higher stability, peanut oil is used for the preparation of biscuits, cakes, confectionery, margarine, and canned fish. Low-quality peanut oils, on the other hand, can be used for soap, fuel, etc. Peanut oil is also used as a raw material in many industries. In addition, 30% of peanut oil is mixed with diesel fuels and used as fuel in the operation of diesel engines (Matthäus and Musazcan Özcan, 2015).

Peanut oil is rich in unsatured fatty acids. In vegetable oils, the quality of the oil increases in parallel with the increase in the unsaturated fatty acid ratio. Unsaturated fatty acid/saturated fatty acid ratio in peanut is 4.6. The fact that it contains eight of the fatty acids that are very important in terms of nutrition (Palmitic, Stearic, Oleic, Linoleic, Arachidic, Eicoseonic, Behenic and Nervolic fatty acids) that increases the nutritional value of the oil, and the presence of traces of linolenic acid, which is an undesirable fatty acid, increases the quality of the oil. Peanut oil is superior to other vegetable oils in terms of taste and durability (Davis et al., 2016).

This study aimed to determine the saturated and unsaturated fatty acids ratio and oil quality of different peanut varieties grown in the Eastern Mediterranean region in the second crop season peanut condition.

2. Material and Methods

Peanuts varieties tested in the present study were Runner (Georgia Green) Virginia (NC-7-, Masal, Halisbey, Wilson, Çom, Brantley, Düzici-1) Spanish (Florispan, Nigeria-1) market types. The trial was established in as second crop season after wheat at Cevdetiye locations belonging to Osmaniye Oil Seeds Research Institute in 2020 (37°07'28" N, 36°11'38" E; 50 m) and 2021 (37°07'89" N, 36°11'33" E; 50 m).

Trials were set up as 3 replications according to the randomized blocks design. Each plot consisted of 4 rows of 5 m length with inter and intra row spacing of 70 and 15 cm, respectively. Each plot was 14 cm^2. Before planting 25 kg da^{-1} of DAP was applied. Before the first irrigation, 15 kg da^{-1} of urea was applied and 10 kg da^{-1} of urea was applied before the second irrigation. Irrigation was done 5 times during both growing seasons with the sprinkler system. Harvesting was done by hand, side effects were discarded, and harvest parameters were evaluated on ten randomly selected plants in the two mid-rows of plots. Harvest was done on September 15, 2020, and on September 25, 2021.

In Osmaniye, has the Mediterranean type of climate, with warm and rainy winters and hot and dry summers. Some important climatice parameters for long seasons and the climate parameters of growing years 2020 and 2021 were given in (Table 1).

As can be seen from the Table 1 that the total amount of precipitation in 2020 was lower than in 2021. It has been determined that the average temperature in 2020 was lower than 2021 and higher than the average of long season values. It has been determined that the average relative humidity of long seasons was lower than 2020 and higher than 2021.

Table 1. Climate parameters of the research field (2020, 2021, and long-year average)

Months	Precipitation (mm)			Temperature (°C)			Relative Humidity (%)		
	LY	2020	2021	LY	2020	2021	LY	2020	2021
April	86.5	123.9	32.3	17.0	17.1	17.7	64.2	69.4	64.8
May	72.6	83.5	4.6	21.3	22.1	22.9	63.2	62.4	59.8
June	42.4	5.5	1.8	25.2	24.0	25.0	62.7	68.7	65.9
July	19.8	2.0	15.7	27.9	28.4	28.9	66.4	71.7	64.6
August	10.7	21.5	19.7	28.6	28.6	29.3	64.9	64.0	62.8
September	34.5	0.9	14.0	25.7	28.6	25.9	60.7	61.8	60.8
Total/Av	266.5	237.3	88.0	24.3	24.8	25.0	63.7	66.3	63.1

Av= average; LY= long year.

Determination of fatty acids composition: Fatty acid methyl esters were prepared according to AOCS (1989), method Ce 2-66 and analyzed with HP 6890 Series II Gas Chromatograph (GC) (Hewlett-Packard Company), (Agilent Technologies, Wilmington, DE, USA) equipped with a flame ionization detector and autosampler. A fused silica capillary column SP 2340 (60 m × 0.25 mm i.d.) with a film thickness of 0.25 µm (Supelco, Taufkirchen, Germany) was used. Injection, detector, and oven temperatures were 250, 260, and 190°C, respectively. Nitrogen was used as a carrier gas at a flow rate of 1.0 mL min^{-1}. Individual peaks were identified by comparing the retention times with grain fatty acid methyl esters.

Iodine values (IV) = [(% oleic acid x 0.8601) + (% linoleic acid x 1.7321)] and Oleic acid/Linoleic acid (O/L) ratio = [% oleic acid (18:1)/linoleic acid (18:2)] of the peanut oils were calculated using the equation given by Chowdhury et al. (2015).

Experimental data were subjected to analysis of variance by RCBD separately for each year with the aid of R v4 software. Means were compared with the aid of Duncan's multiple range test (Genç and Soysal, 2018).

3. Results and Discussion

Arachidonic acid was statistically significant (P<0.01) between years and cultivars. Palmitic acid was statistically significant (P<0.01) between years and cultivars. Stearic acid was statistically significant (P<0.01) between years, cultivars, and year x cultivars interaction was significant. Oleic acid was statistically significant (P<0.01) between years and cultivars. Linoleic acid was statistically significant (P<0.01) between years and cultivars. It was determined that oleic/linoleic (O/L) acid was statistically significant (P<0.01) among cultivars. Behenic acid was statistically significant (P<0.01) between years and cultivars. Lingoseric was statistically significant (P<0.01) interactions between years, cultivars, and year x cultivars. Iodine value was statistically significant (P<0.01) between years, cultivars, and year x cultivars interaction were significant (Table 2).

According to a two-year data, the highest arachidic acid ratio was found in the Masal variety with 1.65, while the Florispan variety was found the least with 1.26% (Table 3). Salamatullah et al. (2021) reported that the arachidic acid ratio varied between 1.30-1.43%; Gölükçü et al (2016) reported that the arachidic acid value varied between 1.17% and 1.66%; Söğüt et al. (2016) determined that the ratio of arachidic acid varies between 0.102% - 0.123%. The difference in arachidonic acid was thought to be due to environmental factors and genotypic make-up of the cultivars.

Table 2. Results of the analysis of variance for characteristics studied in the experiment

SV	df	PA	SA	AA	OA	LA	IV	O/L	BA	LS
Block	4	ns	ns	ns	ns	ns	ns	ns	ns	Ns
Year	1	**	**	**	**	**	**	ns	**	**
Varieties	14	**	**	**	**	**	**	**	**	**
Y x V	14	ns	**	ns	ns	ns	**	ns	ns	**
CV (%)		4.43	4.20	4.98	2.43	4.50	1.41	22.25	5.03	5.74

SV= source of variation, df= degree of freedom, CV= coefficient of variation, PA= palmitic acid, SA= stearic acid, OA= oleic acid, LA= linoleic acid, O/L= oleic/linoleic ratio, IV= iodin value. **P<0.01.

Table 3. Means of palmitic acid, stearic acid and arachidonic acid

	Palmitic Acid (%)			Stearic Acid (%)			Arachidonic Acid (%)		
Varieties	2020	2021	Average	2020	2021	Average	2020	2021	Average
Nigeria-1	9.12 ^{cde}	9.80 ^{cde}	9.46 ^{cd}	3.29 ^{a}	3.86 ^{a}	3.58 ^{a}	1.48 ^{b}	1.55 ^{bcd}	1.52 ^{bc}
NC-7	9.27 ^{cd}	9.75 ^{cde}	9.51 ^{c}	3.07 ^{a}	3.13 ^{c}	3.10 ^{c}	1.60 ^{a}	1.57 ^{abc}	1.59 ^{ab}
Masal	5.05 g	5.95 ^{g}	5.05 ^{g}	3.19 ^{a}	3.66 ^{b}	3.42 ^{b}	1.61 ^{a}	1.69 ^{a}	1.65 ^{a}
Georgia Green	10.11 ^{ab}	11.02 ^{ab}	10.56 ^{b}	1.82 ^{e}	1.92 ^{g}	1.87 ^{h}	1.12 ^{d}	1.87 ^{f}	1.56 ^{g}
Halisbey	8.73 ^{de}	9.27 ^{e}	9.00 ^{de}	2.51 ^{bc}	2.61 ^{e}	2.56 ^{ef}	1.39 ^{bc}	1.41 ^{de}	1.40 ^{de}
Wilson	8.43 ^{e}	9.08 ^{e}	8.76 ^{e}	2.57 ^{b}	2.72 ^{de}	2.64 ^{de}	1.38 ^{bc}	1.42 ^{de}	1.39 ^{de}
Com	9.78 ^{bc}	10.43 ^{bc}	10.11 ^{b}	2.15 ^{d}	2.35 ^{f}	2.25 ^{g}	1.17 ^{d}	1.35 ^{e}	1.26 ^{f}
Sultan	8.67 ^{de}	9.56 ^{de}	9.12 ^{cde}	2.54 ^{bc}	2.62 ^{e}	2.58 ^{ef}	1.36 ^{bc}	1.45 ^{cde}	1.41 ^{de}
Brantley	7.19 ^{f}	7.99 ^{f}	7.59 ^{f}	3.07 ^{a}	3.18 ^{c}	3.13 ^{c}	1.43 ^{bc}	1.46 ^{cde}	1.45 ^{cd}
Florispan	10.60 ^{a}	11.52 ^{a}	11.06 ^{a}	2.41 ^{c}	2.58 ^{e}	2.50 ^{f}	1.33 ^{c}	1.31 ^{ef}	1.32 ^{ef}
Duzici-1	8.91 ^{de}	10.17 ^{cd}	9.54 ^{c}	2.68 ^{b}	2.83 ^{d}	2.76 ^{d}	1.42 ^{bc}	1.63 ^{ab}	1.53 ^{bc}
Average	8.71	9.50	9.07	2.66	2.86	2.76	1.39	1.52	1.46

$^{a-f}$Different letters shows statistical differences (P<0.05) in same column.

It was determined that the average palmitic acid ratio in the second crop peanuts varied between 5.05-11.06% (Table 3). Yu et al. (2020) reported that oleic and palmitic acid ratio were 2.92 and 5.5% respectively; Shibli et al. (2019) found that the palmitic acid ratio varied between 9.32-12.03%; Kamdar et al. (2021) reported palmitic acid values between 8.1-14.2%. Shibli et al. (2019) and Yu et al. (2020) reported that there was a linear relationship between the palmitic acid ratio and the average temperature from flowering to harvest. The palmitic acid in 2020 was lower than 2021. When we look at the climate values, it is seen that 2021 is warmer than 2020 (Table 1). Our findings were similar to the findings reported in the literature.

It was determined that the average stearic acid ratio varied between 1.87-3.58% (Table 3). Salamatullah et al. (2021) found that the rate of stearic acid varied between 4.01–4.59%; the amount of stearic acid in 2021 was higher than in 2020. As can be seen in Table 1 that the temperature in 2020 was lower than in 2021.

According to the two-year average values, the lowest oleic acid ratio was determined from cultivar Florispan (43.38%), and the highest was determined from Masal (79.71%) (Table 4). The rate of oleic acid in 2020 was lower than in 2021. Gali et al. (2021) and Asibuo et al. (2008) reported that the rate of oleic acid was 55.9% in peanut. Zhang et al. (2009) reported that the rate of oleic acid varied between 45.2-56.4%. Wang et al. (2013) determined that the rate of oleic acid varied between 38.97-62.04%. The oleic acid ratio in our study was similar to other studies.

Table 4. Means of oleic acid, linoleic acid

Varieties	Oleic Acid (%)			Linoleic Acid (%)		
	2020	2021	Average	2020	2021	Average
Nigeria-1	52.78 ^{d}	54.50 ^{d}	53.64 ^{de}	24.28 ^{d}	25.16 ^{d}	24.72 ^{d}
NC-7	56.76 ^{c}	57.56 ^{c}	57.16 ^{c}	21.46 ^{f}	22.38 ^{e}	21.92 ^{e}
Masal	79.39 ^{a}	80.03 ^{a}	79.71 ^{a}	2.24 ^{g}	2.45 ^{g}	2.34 ^{g}
Georgia Green	43.57 ^{g}	44.87 ^{g}	44.22 ^{h}	32.50 ^{a}	33.96 ^{a}	33.23 ^{a}
Halisbey	51.79 ^{d}	53.53 ^{d}	52.66 ^{e}	26.69 ^{c}	27.33 ^{c}	27.01 ^{c}
Wilson	53. 78 ^{d}	55.82 ^{cd}	54.80 ^{d}	24.35 ^{d}	25.08 ^{d}	24.72 ^{d}
Com	45.71 ^{f}	48.03 ^{f}	46.87 ^{g}	29.90 ^{b}	31.16 ^{b}	30.53 ^{b}
Sultan	48.65 ^{e}	50.79 ^{e}	49.72 ^{f}	27.92 ^{c}	28.55 ^{c}	28.24 ^{c}
Brantley	69.15 ^{b}	70.15 ^{b}	69.65 ^{b}	11.10 ^{f}	11.73 ^{f}	11.41 ^{f}
Florispan	42.39 ^{g}	44.36 ^{g}	43.38 ^{h}	33.66 ^{a}	34.50 ^{a}	34.08 ^{a}
Duzici-1	53.67 ^{d}	54.66 ^{d}	54.17 ^{de}	23.60 ^{d}	24.81 ^{d}	24.20 ^{d}
Average	51.91	53.60	52.76	25.24	26.15	25.69

$^{a-h}$Different letters shows statistical differences (P<0.05) in same column.

Mean linolenic acid ratios were between 2.34% (Masal) and 34.08 (Florispan) (Table 4). Gali et al. (2021) reported that the ratio of linoleic acid varied between 27.87-31.16% and there was a negative correlation between the percentage of oleic and linoleic acid. Bishi et al. (2015) reported that the ratio of linoleic acid varied between 22.4-41.4% and there was an inverse relationship between mean temperature and oleic and linoleic acid. The study is similar to other studies. According to two-year climate data, the monthly average temperature in 2021 was higher than in 2020, so the linoleic ratio was thought to be high in 2021 (Table 1).

The determined iodine value was between 72.62% (Masal) and 96.35% (Florispan) according to the two-year average data (Table 5).

It has been reported that the two-year average O/L value rate varied between 1.27% and 34.35% (Table 5). Gali et al. (2021), reported that O/L value rate varied between 1.20 and 27.52; Lopez et al. (2001) It has been determined that the O/L value rate varies between 0.8-2.5%. O/L values rate were similar to other studies.

Cultivar Wilson had lowest behenic acid rate with 2.51%, and Georgia Green was the highest with 3.14% in two years average data (Table 6). Shibli et al. (2019) reported that the ratio of behenic acid varied between 2.69-2.89%; Konuskan et al. (2019), reported that the ratio of behenic acid is 3.25%; Candela et al. (2019), the ratio of behenic acid is 5.82%; Akcura et al. (2021) determined that the ratio of behenic acid varied between 3.02-3.64%. Our study was performed by Shibli et al. (2019) and Akcura et al. (2021) similarity to the study; Konuskan et al. (2019) and Candela et al. (2019) differed with the studies conducted by. Akcura et al. (2021) reported that the fatty acid composition of peanut oil depends on the genotype, seed maturation, climatic conditions, the region where it is grown, and the interaction between these factors.

It was determined that the average lignoceric acid ratio for two years varied between 1.07% and 1.78% (Table 6). The amount of lignoceric acid varied between 1.01-1.88%; Konuskan et al. (2019), ligneceric 1.62%; Candela et al. (2020) reported that the rate of lignoceric acid varies between 1.0-1.86%. The ratio of behenic acid in the studies was similar to our study.

Table 5. Means of iodine value (IV), oleic/linoleic ratio (O/L)

	Iodin Value (IV)			Oleic/Linoleic Ratio (O/L)		
Varieties	2020	2021	Average	2020	2021	Average
Nigeria-1	87.45 ^{c}	90.46 ^{d}	88.96 ^{c}	2.18 ^{c}	2.17 ^{c}	2.17 ^{c}
NC-7	85.99 ^{c}	88.28 ^{e}	87.13 ^{d}	2.65 ^{c}	2.57 ^{c}	2.61 ^{c}
Masal	72.16 ^{f}	73.08 ^{g}	72.62 ^{f}	35.76 ^{a}	32.93 ^{a}	34.35 ^{a}
Georgia Green	93.77 ^{a}	97.41 ^{a}	95.59 ^{a}	1.34 ^{c}	1.32 ^{c}	1.33 ^{c}
Halisbey	90.77 ^{b}	93.38 ^{c}	92.08 ^{b}	1.94 ^{c}	1.97 ^{c}	1.96 ^{c}
Wilson	88.42 ^{bc}	91.45 ^{d}	89.94 ^{c}	2.22 ^{c}	2.24 ^{c}	2.23 ^{c}
Com	91.10 ^{b}	95.28 ^{b}	93.19 ^{b}	1.53 ^{c}	1.54 ^{c}	1.54 ^{c}
Sultan	90.21 ^{b}	93.13 ^{c}	91.67 ^{b}	1.74 ^{c}	1.78 ^{c}	1.76 ^{c}
Brantley	78.71 ^{d}	80.64 ^{f}	79.67 ^{e}	6.29 ^{b}	6.07 ^{b}	6.18 ^{b}
Florispan	94.78 ^{a}	97.92 ^{a}	96.35 ^{a}	1.26 ^{c}	1.28 ^{c}	1.27 ^{c}
Duzici-1	87.03 ^{c}	89.98 ^{d}	88.51 ^{cd}	2.28 ^{c}	2.21 ^{c}	2.42 ^{c}
Average	87.31	90.09	88.70	5.35	5.10	5.26

$^{a-f}$Different letters shows statistical differences (P<0.05) in same column.

Table 6. Means of behenic acid, lignoceric acid

	Behenic Acid (%)			Lignoceric Acid (%)		
Varieties	2020	2021	Average	2020	2021	Average
Nigeria-1	2.71 ^{b}	2.77 ^{b}	2.74 ^{b}	1.21 ^{f}	1.29 ^{c}	1.25 ^{c}
NC-7	2.56 ^{cd}	2.54 ^{c}	2.55 ^{d}	1.25 ^{ef}	1.54 ^{b}	1.39 ^{b}
Masal	2.65 ^{bc}	2.73 ^{bc}	2.69 ^{bc}	1.39 ^{bcd}	1.41 ^{bc}	1.40 ^{b}
Georgia Green	3.12 ^{a}	3.17 ^{a}	3.14 ^{a}	1.76 ^{a}	1.80 ^{a}	1.78 ^{a}
Halisbey	2.57 ^{cd}	2.66 ^{bc}	2.61 ^{cd}	1.45 ^{bcd}	1.49 ^{b}	1.47 ^{b}
Wilson	2.48 ^{d}	2.54 ^{c}	2.51 ^{d}	1.41 ^{bcd}	1.43 ^{bc}	1.42 ^{b}
Com	2.67 ^{bc}	2.70 ^{bc}	2.68 ^{bc}	1.47 ^{bcd}	1.55 ^{b}	1.51 ^{b}
Sultan	2.65 ^{bc}	2.74 ^{bc}	2.69 ^{bc}	1.50 ^{bc}	1.51 ^{b}	1.51 ^{b}
Brantley	2.21 ^{e}	2.27 ^{d}	2.24 ^{e}	1.04 ^{g}	1.09 ^{d}	1.07 ^{d}
Florispan	3.11 ^{a}	3.10 ^{a}	3.11 ^{a}	1.56 ^{b}	1.44 ^{b}	1.50 ^{b}
Duzici-1	3.04 ^{a}	3.09 ^{a}	3.07 ^{a}	1.33 ^{def}	1.50 ^{b}	1.42 ^{b}
Average	2.71	2.60	2.73	1.40	1.46	1.43

$^{a-g}$Different letters shows statistical differences (P<0.05) in same column.

4. Conclusion

As a result of 2-year field experiment, the oil composition and oil content of peanut varieties were affected by the environmental conditions, genotypic differences and years. Cultivar Masal, belonging Virginia market type, had the highest unsaturated fatty acid compositions with %82.05 which consisted of 79.71% oleic acid and 2.34% linoleic acid in two-year average. Besides, the highest O/L value ratio was observed from cultivar Masal with 34.35. These results showed that cultivar Masal may be the best option because of its nutritional quality, storability, and shelf-life in second crop season in Osmaniye, Eastern Mediterranean Region of Türkiye.

Author Contributions

All task made by M.Y. (100%) data acquisition and analysis, writing up, submission and revision. The author reviewed and approved final version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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Research Article
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NON-DESTRUCTIVELY DETERMINING BLUEBERRY (*Vaccinium corymbosum* L.) LEAF AREA USING DPI-BASED SOFTWARE

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Abstract: Blueberries (*Vaccinium* spp.) are a popular crop all throughout the world. Blueberry (*Vaccinium corymbosum* L.) leaves were randomly selected from the experimental area of Ondokuz Mayis University, Faculty of Agriculture as a research material. Blueberries are high in polyphenolic chemicals, particularly anthocyanins, which are antioxidants and anti-inflammatory. A total of 1500 leaves were collected, with 100 for each cultivar, to represent the variety of sizes found on 15 plants of each cultivar. Manual leaf area measurement was made with a digital planimeter. The area was measured using a 300dpi resolution image read from the relevant file in such a way that every one of them is defined by 8bits in RGB color space. A weighted sum of the RGB components of the image is used to convert RGB values to grayscale values. This three-dimensional gray image generates a binary image. The Otsu method was used to determine the threshold value required to minimize the in-class variation of the threshold black and white pixels. Simulink was used for easy use of the end-user with the developed software. This software can be used for the area measurement of all plant leaves.

Keywords: Blueberry, Leaf area, Software, *Vaccinium corymbosum* L.

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1. Introduction

Leaves are one of the fundamental physiological organs of a plant which photosynthesis and transpiration take place and they are the most important organs that plants have. They contribute to the absorption of nutrients and water from the soil through the roots and allow the translocation of photosynthetic products from the source to the sink organs. Moreover, within a life cycle of a plant, leaves pass from a juvenile heterotrophic state to a mature autotrophic condition determining source-to-sink transitions (Turgeon, 1989; Shakya and Lal, 2018). Since leaf size is an adaptation and response to the environment, it can be considered an indicator of the conditions plants grow in: climate, topography, soils, etc. These relationships are interesting for geneticists, ecologists, and agronomists. Agricultural practices aim to improve growing conditions for plants. Leaf area is one of the aspects measured by agricultural scientists, especially agronomists, to establish the best agricultural practices. Similarly, farmers can check if they are creating an ideal environment for their annual and perennial crops (Uzun and Çelik, 1999; Çelik and Odabas, 2011; Trimble, 2019).

Leaf area measurement is a reliable parameter in studying the impact of the environment on plants in the disciplines of ecology, genetics, and crop management. Eco-physiologists, geneticists, botanists, ecologists, environmental scientists, and agronomists are some of the occupations that use leaf area measurements. Leaf area measurement is extremely useful to scientists and growers. Therefore, an understanding of leaf area and the different methods to measure it is important (NeSmith, 1991; Montero et al., 2000). There are destructive and non-destructive methods of measuring leaf area. Some of the common methods are direct measurements, millimeter graph paper method, planimeter, image processing and digital scanners. In the past decade, scientist developed several equations for estimating the actual leaf area nondestructively by several linear measurements (Uzun and Çelik, 1999; Pandey and Singh, 2011).

A non-destructive leaf area calculation method is essential as the blueberry leaves cannot be used for scientific studies. Blueberry leaves are also important both for berry enlargement and ripening as well as their chemical properties. According to the Retamales and Hancock (2018), the whole-canopy leaf area affects fruit quality. They also revealed that the rate of leaf area development of blueberry bushes is greater than in annual crops. And it is clear that maintaining a balance between vegetative and reproductive growth is needed

to optimize blueberry yield and quality. Carbohydrates are produced, even after harvesting to color change and leaf fall (Goncalves et al., 2002).

According to leaf size and shape, direct leaf area determination in the field is generally unrealistic because it needs sophisticated leaf area meter devices (Uzun and Çelik, 1999; Odabas et al., 2009). The non-destructive leaf area prediction via simple equations is an inexpensive and rapid tool, and can provide many advantages to researchers for horticultural experiments. Non-destructive methods, which do not require the leaves to be detached, are useful because they allow measurements to be repeated during the plant's growth period, and reduce the variability associated with destructive sampling procedures (Çelik and Uzun, 2002). Moreover, the simple mathematical models enable us to measure leaf area on the same plants during growth periods and may reduce variability in experiments.

Development of a mathematical model to predict leaf area with the dimensions of leaf length, width and petiole length, or some combinations of these variables, which usually have high accuracy, has been a common approach (Kersteins and Hawes, 1994; Montero et al, 2000). Various mathematical models for indirect estimation of leaf area can be revealed separately for cultivars, species and genotypes, or a model can be applied for several cultivars and different species (Potdar, 1991; Öztürk et al., 2019). It is obvious that without proper experimental and statistical validation, models which attempt to predict leaf area of the plants must be viewed with caution (Rao et al., 1978).

The importance of rapid and accurate measurements of leaf area in agronomic and physiological studies is well known, but literature revealed little information available for highbush blueberries. A leaf area estimation model on rabbiteye blueberry was reported only by Nesmith (1991). Northern highbush blueberries have been getting the most popular small fruits in the northern part of Anatolia and several physiological and quantitative studies are going to be adjusted on it. Therefore, the objective of this study was to develop regression models that would accurately predict northern highbush blueberry cultivars leaf area using physically determinable points of measurement. Area measurements of leaves are generally used with devices that take up a large area or portable (measuring according to the sensitivity of the user) devices. However, the costs of these devices are high.

In the present study, a software has been developed to measure the leaf width, height and area of the plant with high accuracy using a database of blueberry leaves. Image processing and artificial neural networks are used in this software. This software can be used not only for blueberry leaves, but also for calculating leaf width, height and area of other plants.

2. Material and Methods

2.1. Plant Material

Pot grown 6 years old blueberry plants were randomly selected from the experimental area of the University of Ondokuz Mayis, Faculty of Agriculture. Twelve northern highbush (*Vaccinium corymbosum* L.) (Bluecrop, Denise Blue, Bluegold, Patriot, Nui, Brigitta, Hortblue Poppins, Goldtraube, Aurora, Liberty, Northland and Reka) and 3 southern highbush (*Vaccinium corymbosum* x *Vaccinium darrowii* hybrids) Misty, O'Neil and Jubilee were used to develop leaf area prediction models. Leaves on the first flushes were considered, and a total of 1500 leaves, 100 for each cultivar, were collected representing the range of sizes present on 15 plants of each cultivar. The leaves were processed in the following manner in order to develop the best fitting model for predicting the highbush blueberry leaf area individually and overall. After collecting the leaves, they were placed in sealed plastic bags, stored at 15 °C, moved to laboratory conditions, finally placed on the photocopier desktop by holding them flat and secure, and scanned with 300 dpi.

In agricultural trials, leaf area estimate algorithms that try to forecast leaf area non-destructively can bring various advantages to researchers. Furthermore, these models allow researchers to measure leaf area on the same plants over the duration of a study, resulting in lower experimental variability. Expensive tools and/or predictive methods can be used to determine leaf area (Figure 1).

Figure 1. Digital planimeter.

2.2. Image Processing

The 300dpi resolution image required to calculate the area was read from the relevant file in a way that each of them is represented by 8bits in RGB color space. RGB values are converted to grayscale values using a weighted sum of the RGB components of the image (0.2989 x R + 0.5870 x G + 0.1140 x B). A binary image is obtained from this 3-dimensional gray image. Here, the threshold value required to minimize the in-class variance of the threshold black and white pixels was determined by the Otsu method (Otsu, 1979).

It is aimed to separate the compound leaves from each other by using the morphological image processing method on this binary image. For this purpose, erosion

and then dilation is applied to the image with a disk-shaped structural element. Any areas with small pixels from the image are extracted and the gaps (white areas) are filled. Thus, an image is obtained in which the leaf areas are white and the other areas are black. The number of leaves is determined by labeling the connected components from the pixels of the image. Also, the actual number of pixels in this linked white region is calculated as a scalar. Since 1 Inch=2.54 cm, the area for a DPI_X=DPI_Y=300 image is calculated with the equation 1.

$$LA=PN/[(DPI\_X/2.54)x(DPI\_Y/2.54)] \quad (1)$$

where; LA is leaf area, PN is pixel number, X is leaf width and Y is leaf length. The Otsu method is used for automatic image thresholding in computer vision and image processing. The method returns a single intensity threshold that divides pixels into two classes: foreground and background, in its most basic form. The density within-class variance is minimized, or the variance between classes is maximized, to calculate this threshold.

2.3. Graphical User Interface (GUI)

Graphical User Interface (GUI) designs are created to assist electronic devices in using icons, icons, and other visual graphics. On older computers and electronic devices with GUI, pre-command-based operating systems, the command line was utilized to accomplish any task. Users used the keyboard and commands to carry out all computer operations. Within the computer screen, the GUI consists of windows, icons, and control windows. Users can use computers without having to input even a single command line in this manner.

The computer can be handled with a mouse and operations may be completed rapidly using shortcuts and keys thanks to the GUI. Unlike command-based systems like IOS, Unix, or MS-DOS, the GUI is significantly easier to learn and use. To accomplish actions in command-based systems, users must both write and memorize codes. In GUI operating systems, however, the computer can be utilized without knowing a single line of command code. Another benefit that graphical interfaces provide to consumers is that they do not require knowledge of any programming languages. Because all systems with a graphical user interface are now created with the needs of the end-users in mind (Anonymous, 2022).

For leaf and fruit measurements, new equipment, tools, and machines, such as hand scanners and laser optic apparatuses, have recently been developed. For both basic and simple studies, these are quite expensive and sophisticated apparatus. Furthermore, when compared to geometric measurements, non-destructive calculation of leaf area saves time (Odabas et al., 2009).

Artificial neural networks, image processing, and GUI were implemented using the program MATLAB software (Matlab ® R2013a). Whether the developed software measures correctly or not has been verified with a square with a certain width and length (Figure 2).

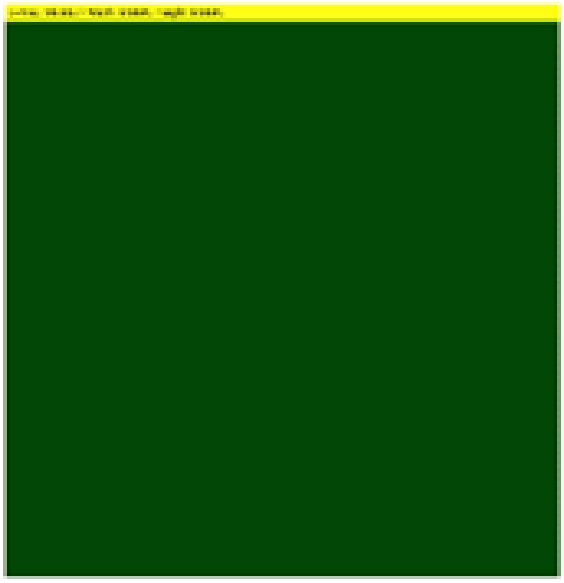

Figure 2. Square used for software area measurement verification (10cm × 10cm).

With the help of the developed interface, the areas of all leaf types can be easily measured. For the system, first of all, the leaves must be scanned at 300dpi resolution. The scanned image should be saved as a jpeg or tiff file. Then the program processes the raw image first. Image processing application is used by the software for this process. (Figure 3).

3. Results and Discussion

The leaf area is a measurement of plant health and potential crop yields that is directly tied to time-dependent crop growth (Baar et al., 2022). Leaf width, leaf length, and leaf areas are automatically calculated for each leaf, with precise borders determined by the software (Yin et al., 2022). The photosynthetic rate, dry matter buildup, and crop development are all influenced by the leaf. One of the criteria used to evaluate plant vegetative growth is leaf area. Leaf area index is used in crop modeling, as well as crop model calibration and validation (Shabani et al., 2017).

Leaf width and leaf length were used as input parameters for the ANN that calculated leaf area, while leaf area was used as an output parameter. A comparison of the results obtained with the software with the planimeter measurements is shown in Table 1. The software developed in terms of measurement values gives higher accuracy and faster results. Measuring with a planimeter is both time-consuming and makes a significant difference in precision. There is no uniformity between measurements. This causes errors in the leaf area calculation.

The planimeter measurements shown in Table 1 took approximately 20 minutes. The same process was carried out in less than one second with the help of software. This is another proof that the developed software is quite effective.

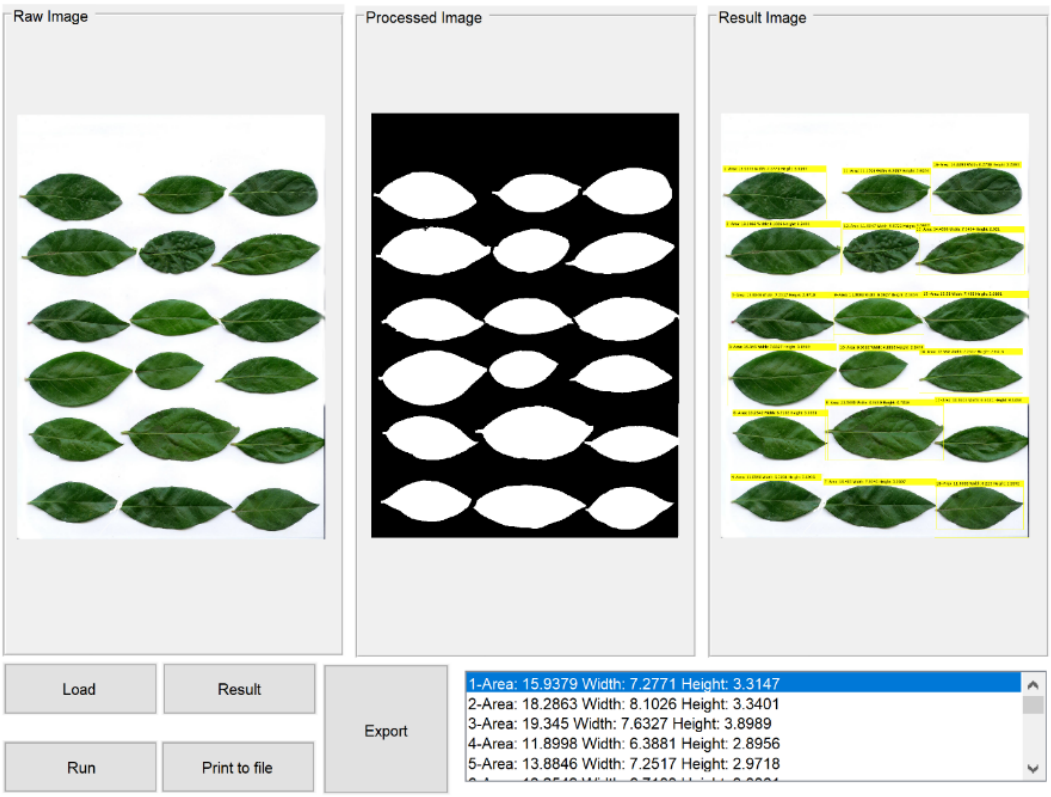

Figure 3. The developed GUI was used to calculate the leaf width, leaf length and leaf area of the samples.

Table1. Comparison of software results and digital planimeter measurement

Software			Digital planimeter		
Area (cm²)	Width (cm)	Height (cm)	Area (cm²)	Width (cm)	Height (cm)
15.8	7.2	3.3	14.0	6.3	3.0
18.2	8.1	3.3	16.4	7.2	3.1
19.3	7.6	3.9	16.1	6.8	3.5
13.7	7.2	3.0	11.8	6.5	2.7
11.6	6.3	2.9	9.7	5.7	2.6
13.1	6.7	3.1	11.1	6.0	2.7
16.1	7.9	3.1	14.4	7.3	2.7
20.8	8.3	3.8	18.3	7.2	3.5
10.9	6.3	2.6	9.5	5.7	2.3
8.9	4.8	2.7	8.0	4.5	2.4
11.1	6.2	2.7	10.0	5.6	2.5
11.7	5.4	3.0	10.4	5.0	2.7
14.1	7.6	2.9	12.4	6.7	2.5
12.3	7.2	2.6	10.7	6.4	2.4
15.3	7.3	3.1	13.0	6.9	2.7
14.6	6.2	3.3	12.1	5.6	3.0
11.6	6.7	9.3	9.7	5.8	2.3
11.5	6.1	2.9	10.3	5.0	2.7

One of the similar research, the proposed enhancement was to automate the image segmentation process using a two-level thresholding stage, removing the need to manually enter setup values. The findings imply that this technology is cost-effective and simple to execute in field situations, paving the way for future deployment on portable and traditional devices such as smartphones and tablets (Mora et al., 2016).

4. Conclusion

A plant's leaf area is a key factor of its growth. The material produced by the plant in an interval of time is dependent on the size of the leaves assimilating system. Particularly in the early stages of growth, it is comparatively easy to measure leaf area directly allows for the use of a small number of plants. In the later stages, however, when the leaves become numerous and large,

measuring the leaf area of every leaf may become extremely laborious. This difficulty is amplified when working with field crops. Because of the crop's high variability, all observations must be done on a large number of random samples, each including numerous plants, in order to correctly quantify growth changes and the degree of experimental errors. Directly measuring the leaf area of such large plant samples would be impractical due to the time and effort required to measure hundreds, if not thousands, of leaves at each sampling interval. In this study, it is aimed to calculate the leaf area, which is important in plant physiology studies. In the calculation of the leaf area, the width and length of the leaf are considered as the basic criteria. Area calculation was carried out by using artificial neural networks in the leaf database created by image processing. The results obtained have created an interface that end users can use more easily via the GUI. Computer-assisted prediction is possible when image processing and a neural network technique are coupled. As a result, the image processing and neural network technique is more reliable, faster, and allows for high-precision prediction.

Author Contributions

M.S.O. (25%), G.K. (25%), H.Ç. (25%) and R.O. (25%) design of study. M.S.O. (25%), G.K. (25%), H.Ç. (25%) and R.O. (25%) data acquisition and analysis. M.S.O. (25%), G.K. (25%), H.Ç. (25%) and R.O. (25%) writing up. M.S.O. (25%), G.K. (25%), H.Ç. (25%) and R.O. (25%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Research Article
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EVALUATION OF SEEDLING DEVELOPMENT AND PHYSICAL CHARACTERISTICS, VIABILITY AND GERMINATION OF SEEDS IN SOME LAMIACEAE TAXA

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Abstract: The Flora of Türkiye is very rich in the members of the Lamiaceae family. This study, it was aimed to investigate some physical characteristics, viability, germination performance of their seeds, and initial seedling development of 16 taxa belonging to the Lamiaceae family collected from the natural area. According to the results, the highest and lowest viability value was recorded in *S. aethiopis* (82.86%) and in *S.yosgadensis* (10.71%), respectively. The root length values of the taxa changed between 25.57mm (*T.sipyleus*)-66.82mm (*S.aethiopis*) at the end of the 21st day. The shoot lengths of the seedlings were determined to vary between 3.12mm (*T.sipyleus*)-22.41 mm (*P.armeniaca*). The seed width and length values of taxa varied between 0.911-2.788mm and 0.999-5.055mm, respectively. Values varying between 0.432-0.865 were calculated according to the geometric properties determined by proportioning the width and length dimensions of the seeds. The obtained results will be useful for studies on the cultivation of these taxa.

Keywords: Lamiaceae, Tetrazolium, Shoot length, Vigor index, Sphericity

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1. Introduction

Türkiye is situated at the intersection of the most important gene centers of the world (the Mediterranean and the Near East) due to its geographical location. At the same time, it is rich in terms of both plant species number and habitat types as a natural result of its wide variety in topography, climate, and geomorphology. According to the research results made in recent years, 9753 shows native species distributed in Türkiye. 3 035 of them are endemic, and when subspecies taxa are added, it is stated that there are 11 707 taxa, 3 649 of which are endemic (31.82%) (Guner et al., 2012). Türkiye is one of the important gene centers of the Lamiaceae family. The most important genuses of this family are *Thymbra*, *Thymus*, *Origanum*, *Satureja*, *Mentha*, *Teucrium*, *Ballota*, *Stachys*, *Salvia*, *Ajuga*, *Prunella*, *Melissa*, *Lamium*, *Sideritis,* and *Marrubium*. Most of the members of the Lamiaceae family are rich in essential oils and other secondary metabolites; they have usage areas in medicine, pharmacy, food, cosmetics, and perfumery, etc. (Nieto 2017, Karpiński 2020).

Knowing the physical properties of seeds is necessary for designing and developing the equipment necessary for the transportation, storage and processing of seeds (Zewdu 2011). It is of great importance to know the seed properties of plants especially used in food, treatment, cosmetics, etc. On the other hand, information on seed viability is of great importance for both producers and conservation of seeds in gene banks. Seed quality is a general term that includes the genetic, and physiological properties of the seed and determines the product performance and yield in relation to the potential of the variety. In measuring seed quality; various parameters are used such as genetic and physical purity, vitality, vigor, uniformity of seed size, seed-borne diseases and pests ,and other factors affecting seed performance in the field (Sun et al., 2007). Of these parameters, seed viability has a very important place in the concept of seed quality. Because, in a non-viable seed, talking about other quality features will not matter in practice. Depending on the ability to germinate and form a normal seedling, any seed is considered alive or lifeless.

There are many tests used in determining seed viability. However, the germination test and tetrazolium test are the most important vitality tests used in seed laboratories worldwide. The purpose of the germination test; is to learn about the viability of that population by using a seed sample that was randomly taken from a seed population. According to seed physiologists, germination is defined as the exit of the radiculate (root) from the testa (seed coat). However, the seed that is considered to

have germinated (the radicula extending from the testa to a few mm or up to 1 cm) can form a normal or abnormal seedling in the future (Rajjou et al., 2012). Among the biochemical tests used to determine seed viability, the tetrazolium test is the most popular and provides faster predictions of viability compared to a standard germination test. The standard germination test evaluates seedling growth and development, and also proves photosynthetic activity. In contrast, the tetrazolium test is a measure of respiratory activity and reveals signs of life or metabolic activity in seeds (Elias et al., 2012).

In this study, it was aimed to investigate some physical characteristics, viability, germination performance of their seeds, and initial seedling development of 16 taxa belonging to 6 genera (*Salvia*, *Marrubium*, *Teucrium*, *Phlomis*, *Thymus,* and *Stachys*) from the Lamiaceae family collected from the natural area.

2. Materials and Methods

2.1. Plant materials

The aerial parts of the taxa (herbage) in the research were collected from the natural area during the full flowering period. The location information and gathering date of taxa are given in Table 1. Species identification of the collected plants was made at Yozgat Bozok University, Faculty of Arts and Sciences, Department of Biology. According to the species identification results, mature seeds were collected from taxa in the natural area during the seed maturity period. Until the analyzes were carried out, the seeds were placed on paper bags and kept in closed cabinets under room conditions.

Table 1. Location information and collection dates of taxa

Taxa	Location	Full flowering
Salvia absconditiflora (sin: *Salvia cryptantha*) (Montbret&Aucher ex Benth.) Greuter&Burdet *	35° 31' 1.596"E-39° 23' 52.5834"N 1324m	May 30
Salvia aethiopis L.	35° 14' 4.6314"E-39° 24' 47.52"N 1130m	June12
Salvia ekimiana Celep & Doğan*	35°48'28.55" E-39° 34'32.42"N 1810 m	June17
Salvia verticillata L. subsp. *amasisca* (Freyn&Bornm.) Bornm.	35° 26' 40.4514"E-39° 24' 44.0634"N 1217m	June13
Salvia sclarea L.	7° 30' 56.0874"E-43° 56' 16.4394"N 1837m	June 23
Salvia viridis L.	35°09' 34"E-39°35' 13" N 1135m	May 28
Salvia candidissima Vahl. subsp *occidentalis* Hedge	34° 29' 42.1074"E-39° 29' 38.4714"N 1323m	June 23
Salvia virgata Jacq.	34° 27' 50.688"E-39° 28' 28.1994"N 1216m	July 05
Salvia dichroantha Stapf	31°12' 23"E-40°28' 16" N 853m	June 12
Salvia tomentosa Mill.	31°11' 57"E-40°29' 00" N 816m	June 15
Salvia yosgadensis Freyn & Bornm.*	34° 30' 32.6154"E-39° 30' 5.976"N 1517m	May 30
Marrubium parviflorum Fisch. & C.A.Mey. subsp. *parviflorum*	34° 27' 38.412"E-39° 28' 38.316"N 1238m	June 02
Teucrium chamaedrys L. subsp. *chamaedrys*	39° 28' 3.252"E-34° 28' 40.4394"N 1349m	June 22
Phlomis armeniaca Willd.	35° 26' 40.632"E-39° 24' 46.296"N 1207m	June 23
Thymus sipyleus Boiss.	35° 26' 40.632"E-39° 24' 46.296"N 1207m	June 22
Stachys annua (L.) subsp. *annua*	34° 28' 46.6314"E-39° 28' 7.5714"N 1391m	June 22

*Endemic

2.2. Determination of weights and size properties of seeds

Damaged seeds and foreign materials in all seed samples were eliminated before starting the measurements. The seeds were weighed on a sensitive scale and their weight was recorded. The length and width of the seeds were measured with a LEICA M125 (KL1500 compact) stereo microscope. Using the data obtained through length and width measurements, the seeds of taxa; arithmetic mean diameter (equation 1), geometric mean diameter

(equation 2), volume (equation 3), spheroid (equation 4), and sphericity (equation 5) values were calculated (Lorestani 2012, Bayram et al., 2016).

$$Da = (L+2D)/3 \quad (1)$$

$$Dg = (LD2)1/3 \quad (2)$$

$$AVk = \pi.L.D2/6 \quad (3)$$

$$\vartheta = 1-D/L \quad (4)$$

$$Ø = (LD2)1/3/L \quad (5)$$

Where; L= length; D: width

2.3. Classification of Seeds According to Their Geometric Properties

Seed width (W) / seed length (L) values of seeds belonging to each taxon were determined and classifications were made according to Table 2.

Table 2. Classification of seed samples according to their geometric properties (Dumanoglu et al., 2019).

Classification	W / L (mm)
Long (L)	0.6
Medium (M)	0.6-0.7
Short (S)	>0.7

2.4. Viability and Germination Tests

Tetrazolium test and germination tests were carried out to determine the viability rates of seeds collected from the natural area.

2.5. Tetrazolium Test

The viability of the seeds of the taxa Tetrazolium test has been determined and 50 seeds have been used (AOSA, 2000). The seeds were first swollen at room temperature for 1-2 days in water and kept at 25 °C for 2 hours in 1% 2, 3, 5- Triphenyl Tetrazolium Chloride (TTC) solution (pH 6-7). At the end of the period, the seeds were washed several times with pure water and examined under a stereo microscope. The viability of the seeds according to the staining of red was determined (AOSA 2000, Sagsoz 2000, Baydar 2013). Loose-structured, embryos, endosperm, and seeds with an unpainted region of more than 50% at the root end were considered lifeless (Sagsoz 2000, Santos et al., 2007). The viability of the seeds belonging to the species was determined as %.

2.5. Germination Test

Standard germination tests were set up in petri dishes with 4 replicates using 20 seeds each. Drying paper was cut under and over the Petri dishes and the seeds were germinated in a humid environment. Before the germination test, seeds were surface sterilized with 1% sodium hypochlorate (Subası and Guvensen 2010). The seeds were left to germinate in the petri dishes at 16 ± 2 °C in a dark 16 ± 2 °C and 8-hour light environment at 24 ± 2 °C in the climate cabinet. Counts were made at the end of 7, 14, and 21 days and the average germination rate was determined. The root of the root (radicule) 2 mm is considered as germination criteria and germination percentages are determined. At the end of the 21st day, the shoot length and root length were measured and leaf numbers were recorded. The seedling vigor index (SVI) of taxa was calculated using the equation (equation 6) below.

$$SVI = [\text{average shoot length} + \text{average root length}] \times \text{germination rate} \quad (6)$$

All analyzes and measurements were made in four replicates and the results are given as the mean. The important differences recorded as a result of the analysis of variance were subjected to the Least Significant Difference (LSD) Test and correlation analysis was used in bilateral relations. The MINITAB 19 package program was used in the statistical analysis.

3. Results

The viability and germination test results of taxa are presented in Table 3. According to the viability test results, no vitality was observed in any of the *S. cryptantha* seeds used in the study. Among the other 15 taxa, the highest viability value (82.86%) was recorded in *S. aethiopis* and the lowest value (10.71%) was recorded in *S. yosgadensis*. No germination occurred in the seeds of *S.ekimiana* and *S. tomentosa* species whose viability values were 30.77% and 27.78% respectively. On the other hand, no germination was observed in the seeds of *S. candidissima* subsp. *occidentalis*, *M. parviflorum* subsp. *parviflorum* and *T. chamaedrys* subsp. *chamaedrys* taxa on the 7th day when the first count was made. In *S. verticillata* subsp. *amasisca*, *S. candidissima* subsp. *occidentalis* and M. parviflorum subsp. parviflorum taxa, the first recorded germination value, and the last recorded value were the same. In other words, the values recorded on the 7th or 14th day did not change on the 21st day. In *S. aethiopis*, *S. dichroantha*, *S. virgata,* and *T. sipyleus* species, it was determined that the germination values on the 7th day changed very little on the 21st day. The germination values of *S. sclarea*, *S. viridis*, *S. yosgadensis*, *T. chamaedrys* subsp. *chamaedrys*, *P. armeniaca* and *S. annua* subsp. *annua* taxa increased significantly from the first count (7th day) to the last count (21st day). For example, in *P. armeniaca*, the germination value of 11.25% on the 7th day increased to 58.75% on the 21st day (Table 3).

Except for three taxa that could not be measured, root length values changed between 25.57 mm (*T. sipyleus*) - 66.82 mm (*S. aethiopis*) at the end of the 21st day. The shoot lengths of the seedlings were determined to vary between 3.12 mm (*T. sipyleus*) -22.41 mm (*P. armeniaca*). The number of leaves in shoots was recorded as 2.0 (*T. sipyleus*) -5.6 (S. annua subsp. annua) (Table 4).

Table 3. The viability and germination values of taxa

Taxa	Viability (%)	Germination Rate (%)		
		7th day	14th day	21st day
S. cryptantha	-	-	-	-
S. aethiopis	82.86	68.75	70.00	70.00^{BC}
S.ekimiana	30.77	-	-	-
S. verticillata subsp. *amasisca*	60.00	20.00	20.00	20.00^{E}
S. sclarea	64.29	78.75	81.25	82.50^{AB}
S. viridis	76.00	86.25	93.75	93.75^{A}
S. candidissima subsp. *occidentalis*	26.32	-	1.25	1.25^{G}
S. virgata	34.62	36.25	37.50	37.50^{D}
S. dichroantha	78.72	32.50	35.00	37.50^{D}
S. tomentosa	27.78	-	-	-
S. yosgadensis	10.71	1.25	2.50	2.50^{G}
M. parviflorum subsp. *parviflorum*	65.52	-	1.25	1.25^{G}
T. chamaedrys subsp. *chamaedrys*	14.29	-	1.25	3.75^{FG}
P. armeniaca	63.64	11.25	42.50	58.75^{C}
T. sipyleus	47.83	15.00	16.25	16.25^{EF}
S. annua subsp. *annua*	63.64	13.75	17.50	17.50^{E}

-no germination occurred in seeds

$^{A-G}$The difference between the averages shown with the same letter is statistically insignificant at the 5% level. (LSD (0.05) =13.482).

Table 4. The observations on initial seedling development of taxa

Taxa	Root length (mm)	Shoot length (mm)	The number of leaves
S. aethiopis	66.82^{A}	5.50^{F}	4.2^{B}
S. verticillata subsp. *amasisca*	55.88^{BC}	9.16^{CD}	2.5^{CD}
S. sclarea	47.75^{D}	13.53^{B}	4.4^{B}
S. viridis	51.02^{CD}	7.07^{E}	2.9^{C}
S. candidissima subsp *occidentalis*	-	-	-
S. virgata	61.46^{AB}	12.73^{B}	4.1^{B}
S. dichroantha	57.37^{BC}	7.92^{DE}	2.1^{D}
S. yosgadensis	39.03^{E}	9.47^{C}	4.0^{B}
M. parviflorum subsp. *parviflorum*	-	-	-
T. chamaedrys subsp. *chamaedrys*	-	-	-
P. armeniaca	57.56^{BC}	22.41^{A}	2.7^{C}
T. sipyleus	25.57^{F}	3.12^{G}	2.0^{D}
S. annua subsp. *annua*	60.88^{AB}	10.45^{C}	5.6^{A}

-The observation could not be obtained since there was not enough germination.

$^{A-G}$The difference between the averages shown with the same letter is statistically insignificant at the 5% level.

($LSD_{(Root\ Length)\ (0.05)}$=7.891; $LSD_{(Shoot\ Length)\ (0.05)}$=1.378;$LSD_{(The\ number\ of\ leaves)\ (0.05)}$=0.549)

A moderate positive relationship was found between root length (RL) and shoot length (SL, r=0.330), and leaf number (LN, r=0.377) (Figure 1). According to the result of the research, seedling vigor index values were between 121.3% and 5445.9%. Among the taxa, the lowest SVI value was determined in *S. dichroantha* species. The highest value was recorded in *S. viridis* species (Figure 2).

The seed width and length values of taxa varied between 0.911-2.788 mm and 0.999-5.055 mm, respectively. The highest seed weight was 0.00910g, followed by *P. armeniaca* with 0.00868 g (Table 5). It has also been found that seed length and width are positively correlated with seed weight (Figure 3).

The highest volume value was recorded in *S. cryptantha* species. This species was followed by *P. armeniaca* with a difference of 0.9578 mm3. Apart from these two species, the highest volume value was found in *S. tomentosa* species. The volume values of five taxa (*S. viridis*, *S. candidissima* subsp. *occidentalis*, *S. dichroantha*, *M.*

parviflorum subsp. *parviflorum*, *T. chamaedrys* subsp. *chamaedrys*, *T. sipyleus*) were found to be less than 2 mm in average. The volume values of the seven taxa (*S. aethiopis*, *S.ekimiana*, *S. verticillata* subsp. *amasisca*, *S. sclarea*, *S. virgata*, *S. yosgadensis*, and *S. annua* subsp. *annua*) took values between 2-6 mm on average. In the taxa included in the study; geometric mean diameter, arithmetic mean diameter, and sphericity values were recorded in the range of 0.9068-2.9619 mm, 0.9090-3.1423 mm, and 0.5718-1.6287%, respectively. When the spheroid values of taxa were examined, only the value of *T. sipyleus* species was positive (0.1361%), while the values of other taxa were negative and varied between -0.1280 and -0.5348%. (Table 6).

Values varying between 0.432-0.865 were calculated according to the geometric properties determined by proportioning the width and length dimensions of the seeds. *S. aethiopis* (0.614) and S. yosgadensis (0.617) medium (M), *S. viridis* (0.536), *M. parviflorum* subsp. *parviflorum* (0.498) and *P.armeniaca* (0.432) are long (L) seeds and other taxa with values between 0.729-0.865 are in the short (S) seeds class (Table 7).

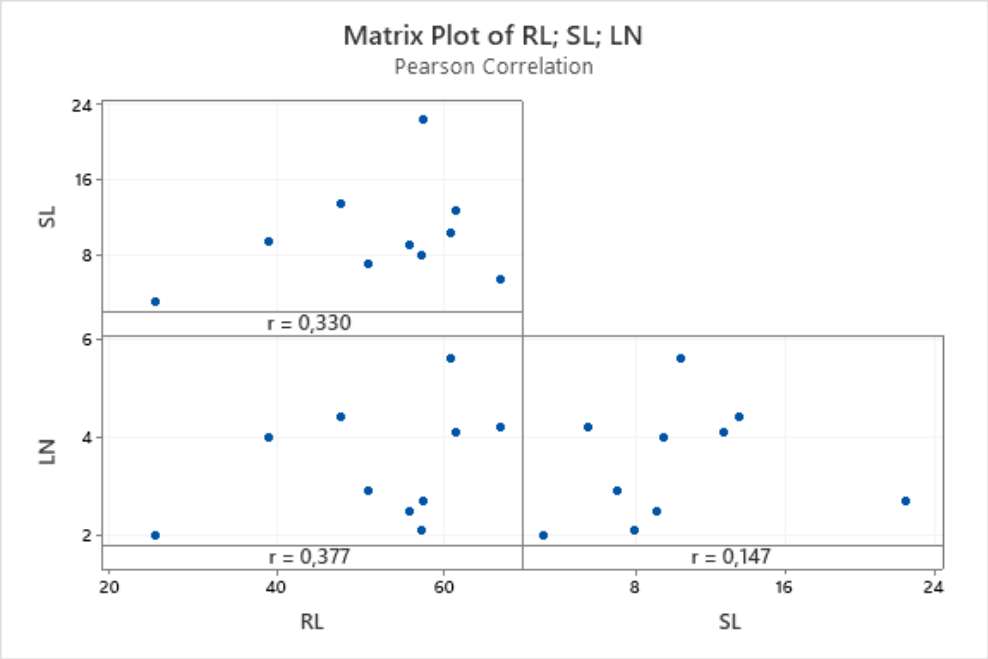

Figure 1. Pearson's correlation coefficients between root length (RL), shoot length (SL),) and leaf number (LN).

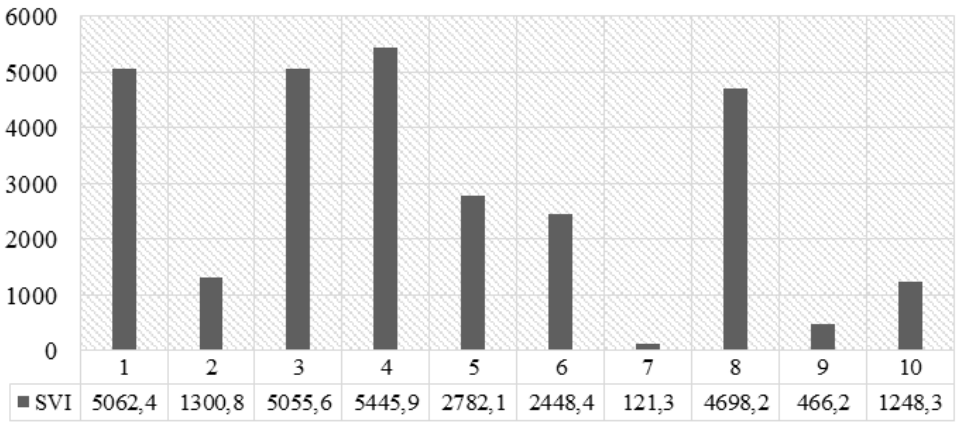

Figure 2. Seedling vigor index (SVI) values of taxa (%). 1= *S. aethiopis,* 2= *S. verticillata* subsp. *amasisca,* 3= *S. sclarea,* 4= *S. viridis,* 5= *S. virgata,* 6= *S. dichroantha,* 7= *S. yosgadensis,* 8= *P. armeniaca,* 9= *T. sipyleus,* 10= *S. annua* subsp. *annua.*

Table 5. The length, width, and weight values of the taxa seeds

Taxa	Width (mm)			Length (mm)			Weight (g)
	Min.	Max.	Mean	Min.	Max.	Mean	Mean
S. cryptantha	2.284	3.618	2.788	2.362	4.081	3.343	0.00662
S. aethiopis	1.366	2.076	1.593	2.250	2.736	2.594	0.00278
S. ekimiana	1.565	2.372	2.051	2.294	3.199	2.740	0.00341
S. verticillata subsp. *amasisca*	1.032	1.683	1.450	1.697	2.106	1.964	0.00062
S. sclarea	1.645	2.366	1.970	1.589	2.919	2.596	0.00433
S. viridis	0.900	1.126	1.048	1.821	2.077	1.954	0.00256
S. candidissima subsp *occidentalis*	1.646	2.041	1.861	2.174	2.612	2.409	0.00150
S. virgata	1.194	1.693	1.527	1.190	2.173	1.989	0.00910
S. dichroantha	0.955	1.474	1.213	1.467	1.879	1.664	0.00029
S. tomentosa	2.003	2.623	2.357	2.392	2.986	2.756	0.00474
S. yosgadensis	1.300	2.695	1.907	2.458	3.066	2.841	0.00292
M. parviflorum subsp. *parviflorum*	0.722	1.162	0.911	1.521	1.960	1.829	0.00037
T. chamaedrys subsp. *chamaedrys*	0.924	1.548	1.216	1.079	1.795	1.480	0.00114
P. armeniaca	1.770	2.466	2.186	4.366	5.958	5.055	0.00868
T. sipyleus	0.766	0.963	0.864	0.688	1.175	0.999	0.00023
S. annua subsp. *annua*	1.256	1.665	1.512	1.704	2.102	1.890	0.00269

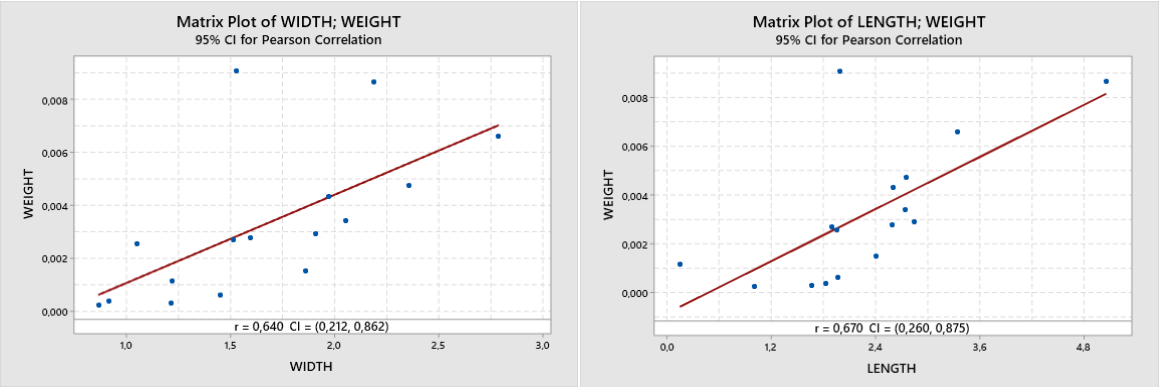

Figure 3. Pearson's correlation coefficients between seed weight, seed wight and seed length.

Table 6. Some physical properties of taxa seeds

Taxa	V (mm^3)	GMD (mm)	AMD (mm)	Sphericity (%)	Spheroid (%)
S. cryptantha	13.6057	2.9619	2.9730	0.8756	-0.5348
S. aethiopis	3.4466	1.8741	1.9266	0.7224	-0.2286
S. ekimiana	6.0350	2.2588	2.8806	0.8244	-0.3835
S. verticillata subsp. *amasisca*	2.1621	1.2523	1.6213	0.8168	-0.2291
S. sclarea	5.2751	1.3743	2.1786	0.8319	-0.3736
S. viridis	1.1236	1.2898	1.3500	1.2898	-0.0245
S. candidissima subsp *occidentalis*	1.2613	2.0281	2.0436	0.8419	-0.3574
S. virgata	2.4283	1.6676	1.6810	0.8384	-0.2649
S. dichroantha	1.2819	1.3477	1.3633	0.8099	-0.1280
S. tomentosa	8.0167	2.4831	2.4900	0.9009	-0.4923
S. yosgadensis	5.4096	2.1779	2.2183	0.7666	-0.3192
M. parviflorum subsp. *parviflorum*	0.7947	1.1492	1.2170	0.6283	0.0486
T. chamaedrys subsp. *chamaedrys*	1.1458	1.2983	1.3040	0.8772	-0.1459
P. armeniaca	12.6479	2.8907	3.1423	0.5718	-0.2346
T. sipyleus	0.3904	0.9068	0.9090	0.9077	0.1361
S. annua subsp. *annua*	2.2623	1.6287	1.6380	1.6287	-0.2708

V= volume GMD= geometric mean diameter, AMD= arithmetic mean diameter

Table 7. Classification according to the geometric characteristics of seeds belonging to taxa

Taxon	W/L	Classification	Taxon	W/L	Classification
S. cryptantha	0.834	S	*S. dichroantha*	0.729	S
S. aethiopis	0.614	M	*S. tomentosa*	0.855	S
S. ekimiana	0.749	S	*S. yosgadensis*	0.671	M
S. verticillata subsp. *amasisca*	0.738	S	*M. parviflorum* subsp. *parviflorum*	0.498	L
S. sclarea	0.759	S	*T. chamaedrys* subsp. *chamaedrys*	0.822	S
S. viridis	0.536	L	*P. armeniaca*	0.432	L
S. candidissima subsp *occidentalis*	0.773	S	*T. sipyleus*	0.865	S
S. virgata	0.768	S	*S. annua* subsp. *annua*	0.800	S

4. Discussion

Viability and germination tests were carried out in taxa whose seeds can be collected from the natural environment. A tetrazolium test was used to determine the viability of seeds. Tetrazolium test is a method that provides practical determination of seed viability and can get results in less time than the germination test (McDonald and Contrrnas 2002). As a result of the Tetrazolium test, the vitality values of the taxa varied between 0-82.86% and no viable seeds were found in *S. cryptantha* species. In the germination experiment, no germination occurred in the seeds of the two Salvia taxa (*S. ekimiana* and *S. tomentosa*). Contrary to our findings, 31.33% germination was recorded in the seeds of the S. tomentosa species (Ozcan et al., 2014). As is known, the seeds belonging to the Salvia genus have a mucilaginous seed coat causing dormancy. Dormancy is an important factor in preventing germination (Finkelstein et al., 2008). In studies performed with different sage species, the germination strength value was 31% in *S. fruticosa* (Sonmez et al., 2019), 25.50% in *S. pomifera* (Ozcan et al., 2014), 76% in S. cyanescens, 29% (Yucel and Yılmaz 2009), in *S. verticillata* (Tursun 2020) and in 41% *S. sclarea* (Joshi and Pant, 2010) was recorded. While the germination value of *M. vulgare* was determined as 88% (Dadach et al., 2015), this value was very low 1.25% in *M. parviflorum* subsp. *parviflorum*. There is a great variation in germination values among the species. For example, while the germination value of *Phlomis armeniaca* included in this study was 58.75%, the germination value of the *P. italica* species used in the study carried out by Galmés et al., (2006) was recorded as 8.7%. In our study, the highest germination values were recorded on the 21st day.

Seed quality can be expressed as the degree of excellence of the characters or traits that determine the performance of the seed when it is sown or stored. The standard germination test is a basic and acceptable test in quality controls. This test can be used to predict germination under optimum field conditions (Hampton 2002). However, optimum conditions for germination rarely occur in the field. For this reason, there is a need to use tests that can evaluate the seed/seedling vigor, which is an expression of the characteristics that enable seeds to perform germination activities and performances in different environmental conditions and especially in conditions below optimum (ISTA 2003). In general, the seed with high vigor shows a uniform and rapid germination and emergence in the field in unsuitable soil conditions or cold early sowing periods. In our study, the SVI values of taxa varied greatly and the highest value was obtained from *S. viridis* (5445.9%).

Information on the physical and aerodynamic properties of agricultural products is needed for the design and adjustment of machinery used during harvesting, separation, cleaning, transportation and storage of agricultural materials and for their transformation into food and feed (Nalbandi et al., 2010, Tavakoli et al., 2014). For this reason, features useful during design should be known and these properties should be determined under laboratory conditions. The most important physical properties considered during the separation and cleaning of medicinal and aromatic plant seeds as other field crop seeds are geometric properties such as size and shape (Tavakoli et al., 2014). In theoretical calculations, medicinal plant seeds are assumed to be spherical or elliptical due to their irregular shape (Nalbandi et al., 2010). It was determined that the physical properties of seeds belonging to medicinal and aromatic plant species such as Salvia sp. (Ixtaina et al., 2008; Bayram et al., 2016, Tavakoli et al., 2014, Yılar and Altuntas, 2017), *Foeniculum vulgare* L. (Ahmodi et al., 2009), *Momordica charantia* L. (Golukcu et al., 2014), *Cuminum cyminum* L. (Singh and Goswami 1996), and *Coriandrum sativum* L. (Coskuner and Karababa 2007) were examined in the literature reviews.

5. Conclusion

Taxa included in this study are not cultured, however, they show naturally occurring in Türkiye. These taxa in the natural flora are used by the local people for various purposes. In the future, the possibility of using these taxa in different areas may come to the fore. There are many medicinal and aromatic plants, but there is limited research on the physicochemical properties of their seeds. However, it is important to determine the physical

properties of medicinal and aromatic plant seeds due to their potential use in industries such as food, cosmetics, medicine, chemistry, etc. For this reason, we are of the opinion that these findings will make important contributions to the current literature.

Author Contributions

B.C.Ş. (100%) wrote the manuscript and conceived the original idea, organized, analyzed, and interpreted the data. T.U. (100%) carried out the experiment and structured the paper and edited the manuscript. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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DETERMINATION OF THE GROWTH PATTERN OF ROSS 308 BROILER STRAIN REARED IN A HUMID TROPICAL ENVIRONMENT

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Abstract: The current study was aimed to investigate the growth pattern of Ross 308 broilers reared in a humid tropical environment Data on body weight and other linear body traits (shank length, body length, wing length, breast width and drumstick were obtained weekly from 60 day old birds for a period of 8weeks. Data collected were subjected to Analysis of Variance (ANOVA), using the general linear model procedure. Means were separated for significance, using Least Significant Difference (LSD). The regression model, $Y_I = B_IX_I$, was used for the regression analysis and the allometric growth equation used was $Y = aW^{ß}$. A marked difference ($P<0.05$) for the body weight of broiler across the various weeks was observed, with the lowest value (139.40±5.48) 0bserved in week 1 and the highest value (2.440.33±51.66) in week 8. The linear regression of age on body parameters showed a highly significant difference ($P<0.01$) for the morphometric traits studied. The percentage of the regression coefficient for all the traits under consideration were high (above 50%), except for DL that has a low value of 19.2% and the various coefficient of allometry with coefficient of isometric (0.33) indicates that DS and SL grew faster than other components of the Ross 308 broiler body. With the high R^2-value obtained for the regression analysis, the study therefore recommends age at 8weeks and all other morphometric traits except DL are the best for the selection of Ross 308 strain of broilers for market weight. Hence, all the studied morphometric traits nice predictors of body weight of broilers, except the drumstick length.

Keywords: Broilers, Ross308, Growth pattern, Allometric

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1. Introduction

The broiler industry in order to meet up with the world poultry meat consumption rate need to produce birds with fast growth rate and high carcass yield in a short time (Prince, 2002). The high demand for fast growing broilers has been an issue that most farmers are yet to overcome due to the choice of strain which the farmer uses. According to Essien and Adiyimi (2009), the Nigerian poultry industry has over the years witnessed the introduction of Abor Acres, Ross 308, Hubbard and other strains of broilers. The realization of the full potentials of these strains is largely expected to depend on the nutritional and climatic variables subject to the genotypic traits which in turn set a ceiling on the productive capacity of the strains. However, the implication of this is that the broiler producers select stocks which have the genetic potential for fast growth and attainment of market weight early enough under the Nigerian climatic conditions.

Therefore, with the influx of different strains of broilers in Imo state and other states in Nigeria, it has become necessary for the farmers to know the pattern of growth of the various strains they encounter. This could assist them in choice of the feeding regime to attain optimum market weight at a short interval of time

The Ross 308 is a robust, fast growing feed efficient broiler with good meat yield. It is designed to satisfy the demands of customers who require consistency of performance and the versatility to meet a broad range of end product requirements. It is one of the most common strains available in commercial farms found in Owerri, Imo State. According to Alkano et al. (2007), defining growth with respect to each feature of the animal is not sufficient, since it gives limited information on the animal's growth performance in general. Therefore, to ascertain data for growth performance of different body parts of an animal with respect to each other and the whole body, prediction of allometric growth parameters becomes an essential tool. It therefore becomes necessary to ascertain the growth pattern of this strain, as this information could be exploited and used in breeding programme to develop fast growing indigenous strain adaptable to the humid tropic environment as well

as get the farmers well informed for easy prediction. The study tries to ascertain the allometric growth pattern of Ross308 strain of broiler in Owerri, Imo state, determine the growth characteristics of Ross 308 strain of broiler chicken and determine growth rates of the body and other linear body parameters.

2. Materials and Methods

2.1 Experimental Site and Animals

This experiment was conducted at the Teaching and Research Farms of the Department of Animal Science and Technology, Federal University of Technology Owerri. Federal University of Technology Owerri is located within South-East agro-ecological zone of Nigeria. It is located in the rainforest belt of Nigeria, with altitude of 90m and the mean annual rainfall, temperature and humidity of 2500mm, 26.5 27.50 ˚C and 70-80% respectively. It is located 25 kilometers south of Owerri, latitude and longitude of 5˚29N and 7˚02E respectively. 60 Day old Ross 308 strain of broiler chicks were purchased from a reputable hatchery. The brooding and rearing of the birds were done on a deep litter system. On arrival, the Day Old Chicks, permanent marker was used to mark each of them for identification. Feeders and drinkers were provided for the supply of feeds and water respectively. The birds were fed formulated starter and finisher diet ad libitum for the starter and finisher phases respectively. Water was also served *ad-libitum*, throughout the experimental period. Poultry routine management and vaccinations were maintained throughout the experiment, and the experiment lasted for 56days (March – May). Brooding was carried out for a period of 2 weeks and the birds were separated into five (5) different pens of 15 birds per pen. At this point, permanent tags were placed on the shanks of each bird for identification. The body weights of the individual birds and other body linear traits (Shank length, body length, keel length, wing length, breast width, and drumstick length and chest circumference) were measured to obtain their initial body weight, prior to the commencement of the experiment and thereafter recorded on weekly basis before the birds are fed.

2.1. Statistical Analysis

Data collected were analyzed using the general linear model procedure of statistical package for the social sciences (SPSS, 2011). Simple linear regression of each of the measured parameters on age was carried out to determine the rates of growth of the body and each of the component parts.

The regression model is given in the expression (equation 1);

$$Y_i = a + b_i x_i \quad (1)$$

Where; Y_i = intercept, a = Y-intercept, b_i = slope, x_i = ith independent variable, i.e., age.

Allometric growth equation that will be fitted to the data on body weight (BWT) and linear structural body parameter (Y) is of the form in expression (equation 2);

$$Y = aW_{\beta} \quad (2)$$

Where; Y = linear structure body parameter, i.e. body length (BLT), a = initial growth constant, β = coefficient of allometry

Least estimate of a and β was obtained by fitting the log-transformed linear equation in the expression (equation 3);

$$\log_{10}Y = \log_{10}a + {}_{\beta}\log_{10}W \quad (3)$$

3. Results and Discussions

The mean (±SE) of body weight and body linear measurements of Ross 308 from week 1 to 8 as shown in Table1, reviewed a significant progression ($P<0.01$) in the mean body weight and linear body parameters with age. The lowest values (139.40±5.48, 8.0±0.15, 3.76±0.08, 5.40±0.16, 7.73±1.89 and 10.37±0.20) for body weight, wing length, shank length, drumstick length, breast width and body length respectively, were in week one while the highest values (2440.33±51.66, 18.99±0.33, 8.39±o.14, 16.6±0.3, 18.48±0.32 and 29.49±0.47) for body weight, wing length, shank length, drumstick length, breast width and body length respectively, were in week eight. The increase with advancement in age as observed in this study is in tandem with the reports of Adeyinka et al. (2004) and Udeh et al. (2011) who reported that age is a major determinant of growth and physiological development. The mean final body weight (2562 ± 52.55) of ROSS 308 at 8weeks of age obtained is equivalent to the findings of Afolayan et al. (2012), who ascertained a market weight of 2310.0g for Ross308 at 8weeks of age. The attainment of market weight greater than 2kg gives the impression that Ross308 has a great potential for weight gain and growth rate in the humid tropics of South East, Nigeria.

Table 1. Weekly mean (Mean ± SE) of body weight and body linear measurements of ROSS 308

Traits	WK I(60)	WK 2(60)	WK 3(60)	WK 4(60)	WK 5(60)	WK 6(60)	WK 7(60)	WK 8(60)
BW (g)	134.45 ± 5.66	317.80 ± 15.80	513.32 ± 11.93	820.68 ± 25.88	984.83 ± 12.4	1184.88 ± 30.57	1816.12 ± 43.99	2562 ± 52.55
WL (cm)	8.13 ± 0.19	10.94 ± 0.22	13.90 ± 0.12	14.78 ± 0.23	16.10 ± 0.24	18.60 ± 0.14	21.10 ± 0.13	24.57 ± 0.29
SL (cm)	3.98 ± 0.05	5.18 ± 0.12	5.10 ± 0.06	5.46 ± 0.09	6.00 ± 0.05	6.57 ± 0.06	7.30 ± 0.07	7.08 ± 0.09
DS (cm)	5.15 ± 0.08	7.56 ± 0.17	7.65 ± 0.08	9.51 ± 0.19	10.14 ± 0.32	12.07 ± 1.59	12.00 ± 0.10	19.16 ± 0.24
BW (cm)	7.45 ± 0.17	9.90 ± 0.22	10.73 ± 0.14	13.90 ± 0.38	15.53 ± 0.11	16.50 ± 0.19	19.99 ± 0.17	16.74 ± 0.22
BL (cm)	10.47 ± 0.19	13.22 ± 0.28	15.74 ± 0.29	17.81 ± 0.28	19.04 ± 0.03	20.94 ± 0.23	24.17 ± 0.24	31.86 ± 0.32

Table 2 shows the regression of body parameters on age in Ross308 broiler. The result shows that all the regression equations were highly significant (P<0.01), considering the comparative goodness of fit of the considered model, judging from the criteria like R2-values, levels of significance error of estimate and signs and size of regression coefficient. High R^2-values ranging between 68.5% and 89.4% were obtained for BR and WL respectively. However, DL has a very low R^2-value of 19.2%. The co-efficient were generally positive ranging between 2.82 for drumstick length to 7.14 for body weight respectively. The linear regression of age on body parameters in Ross 308 strains of broilers showed a highly significant difference (P<0.01) for all traits across various ages. The high R^2-values (81.8%, 89.4%, 70.8%, 68.5% and 82.1%) for BW, WL, SL, BW and BL respectively, obtained in the relationships shows that the linear traits of Ross 308 can be predicted if the animal's age is known. Except for the DL, this has a very low R^2-value (19.2%). This saves the farmer additional cost of buying a weighing scale, thus predicting the body weight of the bird at every age stage using values of any morphometric trait, as described by Chineke et al., (2006). The highly significance (P<0.01) F-value shows that the data fits in very well in the model used.

Table 2. Linear Regression of Age on Body Parameters in ROSS 308 broilers

Traits	Equation	R^2 (%)	S.E	Sig
BW	BW = -502.182 + .905Age	81.8	334.04	0.00
WL	WL = 6.69 + .946Age	89.4	1.52	0.00
SL	SL = 3.94 + .841Age	70.8	0.59	0.00
DL	DL = 2.82 + .441Age	19.2	7.21	0.00
BR	BW = 7.14 + .828Age	68.5	2.17	0.00
BL	BL = 7.01 + .906Age	82.1	2.64	0.00

Table 3 shows the log linear and allometric growth equations and distribution co efficient for linear growth parameters for 8-week period in Ross 308 strain of broilers. The degree of reliability of the allometric equation was measured by the R^2 values. Very high (91.5% and 92.8%) R^2 values were obtained for the allometric equation relating body weight and shank length and body weight and drumstick respectively. Whereas, a relatively high (80.6%, 80.8% and 76.9%) R2 values were obtained for body weight and breast width, body weight and wing length and body weight and body length respectively. The R^2 values obtained from the results showed that the model used fits the data. The former shows a very high reliable equation whereas, the later showed a relatively high reliability of allometric equation. Hence, this confirms the postulate of Palsson (1955), that different components of the body have a different growth rate, thus explaining the differential growth patterns observed between body weight and different body components. Different body parts develop at varying rates and these changes determine the shape, conformation and body proportion of the animal at a given time (Olutogun et al., 2003).

Table 3. Log Linear and Allometric Growth Equations and Distribution coefficient for Linear Growth for 8 weeks

Linear parameters	log linear	R^2 (%)	SEM	Allometric
Shank length (SL)	Y = 0.144 + 0.957BW	91.5	0.176	$BL = 0.31W^{0.975}$
Drum stick (DS)	Y = 0.49 + 0.96BW	92.8	0.115	$DS= 1.63W^{0.96}$
Breast width (BR)	Y=1.246 + 0.90	80.6	0.171	$BR= 3.48^{0.90}$
Wing length (WL)	Y=0.986 + 0.90BW	80.8	0.177	$WL= 2.68W^{0.90}$
Body length (BL)	Y=5.001+ 0.90BW	76.9	0.118	$BL=148.56W^{0.88}$

BL= body length, Sl= shank length, BR= breast width, DS= drumstick length, WL= Wing length, BW= body weight

4. Conclusion and Recommendations

The quest for broiler production in Nigeria cannot be possible without strategizing the possible breeding pattern for improvement of the various strains of broilers that are peculiar to the environment. To ensure the sustainability of the poultry enterprise, the productive capability of the animal (broilers) must be improved. This cannot be achieved without considering the pattern of growth of the various strains of broilers, measurement of correlations among body parameters (traits) and age of the birds for development of selection programs for effective planning. This results from this study therefore reviewed a marked difference (P<0.05) for the body weight of broiler across the various weeks, hence, as the chicken increase in age, there is a concordance increase in the body parameters. The linear regression of age on body parameters showed a highly significant difference (P<0.01) for the morphometric traits studied, the percentage of the regression coefficient for all the traits under consideration were high (above 50%), except for DL that has a low value of 19.2% and the various coefficient of allometry with coefficient of isometric

(0.33) indicates that DS and SL grow faster than other components of the Ross 308 broiler body.

With the high R^2-value obtained for the regression analysis, the study therefore recommends age at 8weeks and all other morphometric traits except DL are the best for the selection of Ross 308 strain of broilers for market weight and size. It is a nice predictor for the linear body parameters.

Author Contributions

O.K. (25%), I.A. (25%), C.U. (25%) and C.I. (25%) design of study. O.K. (25%), I.A. (25%), C.U. (25%) and C.I. (25%) data acquisition and analysis. O.K. (25%), I.A. (25%), C.U. (25%) and C.I. (25%) writing up. O.K. (25%), I.A. (25%), C.U. (25%) and C.I. (25%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The experimental procedures approved by local Animal Care and Ethics Committee of Animal Science and Technology, Federal University of Technology Owerri, Imo state (Decision Number/Date: 2021/03-02, March 27, 2021).

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FUNGICIDE AND ARBUSCULAR MYCORRHIZA FUNGI APPLICATIONS IN TOMATO

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Abstract: Tomato is one of the important food crops of the world. It has rich essential nutrients features. However, tomato plants are sensitive to certain diseases and pests. This situation causes intense and unconscious pesticide use to avoid crop losses. It is known that mycorrhiza provide many advantages to plant. In this study, the effects of different doses of fungicide applications on some physiological parameters were examined in mycorrhiza applied and non-applied mycorrhiza tomato plants. A pesticide was applied at different doses which were, namely, recommend (R), half of recommend (R/2), and two-fold recommend (R×2). The content of proline, chlorophyll and carotenoid analysis were conducted in the plant samples. Proline values were found low in mycorrhizal than non-mycorrhizal plants in all pesticide doses ($P<0.05$). However, mycorrhiza*dose interaction was statistically significant ($P<0.01$). It was found statistically significant in chlorophyll-a ($P<0.01$), chlorophyll-b ($P<0.05$), total chlorophyll ($P<0.01$), and carotenoid ($P<0.05$) values in terms of mycorrhiza*dose interaction. We suggest that studied arbuscular mycorrhiza may increase at highly the resistance tolerance to fungicide. AMF is suitable option for low chemical input and nature conservation based sustainable agriculture.

Keywords: Tomato, Proline, Chlorophyll, Carotenoid, Mycorrhiza, Fungicide

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1. Introduction

Recent rapid population growth causes nutrition problem that is one of the biggest problems faced by humanity. To resolve this problem, studies focusing on the maximum product uptake in agriculture have been increasing. Almost all the cultivated plants in the world have been threatened by diseases, pests, and weeds (Tiryaki et al., 2010). These cause crop losses around the world (Capinera, 2005; Paini et. al., 2016). The cultural, mechanical, physical, biotechnical, and biological methods are used to solve the problems in crop production, today. Pesticide use is the most preferred chemical control method. In recent years, the pesticides are widely used in the crops are grown both in the greenhouse and in the field (Hazra and Purkait, 2019). However, while these applications cause an increase in the quality and efficiency of the products, agroecosystems and environmental protection is very important to sustain ecological balance. Unconscious and excessive applications of plant protection products in plants cause problems including phytotoxicity, residues in agricultural products with the problem in domestic and foreign markets. The need for studies to minimize the damage of these chemicals is a common problem to the world (Delen et al., 2010; Appah et al., 2020). Mycorrhizal fungi are used in the fight against plant diseases and damages in our country and world (Öztekin and Ece, 2014).

One of the most important mutualistic relationships that increase productivity and nutrient cycles between plants and microorganisms is mycorrhiza (Tilak et al., 2005). Arbuscular mycorrhizal fungi (AMF) form a mutually symbiotic with the roots of land plants and play an important role in regulating community and ecosystem functioning (Wu et al., 2021).

Mycorrhizal fungi are important in agriculture and forestry as bidirectional nutrient transfer between host and fungal endophyte (i.e., drain of host carbon and uptake of soil mineral drive many nutrient cycling processes in soil) (Xavier and Germida, 1999; Alaux et al., 2021). In community with decreased mycorrhizal fungi, weed species that are characterized by non-mycorrhizal relationships increase and the nutrient cycle can be broken.

There are separate studies showing positive or negative effects of plant-mycorrhizal relationships and fungicide use on the plant (Cordier et al., 1996; Al-Karaki, 2000; Hajiboland et al., 2010; Song et al., 2010; Abdel Latef and Chaoxing, 2011; Çekiç and Yılmaz, 2011; Öztekin and Ece, 2014; Almaca, 2014; Abdulhadi et al., 2017). Hage-Ahmed (2018) reported that there is a need to investigate the combined effects of AMF and pesticide

applications on plants. Özbucak and Kabul (2019 and 2020) was determined mycorrhizal tomato had positive effect on fruit and growth parameters despite pesticide application.

In this study, we examined the tomato plants that are widely in human nutrition in Türkiye and in the world (Qasid et al., 2022), with frequently applied fungicides. Fungicide application may change proline, chlorophyll, and carotenoid metabolic activity. For this purpose, we compared the effects of different doses pesticide use on proline, chlorophyll, and carotenoid parameters in mycorrhizal and non-mycorrhizal tomato seedlings.

It is necessary to develop alternative strategies to reduce the negative effects of chemical inputs such as pesticides which are widely used in agriculture, on nature and living things. We believe that the encouraging results obtained from this study can contribute to the sustainability of the agricultural production and the promotion of the commercial use of these products.

2. Materials and Methods

2.1. Materials

In this study was used commercially purchased tomato seeds (*Solanum lycopersicum* L.) and mycorrhiza preparation (*Glomus fasciculatum, Glomus intraradices, Glomus mosseae* mixture). Antracol WP 70 (% 70 Propineb) fungicide used as pesticide.

2.2. Experimental Design

100 seeds of tomato were surface sterilized with 70% ethyl alcohol for 1 min. and 10% NaClO for 5 min., followed by rinsing 10 times with sterile-distilled water. Afterwards seeds were hold in sterile-distilled water for 20 min. and then were filtered through filter paper (Battke et al. 2003). Sterilized tomato seeds were germinated to 100 plastic cups with peat: perlite: soil mixture (2:1:1). The characteristics of the soil sandy-clay-loam (60%, 25%, 15%). 50 of plastic cups were planted with 2gr mycorrhiza and then were placed in a climate cabinet with a 14: 10 h light: dark cycle with 23.5°C-60% temperatures and humidity, respectively. and watered every other day to 60% water holding capacity (Figure 1).

Figure 1. The germination of tomato seeds in climate cabinet.

After one-month, plastic cups were removed from climate cabinet. They remained in the laboratory for 15 days. Healthy seedlings were transplanted to 20 L. pots in a greenhouse. Two seedlings were planted in each of the 24 pots. 12 pots were planted inoculum with mycorrhiza seedlings. The other 12 pots were planted with non-mycorrhiza seedlings (Figure 2, 3). Fungicide (Antracol WP 70- Propineb) application was made by spray in case of four doses, namely: (a) control, (b) recommended dose (R=0.75 g/250 ml water) (c) half of recommended dose (R/2=0.375g/250 ml water), (d) two-fold recommended dose (R*2=1.5 g/250 ml water). Pesticide sprayed to plants with days by 5 times after 24 days seedling planting. In the first flowering period was applied natural manure to plants. It was used peat, perlite, soil, fertilizer (2:1.1:1/2) for each pot. Approximately 7 days after the fifth spraying, leaf samples were taken from the different pots in each experimental group for proline, chlorophyll and carotenoid analyses.

2.3. Plant Analyses

A week after the fifth spraying treatment, leaf samples were taken from experimental groups for proline, chlorophyll, carotenoid analyses. Proline content was determined Bates et al. (1973). The leaf samples (1 g) were homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid solution. Supernatants were transferred to test tubes and mixed with equal volumes of glacial acetic acid and ninhydrin reagent. Test tubes were incubated in the oven for 1 h at 100 °C. The test tubes were then placed in an ice bath and thus the reaction was stopped. The samples were rigorously mixed by using a vortex after 4 mL of toluene was added to the tubes. After 50 min, toluene phases were obtained. The absorbance was measured at 520 nm on a UV-visible spectrophotometer. Photosynthetic pigment (chlorophyll a, chlorophyll b and carotenoid) contents were detected according to the method of Kaçar (1984). Fresh leaf samples (1 g) were extracted overnight with 80%

acetone at 0–4 °C. The extracts were centrifuged at 3,000 × g for 5 min. Supernatant was obtained and absorbance was read at 645 and 663 nm for chlorophyll, at 470nm for carotenoid using a spectrophotometer. The results were calculated according to Lichtentaler and Wellburn (1985) (equations 1, 2, 3 and 4).

$$\text{Chlorophyll-a} = 11.75 \times A_{662} - 2{,}35 \times A_{645} \quad (1)$$

$$\text{Chlorophyll-b} = 18.61 \times A_{645} - 3.96 \times A_{662} \quad (2)$$

$$\text{Carotenoid} = 1000 \times A_{470} - 2.27 \times \text{Klorofil-a} - 81.4 \times \text{Klorofil-b} / 227 \quad (3)$$

$$\text{Total Chlorophyll} = \text{Chlorophyll-a} + \text{Chlorophyll–b} \quad (4)$$

The assumptions of data were tested with the Kolmogorov Smirnov and the Levene's tests, respectively. The variables were analyzed by two-way ANOVA/Kruskal-Walli's test. The means compared with Tukey's HSD/Dunn post-hoc test and the results were displayed by letters. The alpha level was set at 5%. All calculations were performed with Minitab 17 statistical software.

Figure 2. The planting seedlings in pots.

Figure 3. Growth of seedlings in pots.

3. Results

3.1. Proline Concentration (mM/gr/) in Leaf

The mean of proline concentration values in mycorrhizal tomato were determined lower than non-mycorrhizal tomato ($P<0.05$) (Table 1). Proline content of tomato leaf was found statistically significant in terms of mycorrhiza*dose interaction ($P<0.001$). According to Tukey test results, there were no statistical differences between control, R/2, R ($P>0.05$) dose in mycorrhizal plant in terms of proline concentration. However, it was statistically significant in R* dose ($P<0.05$). While control group has low proline content in non- mycorrhizal plant control ($P<0.05$), there is no significant differences between R dose ($P>0.05$).

3.2. Chlorophyll-a Content (mg/ml) in Leaf

The chlorophyll-a content was found statistically significant in terms of mycorrhiza*dose interaction ($P<0.01$) (Table 2). The mean values of chlorophyll-a content were found higher in mycorrhizal than non-mycorrhizal plant. Chlorophyll-a content was not found significant in mycorrhizal plant in all doses to Tukey ($P>0.05$). However, chlorophyll content in R dose was statistically significant in non-mycorrhizal plant ($P<0.05$). The chlorophyll-a content of R dose was found

lower in non-mycorrhizal than mycorrhizal plant (P<0.05).

3.3. Chlorophyll-b Content (mg/ml) in Leaf

Chlorophyll-b content was found statistically significant in terms of mycorrhiza*dose interaction (P<0.05) (Table 3). Chlorophyll-b content was found significant in control, R/2 and R doses of mycorrhizal plant, R/2 and R doses in non-mycorrhizal plant to Tukey (P<0.05). The mean values of chlorophyll-b content were found lower in mycorrhizal than non-mycorrhizal plant. There was found statistically significant in R* dose (P<0.05).

3.4. Total Chlorophyll Content (mg/ml) in Leaf

Total chlorophyll content was found statistically significant in terms of mycorrhiza*dose interaction (P<0.01) (Table 4). In mycorrhizal and non-mycorrhizal plants were found statistically significant in R* dose than R/2 and R doses to Tukey (P<0.05). Total chlorophyll and carotenoid contents were found higher in non-mycorrhizal plant than mycorrhizal plants in R* dose (P<0.05).

3.5 Carotenoid Content (mg/ml) in Leaf

Mycorrhiza*dose interaction was found statistically significant in terms of carotenoid content (P<0.05). Carotenoid content was found higher in R dose than control and R* doses in mycorrhizal plant (P<0.05). Control and R* doses were found statistically significant from R and R/2 doses (P<0.05). The lowest content was found in R dose (P<0.05) (Table 5).

Table1. Proline concentration (mM/gr) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	0.063^{Bb}	0.006	0.010	0.055	0.075	0.108^{Ba}	0.003	0.004	0.105	0.113	0.086	0.010	0.026	0.055	0.113
R/2	0.060^{Bb}	0.005	0.009	0.054	0.071	0.143^{Aa}	0.003	0.006	0.137	0.148	0.101	0.019	0.046	0.054	0.148
R	0.074^{Bb}	0.013	0.023	0.060	0.101	0.137^{ABa}	0.002	0.003	0.135	0.141	0.106	0.015	0.037	0.060	0.141
R*	0.143^{Aa}	0.005	0.009	0.133	0.149	0.160^{Aa}	0.003	0.005	0.156	0.165	0.151	0.005	0.011	0.133	0.165
G	0.085	0.011	0.037	0.054	0.149	0.137	0.006	0.020	0.105	0.165					
P-Value	Mycorrhiza: 0.000; Dose: 0.000; Mycorrhiza×Dose:0.000***														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum

*statistically significant (p<0,05); ***statistically significant (p<0.001)

In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).

In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

Table 2. Chlorophyll-a content (mg/ml) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	31.824^{Aa}	0.391	0.678	31.067	32.374	32.631^{Aa}	0.117	0.203	32.454	32.853	32.227	0.257	0.629	31.067	32.853
R/2	32.701^{Aa}	0.265	0.459	32.188	33.072	32.518^{Aa}	0.071	0.123	32.376	32.590	32.610	0.129	0.317	32.188	33.072
R	32.242^{Aa}	1.299	2.251	29.704	33.995	27.586^{Bb}	0.612	1.060	26.503	28.621	29.914	1.223	2.996	26.503	33.995
R*	31.709^{Aa}	0.394	0.682	31.206	32.486	31.707^{Aa}	0.264	0.457	31.314	32.209	31.708	0.212	0.520	31.206	32.486
G	32.119	0.328	1.137	29.704	33.995	31.110	0.640	2.215	26.503	32.853					
P-Value	Mycorrhiza:0.022; Dose:0.001; Mycorrhiza×Dose:0.001**														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum

**statistically significant (p<0.01)

In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).

In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

Table 3. Chlorophyll-b content (mg/ml) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	24.608^{Aa}	1.839	3.185	22.226	28.226	26.296^{Aa}	0.532	0.922	25.455	27.282	25.452	0.936	2.292	22.226	28.226
R/2	15.760^{Ba}	1.379	2.388	14.304	18.516	18.696^{Ba}	0.952	1.648	16.956	20.234	17.228	0.996	2.440	14.304	20.234
R	14.762^{Ba}	0.295	0.511	14.400	15.347	15.494^{Ba}	0.234	0.405	15.228	15.960	15.128	0.235	0.575	14.400	15.960
R*	19.693^{ABb}	0.078	0.136	19.538	19.791	28.918^{Aa}	2.485	4.304	24.674	33.279	24.305	2.343	5.740	19.538	33.279
G	18.706	1.268	4.394	14.304	28.226	22.351	1.744	6.043	15.228	33.279					
P-Value	Mycorrhiza:0.001; Dose:0.000; Mycorrhiza×Dose:0.017*														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum

*statistically significant (p<0.05)

In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).

In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

Table 4. Total Chlorophyll content (mg/ml) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	56.432^{Aa}	2.039	3.532	53.293	60.257	58.927^{Aa}	0.423	0.733	58.308	59.736	57.679	1.086	2.660	53.293	60.257
R/2	48.461^{Ba}	1.127	1.952	47.148	50.704	51.214^{Ba}	0.897	1.553	49.543	52.613	49.838	0.891	2.182	47.148	52.613
R	47.004^{Ba}	1.451	2.513	44.104	48.534	43.080^{Ca}	0.407	0.705	42.463	43.849	45.042	1.106	2.710	42.463	48.534
R*	51.402^{ABb}	0.316	0.547	50.997	52.024	60.625^{Aa}	2.229	3.860	56.883	64.593	56.013	2.295	5.621	50.997	64.593
G	50.825	1.236	4.282	44.104	60.257	53.461	2.165	7.500	42.463	64.593					
P-Value	Mycorrhiza:0.012; Dose:0.000; Mycorrhiza×Dose:0.001**														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum
*statistically significant (p<0.05)
In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).
In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

Table 5. Carotenoid content (mg/ml) of mycorrhizal and non-mycorrhizal plants in different pesticide doses (n=12)

Dose	Mycorrhiza (n=3)					Non-Mycorrhiza (n=3)					General (n=6)				
	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max	$\bar{X}$	$S_{\bar{X}}$	S_X	Min	Max
C	8.226^{BCa}	0.527	0.912	7.179	8.847	7.866^{Ba}	0.190	0.329	7.514	8.165	8.046	0.263	0.644	7.179	8.847
R/2	10.938^{ABa}	0.386	0.668	10.281	11.617	10.319^{Aa}	0.143	0.248	10.041	10.519	10.629	0.230	0.564	10.041	11.617
R	12.005^{Aa}	0.112	0.194	11.788	12.160	10.681^{Aa}	0.244	0.422	10.206	11.015	11.343	0.320	0.783	10.206	12.160
R*	9.830B^{Ca}	0.428	0.742	8.974	10.290	6.895^{Bb}	0.855	1.480	5.375	8.332	8.362	0.783	1.918	5.375	10.290
G	10.250	0.454	1.573	7.179	12.160	8.940	0.521	1.806	5.375	11.015					
P-Value	Mycorrhiza:0.012; Dose:0.000; Mycorrhiza×Dose:0.001**														

G= general, C= control, $\bar{X}$= mean, $S_{\bar{X}}$= standard error, S_X= standard deviation, Min= minimum, Max= maximum
*statistically significant (p<0.05)
In the same column, the difference between means without a common capital letter is statistically significant (p<0.05).
In the same column, the difference between means without a common lowercase letter is statistically significant (p<0.05).

4. Discussion

In this study, proline concentration, chlorophyll a, b, total chlorophyll, and carotenoid quantity values were found statistically significant. It has been found that proline concentration of plant leaf statistically significant in terms of mycorrhiza*dose interaction (P<0.01) (Table 1). However, the mean values of proline concentration were found lower in mycorrhizal than non-mycorrhizal plant in all pesticide doses. Most plant synthesizes proline amino acid from glutamine when exposed to stress. (Tort et al., 2004). It is organic compound that is synthesized and accumulated in plant's stress condition. Claussen (2005) was reported that proline is a reliable indicator of the environmental stress in tomato. It has been reported that short-term AZX exposure to the aquatic macrophyte *Myriophyllum quitense* Kunth. occurred oxidative stress and DNA damage occurred (Garanzini and Menone, 2015).

Proline might play a critical role in protecting plants under stress (Velázquez, et al., 2010). Matysik et al. (2002) reported that proline is organic indicator substance which increases the resistance of plants to stress conditions. Many studies have shown a positive correlation between stress tolerance to synthesis of proline (Asraf and Foolad, 2007; Topaloğlu 2010; Özdener and Kutbay, 2011; Yıldıztekin and Tuna, 2015). Ghosh et al. (2022) reported that proline is an antioxidative defense molecule that scavenges reactive oxygen species (ROS) with its metal chelator properties. In our study, proline concentration was found to be higher than the control at all doses applied to fungicide in non-mycorrhizal plant. This increase may be evidence that the stress of tomato from fungicide applications. It has been suggested that the toxic substances which were produced using fungicides inhibits protein synthesis induces change in the enzymatic system and disturbs nitrogen metabolism. Fungicide treatments in cotton (*Gossypium hirsutum* L.) caused accumulation of reactive oxygen species (ROS) (Mohamed et. al. 2018). However, mycorrhizal plants have low proline concentration in all doses. This show that mycorrhizal plants are less affected by fungicide application.

Many studies on abiotic stresses have shown that human activities such as excessive use of pesticides and fertilizers, deforestation and irrigation negatively affect plant growth, development, and yield. However, it has been reported that several studies have confirmed that plants infected with AMF is more resistant to abiotic stress such as drought, salinity, fungicide, and heavy metal contamination (Claussen, 2005; Çetinkaya and Dura, 2010; Erzurumlu and Kara, 2014; Ganugi et al., 2019). Diagne et al. (2020) reported that AMF improved plant growth parameters some species such as *Solanum lycopersicum* L. (Bona et al., 2016), *Cucurbita maxima* Duchesne (Al-Hmoud et. al., 2017), *Piper longum* L. (Gogoi, 2011), *Phaseolus vulgaris* L. (Ibijbijen et al., 1996) in stressed conditions. *Glomus* genera have different reproductive organs which are compatible to unstable environment conditions (Azimi et al., 2018). Gonzalez-Chavez et al. (2002) reported *Glomus intraradices* and

Glomus mosseae vesicular arbuscular mycorrhiza could be suitable for the reconstruction and rehabilitation of plant communities in harsh environmental conditions (Gonzalez-Chavez et al., 2002). Zhu et al. (2009) was reported that the *Zea mays* leaf proline content in was lower in mycorrhizal plant with AM fungus than nonmycorrhizal plants under temperature stress.

Proline results were found like chlorophyll-a, b, total chlorophyll, and carotenoid quantity values in present study. Abiotic stresses factors such as heavy metals, nutrient deficiency and pesticides have negative effect on chlorophyll biosynthesis (Sharma et al., 2020). It was known that the use of pesticide to reduce the amount of chlorophyll and negative effect on the CO2 fixation, Hill reaction and electron transport system (Hopkins, 1995; Sharma et al., 2016). It has been reported plants infected mycorrhiza have higher chlorophyll content (Akay and Kararslan, 2012). Chlorophyll and carotenoid contents were decreased in line with dose increase. On the other hand, it was reported that Antracol WP 70 (Propineb) fungicide cause a reduction in the chlorophyll content (Özörgücü et al., 1990). Sharma et al. (2020) was reported that fungicide reduce photosynthesis by reducing amount photosynthetic pigments. Also, similar results have been reported by Tort et al. (2004). However, all chlorophyll and carotenoid values were found higher in infected mycorrhizal plants in all pesticide doses in our study. AMF watermelon plants higher photosynthetic rate, chlorophyll contents, and biomass accumulation showed to non-AMF watermelon plants, and they are enhancing resistance to soil borne fungal diseases (Wu et al., 2021). It shows that, chlorophyll-a, and carotenoid quantity values such as proline of mycorrhizal plants not affected by pesticide application.

In the present study was investigated fungicide resistance of mycorrhiza in tomato plant in terms of some physiological parameters. In the comparison of these parameters, positive results were determined on resistance of mycorrhiza against pesticide. It is well-known that AM fungi not only stimulate the growth of plants but also contribute to enhancing plant tolerance to abiotic stresses factors (Charest et al., 1993; Augé, 2001). Mycorrhiza is considered as a stimulant for superoxide dismutase, catalase, and peroxidase in leaves. AMF symbiosis can alter plant physiology in a way to cope with stresses under stressful conditions (Miransari et al., 2008). It has reported that knowledge on the mechanisms of dealing with pesticides is limited (Hage-Ahmed et al., 2018). Murrel et al. (2020) reported that AMF colonization can also increase secondary metabolite and defense gene regulation in crop plants. AMF have different strategies as morphological adaptation, protective molecules, and changes in gene expression to deal with organic pollutants (Lenoir et al., 2016; Diagne et al., 2020). It has been documented that some herbicide applications in some crop plants affect AMF root colonization within a few days, reaches balance within a few weeks (Santos et al., 2006).

Today, the damages to the environmental health of fungicide widely used in the agriculture has been scientifically proven. The biggest problem related to pesticides used in the prevention of bacterial and fungal diseases of the damage is irrational and uncontrolled use. The unconscious use of pesticides leads to the accumulation of this in the nature that are not tolerated its damages. Therefore, we must develop alternative applications or methods that will reduce the damages that may occur due to the use of fungicide. Recent mycorrhiza studies indicate that AMF applications that reduce the effects of abiotic stress can be an alternative for sustainable agriculture. The use of arbuscular mycorrhizal fungi (AMF) may be an alternative to improve the defense mechanisms of plants. It has been reported that arbuscular mycorrhizal fungi (AMF) effectively induce phenolic profiles and antioxidant activities in leaves of Potato (*Solanum tuberosum* L.) (Fritz et. al., 2022). Kaymak (2022) stated that alternative environment-friendly methods should be applied in agriculture.

5. Conclusion

In this study, a potential fungicide resistance was tested in mycorrhizal applications. Arbuscular mycorrhiza fungus affected plant growth-promoting traits despite fungicide application. Studied arbuscular mycorrhiza may increase at highly the resistance tolerance to fungicide. Therefore, AMF is suitable option for low chemical input and nature conservation based sustainable agriculture. Thus, it is necessary to conduct further studies on the mechanism of AM fungi in terms of enzymatic.

Author Contributions

T.Ö. (100%) supervised the research, suggested the research methods, structured the paper and edited the manuscript. D.K. (100%) initiated the research idea, developed, organized, analyzed, and interpreted the data and wrote the manuscript. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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EVALUATION OF AN INTERCROPPING SYSTEM: LETTUCE AND RADISH GROWING IN FRUIT SAPLING PRODUCTION

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Abstract: Agricultural production is one of the most negatively affected sectors from increasing population and global warming. Increasing food demand along with narrowing agricultural production areas increased the need for sustainable agricultural approaches where the unit area is better utilized. Intercropping systems are of those approaches based on the principle of growing more than one crop in the same area. In this study, it was aimed to analyze the opportunities of increasing land-use efficiency in open field fruit sapling production. For this aim, lettuce and radish were grown on the inter-rows of almond, apple, apricot, cherry, and pear sapling growing lines. When compared with control plants, results indicated a slight negative effect of intercropping systems on sapling quality. Yield and growth characteristics were lower in the vegetables subjected to intercropping. On the other hand, Land Equivalent Ratio (LER) and Net Economic Profit (NEP) were higher in intercropping systems. LER value varied between 1.86 and 1.97, and NEP value between 3328 and 6962 USD/da. These results indicated that land-use efficiency was increased with the examined intercropping systems. As a result of the study notwithstanding the quality and yield loses, it was concluded that intercropping of lettuce and radish in fruit sapling production is a beneficial growing application for the mentioned aims.

Keywords: Sapling growing, Intercropping, Land use, Sustainable agriculture, Vegetable growing

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1. Introduction

The main goal of agricultural production is to achieve high yield and quality products at the lowest possible cost. There are different ways of reducing costs in agricultural production, such as using less inputs or using lower-cost inputs. The best use of the unit area is also important in reducing costs in agricultural production. Thus, in spite of the increasing population in the world, the limited possibilities of increasing agricultural production areas reveal the importance of the issue. Additionally, global warming and consequently occurring climate change constitutes another restricting factor on agricultural production that compels to produce more with less together with sustainable agriculture approaches.

The yield obtained from the unit area can be increased by growing more than one crop species in the same area in one year, depending on the ecological conditions. Intercropping idea was put forward in this context and is defined as cultivating more than one plant species in the same area simultaneously (Midmore, 1993). Intercropping applications have been successfully implemented in different ways with different plant species, so that better utilization of the unit area and more efficient use of inputs such as water and fertilizer have been possible to generate higher income (Li et al., 1999). Furthermore, the control of biotic factors such as diseases, pests and weeds in the intercropping production system can be performed more effectively (Theunnissien, 1997; Baumann et al. 2001). In addition, the overall yield and net income from intercropping generally increases in comparison to normal cultivation, and the risk of yield loss or price fluctuations of one species can be compensated by the yield from other product or products (Nissen et al., 2001, Ojeifo et al., 2007a). In addition, it contributes to the reduction of erosion as a result of a more intensive plant cover (Zimmermann, 1996; Nissen et al., 2001). In intercropping systems, in order to achieve these advantages, it is necessary to choose the correct combinations of species and varieties (Hauggaard-Nielsen and Jensen, 2001). In this sense, it is important to consider the morphological properties, nutrient requirements and chemical interactions (allelopathy) of the plants to be selected (Davis and Wolley, 1993).

Fruit sapling production is a two-year activity without any income till the end and this poses a significant disadvantage in terms of sapling cost. Therefore, the

possibilities of intercropping application for the evaluation of the land by earning income during this period, which requires a long process for sapling producers, were investigated in some limited previous studies conducted to determine the applicability of intercropping systems for better evaluation of the area by earning income during the sampling production period. Chifflot et al. (2006) indicated that the intercropping system of cherry and walnut saplings and wheat, barley and sunflower had positive effects on stem diameter, shoot height and stem volume index of saplings. Ojeifo et al. (2007a) cultivated watermelon among the mandarin saplings and at the end of the research stated that the intercropping system significantly increases the income. In another study conducted by the researchers, melons were cultivated among the saplings and obtained similar positive results (Ojeifo et al., 2007b). Karlıdağ and Yıldırım (2009a) cultivated lettuce and radish in apricot and cherry sapling production and reported that more income could be obtained from the unit area with this system. Song et al. (2020) have grown sweet potatoes among walnut saplings and similarly reported positive results.

In this study, the possibilities of obtaining the mentioned advantages of intercropping during the saplings production of different fruit species were investigated. For this purpose, saplings belonging to five different fruit species were produced and lettuce and radish were grown among the saplings in two years of production. The obtained results were of guidance for the producers of saplings of the mentioned fruit species.

2. Material and Methods

This study was conducted in research fields of Malatya Turgut Özal University located in Battalgazi district of Malatya in Türkiye (N 38°27'56.12", E 38°21'29.05", 721 m above sea level). The climate of the study area is characterized with hot and dry summer, and cold and long winter periods. The mean temperature ranges between -3.4 and 33.9 °C and mean annual precipitation is 376 mm in the area (MGM, 2020). The climatic conditions were in normal ranges of the experimental area during the study (Table 1).

Table 1. Meteorological data of the experimental area recorded during the study

	Months									
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
	2017									
MT	0	2.9	8.9	12.6	17.7	24.7	29.6	30.3	23.5	14.2
MMT	5.8	10.3	16.5	20.4	25.5	32.6	37.7	37.8	34.5	23.1
MNT	-5.2	-6.1	0.8	4.3	9.5	13.9	17.1	17.2	11.5	5.4
	2018									
MT	4.2	6.35	11.5	15.1	18.9	23.7	27.7	28.1	22.4	15.5
MMT	9.5	12.7	18.9	25.1	26.8	33.1	37.6	37.0	29.4	21.3
MNT	-1.1	0.0	4.2	5.2	11.1	14.4	17.8	19.2	15.5	9.9

MT= mean temperature, MMT= maximum temperature, MNT= minimum temperature.

As part of the study, fruit saplings of almond, apple, apricot, cherry, and pear were produced between the years of 2016 and 2018. Radish and lettuce were grown between the produced saplings in 2017 and 2018. Sapling production process was started by sowing the rootstock seeds of almond (*Prunus dulcis* var. amara) and apricot (wild bitter common apricot, *Prunus armenica* L.) in November 2016. The clonal rootstocks of 'MM-106', 'OHF-333', and 'MaxMa 14' were used for apple, pear, and cherry saplings, respectively. Clonal rootstocks were obtained from commercial rootstock sapling producer companies and planted in March 2017. Grown rootstocks were grafted in September 2017, and the cultivars grafted on almond, apple, apricot, cherry, and pear rootstocks were 'Ferragnes', 'Fuji Kuki', 'Hacıhaliloğlu', '0900 Ziraat', and 'Santa Maria', respectively. The saplings were planted at 110 cm × 25 cm spacing (3636 saplings/da). 'Adranita' lettuce (*Lactuca sativa* L. var. Longifolia) and 'Cherry Bella' radish (*Raphanus sativus* L.) cultivars were the vegetable materials grown on a single line (intrarow spacing of 10 cm for radish and 25 cm for lettuce) at the center of inter space of sapling lines. Control plots of vegetables were planted in recommended grid, 30 × 30 cm for lettuce, and 15 × 5 cm for radish (Günay, 1992; Günay, 1993). In both years of fruit sapling production, the vegetables were grown in the autumn season (harvest mid-November).

Irrigation and fertigation was carried out via drip irrigation. There was no serious drought and nutrient deficiency that would disturb the experiment observed. Weed control was carried out in the form of hoeing and no pesticides applied.

In two consequent study years, heights (cm) and stem diameters (mm) of the saplings measured at 5 cm above the budding union via digital calipers. Similarly, some physical but also some chemical assessments were performed in lettuce and radish plants in both years of the study. As part of physical properties which were measured by using precision scales, tape line and digital calipers; plant weight (g), plant height (cm), stem diameter (mm), stem length (mm), head diameter (cm) were measured in lettuce plants. The growth parameters

of radish were root weight (g), root length (cm), root diameter (cm), plant weight (kg) (whole plant weight including root and leaves), and dry matter (%) which were calculated according to the ratio of fresh root weight and dry root weight after drying of radish root samples in drying oven at 65 °C until the weight was fixed. In both lettuce leaf and radish root juice samples; pH, Total Soluble Solids (TSS) (%), Titratable Acidity (TA) (%), and TSS/TA values were detected. The pH value of juice samples were measured by using digital pH meter (Hanna HI99141) and TSS were determined via hand refractometer (ATC 0-32). Titratable Acidity (TA) was measured according to Haffner and Vestrheim (1997). TSS/TA value, an important index of taste, was calculated according to the ratio of TSS and TA values (Ledbetter et al., 2006).

In order to determine the effectiveness of intercropping systems in terms of unit area usage, Land Equivalent Ratio (LER) was calculated for each treatment combination according to below formula (Vandermeer, 1989). In the formula; A represents the main crop and B is the intercropped crop. I and S indicate the yield of main (AI and AS) or intercropped crop (BI and BS) under intercropping system or sole-cropping. Since the yield of sapling was not depended on intercropping, the ratio of AI/AS was accepted as "1" in the calculation of LER value (Equation 1) (Ojofo et al., 2007b).

Additionally, Net Economic Profit (NEP) (USD/da) value (Equation 2) was calculated to determine the profitability of the applied intercropping systems according to the below formula (Miller, 1982; Karagölge, 1996). Production Value (PV) was calculated by multiplication of yield and unit price of the obtained products (vegetables and saplings) in the study area at the harvest.

$$LER = \frac{AI}{AS} + \frac{BI}{BS} \quad (1)$$

$$NEP = PV - (\mathrm{DC} - \mathrm{IDC}) \quad (2)$$

The experiment was conducted according to random block design in four replicates. Obtained results were statistically analyzed according to Duncan's Multiple Range Test using SPSS 23.0 for Windows. Variations and differences among treatments were determined at the P≤0.05 significance level.

3. Results

3.1. Sapling Quality Evaluations

The effects of different intercropping systems on the diameter and length of the saplings are given in Table 2. When the table is analyzed, it can be seen that intercropping significantly affected the height of apple and pear saplings, but not the height of apricot, cherry and almond saplings in the first year of the study. The height of the apple saplings varied between 118.7 and 130.7 cm and especially the combination with radish decreased the height of apple saplings. In the intercropping combination made with lettuce, the average apple sapling height was determined as 128.8 cm, but it was concluded that the difference was not significant compared to the control group seedlings. Similarly, the length of pear saplings was measured between 122.2 and 130 cm, and it was found that the intercropping system with both species significantly reduced the pear sapling lengths compared to the control. In the same study year, the stem diameter values of the seedlings belonging to any species were not affected by their breeding systems together.

When the results obtained from the second trial year are analyzed, it can be seen that the lengths of the saplings of all species are affected by the intercropping systems evaluated.

Table 2. Height and stem diameter results of intercropped saplings under different intercropping combinations*

Intercropping Combination		Sapling Height (cm)		Stem Diameter (cm)	
		2017	2018	2017	2018
Almond	Control	99.9	167.3[a]	1.11	1.50[a]
	Lettuce	96.5	142.8[b]	1.07	1.23[b]
	Radish	93.7	145.4[b]	1.01	1.31[b]
Apple	Control	130.7[a]	168.0[a]	1.53	1.66[a]
	Lettuce	128.8[a]	159.6[b]	1.51	1.61[ab]
	Radish	118.7[b]	161.4[b]	1.48	1.51[b]
Apricot	Control	159.4	197.2[a]	1.36	1.62
	Lettuce	156.2	168.0[b]	1.24	1.60
	Radish	155.1	165.5[b]	1.33	1.59
Cherry	Control	161.6	177.6[a]	1.19	1.71
	Lettuce	158.6	161.6[b]	1.15	1.65
	Radish	157.8	168.5[b]	1.14	1.68
Pear	Control	130.0[a]	191.2[a]	1.19	1.61[a]
	Lettuce	122.2[b]	165.9[b]	1.12	1.46[b]
	Radish	128.9[b]	178.5[b]	1.17	1.41[b]

[a,b]Differences among the values of a particular character and year signed with different letters are significant at P≤0.05, *years were evaluated separately.

The average height of saplings was measured between 142.8 and 167.3 cm, 159.6 and 168.9 cm, 165.5 and 197.2 cm, 161.5 and 177.6 cm, 165.9 and 191.2 cm for almond, apple, apricot, cherry and pear saplings. Regardless of the species and the plants grown together, sapling height was affected negatively from different plant combinations. Stem diameter values of almond, apple, and pear seedlings varied between 1.41 and 1.61 cm, 1.61 and 1.66 cm, and 1.23 and 1.50 mm, respectively, and were significantly affected by intercropping systems. On the other hand, the diameter values of apricot and cherry saplings were not significantly changed when compared to control saplings.

Even though there were some decreases in sapling height and stem diameter in the intercropping combinations when compared to sole-cropping control lines, overall quality of the obtained saplings at the end of the study was not significantly different as no price difference were occurred during sales of the obtained saplings.

3.2. Vegetable Yield and Quality Evaluations

The yield values of lettuce and radishes grown between the sapling rows of different species are shown in Table 3. When the table is examined, it can be understood that the yield values of both lettuce and radish plants were significantly affected by the intercropping systems in both trial years.

In the first study year lettuce yield values ranged from 2.78 to 3.29 kg/m^2. The highest yield was obtained from sole-cropping plants, while the lowest was obtained from plants grown among pear seedlings. In the second year, the highest lettuce yield which was 2.92 kg/m^2 in average obtained from the control plants and the lowest value was obtained from the plants grown with apple saplings with 2.47 kg/m^2.

When the data of the first year of the radish plants in the table are examined, it is seen that the values varied between 1.44 and 1.60 kg/m^2. The highest value was obtained from the radishes solely grown and the lowest value was obtained from the plants grown among apple trees. The highest yield value for radish in the second trial year was obtained from the control group with 1.24 kg/m^2 and the lowest value was obtained from the plants grown among apple trees with 1.12 kg/m^2.

Data showing the physical properties of lettuce and radishes subjected to different intercropping combinations are presented in Table 4 and Table 5, respectively. Results indicated that the vegetables subjected to intercropping presented worse results in most of the physical characteristics when compared to control plants. On the other hand, there was no significant difference in chemical parameters in both of the study year. For that reason, the results of the chemical parameters are not presented.

Table 3. The effects of different intercropping combinations on lettuce and radish yield*

Intercropping Combination		Yield (kg/m^2)	
		2017	2018
Lettuce	Control	3.29 ^{a}	2.92 ^{a}
	Almond	3.19 ^{ab}	2.57 ^{c}
	Apple	2.89 ^{c}	2.47 ^{d}
	Apricot	3.09 ^{b}	2.52 ^{c}
	Cherry	2.84 ^{c}	2.84 ^{b}
	Pear	2.78 ^{d}	2.75 ^{b}
Radish	Control	1.60 ^{a}	1.24 ^{a}
	Almond	1.53 ^{ab}	1.20 ^{ab}
	Apple	1.47 ^{b}	1.12 ^{b}
	Apricot	1.55 ^{ab}	1.17 ^{b}
	Cherry	1.44 ^{b}	1.14 ^{b}
	Pear	1.51 ^{ab}	1.19 ^{ab}

^{a,b}Differences among the values of a particular character and year* signed with different letters are significant at P≤0.05, *years were evaluated separately.

Table 4. Growth characteristics of intercropped lettuce as affected by different main crops*

Main Crop	Plant Weight (g)		Plant Height (cm)		Stem Diameter (mm)		Stem Length (mm)		Head Diameter (cm)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Control	1048.4 ^{a}	931.0 ^{a}	32.9 ^{b}	29.2	53.6 ^{a}	44.3 ^{a}	47.3 ^{c}	42.0 ^{c}	10.5	9.3 ^{d}
Almond	1029.2 ^{a}	913.9 ^{a}	34.1 ^{a}	30.3	42.0 ^{b}	37.3 ^{b}	85.9 ^{b}	76.3 ^{b}	15.1	13.4 ^{a}
Apple	654.0 ^{d}	580.7 ^{c}	33.5 ^{a}	29.7	32.5 ^{cd}	28.9 ^{d}	89.3 ^{b}	79.3 ^{b}	12.2	10.8 ^{c}
Apricot	560.0 ^{e}	497.3 ^{d}	33.8 ^{a}	30.1	28.3 ^{d}	25.1 ^{d}	92.4 ^{a}	82.0 ^{a}	14.3	12.7 ^{b}
Cherry	912.5 ^{b}	810.3 ^{b}	34.5 ^{a}	30.7	35.9 ^{c}	31.8 ^{c}	92.5 ^{a}	82.2 ^{a}	14.8	13.1 ^{a}
Pear	931.7 ^{c}	827.4 ^{ab}	33.7 ^{a}	29.9	37.8 ^{c}	33.6 ^{bc}	87.7 ^{b}	77.9 ^{b}	14.2	12.6 ^{b}

^{a,b}Significant differences (P<0.05) are indicated by different letters, *years were evaluated separately.

Table 5. Physical assessment results of intercropped radish samples as affected by different main crops

Main Crop	Root Weight (g/plant)		Root Length (cm)		Root Diameter (cm)		Plant Weight (g/plant)		Dry Matter (%)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Control	200.1 a	177.7 a	7.71 a	6.85 a	6.80 a	6.04 a	6.5 ab	5.8 ab	3.77 b	6.04 a
Almond	213.2 a	189.3 a	7.79 a	6.92 a	6.98 a	6.20 a	6.0 b	5.3 b	3.19 c	6.20 a
Apple	109.4 d	97.1 d	6.22 b	5.52 c	5.37 c	4.77 c	4.9 c	4.5 c	3.79 b	4.77 c
Apricot	82.8 e	73.6 e	5.55 c	4.93 d	4.77 d	4.24 c	4.7 c	4.6 c	4.52 a	4.24 c
Cherry	148.7 c	132.1 c	6.94 b	6.16 b	6.17 b	5.48 b	6.1 b	5.4 b	3.07 c	5.48 b
Pear	169.9 b	150.8 b	7.37 a	6.54 ab	6.49 b	5.76 b	7.4 a	6.5 a	2.97 d	5.76 b

Significant differences (P<0.05) are indicated by different letters, *= years were evaluated separately.

3.3. LER and NEP Results

Table 6 presents the LER results calculated for different intercropping combinations. The highest LER value was obtained from the almond seedlings and radish plants (1.97). The lowest value was obtained with the combination of apple saplings and lettuce with 1.86. LER values of other intercropping systems varied between 1.89 and 1.96.

Table 6. Land Equivalent Ratio (LER) and Net Economic Profit (NEP) values in different sapling+vegetable intercropping systems

Intercropping Combination		LER	NEP (USD/da)
	Sole	1.00^{d}	3328^{f}
Almond	Lettuce	1.93^{ab}	4409^{ef}
	Radish	1.97^{a}	4055^{e}
	Sole	1.00^{d}	5624^{b}
Apple	Lettuce	1.86^{b}	6962^{a}
	Radish	1.92^{ab}	6275^{ab}
	Sole	1.00^{d}	4509^{def}
Apricot	Lettuce	1.90^{ab}	5878^{ab}
	Radish	1.96^{a}	5201^{b}
	Sole	1.00^{d}	5191^{bc}
Cherry	Lettuce	1.92^{ab}	6349^{ab}
	Radish	1.91^{ab}	5901^{ab}
	Sole	1.00^{d}	4759^{d}
Pear	Lettuce	1.89^{ab}	5941^{ab}
	Radish	1.95^{ab}	5543^{b}

^{a,b}Significant differences (P < 0.05) are indicated by different letters.

4. Discussion

When the results of the saplings subjected to both sole-cropping and intercropping are examined, it can be observed that the length of the saplings was negatively affected especially in the second year of the study in both the lettuce and the radish grown saplings. Similarly, although the sapling diameter was not negatively affected in the first year of the study, in the second year it decreased in almond, apple and pear saplings with intercropping.

Findings reported in previous studies were mostly different in terms of quality of saplings. Chifflot et al. (2006) found that wheat, barley and sunflower cultivation among cherry and walnut saplings had a positive effect on sapling diameter, shoot length and stem volume index in saplings. Similarly, Ojeifo et al (2007a, 2007b) reported that intercropping of melon and watermelon with mandarin saplings do not have a negative effect on plant growth in saplings or even positively affect them. Karlıdağ and Yıldırım (2009b) also stated that lettuce and radish grown between apricot and cherry sapling production rows do not have a negative effect on seedling height and stem diameter. The main reason of the emergence of a different result in this study compared to previous studies would be the differences in cultivars and planting intervals. Nevertheless, the quality changes in the saplings that were subjected to intercropping were limited, and were not at a level that would affect the general quality of the seedlings and therefore the sales price. As a matter of fact, the obtained NEP results confirmed this situation. Additionally, higher LER values were obtained from all intercropping applications when compared with sole-cropping. Both NEP and LER results were in accordance with the results reported by Karlıdağ and Yıldırım (2007), Karlıdağ and Yıldırım (2009a, 2009b).

The fact that the LER value is greater than 1 indicates that intercropping is more effective in terms of yield and land use than sole-cropping; adversely LER value is less than 1 means that intercropping is less effective than sole-cropping (Vandermeer, 1989). It has been reported that this situation caused by more efficient use of resources such as light, plant nutrient and water in the unit area compared to sole-cropping in intercropping practices consisting of plants with different morphological structure and development time (Ojeifo ve ark., 2007a, 2007b). Tripati et al (2019) reported that in the intercropping of some medicinal-aromatic plants with peach trees, the fruit yield increased in the trees subjected to intercropping compared to the control plants, and the income from the unit area increased with the grown medicinal-aromatic plants. In another study, intercropping of watermelon and melon with mandarin saplings was tried and it was reported that it significantly increased the income obtained from the intercropping unit area (Ojeifo et al. 2007a, b). Song et al. (2020) reported similar findings in their study conducted on intercropping of sweet potato with walnut saplings.

When the obtained vegetable yield results are analyzed, significant yield decreases were observed in most of the

intercropping applications compared to control plants especially in the second year of the study, but also in the first year. Similarly, in a general point of view, almost all values of plant characteristics decreased in both radish and lettuce plants subjected to intercropping compared to control. Besides plant and stem height values of lettuce were higher in intercropping applications. Similar results were also reported by Karlıdağ and Yıldırım (2009a), and this is thought to have occurred as a result of the shading effect of the saplings. The effects were relatively lower in vegetables grown with almond saplings. This was probably due to the relatively shorter length of the almond seedlings and the smaller shading effect due to the smaller canopy volume.

In intercropping systems consisting of different underground and aboveground structures, cross-species competition for light and ground resources may decrease or disappear altogether. It has been suggested that intercropping systems occupy a wider soil area because of the different root structures of the species that make up the system compared to sole-cropping and they need more resources at different times, and therefore they benefit from soil resources such as plant nutrients and water more effectively (Francis, 1989; Woolley and Davis, 1991; Morris and Garrity, 1993).

Likewise, in intercropping systems consisting of plant species having different growth rates and periods of demand to growth resources, the resources are used in the best way, so land-use efficiencies are very high (Fukai and Trenbath, 1993). As a matter of fact, the growth and development of the saplings towards the autumn slow down and the amount of used resources also decreases which provided a more suitable environment for vegetable growth in the intercropping systems examined within the scope of the study.

5. Conclusion

Sapling production is an important and sensitive field of agricultural production because of the influence the orchard quality by which they are planted. On the other hand, especially open field sapling production takes two years of production without any income until the end of the production period. Besides, increasing population required increasing amounts of food supply. For all those reasons, this study was conducted to evaluate the efficiency of growing lettuce and radish in the sapling production parcels. In order to determine the efficiency of assessed intercropping systems sapling and vegetable quality attributes, LER and NEP values were compared with sole-cropping control plants. Results indicated significant yield and physical quality decreases in vegetables together with no effect on chemical attributes. Sapling height and stem diameter slightly decreased in some of the combinations, whereas this was not found significant on overall sapling quality. Thus, LER and NEP values were significantly increased by the intercropping systems applied as part of the study. As a result of the study it was concluded that intercropping of lettuce and radish in fruit sapling production would be beneficial for sapling producers and for increasing the food supply.

Author Contributions

H.K. (100%) designed the study and set up the trials. H.K. (20%), İ.K.K. (20%), F.E.K. (20%), R.K. (20%) and T.K. (20%) conducted the study. H.K. (34%), İ.K.K. (33%) and F.E.K. (33%) analyzed the data. F.E.K. (34%), İ.K.K. (33%) and H.K. (33%) wrote the manuscript. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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EFFICACY OF NATIVE *BEAUVERIA BASSIANA* AND *B. PSEUDOBASSIANA* ISOLATES AGAINST INVASIVE BROWN MARMORATED STINK BUG, *HALYOMORPHA* HALYS (STÅL) (HEMIPTERA: PENTATOMIDAE)

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Abstract: Invasive brown marmorated stinkbug (BMSB), [*Halyomorpha halys* (Hemiptera: Pentatomidae)] are caused significant yield and quality losses in hazelnut orchards. This study evaluated the efficacy of 7 native *Beauveria bassiana* and *B. pseudobassiana* isolates against BMSB adults at 1×10^8 conidia mL^{-1} concentration under laboratory conditions. The LT_{50} and LT_{90} values for all isolates used in the study ranged between 5.37-7.74 and 9.85-18.35 days, respectively. Moreover, the mortality rates caused by all isolates were between 72 and 96%. The lowest LT_{50} value (5.37 days) was recorded for TR-SM-11, whereas the lowest LT_{90} (9.85 days) value was noted for TR-D-1 isolate. Similarly, the LT_{90} and LT_{50} values were 10.82 and 7.74 days for TR-SM-11 and TR-D-1, respectively. The LT_{90} and LT_{50} values for TR-SK-1 isolate were 6.16 and 10.25 days, respectively. These isolates (TR-D-1, TR-SK-1, TR-SM-11) caused the highest mortality rates (96, 96 and 92%, respectively) at the end of the 11th day. TR-SM-11, TR-D-1 and TR-SK-1 isolates of *B. bassiana* seemed to be one of the most promising and potential biological control agents against BMSB. However, further studies are needed to evaluate the efficacy of these isolates against BMSB under field conditions.

Keywords: Hazelnut, Invasive pest, BMSB, Entomopathogenic fungi, *Beauveria*, Biocontrol

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1. Introduction

Invasive brown marmorated stinkbug (BMSB), [*Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae)] is a well-known and harmful insect. It is indigenous to China, Japan, Korea and Taiwan. It has a high infestation and spread rate throughout the world (Zhu et al., 2012). The BMSB was first recorded in North America during the mid-1990s in Pennsylvania (Hoebeke and Carter, 2003) and then spread in 44 states of the USA and 4 regions of Canada (Anonymous, 2018). In Europe, it was first detected in Switzerland in 2004 (Haye et al., 2014). Afterwards, it has been recorded in several European countries.

The BMSB is a polyphagous pest with a wide host range; therefore, capable of causing significant damage to approximately 300 plant species, including ornamental plants, agricultural crops, tree nuts and vegetables (Nielsen and Hamilton, 2009). Both adults and nymphs of the pest cause damage to plants by piercing the surface, injecting digestive enzymes and sucking plant fluids (Rice et al., 2014). Damaged products lose market value to significant extent. Moreover, significant economic losses are caused by the pest in pome and stone fruit, corn and hazelnut in the invaded areas (Bariselli et al., 2016). For this reason, excessive amounts of insecticides are applied against BMSB, which pose negative impacts to environment and human health. Moreover, excessive of insecticides disrupt integrated pest management (IPM) programs. Hence, the current studies concentrated on alternative, effective and eco-friendly management methods such as biological control (Moraglio et al., 2019).

Several natural enemies of BMSB have been identified from Asia (Leskey et al., 2012). The most promising biological control agents recorded are hymenopteran egg parasitoids, i.e., *Trissolcus japonicus* (Ashmead) and *T. mitsukurii* (Ashmead) (Hymenoptera: Scelionidae) (Yang et al., 2009). In addition, adventive populations of *T. mitsukurii* and *T. japonicus* were found out in northern and northwest northwest Italy (Peverieri et al., 2018; Moraglio et al., 2019), Switzerland (Stahl et al., 2019) and North America (Talamas et al., 2015). However, limited studies have been conducted regarding the efficacy of

entomopathogenic fungi (EPF) against BMSB globally. Burjanadze (2020) reported that *B. bassiana* sensu lato (Hypocreales: Cordycipitaceae) and *Isaria cf fumosorosea* (Hypocreales: Cordycipitaceae) isolated from BMSB and caused 72-90.5% mortality of BMSB adults in Georgia. Similarly, a study conducted in the USA reported that *B. bassiana* strains were the most effective and caused 100% mortality in 12 days after the treatment (Gouli et al., 2012). In addition, Karun and Sridhar (2013) revealed that BMSB adults were naturally infected by *Ophiocordyceps nutans* (Ophiocordycipitaceae, Hypocreales) in the forests of Western Ghats of India and Japan. EPF infect insects through integument, rapidly colonize the hemocoel and then cause mortality through sporulation on the surface of the host under favorable environmental conditions (Butt et al., 2016).

Currently, the pest has established in many places along the Black Sea coast especially Artvin and Rize that continuing distrubution in Türkiye (Ak et al., 2019; Ozdemir and Tuncer, 2021). Considering the risk map prepared according to ecological requirements of BMSB in Türkiye (Kistner, 2017), hazelnut is under the major threat by BMSB in Black Sea region (Ozdemir and Tuncer, 2021). Erper et al. (2016) reported that EPF are suitable to use in the Black Sea region due to its rainy and humid environmental conditions and low annual temperature. Therefore, this study aimed to evaluate the efficacy of six native *B. bassiana* and one *B. pseudobassiana* isolates against adults of BMSB. The isolates were isolated from green shield bug [*Palomena prasina* L. (Hemiptera: Pentatomidae)], an important pest of hazelnut production areas (Ozdemir et al., 2021). The result will provide valuable insights on the possible use of EPF against BMSB. Furthermore, isolates found to be effective can contribute in the scope of IPM against BMSB in order to reduce insecticide use in hazelnut production areas of Türkiye.

2. Material and Methods

2.1. Collection of Bugs

Adults of BMSB were collected from various hazelnut, citrus and kiwi orchards situated in different districts of Rize province in eastern Black Sea Region, Türkiye. Healthy adults of BMSB were utilized from the adults brought to the laboratory (Ondokuz Mayıs University, Agriculture Faculty, Plant Protection Department, Samsun, Türkiye) in the experiments.

2.2. Preparation of Entomopathogenic Fungi

A total of 7 native EPF isolates isolated from infected *P. prasina* in hazelnuts orchards, Black Sea region of Türkiye in 2018-2019 and they were tested in this study (Table 1) (Ozdemir et al., 2021). The 7 native EPF isolates belonged to *B. bassiana* (TR-SM-10, TR-SM-11, TR-SM-2, TR-SK-1, TR-D-1, TR-D-2) and *B. pseudobassiana* (TR-SM-1). The isolates were incubated on potato dextrose agar (PDA; Merck Ltd., Darmstadt, Germany) at 25±1°C for one week to obtain conidia, which were suspended in sterile distilled water, filtered through 3 layers of sterile cheesecloth and diluted to a concentration of 1×10^8 conidia mL^{-1} plus 0.02% Tween 20. The resulting spore suspension was adjusted at the concentration 1×10^8 conidia mL^{-1} using Neubauer hemocytometer under Olympus CX31 light microscope (Olympus America Inc., Lake Success, NY) (Tuncer et al., 2019).

Table 1. Species, hosts and locations of entomopathogenic fungi isolates used in the study

Species / Isolate denomination	Genbank accession numbers	Host	Location of collection
Beauveria bassiana / TR-SM-10	MT102327	*Palomena prasina*	Hazelnut orchards
Beauveria bassiana / TR-SM-11	MT102328	*Palomena prasina*	Hazelnut orchards
Beauveria bassiana / TR-SM-2	MT102329	*Palomena prasina*	Hazelnut orchards
Beauveria bassiana / TR-SK-1	MT102330	*Palomena prasina*	Hazelnut orchards
Beauveria bassiana / TR-D-1	MT102331	*Palomena prasina*	Hazelnut orchards
Beauveria bassiana / TR-D-2	MT102332	*Palomena prasina*	Hazelnut orchards
Beauveria pseudobassiana / TR-SM-1	MT102333	*Palomena prasina*	Hazelnut orchards

2.3. Application of Entomopathogenic Fungi against *Halyomorpha halys* Adults

Five adults of BMSB were released into one L plastic transparent and lid cups (disinfected by 70% ethanol) containing fresh persimmon (*Diospyros kaki* L.) fruits. Bottoms of the cups were covered by filter paper moistened with sterile-distilled water. Conidial suspension (1×10^8 conidia mL^{-1}) of each EPF isolate (TR-SM-10, TR-SM-11, TR-SM-2, TR-SK-1, TR-D-1, TR-D-2 and TR-SM-1) was applied to BMSB adults (2 mL per cup), using a Potter spray tower (Burkard, Rickmansworth, Hertz UK). The spray tower was cleaned by 70% ethanol and sterile distilled water after each application of EPF suspension. Only sterile-distilled-water containing 0.02% Tween 20 was sprayed to the control group. The cups were incubated at 25±1°C and 70±2% (RH), 16:8 h light: dark photoperiod for 11 days. Mortality rates were monitored for eleven consecutive days and the trial was repeated, using the same number of different individuals (n=25 insects/day/isolate) with 5 replications per day for each isolate to assure that each day's observations were mutually independent (Robertson et al., 2017). Mortality rates were determined for the corresponding day and the dead insects on that day were removed from the cups. The same procedure was repeated every day for the control groups (Ozdemir et al., 2021). Additionally, a procedure was used to study fungus sutructures on dead insects, and mycosis rates were calculated; for further

details, see Kocacevik et al (2016).

2.4. Conidial Germination Assessment

The viability of conidia of the 7 isolates was determined. A spore suspension was adjusted to 1×10^4 conidia mL^{-1} and 0.1 mL was sprayed onto Petri dishes (9 cm diameter, containing PDA), and the dishes were incubated at 25°C for 24 h. After 24 h of incubation, percentages of germinated conidia were counted using an Olympus CX-31 compound microscope (Olympus America Inc., Lake Success, NY) at 400X magnification. Conidia were considered as germinated when they produced a germ tube at least half of the conidial length (Erper et al., 2016).

2.5. Statistical Analyses

Daily mortality rates were corrected according to the Abbott formula when mortality rate in the control exceeded 5% (Abbott, 1925). The LT_{50} and LT_{90} values were determined by probit analysis using log-probit method (POLO-PLUS ver.2.0). The slopes of regression lines were compared with each other using standard errors. The LT_{50} and LT_{90} values of the isolates were compared using confidence intervals (95%) (Ozdemir et al., 2021).

3. Results

Conidia viability of *B. bassiana* (TR-SM-2, TR- SM-10, TR-SK-1, TR-SM-11, TR-D-1, TR-D-2) and *B. pseudobassiana* (TR-SM-1) isolates used in the study before the bioassays and the conidial germination was nearly 100%. In the present study, all native fungal isolates obtained from *P. prasina* were applied against BMSB at 1×10^8 conidia mL^{-1} concentration under the laboratory conditions evaluating their efficacy (Figure 1). LT_{50} and LT_{90} values of these isolates differed ($P<0.05$) and several isolates had high virulence. Especially, TR-SM-11, TR-SK-1 and TR-D-1 isolates were more virulent than the isolates TR-SM-10, TR-D-2, TR-SM-1, TR-SM-2 and rapidly lethal to BMSB and these isolates presented LT values close to each other's (Table 2). The TR-SM-1 isolate was the least virulent among the isolates applied. In addition, approximately 100% mycosis was obtained in all treatments and at 11 days following inoculation, aggressive colonisation on BMSBs by the isolates was visible (Figure 2).

Table 2. Probit analysis data on mortality time of *Halyomorpha halys* adults after application of at 1×10^8 conidia mL^{-1} of *Beauveria bassiana* and *B. pseudobassiana* isolates

Isolates	LT_{50} (95% CI)	LT_{90} (95% CI)	Slope ± SE	Regression	X^2	*Df*	Heterogeneity
TR-SM-1	7.20(6.37-8.31)^{bc}	18.35(14.28-27.85)^{b}	3.15±0.43	y= -2.7+3.15x	36.13	53	0.68
TR-SM-2	6.73(6.12-7.42)^{bc}	13.08(11.20-16.61)^{ab}	4.44±0.54	y= -3.68+4.44x	35.37	53	0.66
TR- SM-10	6.60(6.04-7.19)^{b}	11.85(10.38-14.44)^{ab}	5.04±0.60	y= -4.13+5.04x	36.22	53	0.68
TR-SK-1	6.16(5.68-6.66)^{ab}	10.25(9.19-11-97)^{a}	5.80±0.63	y= -4.58+5.8x	42.19	53	0.79
TR-SM-11	5.37(4.82-5.92)^{a}	10.82(9.38-13.27)^{a}	4.21±0.47	y= -3.07+4.21x	49.37	53	0.93
TR-D-1	7.74(7.37-8.11)^{c}	9.85(9.27-10.74)^{a}	12.21±1.47	y= -10.86+12.21x	42.23	53	0.79
TR-D-2	6.79(6.22-7.40)^{bc}	12.19(10.65-14.96)^{ab}	5.04±0.61	y= -4.19+5.04x	48.75	53	0.92

^{a,b}Different letters in same column show statistical difference ($P<0.05$).

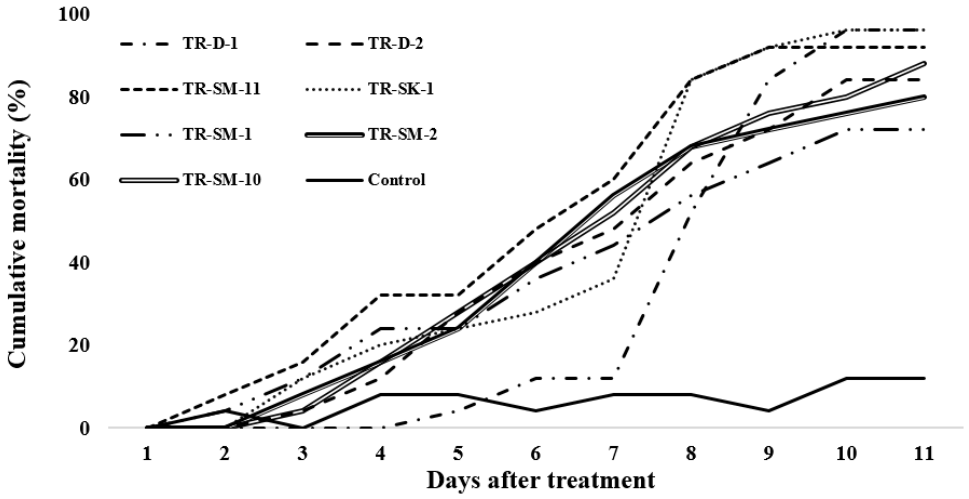

Figure 1. Daily mortality rates of adults of *Halyomorpha halys* treated with 1×10^8 conidia mL^{-1} concentration of *Beauveria bassiana* and *B. pseudobassiana* isolates.

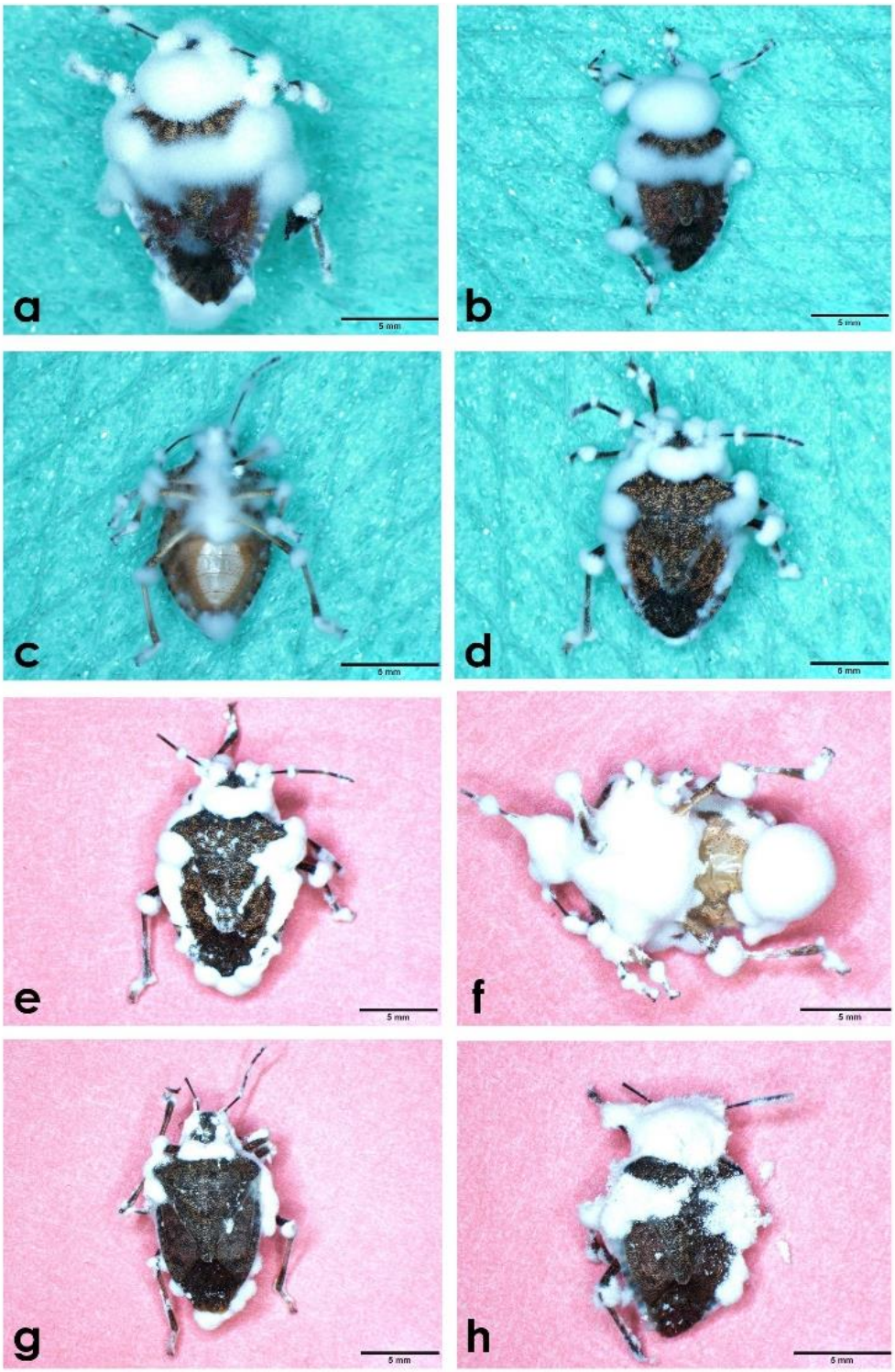

Figure 2. Mycosis of TR-SM-11 (a, b), TR-D-1 (c), TR-SK-1 (d), TR-SM-10 (e), TR-SM-1 (f), TR-SM-2 (g) and TR-D-2 (h) isolates on the body surface of *Halyomorpha halys* adults 11 days after inoculation.

4. Discussion

Some of the tested isolates were highly pathogenic against BMSB adults after 11 days of application in laboratory bioassays. In addition, in the studies conducted on BMSB by Parker et al. (2015), it was reported that adult BMSB was more resistant to the infection than nymphal stages. Therefore, possibly *Beauveria* isolates used in this study would have higher efficacy on the nymphs.

The native *B. bassiana* (TR-SM-2, TR- SM-10, TR-SK-1, TR-SM-11, TR-D-1, TR-D-2) and *B. pseudobassiana* (TR-SM-1) isolates used in the study were isolated from *P. prasina*, which had the highest population density among stink bugs and causes serious yield and quality losses to hazelnut production of Türkiye (Ozdemir et al., 2021). Evaluating the efficacy of the native *Beauveria* isolates,

which are highly pathogenic against *P. prasina* is important as an alternative management method for BMSB. Bioassays in this study revealed that TR-SM-11, TR-D-1 and TR-SK-1 isolates were the most effective against BMSB. These isolates were applied at 1×10^8 conidia mL^{-1} concentration and caused 96, 96 and 92% mortality rates in BMSB adults on the end of 11th day, respectively. The mortality rates of other isolates ranged from 72 to 88% at the same concentration. It is reported that *Beauveria* isolates used in this study caused 100% mortality against *P. prasina* adults at the same concentration within 6-10 days. Moreover, TR-SM-11, TR-SK-1 and TR-D-1 isolates were the most effective isolates against native *P. prasina*, causing 100% death of the bug population 6, 7 and 8 days post application, respectively (Ozdemir et al., 2021). Similarly, in the present study, although these 3 isolates had nearly 100% efficacy against BMSB adults, however the mortality occurred longer times, i.e., 11 days after treatment. While the LT_{50} - LT_{90} values for TR-SM-11, TR-SK-1 and TR-D-1 isolates sprayed at 1×10^8 conidia mL^{-1} concentration on adult *P. prasina* were 3.65 - 6.17, 3.82 - 5.76 and 6.14 - 7.05 days, respectively (Ozdemir et al., 2021), LT_{50} - LT_{90} values of them isolates against BMSB adults were 5.37-10.82, 6.16-10.25 and 7.74-9.85 days, respectively. Considering the LT_{50} - LT_{90} values and mortality rates (%) for *P. prasina* and BMSB, the LT values for BMSB were higher, while mortality rates were lower. Sevim et al. (2010) reported that the native isolates obtained from specific regions have been ecologically compatible with the native pests. In addition, efficacy of the native isolates on non-target organism or the exotic pests such as BMSB decreased significantly. For this reason, the native isolates used in the present study showed relatively lower efficacy against exotic BMSB compared to *P. prasina*.

Gouli et al. (2012) reported that BotaniGard® commercial bio-product (*B. bassiana* strain), from 3 *B. bassiana* and 2 *Metarhizium anisopliae* isolates caused 100% mortality of BMSB adults 12 days post treatment. In a similar study conducted with same bio-product, Parker et al. (2015) reported 95-100% mortality 12 days after treatment against the 2nd nymphal stage of the bug at 1 × 107 conidia/ml concentration. Another *B. bassiana* (ET 10) isolate caused 76.19% mortality, 11 days post treatment on different nymphal stages of BMSB at 5.7×10^5 conidia/ml (Tozlu et al., 2019). Furthermore, prevalence rates of naturally-infected BMSB adults by EPF and collected from several sites, including hazelnut orchards in the Black Sea basin in Georgia had 0.9% infestation of *B. bassiana* and 0.3% of *Isaria fumosorosea*. Moreover, Bover-Ge (*B. bassiana*-024 strain) registered in Georgia as a mycoinsecticide, bioassyed at 1×10^7 and 1×10^8 conidia/ml concentration against BMSB adults under laboratory condition caused 72-90.5% mortality at the end of 12 days (Burjanadze et al., 2020). Similarly, *B. bassiana* strains of TR-D-1 and TR-SK-1 caused 96% mortality rate in this study. Thus, these isolates can be considered as a promising bio-control agent against BMSB. Additionally, *B. pseudobassiana* (TR-SM-1) had the lowest pathogenicity.

Patel et al. (2006) reported that feeding and laying egg of the pest insects infected by *B. bassiana* were decreased during infection period. However for successful field applications, ecological suitability (relative humidity) that will trigger the natural infection chain of the EPF as well as high virulence is important (Bugti et al., 2020). The native isolates that have high virulence used in this study can be used in high infestation areas, which is in hazelnut growing areas in the coastal of Black Sea region, of BMSB. Moreover, climatic suitability of the region for EPF further strengthens this inference. Chemical compounds deposited on integument of pentatomid bugs has potential to act as barrier against microbial infection (Sosa-Gómez and Moscardi, 1998). Additionally, direct contact of metathoracic gland components of some stink bugs have the detrimental effect on conidial germination of some EPFs (Lopes et al., 2015). Therefore, it is of paramount importance that the natural population of the pest can be exposed to EPF with enough amount and prolonged period, with supporting by abiotic factors of the pathogen (Bugti et al., 2020).

5. Conclusion

BMSB is a highly polyphagous invasive pest that threats many agricultural crops, particularly hazelnut in Türkiye. The Black Sea region has very suitable climatic conditions for BMSB. In this study, *Beauveria* isolates obtained from *P. prasina* in main hazelnut production areas of Türkiye were tested, the present results showed that TR-SM-11, TR-D-1, TR-SK-1 isolates belonging to *B. bassiana* seemed to be the most promising and potential biological control agents of BMSB. However, further studies are needed to evaluate their efficacy against BMSB under field conditions.

Author Contributions

I.O.O. (25%), C.T. (25%), M.U. (25%) and E.Y. (25%) designed the study, supervised the work, and wrote the manuscript with input from all authors. I.O.O. (25%), C.T. (25%), M.U. (25%) and E.Y. (25%) carried out the experiments. C.T. analyzed the data. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Ethical Consideration

Ethics committee approval was not required for this study due to the use of research material not included in the definition of experimental animals in the study

(Animal experiment ethics committee regulation on working procedures and principles, Article 4-d).

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Research Article
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BIBLIOMETRIC ANALYSIS FOR GENOME-WIDE ASSOCIATION STUDIES IN ANIMAL SCIENCE

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Abstract: The main idea of the study is to determine the trends in recent years in the field of animal science, by examining 379 studies with the term "genome-wide association studies" in the title of the article published within the scope of SCI-Expanded between 2007 and 2021, within the scope of bibliometric analysis. In this context, the term of "Genome-Wide Association Studies" was searched in the Web of Science database in the study titles and the bibliometric data of the studies were accessed in plaintext format. The bibliometric results show that GWAS within animal science is developing steadily as a field of scientific research and is currently a highly topical issue. GWAS has been one of the most popular research areas due to its application in many different fields such as cell biology, plant sciences, zoology, animal science, etc. In the light of this information, it can be listed as an important contribution that GWAS studies with bibliometric analysis are still up-to-date and that the studies to be done will increase their contribution to animal science.

Keywords: Genome-wide association studies, GWAS, Bibliometric analysis, Animal science

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1. Introduction

Breeding studies aiming to increase genetic capacity and improve environmental factors have come to the fore. Animal breeding is expressed as an effort to increase the relative proportion of economically used animals in the population in the next generations (Wellmann, 2019). Classical animal breeding studies, which have been continuing since the 18th century, continue today. In classical breeding methods, genotypic and phenotypic parameters that will help select individuals that will form future generations can be estimated by using the records of the yield characteristics of animals (Ertugrul et al., 2002). To achieve this goal, there are many methods for estimating the genotypic value. The advancing technology in biology and molecular genetics has allowed the identification of genes and polymorphisms responsible for developing functional traits that are important in breeding studies in farm animals (Smołucha et al., 2020). Modern breeding programs have been received by estimating the genetic values of selection candidates based on phenotypic and pedigree information and then making selection decisions on this information.

In this era, breeding value is achieved by utilizing the knowledge of all the markers in the genome on the contrary using the limited number of marker information of animals. This breeding value has been named genomic breeding value (Genomic Breeding Value: GEBV). The selection made according to the GEBV of animals is called "genomic selection" (Meuwissen et al., 2001). In genomic selection, parameter estimations obtained by using a training population with genetic marker and phenotypic values are also used to estimate the breeding values of individuals in the test population for which only the marker knowledge is available. It was possible to identify genes held by characteristics of economic value to microsatellite markers primarily by QTL mapping in the 90s (Lipkin et al., 1998). Today, however, with the onset of all genome sequencing technologies and the availability of affordable panels of all genomes single nucleotide polymorphisms (SNPs), SNPs are used for planning besides phenotype and pedigree information. To date, many QTLs have been described in several separate research papers (Sharma et al., 2015). However, it is impossible to determine gene effects in a genomic selection where genetic parameters are estimated. The Genome-Wide Association Studies (GWAS) method comes to the fore to overcome this situation.

The first GWAS study published by Klein et al. (2005) discovered a genetic variation relating to a higher risk of age-related macular degeneration. After that, the National Human Genome Research Institute (NHGRI) announced an electronic record of GWAS in 2008 (Mansiaux and Carrat, 2012). GWAS in farm animals have made popularity in mapping QTL to economically essential features such as calving ease, fat and protein content, meat quality and quantity, milk yield, egg production, fertility characteristics, etc. (Sharma et al., 2015). GWAS examines genotyped SNPs in the genome and their relationship to phenotype (Zeng et al., 2015;

Khanzadeh et al., 2020). GWAS has been conducted for many species in livestock (Schook et al., 2005; Zimin et al., 2009; Gu et al., 2011). A GWAS yields by testing each SNP, in order, to associate with the yield trait under consideration. The critical hypothesis in GWAS is that important associations appear because the SNP is in LD with a causative mutation affecting its feature and thus close to it (Hayes and Goddard, 2010).

Since its discovery, GWAS has been one of the most popular research areas due to its application in many different fields such as cell biology, plant sciences, animal science, etc. According to the Web of Science data, there are 20208 publications related to GWAS, and also if we give some examples related to livestock, the first studies were in 2007. Abasht and Lamont (2007) studied the broiler population. Long et al. (2007) also studied GWAS for broilers. In addition, Charlier et al. (2008) reported that they examined fine-scale mapping of the disorder of five recessive genes in cattle using SNP associations. There are hundreds of studies on GWAS, such as these examples. However, as far as we know, no article in the livestock science has presented a general bibliometric perspective of the GWAS.

In this context, bibliometric analysis is a research area that attracts more and more attention in the scientific society and is determined by the rapid improvement of computers and internet (Bar-Ilan, 2008; Merigó and Yang, 2017; Celik, 2021). Bibliometric analysis is a basic approach used to analyze investigation and takes its foundation from the public library and information science.

This paper aims to afford a general indication of GWAS research using bibliometric methods from 2007 to 2021. The Web of Science (WoS) database was used to collect information for this aim. The objective is to identify the most beneficial and effective research in GWAS and make sure the current progression of the area by considering the most prominent papers and authors. Most of the results follow mutual advice. However, we get several specific cases that show in what manner the field of GWAS is increasing, with some topics being very widespread and highly cited. In contrast, some topics do not cite enough citations.

2. Material and Methods

We made a topical query in terms of "Genome-Wide Association Studies" to refine the animal science field using the WoS database. The bibliographic information of 379 animal science studies out of 20208 studies with GWAS was used as a material from 2007 to 2021.

Bibliometric analysis is a scientific computer-assisted analysis method that identifies research, authors, and their relations by covering all the publications published on a particular subject (Han et al., 2020). However, bibliometric analysis can provide comprehensive visualization and relational information on the chosen topic to understand the overall picture. The first bibliometric analysis examines the most influential publications, primarily according to author or citation information. In recent bibliometric analysis, sociometric analysis and network analysis based on keywords, titles and abstract data have been adopted.

Bibliometric analysis is a process from beginning the data collection to visualization of the analysis results. The data collection process begins the search of the issue with the essential keywords in Web of Science (WoS).

The term social network is a structure that shows the interaction, collaboration, and effects between people in a social context (Celik, 2021). Social network analysis examines the social structure that is the subject of research and its effects (Tindall and Wellman, 2001). The primary purpose of social network analysis is to define and visualize the social network structure, model it statistically, and generate information from the network (Celik, 2021).

This study performed a bibliometric analysis for GWAS, widely used in animal science studies in recent years. All statistical evaluations were made using R software with the package of "bibliometrix" (R Core Team, 2020; Aria and Cuccurullo, 2017). The data were obtained bibliographically from the WoS system in Plain text format. After that, the bibliographic data was converted to the data frame by using "convert2pdf" function with the package of "bibliometrix". The bibliometric analysis was performed by the biblioAnalysis function. In this context, this article aims to perform bibliometric, collaboration, and co-citation analysis to determine the importance of GWAS in animal science over the years.

3. Results

The researchers published 379 studies from 38 sources such as journals, books, etc., about "GWAS" by 1741 authors. The annual percent growth rate for scientific production is approximately 7.598. The graphic of the number of publications in terms of yearly scientific output is given in Figure 1. According to Figure 1, while the number of GWAS studies in animal science was 2 in 2007, how much this subject has been used over the years can be seen.

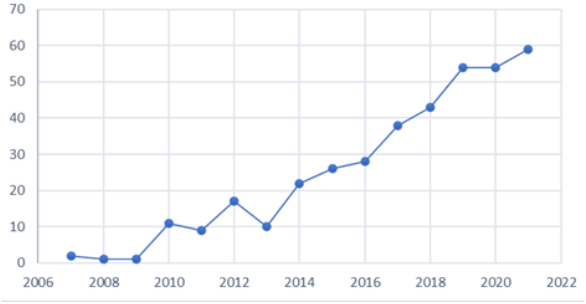

Figure 1. Number of articles per year.

In addition, the preliminary information about the bibliographic data is provided in Table 1. According to Table 1, a total of 379 studies were published in some sources such as journals, books, etc. Only 13 of these studies were done with a single-authored document.

Table 1. The primary information of the data

Information	Number
Documents	379
Sources (Journals, Books, etc)	38
Average years from publication	4.68
Average citations per document	15.09
Average citations per year per document	2.839
References	12702
Authors of single-authored documents	13
Documents per Author	0.221
Authors per Document	4.52
Co-Authors per Documents	6.91
Collaboration Index	4.65

A total of 20208 studies were utilized about the GWAS. However, 379 studies were used about the GWAS in the animal science field (Table 2). According to Table 2, most of these studies consist of articles. In addition, there are two book chapters, three early access studies, four papers, and 29 reviews about GWAS.

Table 2. Document Types for GWAS

Document Types	Number
Article	329
Book chapter	2
Early access	2
Proceeding paper	2
Meeting abstract	13
Review	28
Book chapter (Review)	1

Table 3 provides information on which journals have published the GWAS article. According to Table 3, the researchers published 76 articles in the Genetics Selection Evolution journal as the first chosen journal. The second journal was Animal Genetics journal with the number of 58 articles. The least selected journal was Animal Science Journal with eight articles.

Table 3. The most published articles in journals

Sources	Number
Genetics Selection Evolution	76
Animal Genetics	58
Journal of Dairy Science	52
Journal of Animal Science	34
Animals	29
Livestock Science	19
Animal	16
Journal of Animal Breeding and Genetics	14
Asian-Australasian J of Animal Sciences	12
Animal Science Journal	8

Figure 2 shows the most used keywords in publications. According to Figure 2, among the keywords used in studies about GWAS, expressions such as SNP, candidate genes, dairy cattle, genomic selection appear as the most frequently used keywords, apart from the name of GWAS. Figure 3 shows the most influential authors through the GWAS studies. According to Figure 3, the most effective author was Hayes BJ, with 12 documents about GWAS. Regarding the number of articles, the lowest number of publications so far, with 8, was made by Brito LF, Dekkers JCM, Lund MS and Pryce JE.

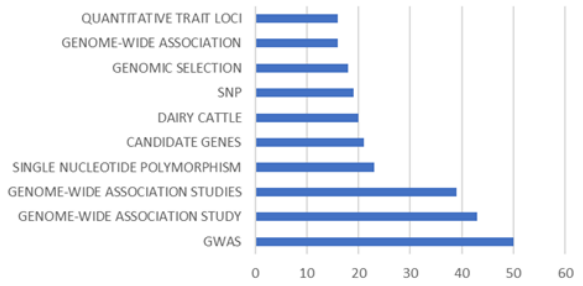

Figure 2. The most chosen keywords for GWAS.

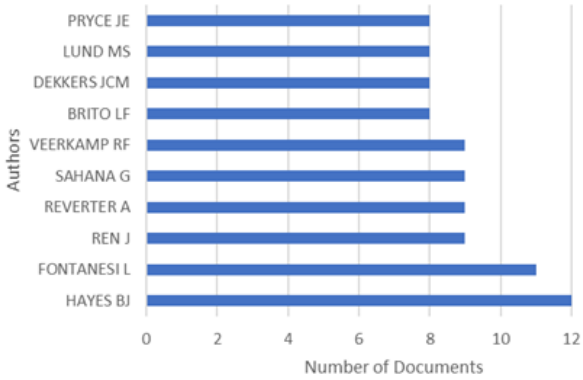

Figure 3. The most productive authors in GWAS generated by bibliometrix package.

Figure 4 provides information about the most cited articles. In this context, it was determined that the article written by Yang ZW received the most citations. This article was published in the journal Anim Biotechnol in 2021. The first article Yang ZW has 311.50 citations per year. In this context, the second widely cited article is the article published by Pryce JE in 2010 in the journal J Dairy Sci. This article has 8.69 citations per year.

According to countries, the most collaborative information about GWAS studies is given in Figure 5. Figure 5 shows that the China and USA is the most productive country according to the number of single (SCP) and multiple (MCP) country publications. However, China was the most productive country in the single country publications.

Figure 6 shows the co-citation network for GWAS studies. Figure 7 shows the conceptual structure of the keywords in the research area for GWAS. There were 3 cluster for the authors' keywords. The cluster showing the greatest similarity of the three clusters consists of terms such as meat quality, milk production, fertility and reproduction, etc.

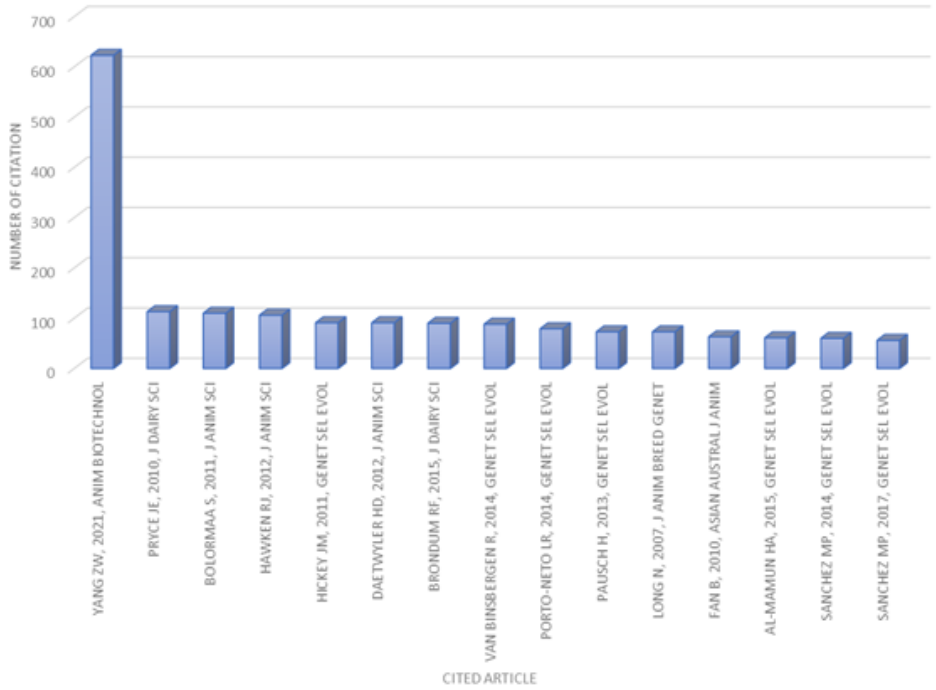

Figure 4. The most cited article.

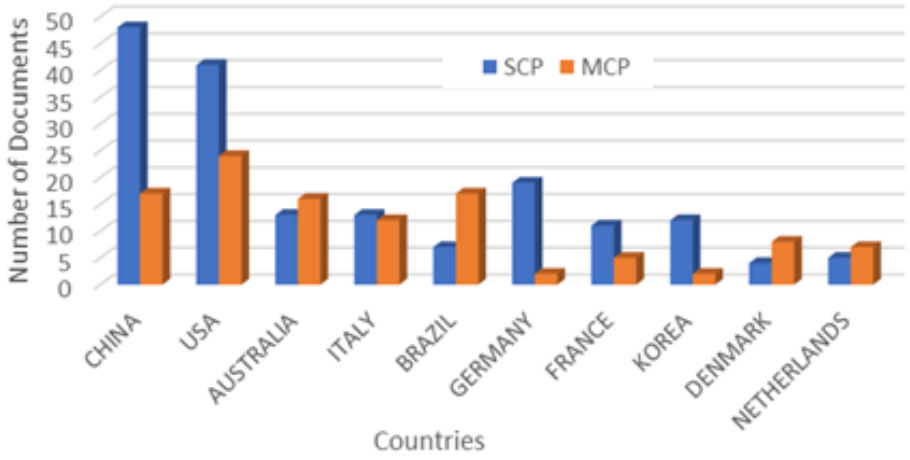

Figure 5. Collaborative information about GWAS studies.

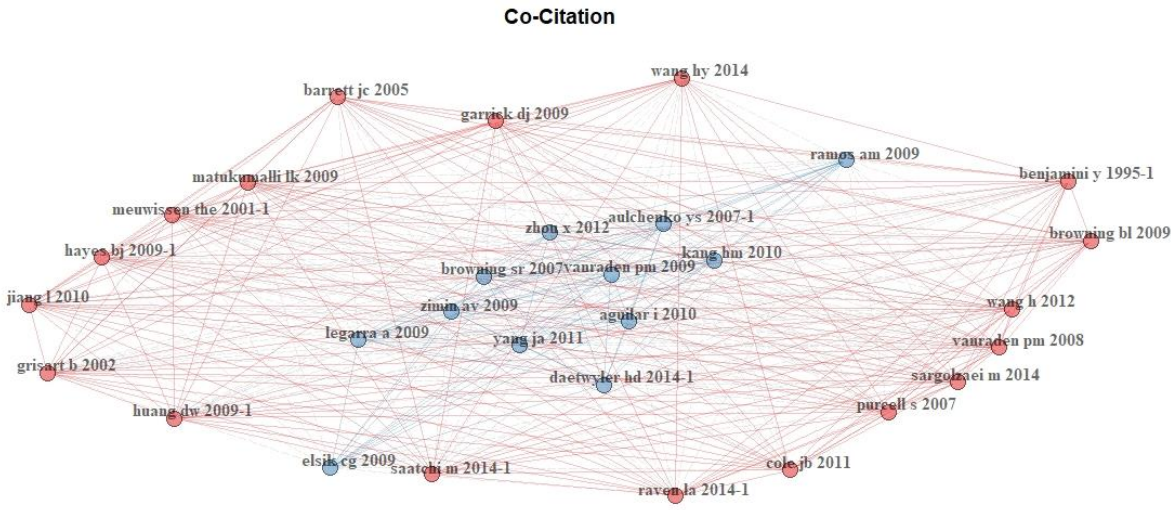

Figure 6. Co-citation report about GWAS.

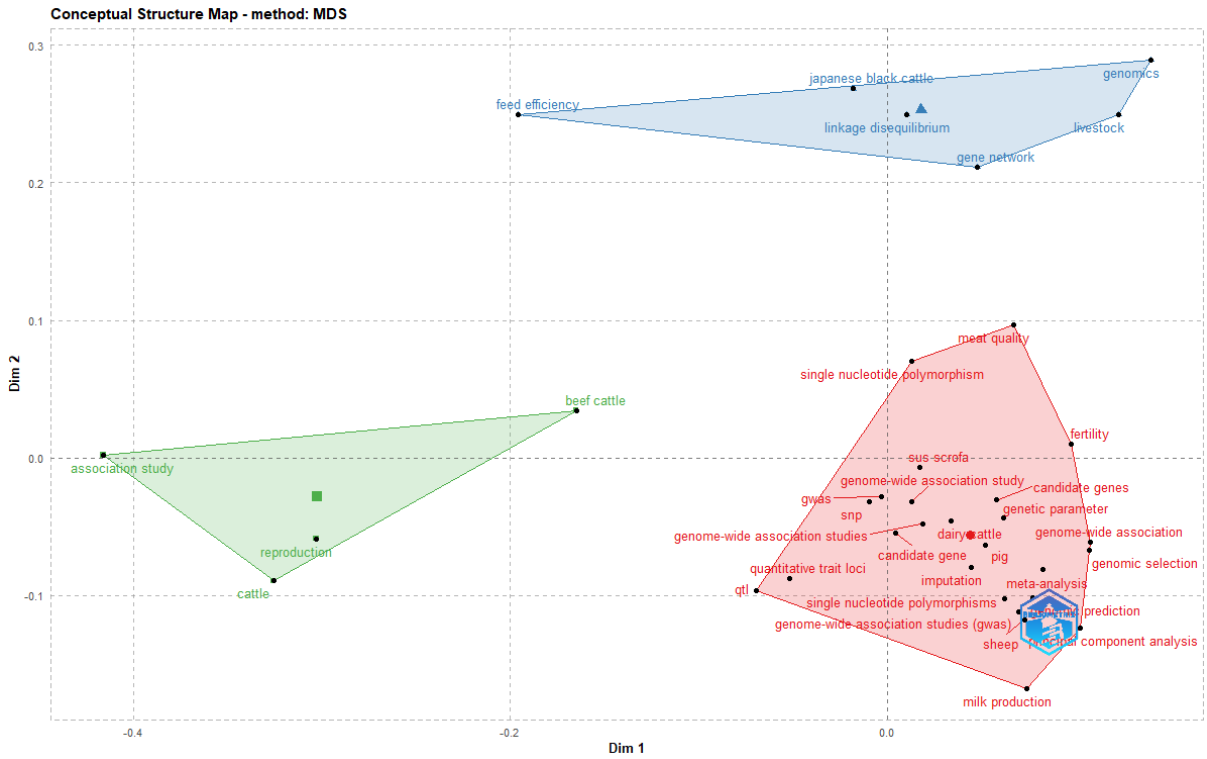

Figure 7. The conceptual structure for GWAS.

Considering the distribution of 379 studies published between 2007 and 2021 on GWAS applications in animal science, it is seen that the most studies were done in 2021. In this context, the issue has not lost its importance and is a current issue. USA and China stand out as the countries with the highest broadcasting rate.

GWAS has been one of the most popular research areas due to its application in many different fields such as cell biology, plant sciences, zoology, animal science, etc. Especially in animal science, it is seen that there are many studies on subjects such as meat quality, milk yield, gene expressions and reproduction, etc. Considering the distribution of 379 studies published between 2007 and 2021 on GWAS applications in animal science, it is seen that the most studies were done in 2021. In this context, the issue still has not lost its importance and stands out as a current issue.

In this context, as a result of the bibliometric analysis of GWAS in animal science, Genetics Selection Evolution has the status of the journal with the most publications on this subject. In addition, when the number of citations was examined, it was determined that the most effective author was Yang ZW. It was determined that the most cited countries were China, Australia and the USA.

In the light of this information, it can be listed as an important contribution that GWAS studies with bibliometric analysis are still up-to-date and that the studies to be done will increase their contribution to animal science.

Author Contributions

All task made by C.T. (100%) data acquisition and analysis, writing up, submission and revision. The author reviewed and approved final version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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THE NUTRITIONAL DYNAMICS OF COMMON WEEDS IN THE RANGELANDS OF THE AKDAĞ MOUNTAINS, SAMSUN

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Abstract: In this study, common weed species (*Anthemis* sp., *Anthemis tinctoria* L., *Pilosella hoppeana* Schultes, *Doranicum orientale* Hoffm, *Muscari neglectum* Guss. ex Ten., *Ornithogalum armeniacum* Baker, *Ornithogalum narbonense* L., *Ornithogalum wiedemannii* Boiss., *Anchusa azurea* Miller, *Echium plantagineum* L., *Echium vulgare* L., *Ajuga orientalis* L., *Stachys germenica* L., *Juncus* sp., *Anacamptis pyramidalis* L., *Ophrys apifera* Huds., *Carex panicea* L., *Ranunculus* sp., *Hypericum perforatum* L., *Primula elatior* L. Hill., and *Galium rotundifolium* L.) of the rangelands of Akdağ mountains, Samsun were evaluated by principal component analysis (PCA) and cluster analysis (CA). These species were collected at least three times in two consecutive years. The proximate nutrients (organic matter, ash, crude protein, ether extract, neutral and acid detergent fibre, non-fibrous carbohydrate and hemicellulose), neutral detergent fibre properties (nitrogen-free neutral detergent fibre and *in vitro* neutral detergent fibre digestibility), and forage quality indicators (digestible dry matter, dry matter intake, metabolizable energy, net energy lactation, estimated net energy, total digestible nutrients, relative feed value, and relative forage quality) were assessed by chemical analysis and empirical equations. There were significant variations in the nutritional dynamics among the weed species. The PCA results demonstrated a relationship between the dietary dynamics assessed. Component 1 (65.5%) and component 2 (14.5%) described 80.0% of the total variation, with eigenvalues of 11.788 and 2.609 in the weed species, respectively. The loadings plot of components shows that most forage quality indicators were distributed to Quadrant 1 and Quadrant 4. Three clusters are observed from the CA for the weeds with significant linkage distance, indicating relatively high independence for each cluster. Due to high variation in their nutritional dynamics, the weed species (*P. elatior, O. wiedemannii, O. narbonense, and G. rotundifolium*) were more similar on component 1 ordination and in Cluster 1 of the dendrogram. In conclusion, our results suggest that the highlighted species have significant potential for grazing livestock as forages and could fulfilling the possible forage gap in the grazing system.

Keywords: Invader species, Weedy forbs, Proximate composition, Nutritive value, Energy value, Forage quality

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1. Introduction

Rangeland-based livestock farming systems, called grazing systems, are still crucial in many countries, including Türkiye (Fırıncıoğlu et al., 2010; Uzun et al., 2015; Diaz-Medina et al., 2021). Sustainable use and maintenance of rangelands has been very considerable because of the vital feed resource for grazing ruminants (Uzun and Ocak, 2019; Diaz-Medina et al., 2021). Early and intensive grazing in grazing systems causes edible or desirable species to be replaced by non-preferred species (Töngel and Ayan, 2005; Uzun and Ocak, 2019). As a result, non-preferred plant species, which are frequently regarded as less desirable or even worthless and harmful plants, have been classified as invaders or weeds (Khan et al., 2013; Koç et al., 2021).

The weeds are indicators of unproductive, unhealthy rangelands (Koç et al., 2021). However, many rangeland weeds, belonging to grasses, legumes, and other botanical families, are consumed by grazing animals (Abaye et al., 2009; Khan et al., 2013; Uzun and Ocak, 2019). Therefore, knowing the potential quality of individual weed species is essential for determining grazing time and range grazing capacity concerning meeting the nutrient requirements of grazing animals (Abaye et al., 2009; Kohl et al., 2012). For farmers in the grazing system, the aerial parts of several weeds are consumed as forage by livestock and play an essential role in the conventional household economy in countries like Türkiye (Gutiérrez et al., 2008; Aydin et al., 2019). We determined that the ratio of species preferred and unpreferred was 70.5% and 29.5%, respectively, in mountainous rangelands (Akdağ) in Samsun, Türkiye. In that study, Aydın et al. (2020) revealed that the other botanical families such as *Asteraceae*, *Lamiaceae*, *Boraginaceae*, *Liliaceae,* and *Scrophulariaceae* dominated most of the weed species that are preferred or somewhat preferred by ruminants. The high percentage of weed species may pose a risk to the quantity and quality of

forages, livestock health, and the floristic patterns of the rangelands (Gutiérrez et al., 2008; Maduro Dias et al., 2020).

Awareness of the forage value of all rangeland species is essential to meet nutritional requirements of grazing ruminants and determine the suitable grazing time and rangeland grazing capacity (Kohl et al., 2012; Aydin et al., 2019). Furthermore, when grazing rangelands containing weed species that belong to the other botanical families, range management has essential importance to successful weed utilization and suppression (Abbaye et al., 2009). All weed species are considered low in quality and yield and harmful to the productivity and health of rangelands and grazing animals (Abaye et al., 2009; Koç et al., 2021). However, it has been stated that the forage quality of perennial weeds varied considerably among species but was equal or superior to that of the most desirable grass and legume species (Frost et al., 2008; Abaye et al., 2009; Kazemi and Valizadeh, 2019). As seen, the importance of weed species concerning their nutritional dynamics has been debatable, mainly proximate nutrients (PN) and forages quality indicators (FQI). Unfortunately, published data addressing the nutritional quality of rangeland weeds in the studied area is almost nonexistent.

Multivariate analysis techniques such as principal component analysis (PCA) and cluster analysis (CA) can assess the complex PN and FQI of weed species by showing the relationship and interdependency among the variables and their relative weights. The PCA and CA techniques have been used in some subjects, such as describing relationships among several quantitative variables in feeds and, according to this, classification of forages (Jayanegara et al., 2011; Uzun and Ocak, 2022). To our knowledge, these techniques have not been used in screening and evaluating weed species in terms of some nutritional dynamics based on acceptable forage quality. Therefore, the objectives of this study were 1) to assess the nutritional dynamics of 21 weed species commonly found in the mountainous rangelands by multivariate analyses and 2) to discuss their relation to the nutrient requirements of livestock.

2. Material and Methods

2.1. Study Area and Weed Species

This study was conducted as part of the major research project (TOVAG - 214O228), namely "Experiments on development of quality index in forage crops based on relative forage quality (RFQ)". In this study, we evaluated 21 weed species, the most dominant species (Uzun and Ocak, 2019; Aydın et al., 2020) for the rangelands of Akdağ Mountains, Samsun, Türkiye (at nearly 1200 m above sea level). These rangelands, open to public grazing, has a climate in which summers are warm and humid, and winters are cool and damp (Aydin et al., 2019). The weed samples were collected at least three times by 15-day intervals from May 5 (before-flowering) to July 5 (after-flowering stage) 2015 and 2016. These weeds, which are non-legumes forbs, belonged to 11 different families (Table 1). The seasonal growth cycle of these species, except for *E. vulgare,* is perennial. *E. vulgare* is a biennial or monocarpic perennial.

Table 1. The common name and family of common weed species in the rangelands of Akdağ Mountains[1]

Scientific name	Symbol in text	Common name	Family
Anthemis sp.	*Anthemis* sp.	Chamomile	Asteraceae
Anthemis tinctoria L.	*A. tinctoria*	Golden chamomile	Asteraceae
Pilosella hoppeana Schultes	*P. hoppeana*	Hawkweed	Asteraceae
Doranicum orientale Hoffm.	*D. orientale*	Leopard's bane	Asteraceae
Muscari neglectum Guss. ex Ten.	*M. neglectum*	Grape hyacinths	Asparagaceae[1]
Ornithogalum armeniacum Baker.	*O. armeniacum*	Saliva grass	Asparagaceae
Ornithogalum narbonense L.	*O. narbonense*	Star of bethlehem	Asparagaceae
Ornithogalum wiedemannii Boiss.	*O. wiedemannii*	Star of bethlehem	Asparagaceae
Anchusa azurea Miller	*A. Azurea*	İtalian bugloss	Boraginaceae
Echium plantagineum L.	*E. plantagineum*	Viper's-bugloss	Boraginaceae
Echium vulgare L.	*E. vulgare*	Blueweed	Boraginaceae
Ajuga orientalis L.	*A. orientalis*	Bugleweed	Lamiaceae
Stachys germenica L	*S. germenica*	Downy woundwort	Lamiaceae
Anacamptis pyramidalis L.	*A. pyramidalis*	Pyramidal orchid	Orchidaceae
Ophrys apifera Huds.	*O. apifera*	Bee orchid	Cyperaceae
Carex panicea L.	*C. panicea*	Carnation sedge	Hypericaceae
Hypericum perforatum L.	*H. perforatum*	St. John's Wort	Hypericaceae
Juncus sp.	*Juncus* sp.	Rush	Juncaceae
Primula elatior L. Hill.	*P. elatior*	True oxlip	Primulaceae
Galium rotundifolium L	*G. rotundifolium*	Bedstraw	Rubiaceae
Ranunculus sp.	*Ranunculus* sp.	Buttercup	Ranunculaceae

[1]These species involved to Asparagaceae are formerly considered to be part of the Liliaceae.

2.2. Proximate Analysis and Cell Wall Constituents

About 300 g of species dried at 60 °C for 72 h were ground in a mill with a 1 mm screen before analyses. These were assessed for proximate analysis, namely dry matter (DM), total ash (Ash), crude protein (CP), and ether extract (EE) determined by standard methods of AOAC International (AOAC, 2005). Cell wall constituents, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the ANKOM A200/220 (ANKOM Technology Corp., Fairport, NY, USA) filter bag technique following Van Soest et al. (1991). The 48-hour *in vitro* NDF digestibility (NDFD, % of NDF) was determined as described by Hoffman et al. (2001). The organic matter (OM), hemicellulose (HC), and non-fibrous carbohydrate (NFC) contents of the weeds were calculated as follows and expressed as % of DM: OM=DM%-Ash%; HC=NDF%-ADF%; NCF=100-(NDFn+CP + EE + ash); where NDFn is nitrogen-free NDF, estimated as NDF×0.93.

2.3. Forage Quality Indicators

Digestible DM (DDM), DM intake (DMI), metabolizable energy (ME), net energy lactation (NEL), estimated net energy (ENE), total digestible nutrients (TDN), relative feed value (RFV) and relative forage quality (RFQ) of weeds were calculated (Undersander et al., 2010; Pflueger et al., 2020; Aydın et al., 2022). The NEL and TNE values were expressed converting to MJ/kg DM. DDM (%)=88.9-(0.799×ADF, % of DM); DMI (% of body weigh [BW])=120/NDF, % of DM; RFV=(DDM, % of DM×DMI, % of BW)/1.29; RFQ=(DMI, % of BW×TDN, % of DM)/1.23; In the RFQ calculation, DMI (% of BW)=120/NDF+(NDFD-45)×0.374/1350×100; ME (Mj/kg DM)=(0.17×DDM, % of DM)-2; NEL (Mcal/kg DM) =1.085+(0.0124×ADF, % of DM); ENE (Mcal/kg DM) =(0.0307×TDN, % of DM)-0.764; TDN=(NFC×0.98) +(CP×0.93)+((EE-1)×0.97×2.25) +(NDFn×(NDFD/100)-7.

2.4. Statistical Analysis

Because the data set in this study is a collection of the data of individual weed species, it used descriptive statistics to describe the basic features of the nutritional dynamics. To identify the species' relationships with each other and with studied traits, the PCA was performed due to the suitability of the data (Kaiser-Meyer-Olkin: 0.816; χ^2: 8507.5, P<0.001). To describe most of the total data variations, the principal components (PCs) that had eigenvalues of >1.0 were considered significant (Jolliffe, 2002). Also, we used the CA to explore the similarities and differences in PN and FQI among the 21 weed species (Zhao et al., 2008). All statistical analyses were performed using the IBM SPSS (SPSS v21.0: IBM Corp.) software package.

3. Results and Discussion

A complete statistical summary of the distribution parameters for the PN and the FQIs of the weed species is given in Table 2 and Table 3, respectively. These tables show that the evaluated species had essential variation in PN, cell wall constituents, and FQI values from species to species, based on descriptive statistics. According to QS suggested by Aydin et al. (2019), the *P. hoppeana, C. panicea* and *Juncus sp.* species had lower values than all other species (Table 3). The QS of *E. vulgare, H. perforatum and S. germenica* species and *Ranunculus sp* were very good and promising, respectively. *A. azura* and *A. tinctoria* species were average and fair, respectively. Other weed species had premium QS. Because chemical composition affects the performance of ruminants, it is one of the critical determinants of animal production based on the grazing system (Abaye et al., 2009; Hassan and Tanveer, 2020). Since there were considerable variations in chemical composition between the weeds (Hassan and Tanveer, 2020), these weeds could affect the performance of animals that consume them to varying degrees. The results on CP, NDF, ADF, ME, and TDN were found to be comparable to the desirable perennial species in rangelands (Algan et al., 2017; Aydin et al., 2019: Aydin et al., 2022) to meet the nutrient requirements of all types of grazing livestock (Abaye et al., 2009; Maduro Dias et al., 2020).

Multivariate analyses showed that the overlap of weed species on the x-axis (Figure 1) and the weed species in the cluster dendrogram (Figure 2) were more similar to each other than either due to high variation in their nutritional dynamics. However, the significant difference between weed species on the y-axis and in subclusters indicates that these invaders had strong, species-specific spatial associations with other species. Similar results have been reported by Uzun and Ocak (2022) for some *Sorghum Bicolor* cultivars. As a result of the PCA, the loading plot of the PCA demonstrated a relationship between the nutritional dynamics evaluated in our study (data not shown). The PCA results revealed the presence of three principal components (PC) with eigenvalues >1, which accounted for 89.1% of the total variance in the weed species. The eigenvalues and % of variance for PC1, PC2 and PC3 were 65.5% and 11.788, 14.5% and 2.609, and 9.1% and 1.630, respectively. However, we only retained PC1 and PC2 that described 80.0% of the total variation for the score and loading plots of the PCA (Figure 1) since the inspection of the scree plot (not presented) showed a clear break after PC2 (Jolliffe, 2002).

Figure 1a presents the factor loadings of the 18 nutritional variables (eight PN, two NDF properties and eight FQI) for the weed species on the PC1 and PC2. The plot of the regression factor scores showed that data points were separated across both the PC1 and PC2 axis. Therefore, the nutritional variables were distributed in all quadrants of the PCA. Based on natural groupings in the PC2 versus PC1 plot, the nutritional dynamics loading on Quadrant 1 (upper right) were ash [0.431 and 0.305], CP [0.348 and 0.433] contents, NDFD [0.001 and 0.954) and some FQIs such as DDM [0.944 and 0.222], ME [0.944 and 0.222] TDN [0.944 and 0.223] and ENE [0.945 and 0.222] with positive loadings for PC1 and PC2.

Table 2. The nutrient contents and the neutral detergent fibre properties of common weeds in the rangelands of Akdağ Mountains

Weed species	Proximate nutrients, % of dry matter								Properties of NDF	
	OM	Ash	CP	EE	ADF	NDF	HC	NFC	NDFn	NDFD
A. orientalis	79.3±0.22	10.3±0.26	11.2±0.28	2.0±0.10	28.4±1.00	33.6±1.98	5.2±0.99	45.4±1.76	31.2±1.84	36.1±0.92
A. pyramidalis	87.2±0.05	5.7±0.29	12.0±0.29	2.3±0.03	28.5±1.00	40.2±2.84	11.7±1.84	42.6±2.03	37.4±2.64	33.0±1.91
A. azurea	77.5±1.31	16.3±1.51	14.8±3.97	1.6±0.31	38.0±0.91	50.1±2.18	12.1±2.02	20.6±0.68	46.6±2.03	55.3±3.49
Anthemis sp.	80.9±2.11	11.1±1.45	11.5±1.38	2.7±0.31	30.0±4.33	42.0±3.41	11.9±2.08	35.7±3.55	39.0±2.96	46.7±4.28
A. tinctoria	86.7±0.64	9.2±0.48	7.7±0.10	2.8±0.03	40.2±1.44	55.8±1.41	15.7±0.03	28.5±0.77	51.9±1.31	39.8±1.57
C. panicea	84.9±0.57	8.2±0.22	12.5±0.36	1.5±0.28	30.8±0.88	65.5±1.83	34.7±1.01	14.0±1.98	60.9±1.71	61.1±1.00
D. orientale	79.4±0.12	11.3±0.25	15.6±0.82	6.0±0.36	27.5±0.64	36.0±2.70	8.5±2.06	33.6±2.28	33.5±2.50	31.3±2.92
E. plantagineum	81.1±0.09	10.4±0.21	15.6±2.03	1.9±0.05	26.8±0.86	37.7±1.45	10.9±0.59	37.1±0.52	35.1±1.35	47.7±0.58
E. vulgare	77.9±1.64	14.5±0.42	12.8±0.54	2.2±0.38	31.0±2.64	43.2±2.20	12.2±1.56	30.3±1.48	40.2±3.91	54.7±1.66
G. rotundifolium	82.0±0.66	10.3±0.13	12.3±0.76	3.3±0.21	22.7±1.06	31.4±2.24	8.6±1.18	45.0±1.23	29.2±2.08	40.4±2.66
H. perforatum	86.4±1.38	6.3±0.92	10.7±0.52	5.5±1.61	34.0±2.70	46.1±2.34	12.2±2.59	34.6±0.08	42.9±1.18	24.5±1.09
Juncus sp.	87.3±0.85	6.1±0.20	11.9±0.71	1.6±0.22	36.6±0.54	68.8±2.13	32.1±1.59	16.4±0.84	64.0±1.98	52.6±0.84
M. neglectum	84.5±0.79	6.9±0.08	11.1±1.14	2.3±0.04	28.3±2.47	39.3±3.28	10.9±1.81	43.2±1.51	36.5±3.77	34.8±0.52
O. apifera	82.8±1.60	8.0±0.17	17.2±1.48	2.8±0.13	27.8±3.56	38.5±3.46	10.7±0.52	36.2±1.55	35.8±3.22	37.3±2.88
O. armeniacum	79.1±0.60	11.2±0.27	15.4±0.38	2.5±0.23	29.5±1.56	38.5±0.32	9.0±1.24	35.1±0.57	35.8±0.30	43.0±2.77
O. narbonense	79.4±0.36	11.6±0.33	13.8±0.34	2.6±0.23	22.6±1.12	30.4±1.87	7.8±0.75	43.8±1.78	28.3±1.74	47.2±0.99
O. wiedemannii	77.3±0.01	12.5±0.12	25.1±0.01	3.1±0.01	18.9±0.01	30.1±0.01	11.1±0.01	31.3±0.02	27.9±0.07	59.2±0.07
P. hoppeana	85.1±1.83	8.6±0.86	10.1±0.52	4.3±0.40	40.9±0.60	55.5±0.05	14.6±0.65	25.5±1.73	51.6±0.05	34.1±0.17
P. elatior	79.4±0.61	13.0±0.17	13.5±1.76	3.2±0.76	22.2±0.83	28.1±1.11	6.0±0.28	44.2±1.64	26.2±1.03	46.5±2.23
Ranunculus sp.	80.2±0.36	9.4±0.21	12.2±0.43	1.5±0.14	33.6±0.02	45.4±0.31	11.8±0.28	34.7±0.08	42.2±0.29	42.4±1.18
S. germenica	84.4±1.91	10.1±1.04	11.2±0.26	2.2±0.44	32.4±1.11	45.5±2.24	13.0±1.56	34.1±1.12	42.3±2.09	44.3±2.29

OM= organic matter, Ash= total ash, CP= crude protein, EE= ether extract, ADF= acid detergent fibre, NDF= neutral detergent fibre, HC= hemicellulose, NFC= non-fibrous carbohydrate, NDFn= nitrogen-free NDF (% of NDF), NDFD= 48-hour *in vitro* NDF digestibility (% of NDF).

Table 3. The forage quality indicators of common weeds in the rangelands of Akdağ Mountains

Weed species	Forage quality indicators								QS
	DDM	DMI	ME	NEL	ENE	TDN	RFV	RFQ	
A. orientalis	66.8±0.78	3.6±0.21	9.4±0.13	1.4±0.01	1.1±0.02	61.3±0.74	186.7±13.25	179.6±12.81	P
A. pyramidalis	66.7±0.78	3.0±0.21	9.3±0.13	1.4±0.01	1.1±0.02	61.2±0.74	156.2±12.95	150.2±12.50	P
A. azurea	59.3±0.71	2.4±0.11	8.1±0.12	1.6±0.01	0.9±0.02	54.2±0.67	110.5±5.61	105.9±5.40	A
Anthemis sp.	65.5±3.37	3.0±0.40	9.1±0.57	1.5±0.05	1.1±0.10	60.0±3.18	153.5±26.98	147.6±16.12	P
A. tinctoria	57.6±1.12	2.2±0.05	7.8±0.19	1.6±0.02	0.9±0.03	52.6±1.06	96.3±4.32	92.2±4.19	F
C. panicea	64.9±0.69	1.8±0.05	9.0±0.12	1.5±0.01	1.1±0.02	59.5±0.65	92.5±3.65	88.9±3.53	U
D. orientale	67.5±0.50	3.4±0.25	9.5±0.08	1.4±0.01	1.1±0.01	61.9±0.47	176.5±14.60	169.8±14.08	P
E. plantagineum	68.0±0.67	3.2±0.12	9.6±0.11	1.4±0.01	1.2±0.02	62.4±0.63	168.6±8.17	162.2±7.90	P
E. vulgare	64.7±2.05	2.8±0.28	9.0±0.34	1.5±0.03	1.1±0.06	59.3±1.93	142.9±18.56	137.4±17.96	V
G. rotundifolium	71.2±0.83	3.9±0.28	10.1±0.14	1.4±0.01	1.2±0.02	65.4±0.78	213.5±17.81	205.7±17.22	P
H. perforatum	62.5±3.66	2.6±0.14	8.6±0.62	1.5±0.06	1.0±0.11	57.2±3.45	127.4±14.23	122.3±13.88	V
Juncus sp.	60.4±0.42	1.8±0.05	8.3±0.07	1.5±0.01	0.9±0.01	55.2±0.39	81.9±3.11	78.5±3.00	U
M. neglectum	66.8±4.26	3.3±0.65	9.4±0.72	1.4±0.07	1.1±0.12	61.3±4.02	174.7±24.79	168.1±13.39	P
O. apifera	67.2±2.77	3.2±0.28	9.4±0.47	1.4±0.05	1.1±0.08	61.6±2.61	166.1±21.25	159.8±15.63	P
O. armeniacum	65.9±1.22	3.1±0.03	9.2±0.21	1.5±0.02	1.1±0.03	60.4±1.15	159.5±4.88	153.3±14.20	P
O. narbonense	71.3±0.87	4.0±0.25	10.1±0.15	1.4±0.01	1.2±0.03	65.5±0.83	220.4±16.34	212.3±15.81	P
O. wiedemannii	74.1±0.05	3.9±0.07	10.6±0.03	1.3±0.01	1.3±0.01	68.2±1.01	229.3±24.03	221.2±18.03	P
P. hoppeana	57.1±0.47	2.2±0.01	7.7±0.08	1.6±0.01	0.8±0.01	52.1±0.44	95.7±5.70	91.6±2.69	U
P. elatior	71.6±0.65	4.3±0.17	10.2±0.11	1.4±0.01	1.3±0.02	65.8±0.61	237.9±11.60	229.2±11.23	P
Ranunculus sp.	62.8±0.02	2.6±0.02	8.7±0.01	1.5±0.01	1.0±0.01	57.5±1.03	128.7±4.91	123.6±10.88	G
S. germenica	63.6±0.86	2.7±0.14	8.8±0.15	1.5±0.01	1.0±0.02	58.3±0.82	131.0±8.63	125.9±8.34	V

DDM= digestible dry matter, DMI= dry matter intake, ME= metabolizable energy, NEL= net energy lactation, ENE= estimated net energy, TDN= total digestible nutrients, RFV= relative feed value, RFQ= relative forage quality, QS= quality scores based on RFQ ranges (P, premium [>138], V= very good [125-137], G= good [115-124], A= average [99-114], F= fair [93-98], U= low/utility [<93]) according to Aydin et al. (2019).

Quadrant 2 (upper left) had the NDF [-0.942 and 0.272], NFDn [-0.942 and 0.272] and HC [-0.652 and 0.591 with negative loadings for PC1 and positive loadings for PC2. The main variables loading on Quadrant 3 (lower left) were OM [-0.636 and -0.285], ADF [-0.944 and -0.223] and NEL [-0.946 and -0.219] with negative loadings for PC1 and PC2. The variables loading on Quadrant 4 (lower right) were EE [0.228 and -0.489] and NFC [0.776 and -0.515] contents and some FQIs (DMI [0.969 and -0.170], RFV [0.983 and -0.077] and RFQ [0.983 and -0.075]) with positive loadings for PC1 and negative loadings for PC2.

The loadings plot of the PC1 and PC2 (Figure 1a) shows that most FQI was distributed to Quadrant 1 and Quadrant 4. Because the position of each variable in the loading plot describes its relationship to the other variables, the grouping of FQIs in the loadings plot suggests their significant mutual positive correlation (Pelletier et al., 2010; Uzun and Ocak, 2022).

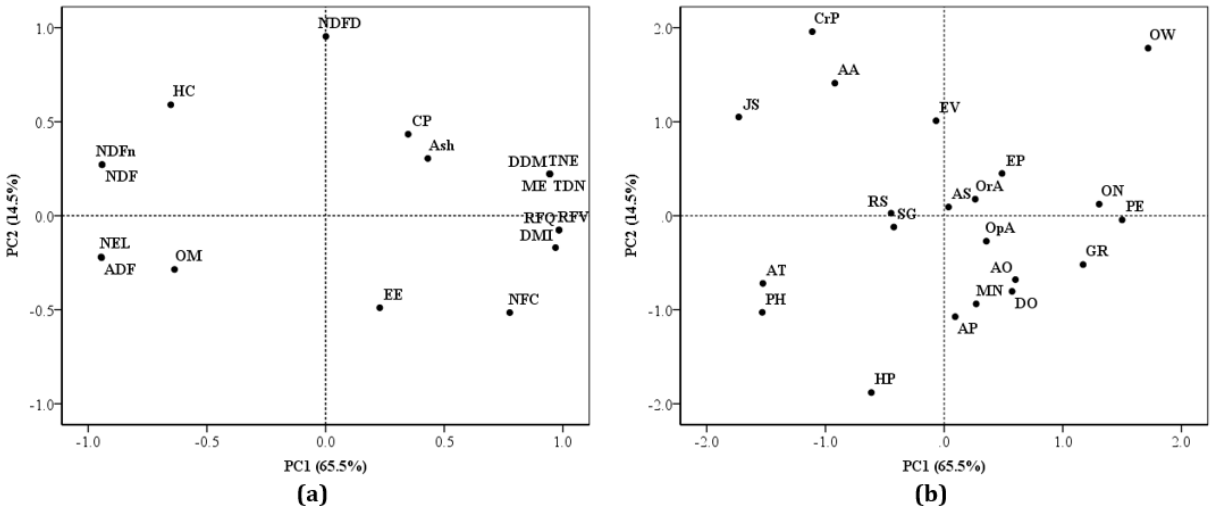

Figure 1. Loading plots (a) and score plots (b) of principal components (PC1 and PC2) for the nutritional dynamics of common weed species in the rangelands of Akdağ Mountains. OM= organic matter, Ash= total ash, CP= crude protein, EE= ether extract, ADF= acid detergent fibre, NDF= neutral detergent fibre, HC= hemicellulose, NFC= non-fibrous carbohydrate, NDFn= nitrogen-free NDF (% of NDF), NDFD= 48-hour *in vitro* NDF digestibility, DDM= digestible dry matter, DMI= dry matter intake, ME= metabolizable energy, NEL= net energy lactation, ENE= estimated net energy, TDN= total digestible nutrients, RFV= relative feed value, RFQ= relative forage quality, AO= *A. orientalis*, AP= *A. pyramidalis*, AA= *A. azurea*, AS= *Anthemis* sp., AT= *A. tinctoria*, CrP= *C. panacea*, DO= *D. orientale*, EP= *E. plantagineum*, EV= *E. vulgare*, GR= *G. rotundifolium,* HP= *H. perforatum*, JS= *Juncus* sp., MN= *M. neglectum*, OpA= *O. apifera*, OrA= *O. armeniacum*, ON= *O. narbonense*, OW= *O. wiedemannii*, PH= *P. hoppeana*, PE= *P. elatior*, RS= *Ranunculus* sp., SG= *S. germenica*.

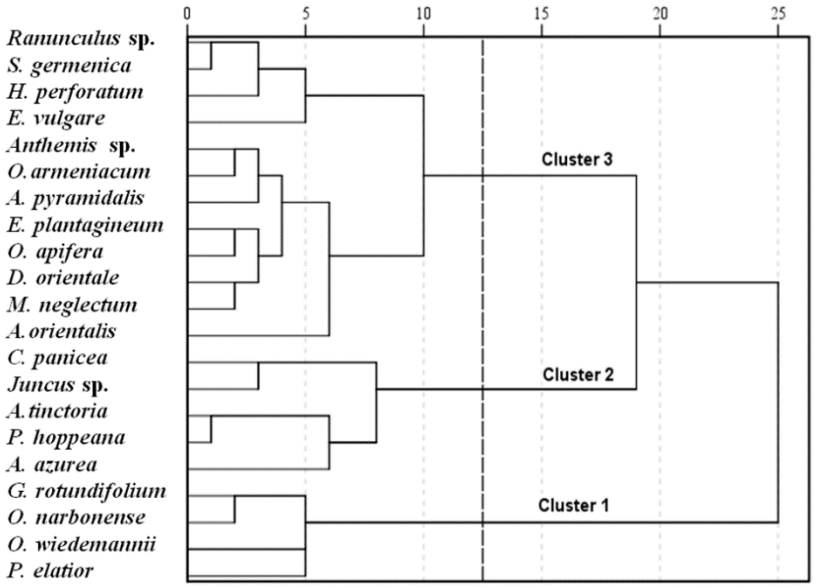

Figure 2. Dendrogram using average linkage (between species) of common weed species in the rangelands of Akdağ Mountains based on a total of 18 nutritional variables (eight proximate nutrients, two neutral detergent fibre properties and eight forage quality indicators).

Morevere, Jayanegara et al. (2011) noted that close variables in any quadrant have high correlations and variables on the opposite side of origin (0.0) are negatively correlated. According to relation matrix loadings (≥0.75 and positive factor loadings) of the variables, these FQIs such as DDM, ME, TDN and ENE contributed most strongly to PC1, while ash, CP contents and NDFD contributed less strongly (Uzun and Ocak, 2022).

The scatter diagram arranged on loading scores of PCs showed that the scatter plots of the weed species were cross-distributed among the quadrants or that there was a contrasted distribution of the species along PC1 and PC2 (Figure 1b). Weeds arranged in the same direction with the nutritional dynamics such as DDM, ME, TDN and ENE were considered good quality compared whit the weed species in the other quadrants. Based on the dataset of other quadrants, weeds were partially related

to low forage quality. On the *x*-axis, the nine species, *S. germenica, C. panicea, A. tinctoria, H. perforatum, E. vulgare, A. azurea, Juncus sp., Ranunculus sp., P. hoppeana*, were opposed to the 12 species, *O. armeniacum A. orientalis, A. pyramidalis, Anthemis sp., D. orientale, E. plantagineum, G. rotundifolium, M. neglectum, O. apifera, O. narbonense, O. wiedemannii, P. elatior*. Pelletier et al. (2010) noted that this contrast is related to the nutritional dynamics arranged on the respective quadrants. Indeed, one end of the axis had higher CP, ash and some FQIs, whereas the other had higher fibre concentrations. This result confirms the idea that forage quality had positively correlated with CP but negatively with NDF and ADF contents (Zhao et al., 2008; Zhai et al., 2018).

Three clusters were observed from the dendrogram (Figure 2) for the nutritional variables in the weed species with significant linkage distance, indicating relatively high independence for each cluster. The four weed species (*P. elatior, O. wiedemannii, O. narbonense and G. rotundifolium*) formed a cluster group (Cluster 1). Cluster 2 consisted of five accessions formed by *A. azurea, P. hoppeana, A. tinctoria and C. panacea* species. The remaining 12 species (*A. orientalis, D. orientale, H. perforatum, E. plantagineum, E. vulgare, Juncus sp., M. neglectum, Ranunculus sp., S. germenica, Anthemis sp., O. armeniacum, O. apifera* and *A. pyramidalis*) were clustered into one group (Cluster 3). Cluster 2 and Cluster 3 had two subgroups. The first subgroup of Cluster 3 was the largest group consisting of eight accessions, representing *A. orientalis, M. neglectum, E. plantagineum, D. orientale, O. armeniacum, O. apifera, Anthemis sp.* and *A. pyramidalis* species.

The results on cell wall constituents showed that the weeds contained favourable levels of NDF, ADL, ADF and HC and, thus, a good and valuable source of these nutrients, as were reported by Khan et al. (2017). Although protein requirement varies with each type and stage of life of grazing animals (Abaye et al., 2009; Kirilov et al., 2016), the dietary adequate-protein level required for maximal growth and activity of ruminal microorganisms is higher than 7% CP (Sampaio et al., 2010; Maduro Dias et al., 2020). The CP content of all the weeds in the present study had more excellent than this value. Hall et al. (2009) noted that acceptable quality is forage of >56% TDN and >10% CP, whereas unsuitable quality is forage of 50-55% TDN and 8-9% CP. As the ADF and NDF contents increases, DMI, DDM and subsequently nutritive value declines due to increasing fibre (Abaye et al., 2009; Zhai et al., 2018). As noted herein, the high CP and low ADF and NDF contents of forages are generally associated with increased energy value or good forage quality (Kirilov et al., 2016; Zhai et al., 2018). Therefore, the highlighted weeds may be relative adequate to meet the nutritional needs of grazing livestock (Gutiérrez et al., 2008; Bunton et al., 2020; Maduro Dias et al., 2020) depending on the ratio of weeds in the rangeland (Uzun and Ocak, 2019). Indeed, a mixture containing 15% weeds and 85% desirable forages did not influence the forage intake or digestibility compared to 100% quality grass and legume mixture (Abaye et al., 2009).

The nutritional dynamics and anti-nutritional factors of forages impact voluntary feed intake of grazing animals. The presence of anti-nutritional factors, which depress digestibility in ruminants and sometimes are toxic, limits the utilization of some weeds (Töngel and İlknur, 2005; Abaye et al., 2009). Unfortunately, we did not determine whether weeds contain anti-nutritional factors or toxins (Burritt and Hart, 2014). However, *Ranunculus* sp., *E. vulgare, E. plantagienum and H. perforatum* species are toxic or poisonous species commonly found in the experimental area (Töngel and İlknur, 2005). Generally, these plants are avoided by all types of livestock because animals learn what to eat and avoid (Abaye et al., 2009; Burritt and Hart, 2014). Otherwise, these species have a significantly higher risk of toxicity to grazing animals eating a single plant species (Töngel and İlknur, 2005; Burritt and Hart, 2014). Even if an animal has eaten any weed evaluated due any reason, this does not mean the animal can survive on a sole diet of that weed. Therefore, any assessed weeds, except for the toxic or poisonous weeds, together with desirable legumes and grasses could be incorporated and satisfactory for grazing without significant problems (Abaye et al., 2009; Burritt and Hart, 2014). Our results conform with the suggestion that not all weeds in a grazing system are detrimental from the standpoint of nutritive value (Abbaye et al., 2009; Bunton et al., 2020). Forage value for the grazing system is the total value of desirable and undesirable (or weeds) forage species in rangeland relative to grazing animal productivity and gain (Khan et al., 2017; Collins and Newman, 2018; Bunton et al., 2020).

The performance of grazing animals in rangelands varies depending on the proportion of high-quality forage species available and accessible. Accordingly, there is an immediate need for forages species with high quality produced abundantly and widely distributed for rangelands subjected to early and overgrazing (Uzun and Ocak, 2019). Annual forage species might meet this instant need (Aydın et al., 2015; Kazemi and Valizadeh, 2019; Uzun and Ocak, 2019), but annual species without autumn to spring cycles are not essential components of sustainable grazing systems (Frost et al., 2008; Abbaye et al., 2009; Uzun and Ocak, 2019). Based on our RFV and RFQ results, weeds had better values than desirable legumes (*Lotus corniculatus, Medicago sativa, Trifolium pratense, Trifolium repens*), grasses *(Dactylis glomerata, Festuca ovina, Lolium perenne)* and other families (*Cichorium intybus and Sanguisorba minör*) collected from same rangelands (Aydın et al., 2022). Similarly, perennial weeds in rangelands have had equal or superior forage quality compared with some desirable grasses and legumes species (Frost et al., 2008; Abaye et al., 2009; Kazemi and Valizadeh, 2019). This situation may be related to grass and legume forages with similar digestibility and voluntary feed intake; there is little

difference in ADF or NDF levels (Collins and Newman, 2018). Most weeds in the present study may be alternatives for forages needed instant if there is high grazing pressure on rangelands (Gutiérrez et al., 2008; Khan et al., 2017; Bunton et al., 2020). It should be forgotten that there is a reduction in the nutritional values of many perennial weeds towards the end of the growing season. Furthermore, some weed species are consumed voluntarily due to greater nutritive values during the early stages of the growing season (Bunton et al., 2020). In such cases, the movement and grazing of animals and forage utilization may differ depending on meeting nutrient requirements (Gutiérrez et al., 2008). Our results indicate that non-preference weeds have the potential to preserve plant diversity and contribute to forage resources in overgrazed rangelands (Uzun and Ocak, 2019).

Because the NDFD is a measure of the digestible rations of NDF (Foster et al. 2009), a weed species with a higher NDFD is a forage with high quality and provided the NDF with more digestible and usable to the animal (Bunton et al., 2020). The NFC that differ from carbohydrates found in NDF is needed to satisfy the activity of rumen microbes and thus animals' health and performance (Tan et al., 2002). The PCA and CA results indicate that the evaluated weeds widely vary in NFC (comprised primarily of starch, sugars, pectin and β-glucans) and depend mainly on the NDF, ADF and CP levels, as in forage grasses (Pelletier et al., 2010). Mayland et al. (2000) observed that the NFC concentration of *Festuca arundinacea* cultivars close relationship to animal grazing preference. As such, grazing animals may likely prefer some weeds (such as *A. Pyramidalis, M. Neglectum, O. Narbonense, P. Elatior, G. rotundifolium* and *A. orientalis*) to others, including all range forage species due to a difference in their NFC concentration. Weeds with high NFC concentration might have relative lower CP, ADF and NDF contents but a relative higher NDFD and, as a result, higher TDN, ME, NET and ENE (Pelletier et al., 2010). Weeds with high NFC concentration might have relative lower CP, ADF and NDF contents but a relative higher NDFD (Pelletier et al., 2010) and thus a higher TDN, ME, NET and ENE. These results and knowledge may explain why the weeds in Quadrant 1 and Cluster 1 are of better quality.

4. Conclusion

Except for four poisonous species (*Ranunculus* sp., *E. vulgare, E. plantagienum and H. perforatum*), the 17 weed species were nutritionally beneficial to grazing livestock and satisfactory for damaged rangelands. Assessed weeds possess a great potential for their utilization as range forage and may be very effective in overcoming the possible shortage of forage. These results may help producers make management decisions based upon the potential benefit or detriment a weed may provide to the overall nutritional value of the grazing system. Thus, weed species not only improve livestock production if there is a forage gap in the grazing system but also benefit biodiversity, a "win-win" solution for farmers and environmentalists. Further research is needed to quantify the anti-nutritional factors and palatability of weedy forage.

Author Contributions

İ.A. (100%) designed the experiment and carried it out. N.O. (100%) analyzed the data and wrote the original draft. İ.A. (50%) and N.O. (50%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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THE ANTAGONISTIC AND SYNERGISTIC COMPARISON OF THE ANTIMICROBIAL CHARACTERISTICS OF EXTRACTS OF SOME HERBS

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Abstract: In the present study, the antagonistic and synergistic effects of *Achillea millefolium* L., *Anthemis cretica* L., *Cichorium intybus* L., *Euphorbia seguieriana* Necker and *Hypericum perforatum* L plant extracts collected from Samsun were investigated. Gram negative bacteria; *Escherichia coli*, *Acinetobacter baumannii*, *Salmonella typhimurium*, Gram positive bacteria; *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* were used as research materials. In the research, methanol and diethyl were used as solvents. The antibacterial activities of the extracts were determined by microbroth dilution method. According to the results of the research; all plant extracts obtained using both methanol and diethyl ether solvent were determined to be more effective against gram positive bacteria. While the whole plant extract showed the most effect on Bacillus cereus bacteria, *Hypericum perforatum* L. methanol extract was the most effective plant against gram positive bacteria. *Achillea millefolium* L.: *Cichorium intybus* L., *Achillea millefolium* L.: *Hypericum perforatum* L. and *Cichorium intybus* L.: *Hypericum perforatum* L. methanol mixture extracts and *Achillea millefolium* L.: *Hypericum perfarotum* L. diethyl ether extract showed a synergistic effect, while other plant mixture extracts showed semi-synergistic or ineffective properties.

Keywords: Combination, Plant extracts, Synergistic effects, Antimicrobial activity, Herbal medicine

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1. Introduction

The treatment with plant extracts, which has survived from the earliest known civilizations to the present, has been the first method that comes to mind in the prevention and cure of many diseases. Treatments with herbal extracts are one of the oldest health care known to mankind (Gupta and Gupta, 2019) and have contributed greatly to people's health needs throughout their existence (Mehmood et al., 2012). It is estimated that there are between 250 and 500 thousand plant species on the planet, and only 1% to 10% are used by humans as food and medicine (Maciel et al., 2002).

Developing living conditions brought with it many diseases. The fact that the diseases experienced in the past become incurable again, and the inadequacy of the treatment of some serious diseases today, has increased the tendency to natural origin drugs. Plants are the most basic products used directly or indirectly in the treatment of such diseases (Çolak et al., 2020). Bacteria have become a serious problem due to their increasing frequency of infection as well as advanced antibiotic resistance (Nilson et al., 2014). Many studies have found that the plant species included in the study have effects on bacteria (Betoni et al., 2006; Stefanovic et al., 2012; Enerva et al., 2015; Leblebici et al., 2016; Gul et al., 2017; Riccobono et al., 2017; Hundur et al., 2018; Darcan et al., 2021; Yanar et al., 2021). Plant-based antibiotics and their synergistic effects could be a useful and practical solution to prevent antibiotic resistance. Studies of synergistic effects of plant extracts are therefore necessary to identify new combinations with highly desirable efficacy (Bahmani et al., 2019). Despite the obtained valuable information about *Achillea, Anthemis, Cichorium, Euphorbia* and *Hypericum* species their synergistic effects have not been sufficiently studied yet (Ma et al., 2009). The ability of plant extracts mixtures to act synergistically could be a new approach to solve the problem of bacterial resistance (Stefanovic and Comic, 2012).

In current study, it was aimed to compare the antimicrobial properties of the extracts of *Achillea millefolium* L. (white yarrow), *Anthemis cretica* L. (mountain daisy), *Cichorium intybus* L. (wild chicory), *Euphorbia seguieriana* Necker (euphorbia), *Hypericum perforatum* L. (St. John's wort) as antagonistically and synergistically.

2. Material and Methods

Achillea millefolium L. (white yarrow), *Anthemis cretica* L. (mountain daisy), *Cichorium intybus* L. (wild chicory), *Euphorbia seguieriana* Necker (spurgery), and *Hypericum perforatum* L. (St. John's wort) were used as research materials (Figure 1). They were collected from Samsun-Alaçam (41 26' 47.88 N°, 35 28' 50.42 E°, elevation: 1657 m) on 8 August 2020. Plants were diagnosed at the flowering time by Prof. Sebahattin Albayrak who is an expert on the rangeland and forage plant management in the Ondokuz Mayıs University, Samsun.

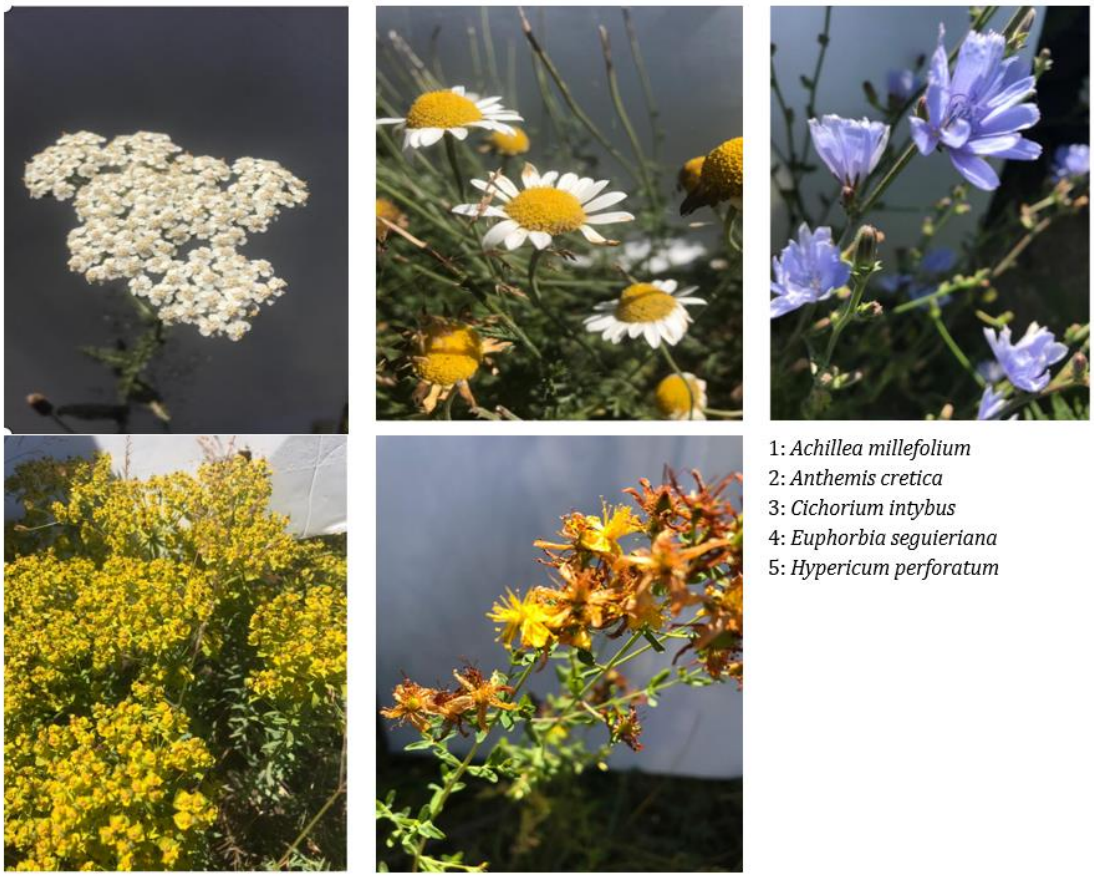

Figure 1. Original plant images used in the present research.

The test strains were obtained from the Faculty of Art and Science, Bilecik Şeyh Edebali University. In the research, gram negative bacteria; *Escherichia coli* W3110, *Acinetobacter baumannii* ATCC19606, *Salmonella typhimurium* ATCC 14028, Gram positive bacteria; *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 7064, *Listeria monocytogenes* ATCC 7644 were used.

The above-ground parts of the plants were dried at room temperature, in a shade and moisture-free environment. The leaf-flower parts of the dried plants were grinded separately with the mill in the laboratory and turned into powder. 5 g of each plant was weighed and treated in two different solvents (80% methanol, 20% water (80ml:20 ml) and (100 ml diethyl ether) and extracted in a Soxhlet device for 7 hours (Del monte et al. 2015).

The antimicrobial activities of the extracts were determined using the microbroth dilution method. U-bottom 96 microtiter plates were used for the experiment. Nutrient broth (NB) medium was used as a growth medium for the growth of bacterial strains. 5.2 g nutrient broth (Biolife) was weighed and dissolved in 400 ml distilled water and sterilized in an autoclave (Core) for 15 minutes at 121 °C. 6 g nutrient broth (Biolife) was poured into petri dishes by applying aseptic techniques when it reached the temperature of 55 °C. According to the method reported by (Aydın and Sevindik, 2018) different medium were added for each bacterial group, the first well in which the decrease in turbidity color in the wells was observed was accepted as the Minimum Inhibitory Concentration (MIC). In MIC experiments, one drop was taken from the wells without growth and allowed to grow on Nutrient Agar media. Therefore, it was determined whether the inhibition was caused by a static or cidal effect. Thus, cidal concentration values were determined (Darcan et al., 2021).

Plants were extracted in a soxhlet device for 7 hours. After using only one extract on each bacterial species, 50-50 were mixed together to evaluate the synergist effectiveness in each bacterial species (Al-Terehi et al. 2015). In vitro interactions between antimicrobial agents were determined by calculating the fractional inhibitory concentration (FIC) index using the following formula: FIC=FICA+FICB; FIC A=Combination effect/MIC A: The

effect of MIC A alone; FIC B=Combination effect/MIC B: The effect of MIC B alone; FIC≤0.5, total synergism; 0.5<FIC≤0.75, partial synergism; 0.75<FIC≤2, no effect; FIC>2, antagonism (Sharma et al. 2020).

3. Results

The results of antimicrobial activity *Achillea millefolium*, *Anthemis cretica*, *Cichorium intybus, Euphorbia seguieriana* and *Hypericum perforatum* are shown in Table 1-5, synergistic activities of those plants are given in Table 6 and 7.

In research of present study, the effect of methanol solvent on all bacterial groups was more effective than diethyl ether solvent. The control group of diethyl ether was more effective on *E.coli* bacteria than all the plants in the study. In methanol, *A.baumanni* and *L. monocytogenes* in Euphorbia and *A. baumanni* in Hypericum control group were found to be more effective (Table 1 to 5).

All plant extracts were more effective on gram positive bacteria than gram negative bacteria. Among the plants, only *Anthemis* extract showed a very high effect on the gram-negative bacteria *A. baumannii* (5.375 mg ml^{-1}). Extracts of all plants in the study showed the greatest effect on *B. cereus* bacteria (1.321 to 5.562 mg ml^{-1}). The most effective plant extract was *H. perforatum* (1.321 mg ml^{-1}). *Euphorbia* extract showed the lowest effect on both bacterial groups (except for *B. cereus*, 5.562 mg ml^{-1}) (Table 1 to 5).

Table 1. Antibacterial activities of *Achillea millefolium* L. Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	11.85	23.70	25.00	50.00	14.15	14.15	12.50	25.00
A.baumannii	11.85	23.70	12.50	25.00	14.15	28.30	25.00	50.00
S.typhimurium	11.85	23.70	25.00	50.00	14.15	14.15	25.00	25.00
Gram- positive								
S. aureus	5.925	11.85	25.00	25.00	10.61	10.61	25.00	25.00
B. cereus	2.962	5.924	25.00	50.00	10.61	10.61	25.00	25.00
L. monocytognes	5.925	11.85	12.50	25.00	10.61	10.61	12.50	25.00

Table 2. Antibacterial activities of *Anthemis cretica* L. Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	21.50	21.50	25.00	50.00	21.37	14.25	12.50	25.00
A.baumannii	5.375	10.75	12.50	25.00	14.25	14.25	25.00	50.00
S.typhimurium	10.75	21.50	25.00	50.00	14.25	14.25	25.00	25.00
Gram-positive								
S. aureus	5.375	10.75	25.00	25.00	10.68	10.68	25.00	25.00
B. cereus	2.680	5.36	25.00	50.00	10.68	10.68	25.00	25.00
L. monocytognes	10.75	10.75	12.50	25.00	10.68	10.68	12.50	25.00

Table 3. Antibacterial activities of *Cichorium intybus* L. Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	12.35	12.35	25.00	50.00	13.60	13.60	12.50	25.00
A.baumannii	12.35	12.35	12.50	25.00	13.60	27.30	25.00	50.00
S.typhimurium	12.35	12.35	25.00	50.00	13.60	13.60	25.00	25.00
Gram- positive								
S. aureus	6.175	6.175	25.00	25.00	6.82	6.82	25.00	25.00
B. cereus	5.375	10.75	25.00	50.00	6.82	6.82	25.00	25.00
L. monocytognes	6.175	12.35	12.50	25.00	6.82	6.82	12.50	25.00

Table 4. Antibacterial activities of *Euphorbia seguieriana* Necker Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	44.50	44.5	25.00	50.00	18.50	18.50	12.50	25.00
A.baumannii	22.50	22.5	12.50	25.00	18.50	18.50	25.00	50.00
S.typhimurium	44.50	44.5	25.00	50.00	18.50	18.50	25.00	25.00
Gram positive								
S. aureus	11.12	11.12	25.00	25.00	13.90	13.90	25.00	25.00
B. cereus	5.562	5.562	25.00	50.00	13.90	13.90	25.00	25.00
L. monocytognes	22.25	22.25	12.50	25.00	13.90	13.90	12.50	25.00

Table 5. Antibacterial activities of *Hypericum perforatum* L.Methanol and Diethyl ether extracts at different concentrations. Minimal inhibition concentration (MIC, mg ml^{-1}), Minimal cidal concentration (MCC, mg ml^{-1})

Bacteries	Methanol (80:20)		Methanol (Control)		Diethyl ether (100%)		Diethyl ether (Control)	
	MIC	MCC	MIC	MCC	MIC	MCC	MIC	MCC
Gram- negative								
E.coli	21.50	21.50	25.00	50.00	21.55	21.55	12.50	25.00
A.baumannii	21.50	21.50	12.50	25.00	10.75	10.75	25.00	50.00
S.typhimurium	10.57	10.57	25.00	50.00	21.55	21.55	25.00	25.00
Gram positive								
S. aureus	2.643	2.643	25.00	25.00	10.70	10.70	25.00	25.00
B. cereus	1.321	1.321	25.00	50.00	10.70	10.70	25.00	25.00
L. monocytognes	2.643	2.643	12.50	25.00	10.70	10.70	12.50	25.00

Table 6. Binary mixture antibacterial activity FIC values of methanol extracts

	CP	CY	CS	CK	PY	PS	PK	YS	YK	SK
Gram- negative										
E.coli	0.67	0.67	0.90	1.02	0.61	0.84	0.96	0.84	0.96	1.19
A.baumannii	0.83	0.66	1.09	0.97	0.85	1.27	1.16	1.11	0.99	1.41
S.typhimurium	0.65	0.64	0.88	1.06	0.64	0.87	1.05	0.86	1.04	1.27
Gram positive										
S. aureus	0.65	0.49	1.10	0.49	0.65	1.26	0.64	1.10	0.49	1.10
B. cereus	0.61	0.49	0.77	0.48	0.60	0.88	0.59	0.77	0.48	0.76
L. monocytognes	0.57	0.49	0.90	0.48	0.57	0.97	0.56	0.90	0.47	0.89

C= *Achillea millefolium*, P= *Anthemis cretica*, Y= *Cichorium intybus*, S= *Euphorbia seguieriana*, K= *Hypericum perforatum.*

Table 7. Binary mixture antibacterial activity FIC values of diethyl ether extracts

	CP	CY	CS	CK	PY	PS	PK	YS	YK	SK
Gram- negative										
E.coli	1.12	1.12	1.13	1.14	1.57	1.58	1.58	1.58	1.59	1.60
A.baumannii	1.08	1.16	1.16	1.16	1.55	1.55	1.55	1.63	1.63	1.63
S.typhimurium	1.10	1.14	1.14	1.11	1.56	1.56	1.53	1.59	1.59	1.56
Gram positive										
S. aureus	0.73	0.84	1.16	0.24	0.90	1.21	0.64	1.31	0.74	1.06
B. cereus	0.72	0.81	1.18	0.25	0.80	1.17	0.60	1.26	0.69	1.06
L. monocytognes	0.75	0.88	1.15	0.24	0.89	1.17	0.63	1.29	0.75	1.03

C= *Achillea millefolium*, P= *Anthemis cretica*, Y= *Cichorium intybus*, S= *Euphorbia seguieriana*, K= *Hypericum perforatum.*

When the cidal (MCC) and static (MIC) values are compared, it can be found that while the active substances have a static effect on Gram-negative bacteria, a cidal effect on the Gram-positive bacteria at MIC value (Table 1 to 5).

In the study, it was determined whether the inhibition effect of the extracts of 5 plants obtained with methanol and diethyl ether was cidal or static, according to the MIC value seen on the bacteria. For this purpose, reproduction status was tested by sowing on the solid medium from wells that did not show growth.

MIC and MCC values of *A. millefolium* were determined to

be different in all bacteria. The MIC value was 11.85 mg/ml in Gram-negative bacteria, the MCC value was 23.7 (Table 1). The MIC value of S. aureus and *L. monocytogenes* (Gram-positive bacteria) were 5.92. In addition, the MIC value of *B. cereus* was determined as 2.96, the MCC value was 5.92. It was clearly seen that the effect of the methanol extract of this plant on MIC concentrations was a static effect. While the methanol extract of *Anthemis cretica* plant had a cidal effect on *E. coli* and *L. monocytogenes*, the diethyl ether extract had a cidal effect on all bacteria except *E. coli* (Table 2). Cichorium intybus methanol extract had a cidal effect except B. ceraus and L. monocytogenes. Diethyl ether extract obtained from this plant showed a static effect only on *A. baumanni* bacteria, while MIC value was found to be cidal concentration in other bacteria (Table 3). Both methanol and diethyl ether extracts of the 2 plants (*Euphorbia* and *Hypericum*) had cidal effects on gram negative and gram positive bacteria. Therefore, the MIC values of these 2 plants were the cidal concentration (Table 4 and 5).

Binary mixtures of plants were determined as effects of synergistic or antagonistic on bacteria. According to fractional inhibitory concentration (FIC) index; FIC≤0.5, total synergism; 0.5<FIC≤0.75, partial synergism; 0.75<FIC≤2, no effect; FIC>2, antagonism (Sharma et al. 2020).

Partial synergism was observed in the combination of *Achillea* and *Anthemis* (FIC 0.57 to 0.75), except for gram-negative bacteria of Diethyl ether and *A.baumannii* of methanol. In the *Achillea+Cichorium* combination, synergism was found in gram positive bacteria and partial synergism in gram negative bacteria of methanol, ineffectiveness in diethyl ether. *Achillea* and *Euphorbia* mixture was ineffective with both methanol and ether solvents. *Achillea+Hypericum* combinations showed a synergistic effect of both methanol and ether solvents on gram-positive bacteria (FIC: 0.24 to 0.49), but not effect on gram-negative bacteria (FIC: 0.97 to 1.16). Except for the methanol solvent *A. baumannii*, the *Anthemis+Cichorium* mixture showed partial synergism, while the diethyl solvent had no effect on all bacteria. The *Anthemis +Euphorbia* combination did not show any effect on both gram positive and gram negative bacteria (FIC: 0.84 to 1.58). The *Anthemis* and *Hypericum* mixture was semi-synergist against gram positive bacteria (FIC: 056 to 0.64) and ineffective against gram negative bacteria in both solvents. It was determined that the *Cichorium+Euphorbia* combination did not affect any bacterial group (FIC: 0.77 to 1.63). The methanol extract of *Cichorium+Hypericum* combination showed a synergist effect, diethyl ether extracts semi-synergistic effect on gram positive bacteria; it had no effect on other groups. The *Euphorbia+Hypericum* mixture was not effective on any bacteria group (FIC: 0.76 to 1.63) (Table 6 and 7).

4. Discussion

Achillea millefolium, Anthemis cretica, Cichorium intybus, Euphorbia seguieriana and *Hypericum perforatum* methanol and diethyl ether extracts were generally found to be more effective on gram-positive bacteria. On the other hand, the effect of methanol on all bacterial groups was more effective than diethyl ether (Table 1 to 5).

The effect of methanol extracts of *Achillea millefolium* on gram-negative bacteria used in this study was the same (MIC 11.85), the effect on gram-positive bacteria was in the range of 2.96-5.92 mg ml^{-1}. In diethyl ether solvent, the MIC value of *A. millefolium* extract was 14.15 on gram-negative bacteria, and it was 10.612 on gram-positive. Kharma and Hassawi (2006) reported that *Achillea spp*. extract had the greatest effect on *S. aureus* bacteria. Salvagnini et al. (2006) found that *Achillea millefolium* extract was effective only against *Bacillus subtilis* from gram-positive bacteria. Kharma and Hassawi (2006) and Salvagnini et al. (2006)'s findings are consistent with our research results.

B. cereus (MIC: 2.68 mg ml^{-1}) was the most sensitive bacteria compared to other bacteria according to the antibacterial effect of *Anthemis cretica* extracts. The finding shown that *Anthemis* extracts had a greater effect on gram-positive bacteria (Formisano et al., 2012; Riccobono et al., 2017) was consistent with our research results.

In Methanol solvent, *Cichorium intybus* extracts showed similar antibacterial effects with *E. Coli, A.baumannii* and *S.typhimurium* (MIC: 12.35 mg ml^{-1}). MIC values of gram-positive bacteria were found in the range of 5.375-6.175. In diethyl ether solvent, the MIC value was 13.65 gram-negative bacteria, and it was 6.825 in gram-positive. Koner et al. (2011) the effect of chicory root extract had more bacteriostatic effect on Gram-positive bacteria than Gram-negative bacteria; Nandagopal and Kumari (2007) concluded that chicory root extracts showed more inhibitory effect on gram positive (*Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus)* bacteria than gram negative (*Escherichia coli* and *Salmonella typhi*) bacteria. The researchers' findings were in agreement with our results.

It had been observed that *Euphorbia seguieriana* extracts (MIC: >44.5 mg ml^{-1}) had a low antibacterial effect on E.coli and S.typhimurium. Rocha et al. (2021) found *Euphorbia macroclada* had no effect on some bacteria, on the other hand, Enerva et al. (2015) reported that *Euphorbia hirta* extract had high effects on *P. aeruginosa, Staphyloccus aureus, Candida albicans* and *Trichopyton mentagrophytes* bacteria. It could be thought that the variability between the findings of different studies may be due to the differences in the material used or the method applied.

Antibacterial activity of *Hypericum perforatum* extracts on *S. aureus* (MIC: 2.643 mg ml^{-1}), *B. aureus* (MIC: 1.321 mg ml^{-1}) and *L. monocytogenes* (MIC: 2.643 mg ml^{-1}) are more effective compared to gram negative bacteria in methanol solvent. On the other hand, the MIC values of

the diethyl ether solvent on bacteria were found to be less efficient. *H. perforatum* was reported to be good antibacterial agents in many sources (Meral and Karabay, 2002; Okmen and Balpınar, 2017; Önem and Çevik Baş, 2018; Özkan et al., 2018). The finding of our research the antibacterial activity of *Hypericum perforatum* extract on gram-negative bacteria was weaker than gram-positive bacteria was consistent with other research results (Düzgüner and Erbil, 2020; Okmen and Balpınar, 2017).

In Methanol solvent, *Achillea millefolium* and *Cichorium intybus, Achillea millefolium* and *Hypericum perforatum, Cichorium intybus* and *Hypericum perforatum* extracts showed synergistic effects on gram- positive bacteria (*S. aureus, B. cereus and L. monocytoges*) (FIC values ranged from 0.47-0.49). The synergistic effect of *Achillea millefolium L: Hypericum perforatum* mixture was found to be high in the extract obtained by using diethyl ether solvent (FIC 0.24, 025 and 024, respectively). Two or more agents in the combination interact in different manners leading to one of the four possible effects-synergistic, partial synergistic, no effect, and antagonism (Kasrati et al., 2014). Synergistic interactions are the most important because they enhance the antimicrobial and antioxidant activity by utilizing the efficiencies of the combined agents in the best possible manner and thereby result in several fold reduction in the required doses of the combined agents (Sharma et al. 2020).

Bahmani et al. (2019) reported that *Origanum vulgare* and *Hypericum perforatum* had a synergistic effect of 0.5 and that this plant combination could be used as a new antibacterial strategy against *S. aureus*. It was stated that their synergistic studies not only show promise in the fight against drug-resistant pathogens and in the future treatment of infectious diseases, but they could also change the purpose of traditional antibiotics, which were often ineffective when used alone. Gram-negative bacteria were generally more resistant to the antagonistic effects of essential oils than Gram-positive ones, due to the lipopolysaccharide and porin proteins found in the outer membrane (György 2010; Darcan 2012). It was stated that their synergistic studies not only show promise in the fight against drug-resistant pathogens and in the future treatment of infectious diseases, but they could also change the purpose of traditional antibiotics, which are often ineffective when used alone (Fatemi et al., 2020). Synergy was a situation that occurs when two or more herbal ingredients mutually increase the effect of each other more than the simple sum of these ingredients (Ma et al., 2009). Studies examining the interactions of plant extracts in combination increase their antibacterial activity compared to studies examined as single extracts. It should be noted that in addition to the synergistic effects obtained with Gram-positive bacteria, antagonistic effects may also occur in Gram-negative bacteria (Obuekwe, 2020).

5. Conclusion

All plant extracts obtained using both methanol and diethyl ether solvents were determined to be more effective against gram positive bacteria. It was determined that methanol solvent was more effective on bacteria than diethyl ether. *Hypericum perforatum* had been an effective herb against gram-positive bacteria. *Achillea millefolium: Cichorium intybus*, *Achillea millefolium: Hypericum perforatum* and *Cichorium intybus.: Hypericum perforatum* methanol mixture extracts and *Achillea millefolium: Hypericum perforatum* diethyl ether extract showed a synergistic effect, other plant mixture extracts showed semi-synergistic or ineffective properties. It will be useful to conduct new research on the antimicrobial single and mixture extracts of the plants used in the study at the point of combating bacteria.

Author Contributions

A.G.D (50%) and C.D. (50%) design of study. A.G.D (50%) and C.D. (50%) data acquisition and analysis. A.G.D (50%) and C.D. (50%) writing up. A.G.D (50%) and C.D. (50%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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THE EFFECT OF ELEMENTAL SULFUR FERTILIZATION ON GREEN BEANS (*Phaseolus Vulgaris* L.) GROWN IN CALCAREOUS SOIL

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Abstract: In our country, most of the bean cultivation areas, are in the Central Anatolia Region. This study was carried out to determine the sulphureous fertilizer demand of beans grown in the calcareous soils of the region under field conditions in Konya. Early "seat bean" variety was used as a plant material. In the experiment, elemental - sulphur (S) was applied to the parcels at the doses of "0-20-40-80 kg S/da. At the end of the experiment, leaf chlorophyll SPAD values showed a significant increase in the plots where the highest sulfur dose was applied. Parcel yield and leaf surface area increased significantly with increasing sulfur doses ($P<0.05$), and the highest data were obtained from 80 kg S/da application. With sulfur application, the nitrogen content of bean leaves and fruit was partially decreased compared to the control ($P<0.05$). Some element content of the bean product was found as follows, respectively: 1.87-2.38% nitrogen (N), 0.43-0.72% phosphorus (P) and 0.39-0.44% S. Also, some element content of leaf was as follows: 1.97-2.37% N, 0.46-0.51% P and 0.50-0.59% S. The N/S ratios in the plant varied between 3.44-4.93 for the leaf and 4.79-5.60 for the bean. Considering the results obtained, 80 kg/da elemental sulfur application can be recommended to meet the sulfur fertilizer requirement for green beans grown in calcareous soil.

Keywords: Elemental - Sulfur, Beans, Calcareous soil, Yield

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1. Introduction

Bean (*Phaseolus vulgaris* L.), which is grown intensively in Türkiye and in the world, is an important member of legumes and is consumed in fresh and dry forms. Fresh and dry bean cultivation areas are 142.218 ha in the 2020 production period in Türkiye, and green beans have been produced in approximately 1/4 of this area. When the yield situation is examined, it has been increased since 1990 until now. According to the data of 2020, while the green bean yield was 139.434 hg/ha, the dry bean yield was 27.147 hg/ha (FAO, 2022). According to 2019 data, most of the cultivation areas in Türkiye are located in the Central Anatolia Region (49%). This is followed by the Eastern Anatolia Region with 14.6%. The province of Konya, where the study was conducted, meets 22.1% of Türkiye's dry bean production alone (Anonymous, 2022a).

Application of elemental sulfur increases the solubility of phosphorus and micronutrient elements in calcareous and alkaline soils (Gupta and Mehla, 1980; Abd-Elfattah and Hilal, 1985; Neilsen et al., 1993). This situation is due to the oxidation of S to SO_4 form, it occurs by decreasing pH and also increasing nutrient availability (Burns, 1967). As a result of the applications of sulfur alone and in combination with nitrogen, the soil pH has decreased significantly, the availability of micronutrients and plant dry matter yield increased (Soliman et al., 1992). The dry matter yield and phosphorus (P) uptake increased as a result of a 0.11-0.37 unit decrease in soil pH value with sulfur application (Erdal et al., 2000). It has been reported by various researchers that the applications of waste material containing elemental S and S provide a decrease in soil pH and an increase in usable nutrients in the trial soils (Kaya et al., 2009). In studies on elemental sulfur carried out in different countries (Hussain et al., 2011; Ganie et al., 2014; Teame et al., 2020), it was stated that positive results were obtained regarding the effect of sulfur together with other nutrients in bean yield and elemental content. Sulfur has many functions in plants and is a part of proteins and plays an important role in the synthesis of vitamins and chlorophyll in the cell (Marschner, 1995). In sulfur deficiency, growth retardation and reduction are observed in plants (Kacar and Katkat, 2021). Sulfur is a limiting factor in reducing crop yields in many parts of the world (Mascagni et al., 2008).

Soil pH is higher than 7.5 in 63% of agricultural lands in Türkiye, and approximately 59% of them contain more than 5% $CaCO_3$ (Eyüboğlu, 1999). Considering that the soils are calcareous and for the stated reasons, sulfur fertilizer applications have increased in our country and in the world in recent years. It is estimated that the

global sulfur fertilizers market size will grow by 3.57% in 2026 (Anonymous, 2022b; Anonymous, 2022c).

In the working soil where this field trial was carried out, in the previous 10-week incubation experiments it was indicated that the pH value of the soil decreased partially as the incubation time of elemental sulfur with the soil increased (Akay et al., 2019).

In this study, the effects of elemental sulfur added in different proportions on the development, chemical content and sulfur uptake of green beans were investigated in the calcareous and high pH soil covering large areas in Konya. In the study, it was also aimed to determine the appropriate sulfur fertilizer dose for beans.

2. Material and Methods

2.1. Experiment Materials

The research was carried out in Konya- Selcuk University Faculty of Agriculture Sarıcalar farm under field conditions. Bean, whose trade name is "seat bean" (Romano 26), was used as a plant material. The characteristics of this bean variety can be listed as follows: The color of the seeds obtained from the plant is white. The length of the pod is 14-15 cm. It is an awnless and early variety (45-50 days). Its flower color is white, and it is a suitable variety for both fresh consumption and canning (Anonymous, 2021).

2.2. Establishment of the Experiment and the Actions Taken During the Experiment

The experiment was set up in the field in randomized block factorial design with three replications. The parcels were prepared to be 12 m^2 (3mx4m) each. Elemental powdered sulfur (100% S) was applied in different doses (S0-S1-S2-S3) (0-20-40-80 kg/da) to the parcels that were pre-prepared before the experiment was established and mixed with the soil homogeneously.

The experiment, which was established as three replications, was carried out in a total of 12 plots. Taking into account the soil analysis results, phosphorus and nitrogen fertilizers were also applied to all plots (15 kg/da DAP). After these procedures, plant seeds were planted in the plots (in May 2019) and irrigated. After emergence, the plants were regularly irrigated twice a week, considering the field capacity of the soil. In addition, the soil was hoed three times according to the weed situation.

During the flowering period, nitrogen fertilizer (8 kg/da ammonium sulfate) was applied to all parcels from above. At the beginning of flowering, the chlorophyll SPAD value (with Minolta-502 SPAD meter) was determined in mature leaves from each parcel and the surface area was measured with a planimeter by taking leaf samples. During the vegetation period, the beans ripening in each parcel were collected weekly, and their weights were determined on precision scales. Fruit length and width values of bean were measured with a digital caliper.

After the bean development was completed, the plants were harvested. The bean samples harvested, and the leaf samples taken during the flowering period were subjected to wet burning using H_2SO_4-H_2O_2 as digestion reagents (Bayraklı, 1987). In the solutions obtained as a result of wet burning, N analysis was determined by the Kjeldahl method (Bremler, 1965). Phosphorus was determined according to the vanadomolybdophosphoric acid method (Barton, 1948) in the spectrophotometer (UV-VIS). Sulfur concentrations in the solution obtained after combustion, it determined by using spectrophotometer in the mixture obtained by adding ammonium acetate, barium chloride and resin (Fox et al., 1964).

2.3. Soil Analyzes

Before the experiment, soil samples were taken from 0-30 cm from the field and some physical and chemical properties were determined (Table 1). According to the results, soil has clay loam texture, slightly alkaline pH. It is salt free and calcareous. The soil contains moderate organic matter. Phosphorus and potassium content of the soil is sufficient, calcium and magnesium content are high. Fe, Zn, Cu, Mn contents of the soil are high and sulfur content is low.

2.4. Statistical Analyzes

All measurements were performed at three times and the results expressed as mean ± standard deviation (SD). Statistical analyses for all data were performed using Minitab 18 package program. Data were analyzed by General linear model to evaluate significant differences between mean at 95% level of confidence. Significant differences were determined by Tukey's Pairwise Comparison test (Düzgüneş, 1963; Yurtsever, 1984).

3. Results and Discussion

According to the variance analysis results of the data obtained at the end of the experiment, parcel yield increased significantly with increasing S doses compared to the control ($P<0.05$) (Table 2). The highest average parcel yield is 80 kg S/da (1959 g/parcel). In the study in which the effect of sulfur application with bacterial inoculation in soybean was determined, it was found that the application of 30 kg S ha^{-1} provided a significant increase in yield compared to the control. It was also stated that plant height, number of pods per plant, straw yield, seed yield and dry matter yield increased by 14%, 56%, 25%, 20% and 26% compared to the control, respectively (Hussain et al., 2011). Togay et al. (2008) stated that, the combined application of P and S increases the yield in beans, and also the best results were obtained with 80 kg P /ha and 120kg S /ha application.

In the random sampling of the products taken in the experiment, the width and height measurements of the bean fruit were made. According to the results obtained, it was seen that the effect of S application on fruit width and length was not statistically significant (Table 2). Low and high rates of S application to beans do not affect yield, seed number and weight values, as agronomic parameters (Pandurangan and Marsolais, 2015).

Table 1. Physical and chemical properties of experimental soil

Properties	Values	Analysis Methods
Physical properties		
Texture class	Clay loam	(Bouyoucos, 1951)
Field capacity	25	(Cassel and Nielsen, 1986)
Fading point	15	(Cassel and Nielsen, 1986)
Chemical properties		
pH (1:2.5 soil:pure water)	8.19	(Jackson, 1969)
EC(µS/cm) (1:5 soil:pure water)	388.7	(Staff US Salinity Lab, 1954)
Organic matter (%)	3.00	Walkley and Black's modified method (Jackson, 1969)
$CaCO_3$(%)	12.1	(Nelson, 1996)
Available P(mg/kg)	37.8	(Olsen and Sommers, 1982)
Extractable K (mg kg^{-1})	867	
Extractable Ca(mg kg^{-1})	8.378	
Extractable Mg(mg kg^{-1})	703	Extraction method with 1 N neutral ammonium acetate
Extractable Na(mg kg^{-1})	213	
Available Zn(mg kg^{-1})	1.66	DTPA (Diethylene triamine penta acetic acid) ekstraction method (Lindsay and Norvell,1978)
Available Fe (mg kg^{-1})	4.45	
Available Cu (mg kg^{-1})	2.48	
Available Mn (mg kg^{-1})	47.8	

Table 2. Effect of sulfur fertilizer applications on yield, fruit width and height, chlorophyll Spad value and leaf surface area of bean plant

S doses (kg/da)	Yield (gr/parcel)	Bean width(mm)	Bean height(mm)	Chlorophyll Spad value	Leaf surface area (mm^2)
0	1548 ± 56 ^{b}	13.27 ± 0.51	110.34 ± 0.77	43.61 ± 2.11 ^{b}	55.53 ± 12.90 ^{b}
20	1663 ± 180 ^{ab}	13.16 ± 0.30	109.78 ± 7.13	40.79 ± 2.91 ^{b}	58.23 ± 3.77 ^{b}
40	1804 ± 160 ^{ab}	12.48 ± 0.31	107.89 ± 3.36	41.39 ± 0.16 ^{b}	73.03 ± 3.18 ^{ab}
80	1959 ± 56 ^{a}	12.69 ± 0.20	109.34 ± 3.17	52.32 ± 2.04 ^{a}	81.63 ± 3.82 ^{a}

^{a,b}Mean values with different superscripts in the same column indicate a significant difference (P<0.05).

It was stated that the bean responded to S fertilization at a low rate (under 10%) in field conditions (Malavolta et al., 1987).

Leaf chlorophyll SPAD values measured at the beginning of flowering did not increase with increasing S doses compared to the control. However, a statistically significant increase was observed in the parcels where the highest S dose was applied, compared to both the control and other S doses (P<0.05). In field trials conducted in Ethiopia in dry beans, it was found that the combination of 20 kg ha^{-1} P and 30 kg ha^{-1} S was the optimum ratio and maximum vegetative growth was achieved. It has been stated that it can be used to provide earliness and high grain yield for Melka Awash-98 variety (Teame et al., 2020).

In the study, it is noteworthy that there was a partial decrease in the N concentration of the leaf and bean with S application compared to the control, and that the S application had a negative effect (Table 3). The N concentration values of the leaf vary between 1.97-2.37% and these values are within the adequacy limit given for the upper leaf at the beginning of flowering for beans (2.00% sufficient and 1% insufficient) (Jones 2001). N concentrations in both leaves and fruit are highest in control treatments. Sulfur applications negatively affected the N concentration in the plant. On the other hand, considering the amount of N removed from the soil by bean fruit, the highest N uptake was observed in 80 kg S/da application compared to control and other S application doses (P<0.05). Although the N concentration in the bean fruit decreased a little compared to the control, the amount of N taken by the plant from the soil increased (Table 4). Teame et al. (2020) stated that the protein content with the effect of P - S fertilization in different bean varieties varied between 22.5-27.1%.

With increasing doses of elemental S, the P concentration in bean leaves and fruit decreased significantly compared to the control (P<0.05), and S adversely affected the P concentration in the plant (Table 3). The P concentration values in the leaf varied between 0.46-0.51%, and these values are above the proficiency limit (0.2%-0.4% sufficient) (Yıldız, 2008). While the P content in the bean fruit were 0.72% in the control application, they decreased to 0.43% in the 80 kg S/da application. Similar to N concentrations, P concentrations in leaves and bean fruits are highest in control treatments. The amount of P removed from the soil by bean fruit also decreased significantly with increasing sulfur doses compared to

the control (P<0.05). This value was an average of 3.73 kg/da in the control application, and it's decreased gradually with the sulfur application and decreased to 2.79 kg/da in 80 kg S/da application. Sulfur adversely affected phosphorus uptake by the plant (Table 4).

Considering the effect of S application on the S concentration in leaves and bean fruits, there was no statistically significant difference between these values compared to the control. The S concentration of the leaf is between 0.50 - 0.59%, and this value is between sufficient and high in the limit values given for sulfur (Yıldız 2008). In the bean fruit, the S concentration varied between 0.39 and 0.44%. The amount of S removed from the soil by the bean fruit increased with increasing S doses, but there was no statistical difference between these values (Table 4). With the bean fruit, an average of 2.16-2.85 kg/da of S was taken from the soil.

When the N/S ratios in the plant were examined at the end of the experiment, it changed between 3.44-4.93 for the leaf and 4.79-5.60 for the bean fruit. The total N/S ratio for protein synthesis in legume plants is 17 (Kacar and Katkat, 2021). Sulfur deficiency will limit protein formation if the total N/S ratio is above 16. If this ratio is above 20, it is stated that there is a serious sulfur deficiency in plants (Stewart and Porter, 1969). Orman and Kaplan (2017) stated that sulfur and manure applications alone in beans significantly affected the total N/S ratio in shoots, reported that the rate changed as 23.76 and 18.72. The fact that the total N/S ratio in the study is lower than the value stated for legumes is thought to be related to the limit value of N concentration in both leaves and fruits of the plant. Mn, Ni and Mo in plant leaves increased when insufficient S was applied to bush beans (*Phaseolus vulgaris* L. var. Tendergreen) to completely neutralize $CaCO_3$ in calcareous soil. It has been observed that $CaSO_4$, which is used as a sulfur source, does not have the same effect as elemental S (Procopiou et al., 1976).

Table 3. The effect of sulfur fertilizer applications on the N, P and S concentrations in the leaf and grain of the bean plant

S doses (kg/da)	Leaf N(%)	Bean N(%)	Leaf P(%)	Bean P(%)	Leaf S(%)	Bean S(%)
0	2.31 ± 0.088^{a}	2.38 ± 0.038^{a}	0.51 ± 0.009^{a}	0.72 ± 0.014^{a}	0.58 ± 0.211	0.43 ± 0.045
20	2.37 ± 0.048^{a}	1.87 ± 0.066^{c}	0.48 ± 0.012^{ab}	0.65 ± 0.016^{b}	0.50 ± 0.122	0.39 ± 0.011
40	2.11 ± 0.105^{ab}	2.12 ± 0.070^{b}	0.46 ± 0.026^{b}	0.61 ± 0.016^{c}	0.58 ± 0.040	0.39 ± 0.051
80	1.97 ± 0.148^{b}	2.22 ± 0.026^{b}	0.46 ± 0.009^{b}	0.43 ± 0.002^{d}	0.59 ± 0.152	0.44 ± 0.043

^{a,b}Mean values with different superscripts in the same column indicate a significant difference (P<0.05).

Table 4. The effect of sulfur fertilizer applications on the amount of N, P, S removed from the soil by the bean fruit, the N/S ratio in the fruit and the N/S ratio in the leaf

S doses (kg/da)	Bean removed N (kg/da)	Bean fruit removed P (kg/da)	Bean fruit Removed S (kg/da)	N/S ratio in leaf	N/S ratio in bean
0	12.25 ± 0.025 ^{b}	3.73 ± 0.200 ^{a}	2.20 ± 0.220	4.35 ± 1.380	5.60 ± 0.590
20	10.36 ± 0.831 ^{c}	3.59 ± 0.297 ^{a}	2.16 ± 0.183	4.93 ± 1.305	4.79 ± 0.163
40	12.70 ± 0.865 ^{b}	3.69 ± 0.422 ^{a}	2.35 ± 0.401	3.65 ± 0.192	5.50 ± 0.866
80	14.52 ± 0.524 ^{a}	2.79 ± 0.297 ^{b}	2.85 ± 0.311	3.44 ± 0.608	5.13 ± 0.426

^{a,b}Mean values with different superscripts in the same column indicate a significant difference (P<0.05).

4. Conclusion

As a result, in this study carried out with green beans grown in calcareous soil, S applications increased bean yield. Although there was no change in bean sizes and chlorophyll content, it was observed that the leaf surface area of the plant was significantly and positively affected by S doses. N, P concentrations in bean leaves and bean fruit decreased partially, but the N removed from the soil by the product increased. Concentration of sulfur in leaves and bean, S removed by fruit, N/S ratios in leaf and bean did not change with sulfur doses. When considering these results, 80 kg/da elemental sulfur can be recommended for sulfur fertilizer for bean growing in calcareous soil.

Author Contributions

All task made by A.A. (100%) data acquisition and analysis, writing up, submission and revision. The author reviewed and approved final version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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MODELING OF IN VITRO GAS PRODUCTION

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Abstract: In this study, *in vitro* gas production values (hour/ml) of standard, alfalfa and corn plants at 3, 6, 9, 12, 24, 36, 48, 72 and 96 hours in Kahramanmaras Sutcu Imam University Animal Science Department Feeds and Animal Nutrition Laboratory.) used cubic piecewise regression, Richard, Logistics, Gamma, Gompertz, Orscov, Sigmoaidal and Quadratic Piecewise Regression models were used. In the modeling study, mean squares of error, determination coefficient, Akaike information criterion and Durbin-Watson autocorrelation values were taken into account for each model of in vitro gas production values. As a result of the study, it was concluded that Logistic and Gompertz models had the best results in corn and alfalfa, standard, while the Gamma model had the worst results in all feeds in terms of the comparison criteria, mean squares of error, coefficient of determination, Akaike information criterion and Durbin-Watson Autocorrelation values.

Keywords: Feed, *In vitro*, Gas production, Stomach

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1. Introduction

In vitro gas production method is one of the most widely used methods in feed evaluation in ruminants (France and al., 2000; Canbolatandal., 2013; Menkeand al., 1988; Rymerand al., 2005; Van, 1994). In the *in vitro* evaluation of feeds with gas production method, the amount of rumen fluid is affected by the amount of feed used, the feed/rumen fluid ratio and the volume of the incubation medium. These factors are the factors that directly affect the amount of gas production. For this reason, it will be possible to make an accurate interpretation of the gas production method in *in vitro* gas production method, by choosing the right model (Canbolat and al., 2007; Karabulut and al., 2006; Özturk and al., 2006; Çölkesen and al., 2005; Gülboy and Önder, 2018). Many different mathematical equations are used to better model and interpret gas production curves (Orskov and McDonald, 1979). Commonly used models in in-vitro gas production method can be listed as exponential, Cubic, Richards, Logistics, France, Gompertz and Groot models. However, the fact that gas production curves are polynomial and a sigmoidal curve makes model selection very difficult. Gas production in the first stage of fermentation is very low. This situation shows a stable increase until it reaches the asymptote. The important thing here is to be able to choose a model that includes sigmoidal structures with and without bends in the gas production curve. Gas production curves correlate with digestion and microbial density. Therefore, the curves obtained by the in-vitro gas production method will show slight differences each time without disturbing the general structure of the curve. In this study, it was aimed to model in vitro gas production values (hr/ml) of standard, corn and alfalfa in nine different time periods (3, 6, 9, 12, 24, 36, 48, 72 and 96 hours) by using Cubic, Gompertz, Logistics, Gamma, Richard, Cubic Piece, Orscov and Sigmoaidal models.

2. Material and Methods

2.1. Materials

In this study, *in vitro* gas production at 3, 6, 9, 12, 24, 36, 48, 72 and 96 hours for 3 different groups belonging to standard, corn and alfalfa in Kahramanmaras Sutcu Imam University Feeds and Animal Nutrition Laboratory values (hr/ml) were obtained. Four measurements were made for each hour from each feed sample and the averages of these measurements were used in the modeling.

2.2. Methods

In modeling of in-vitro gas production values, cubic piecewise regression, Richard, Logistic, Gamma, Gompertz, Orskov, Sigmoaidal and Quadratic Piecewise Regression models were used. Obtaining the curves and estimating the model parameters were made in the SAS (7.0) package program. The equations and expansions of these models are as follows (equation 1, 2, 3, 4, 5, 6, 7 and 8);

Cubic Piecewise Regression,

$$W_t=\beta_0+\beta_1 t+\beta_2 t^2+\beta_3 t^3+\beta_4(t-a)^3+\beta_5(t-b)^3 \quad (1)$$

Logistic,

$W_t=\beta_0/(1+\beta_1 e(-\beta_2 t))$ (2)

Gompertz,

$W_t=\beta_0 e(-\beta_2 e(-\beta_3 t)$ (3)

Gamma,

$W_t=\beta_0 t^{\beta} 1_{(-\beta} 2t)$ (4)

Orskov,

$W_t=\beta_0(1-e^{-c}t)$ (5)

Richard,

$W_t=1/(\beta_0+\beta_1 e(\beta_2 t)^{(-\beta 3)}$ (6)

Sigmoidal,

$W_t=\beta_0/(1+(\beta_1/t)^{\beta 2}$ (7)

Quadratic Piecewise Regression,

$W_t=\beta_0+\beta_1 t+\beta_2 t^2$ (8)

is in the form. Here, Wt:t. gas production over time, β0, β1, β2, β3, β4, and β5: constants defined for the models, a and b; In the piecewise regression, it represents the node points, e: 2.7182, t: time (hour) (Rodrigues, 2009; Çetinkaya. 2015).

2.2.1. Model comparison criteria

In curve modeling studies in the biological field, comparison criteria such as coefficient of determination, corrected coefficient of determination, mean squared error, Durbin-Watson autocorrelation coefficient, BIC, AIC and root mean square error are taken into account. All of these criteria are equations created to determine how adequate or insufficient the model is to represent the point distribution. These equations test how close the point distribution is to the curve created, whether there is a relationship between the error terms, how close the values obtained with the estimation equations and the values obtained, and whether they are within the statistically acceptable error limits while doing these. In this study, the coefficient of determination, mean square error, Durbin-Watson and AIC were taken into account in the comparison of the conformity of different models of the values obtained with the in vitro gas production technique to the point distribution.

Equality of the coefficient of determination (equation 9),

$R^2=1-(RSS/SST)$ (9)

is in the form. In the equation, RSS: Resudual the sum of squares, SST: Sum of square total. The R^2 value indicates how much of the total variation in the data set can be expressed by the model fitted to the nocturnal distribution. It takes values in the range of $0<R^2<1$. A high coefficient of determination means that the obtained model has a high fit to the point distribution.

Equality of mean squared error (equation 10),

$$MSE = \Sigma(Y_i - \hat{Y}_i)^2/n \quad (10)$$

is in the form. The low mean of squares of error is a strong indication that the model is well suited to the point distribution. Therefore, it is widely used in model comparisons.

The Akaike information criterion is a widely used criterion to choose the statistically most appropriate one among the equations created. As a rule, the model with the smallest Akaike information criteria (AIC) value is considered to be the most appropriate model. Equality of the AIC (equation 11),

$$AIC = nxln\left(\frac{HKT}{n}\right) + 2k \quad (11)$$

is in the form (Üçkardeş and al.,2013; Üçkardeş and Efe, 2014).

Durbin-Watson autocorrelation test is a test to test whether the error terms of the predicted model are related. The fact that the value obtained with this test is around 2 is a strong indication that there is no autocorrelation. Durbin Watson test statistics where e_i = error term, t = time (equation 12),

$$DW = \frac{\sum_{t=2}^{n}(e_t - e_{t-1})^2}{\sum_{t=1}^{n} e_t^2} \quad (12)$$

is in the form. The Durbin Watson value always lies between 0 and 4. If the DW value is 2, it is considered that there is no autocorrelation.

3. Results and Discussion

For the standard; it was concluded that the logistic, Gompertz and Quadratic Piecewise Regression models had the best results, while the Gamma model had the worst results in terms of means of error squares, coefficient of determination, Akaike information criterion and Durbin-Watson Autocorrelation values. For corn, as in the standard, when all comparison criteria are taken into account, it has been determined that the Logistic and Gompertz models have the best results, and the Gamma model has the worst results, as in the standard (Table 1, 2 and 3). In terms of comparison criteria for alfalfa, it was concluded that the logistic and Gompertz models had the best results, while the Gamma model had the worst results, as in standard and maize (Figure 1, 2 and 3).

As a result of the study, it was concluded that the logistic and Gompertz models had the best results in standard, corn and alfalfa feed groups in terms of comparison criteria, while the Gamma model had the worst results in all feeds. On the other hand, it was determined that the quadratic model gave values close to these models. These results are in line with the results obtained from the modeling studies of in vitro gas production values (Rodrigues et al., 2009; Wang et al., 2011; Üçkardeş et al., 2013; Çetinkaya and Erdem, 2015;) are in agreement.

Table 1. Gas values obtained for standard; Mean Squared Error, Coefficients of Determination, Akaike Information Criteria and Durbin-Watson Autocorrelation values of all models

Models	MSE	R^2	AIC	DW
Cubic piecewise regression	39.99	0.9910	-233.5	2.33
Richard	53.04	0.9975	-243.9	2.86
Logistic	37.24	0.9986	-412.6	2.09
Gamma	339.5	0.9625	-29.6	1.12
Gompertz	37.28	0.9986	-429.3	1.92
Orskov	208.8	0.9873	-196.5	2.45
Sigmoidal	62.65	0.9971	-313.7	0.96
Quadratic Piecewise Regression	28.47	0.9909	-429.9	2.11

Table 2. Gas values obtained for corn; Mean Squared Error, Coefficients of Determination, Akaike Information Criteria and Durbin-Watson Autocorrelation values of all models.

Models	MSE	R^2	AIC	DW
Cubic piecewise regression	28.03	0.9894	-213.5	2.43
Richard	42.98	0.9974	-263.1	2.96
Logistic	23.84	0.9982	-429.5	2.11
Gamma	653.1	0.9832	-51.6	1.22
Gompertz	24.90	0.9982	-431.5	1.98
Orskov	189.3	0.9880	-181.5	2.47
Sigmoidal	50.80	0.9969	-343.2	0.81
Quadratic Piecewise Regression	18.87	0.9883	-318.5	2.61

Table 3. Gas values obtained for alfalfa; Mean Squared Error, Coefficients of Determination, Akaike Information Criteria and Durbin-Watson Autocorrelation values of all models

Models	MSE	R^2	AIC	DW
Cubic piecewise regression	37.6	0.9911	-259.1	2.35
Richard	53.85	0.9984	-296.3	2.01
Logistic	50.29	0.9981	-441.5	2.04
Gamma	699.7	0.9731	-72.4	1.33
Gompertz	43.36	0.9983	-422.5	1.97
Orskov	233.1	0.9895	-174.6	2.49
Sigmoidal	43.69	0.9981	-402.6	1.09
Quadratic Piecewise Regression	37.72	0.9867	-208.7	2.98

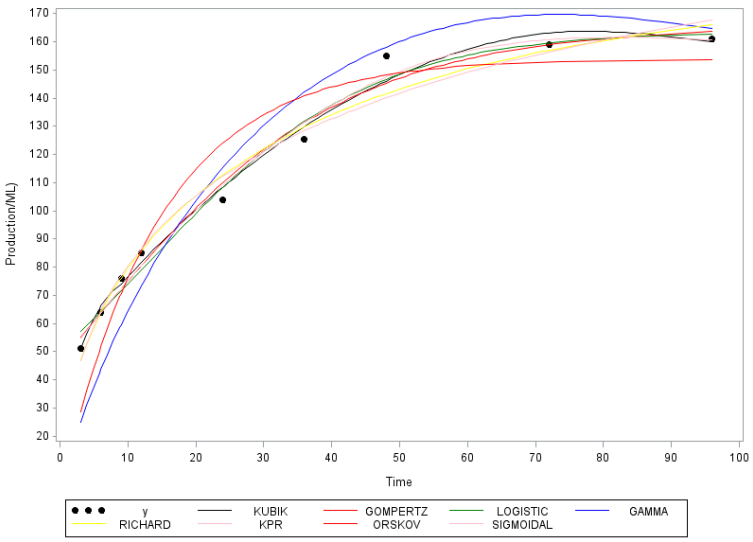

Figure 1. For the standard; gas production curves of cubic piecewise regression, Gompertz, Logistic, Gamma, Richard, Orscov, Sigmoaidal and Quadratic Piecemeal Regression.

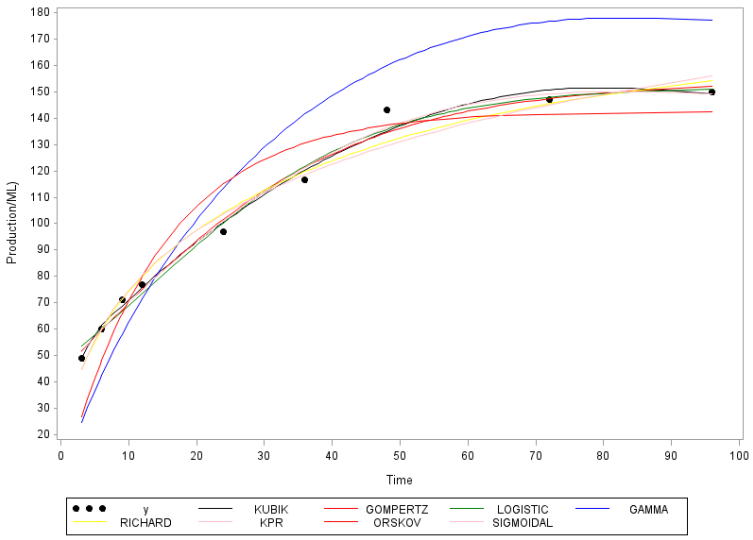

Figure 2. For Corn; gas production curves of Cubic Piecewise Regression, Gompertz, Logistic, Gamma, Richard, Orscov, Sigmoaidal and Quadratic Piecemeal Regression.

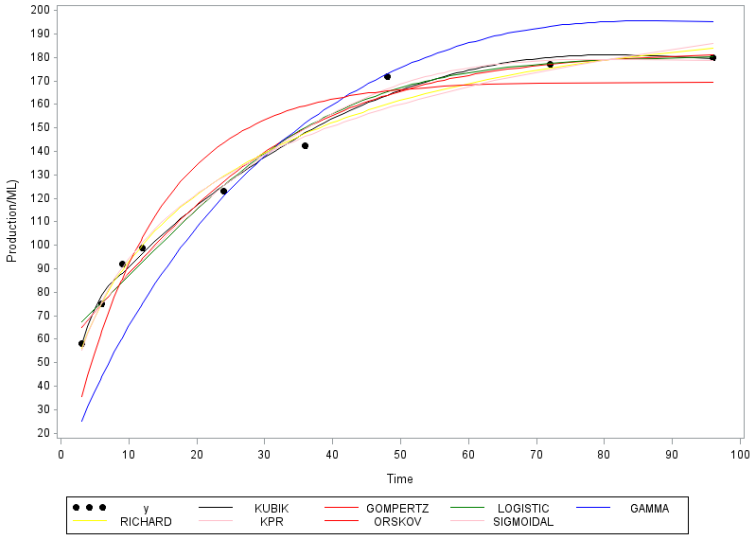

Figure 3. For Alfalfa; gas production curves of cubic piecewise regression, Gompertz, Logistic, Gamma, Richard, Orscov, Sigmoaidal and Quadratic Piecemeal Regression.

4. Conclusion

The point that should not be forgotten here and must be taken into account is the fact that the in-vitro evaluation of feeds by gas production method is affected by the amount of rumen fluid, the amount of feed used, the feed/rumen fluid ratio and the volume of the incubation environment. These factors are the factors that directly affect the amount of gas production. For this reason, accurate interpretation of the gas production method in in-vitro gas production method will only be possible with the selection of the right model. Because of the variability of these factors in each study, the shape of the gas curves obtained, that is, the polynomial structure of the curves, will be slightly different. For this reason, in the modeling of in-vitro gas production curves, it will be the most accurate method to consider more than one model and to consider the biological interpretability, advantages and disadvantages of the models in the evaluation.

Author Contributions

M.B. (34 %), M.Ş. (33%) and T.T. (33%) design of study. M.B. (34 %), M.Ş. (33%) and T.T. (33%) data acquisition and analysis. M.B. (34 %), M.Ş. (33%) and T.T. (33%) writing up. M.B. (33 %), M.Ş. (33%) and T.T. (34%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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RESPONSE OF BREAD WHEAT CULTIVAR TO DIFFERENT SOWING DENSITIES FOR YIELD AND YIELD TRAITS

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Abstract: This study was conducted at Edirne, Tekirdağ and Kırklareli locations in 2017-2018 growing season. The experiment was arranged in a randomized complete block design with four replications. In the study, it was aimed to determine the effects of five different sowing densities (200, 300, 400, 500 and 600 seed m^{-2}) on grain yield and yield components of bread wheat cultivar Aslı. In the experiment, the effect of sowing density and location on grain yield and yield components was found significant. In addition, sowing density × location interaction was found to be statistically significant in terms of grain yield. Grain yield was found among 693.2-849.7 kg da^{-1}, while spike length was ranged from 8.3 to 9.5 cm. However, number of spikelets per spike was in the range of 17.4-19.7, while number of grains per spike was 35.2 to 44.1 and grain weight per spike was 1.43 to 1.75 g. Among the locations, the highest grain yield was determined at Edirne location. In terms of grain yield, the most appropriate sowing density was determined as 400 seed m^{-2} rate.

Keywords: Bread wheat, Grain yield, Sowing density, Yield traits

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1. Introduction

Since the world's population grows each year, the demand for food rises as well. Wheat production in Türkiye ranks first in terms of cultivation area (6.7 million ha) and production amount was 17.65 million tons in 2021 growing season. Bread wheat production took place 82.1% (14.5 million tons) of total wheat production in 2021. Thrace region had a share of 6.5% in wheat agricultural areas and 12.5% in wheat production in Türkiye, with a wheat cultivation area of 445.042 ha and a production of 2.2 million tons. The average yield of bread wheat production at the Thrace region (496 kg da^{-1}) was 1.89 times higher than the average yield of bread wheat production (262 kg da^{-1}) countrywide (TUIK, 2022).

In order to increase the yield per unit area in wheat production, it is necessary to develop varieties with high yielding potential, suitable for the conditions of the region where production will be made and bring them into production (Gungor et al., 2022a, 2022b).

Other factors affecting grain yield in wheat cultivation are good quality seeds, the number of seeds to be utilized per unit area, sowing time and method, fertilization and cultivation practices (Bayramoglu and Gundogmus, 2010).

Sowing density is an important breeding technique that varies depending on genotype and environmental factors. It is necessary to determine the most suitable seed amount to be used per unit area in order to obtain high yield (Chen et al., 2008).

In some studies, high grain yield was obtained as sowing density was increased in wheat (Madan and Munjal, 2009; Costa et al., 2013). Baloch et al. (2010) and Ahmadi et al. (2011) indicated that sowing density had no effect on grain yield, while Carr et al. (2003) reported a grain yield increase until a certain plant density, and then decreased.

This study was carried out to determine the effects of different sowing densities on grain yield and yield components of Aslı bread wheat variety under the conditions of the Thrace region.

2. Materials and Methods

This research was conducted at Edirne, Kırklareli and Tekirdağ locations during 2017-2018 cropping years. The study was arranged in a randomized complete blocks design with four replications and bread wheat cultivar Aslı was used as plant material. The experiments were sown in the first week of November, with 200, 300, 400, 500 and 600 seed m^{-2} rates. The experiment plots were 6

rows with a 20 cm a part and 5 m length. In addition, the plot sizes were 6 m^2 for both planting and harvesting in the trial (6 m x 1 m). Weed control was done manually, and there were no applications for pests.

With sowing, 5 kg da^{-1} of nitrogen and 5 kg da^{-1} of phosphorus were applied and top dressing was divided into two and applied as 9 kg da^{-1} N during tillering and 6 kg da^{-1} N during jointing stages. The harvest of the locations was done in the first week of July. In the study, spike length (SL), number of spikelets per spike (SS), number of grains per spike (GNS), grain weight per spike (GWS) and grain yield (GY) were investigated.

The data obtained over the experiments were subjected to variance analysis in the JMP statistical program, and Duncan test was applied to compare the means (JMP 15.1 SAS Institute Inc, 2020).

3. Results and Discussion

The average spike length and number of spikes per spike are given in Table 1. While the sowing density and locations were found to be statistically significant for spike length and number of spikelets per spike, the interaction of sowing density × location was determined as insignificant for those traits. In terms of sowing density, average spike lengths ranged from 8.3 to 9.5 cm. The longest spike length was obtained from a sowing density of 200 seed m^{-2} (9.5 cm), while the shortest spike length was obtained from a sowing density of 600 seed m^{-2} (8.3 cm). The longest spike length was measured at Edirne (9 cm) location and the shortest was measured at Tekirdağ (8 cm) location (Table 1). As increasing the sowing density, the spike length was decreased (Naveed et al., 2014), and with the increase in sowing density, the amount of nutrients, water and light for the plants decreases and the competition between the plants increases, which shortens the spike length (Mutlu, 2022). Naveed et al. (2014), reported a 10.5-11.9 cm of spike lenght, while Atak et al. (2021), indicated 7.78-8.15 cm and Mutlu (2022) reported a 6.7 to 8 cm variation.

Tekirdağ location had the lowest number of spikelets per spike (17.2), while Edirne location had the highest (19.8) one (Table 1). The average number of spikelets per spike varied between 17.4 and 19.7 for sowing density. The largest number of spikelets per spike was found at 200 seed m^{-2} (19.7), and the lowest number of spikelets per spike was found at 500 seed m^{-2} (17.4). The number of spikelets per spike was determined as 15.9-16.6 by Ulucan and Atak (2020), and there were differences in the number of spikelets with varied sowing density, but these changes were statistically insignificant.

Table 1. Average of sowing density of spike length and number of spikelets per spike

SD (m^{-2})	SL (cm)				SS (no)			
	Edirne	Kırklareli	Tekirdağ	Mean	Edirne	Kırklareli	Tekirdağ	Mean
200	10.1	9.9a	8.5	9.5a	21.0	20.3a	17.7	19.7a
300	8.7	8.4b	7.8	8.3b	19.3	17.0bc	16.3	17.5b
400	8.6	9.2ab	8.0	8.6b	19.7	18.7ab	17.8	18.7ab
500	8.8	8.4b	8.1	8.4b	19.0	16.0c	17.3	17.4b
600	8.8	8.3b	7.9	8.3b	20.0	17.0bc	17.0	18.0b
Mean	9.0a	8.8a	8.0b	8.6	19.8a	17.8b	17.2b	18.3
SD	ns	*	ns	*	ns	*	ns	*
Location (L)			**				**	
SD × L			ns				ns	

SD= sowing densities, SL= spike length, SS= number of spikelets per spike, ** P < 0.01, * P < 0.05, and ns: not significant

The average number of grains per spike and grain weight per spike are given in Table 2. While the differences in number of seeds per spike and grain weight per spike between sowing density and locations were statistically significant, the sowing density × location interaction was found insignificant. According to the sowing density, the largest number of grains per spike was 400 seed m^{-2} (44.1), while the lowest was 600 seed m^{-2} (35.2). The number of grains per spike varied among the locations, ranging from 34.4 to 40.6, with an average of 38.5 grains per spike. The maximum number of grains per spike was obtained from Edirne location, while the lowest amount was determined at Tekirdağ location (Table 2).

In other researches, the number of grains per spike decreased as the sowing density increased: Mutlu (2022), 37.21-44.36, Dinc and Erekul (2010), 47.1-51.7, Ulucan and Atak (2020), 32.2-35.7, Atak et al. (2021), 39-42.1, Yagmur et al. (2021), 26.9-39.6. The grain weight per spike varied among locations, ranging from 1.36 to 1.89 g. The lowest grain weight was found at Tekirdağ location, while the highest grain weight was found at Edirne location. In sowing density applications, average grain weight per spike ranged from 1.43 to 1.75 g. The maximum grain weight values were found at sowing density of 200 seed m^{-2} and 400 seed m^{-2} (1.75 g), while the lowest grain weight per spike was found at 600 seed m^{-2} (1.43 g) (Table 2). Mutlu (2022), 1.66-1.84 g, Atak et al. (2021), 2.04-2.25 g, Yagmur et al. (2021), 0.9-1.32 g, Kazan and Dogan (2005), reported that grain weight in spike varied between 1.65 to 1.99 g.

Table 2. Average of sowing density of grain number/spike and grain weight/spike

SD (m^{-2})	GNS (no)				GWS (g)			
	Edirne	Kırklareli	Tekirdağ	Mean	Edirne	Kırklareli	Tekirdağ	Mean
200	44.0	47.3	34.0b	41.8a	2.02	1.74	1.50a	1.75a
300	38.3	39.0	30.8b	36.0b	1.85	1.36	1.15b	1.45b
400	44.3	46.0	42.0a	44.1a	2.06	1.66	1.54a	1.75a
500	39.7	31.3	35.3b	35.4b	1.74	1.28	1.45a	1.49b
600	36.8	39.0	30.0b	35.2b	1.76	1.35	1.17b	1.43b
Mean	40.6a	40.5a	34.4b	38.5	1.89a	1.48b	1.36b	1.58
SD	ns	ns	*	**	ns	ns	**	*
Location (L)			**				**	
SD × L			ns				ns	

SD= sowing densities, GNS= number of grains per spike, GWS= grain weight per spike, ** P < 0.01, * P < 0.05, and ns: not significant

According to the statistical analysis for grain yield, the difference among sowing density, locations and sowing density × location interaction was found statistically significant (Table 3). The maximum grain yield was obtained at 934.9 kg da^{-1} Edirne location, and the minimum grain yield was obtained at 662.6 kg da^{-1} Tekirdağ location. Grain yield varied between 693.2-849.7 kg da^{-1} and the average grain yield was determined as 768.5 kg da^{-1}. The maximum grain yield (849.7 kg da^{-1}) was obtained at 400 seed m^{-2} rate (Table 3). In similar studies; Dalkilic et al. (2016), reported higher grain yield (506 kg da^{-1}), at 600 seed m^{-2} sowing rate, Ulucan and Atak (2020) indicated a 559.2 kg da^{-1} grain yield at 550 seed m^{-2} rate, while Atak et al. (2021), 566.9 kg da^{-1} at 450 seed m^{-2} rate, Yagmur et al. (2021), 293.3 kg da^{-1} at 650 seed/m^2 rate, Mutlu (2022), 634.17 kg da^{-1} at 650 seed m^{-2} rate.

On the other hand, Dinc and Erakul (2010), stated that different sowing density do not have an effect on grain yield, while Mutlu (2022), reported an increase up to a certain seed density and then decrease. However, Dalkilic et al. (2016) reported that as the sowing density increases, the grain yield in wheat also increases.

Table 3. Average of sowing density and grain yield

SD (m^{-2})	Grain Yield (kg da^{-1})			
	Edirne	Kırklareli	Tekirdağ	Mean
200	918.6a	584.7c	576.4b	693.2c
300	1000.3a	726.4b	692.8a	806.5b
400	1007.5a	841.4a	700.3a	849.7a
500	1020.3a	693.6b	671.1a	795.1b
600	727.8b	694.2b	672.2a	698.1c
Mean	934.9a	708.1b	662.6c	768.5
SD	**	**	**	**
Location (L)			**	
SD × L			**	

SD= sowing densities, ** P < 0.01, * P < 0.05, and ns: not significant

4. Conclusion

One of the most important agricultural practices that determine the grain yield in wheat cultivation is sowing density. Determination of the most suitable seed density of the varieties produced is important to increase the yield.

Among the locations, the highest grain yield was determined at Edirne location. In terms of grain yield, the most appropriate sowing density was 400 seed m^{-2}, and then the grain yield decreased with increasing sowing density. It is concluded that sowing density is a crucial factor in terms of yield and yield components in bread wheat.

Author Contributions

HG (%34), MFC (%33) and ZD (%33) design of study. HG (%34), MFC (%33) and ZD (%33) data acquisition and analysis. HG (%34), MFC (%33) and ZD (%33) writing up. HG (%34), MFC (%33) and ZD (%33) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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DETERMINATION OF THE EFFECTS ON MEAT QUALITY AND FATTY ACIDS OF DIFFERENT BORON SOURCES IN AKKARAMAN LAMBS

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Abstract: In this study, the effects of boron sources such as colemanite, ulexite and etibor-48 supplementation on meat quality and fatty acid composition of Akkaraman lambs weaned at 2.5 months of age were investigated. In the study, 50 single Akkaraman male lambs weaned at the age of 2.5 months were used as animal material. Concentrated containing (17.56% CP and 2600 Kcal/kg ME) and forage (meadow hay) were used as feed material. Colemanite (50.8% B_2O_2, $Ca_2B_6O11.5H_2O$), ulexite (43% B_2O_3, $NaCaB5O_9.8H_2O$) and etibor-48 (48%, $Na_2(OH)_2.8H_2O$) as boron sources were used. The highest protein content of Akkaraman lamb meat was obtained from C and S groups, and the lowest in L group (P<0.05), and there was no significant difference between the groups in terms of ether extract. The ash content of the meats was lowest in the L group (P<0.05). While the Ca level in meat was lowest in L and E groups (P<0.05), P and Water holding capacity (WHC) were found to be high in S and C groups (P<0.05). The Mg content was lowest (P<0.05) in the S group, the B content of the meat was the same in all groups. Among fatty acids, caproic acid in E group, stearic acid in L group, cis-8,11,14-eicosatrienoic acid in U, E and S groups was highest (P<0.05) and the lowest in L group, followed by C group (P<0.05). While oleic acid was numerically higher in C group than other groups, Linoleic acid was found to be low in the L group. Numerically increment was determined in terms of linoleic acid, Tricosanoic acid and Palmitoleic acid in boron groups. The highest a* value in Akkaraman lamb meat was determined in the U group, and there was no difference between the groups in terms of b*, c* and L* values. A numerical increase in b* and L* values in boron groups was determined compared to the S group. While hue value was highest in C, E and L groups and, was lowest in U group (P<0.05). The use of different boron sources in the ration did not have a significant effect on the flexibility, hardness and WBSF properties of meats. However, the addition of boron such as C, U and E to the ration caused a numerical decrease in WBSF.

Keywords: Akkaraman lamb, Meat quality, Fatty acids, Boron sources

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1. Introduction

Red meat has an important place in providing the need for animal protein, which is one of the cornerstones of a healthy diet. The increase in the world population also increases the demand for red meat. Meat and meat products have great importance in human nutrition because they contain protein, fat, essential amino acids, minerals, vitamins, and other nutrients (Olaoye, 2011), in addition, the bioavailability of some macronutrients found only in meat is much higher than those of plant sources (Wyness, 2015).

The main source of red meat production is cattle and small ruminant such as sheep, goats. Sheep and lambs are an important source of meat production among small ruminants. It's known that genetic structure plays an important role in meat quality. Furthermore, to biochemical, physical, histological factors, age, sex, species, race, muscle type, health, and especially nutrition play an important role in determining meat quality (Guerrero et al., 2013). Since the diet is easily manipulated, it significantly affects the nutrient composition of meat (Wyness, 2015).

Minerals are essential for normal animal health, growth, reproduction, and production, in structural, physiological, catalytic and regulatory functions, as components of proteins, enzymes or enzymatic cofactors for a number of biochemical processes (Lavinia et al., 2014). Minerals contribute even more than volatile fatty acids (VFA) to rumen osmolarity. High osmotic pressure may impair digestion in the rumen. Under normal nutritional conditions, the main buffering components in the rumen are Na, K, bicarbonate, and VFA. Therefore, an imbalance or low in mineral level is effective on microbial activity and rumen fermentation (Singh, 2021). In addition, minerals play a very important role in meat quality, as they affect some characteristics such as color and texture of red meat, (Schönfeldt and Hall, 2015, Domaradzki et al., 2016). Regarding energy production, besides the role in

muscle metabolism of minerals, mineral-dependent enzymes also play a role in the softening of meat after death (Bhat et al., 2018).

Boron, a trace mineral, is an essential trace element for plants, humans, and animals. It is known that boron has functions in mineral metabolism, immune and endocrine systems, and low boron intake impairs bone health, brain function and immune response (Nielsen, 2008). Wang et al. (2014) reported that the addition of 160 mg/L boron to water of ostriches improved growth performance and meat quality; determined that high boron concentration decreased both performance and meat quality. The maximum tolerable level in sheep has been determined as 150 mg B/kg diet (NRC, 2007). It is reported that when 0, 35, 52.5 and 70 mg kg^{-1} levels of boron are used in the rams' ration, it does not affect the boron concentration in the rumen fluid, but improves rumen fermentation due to increasing the population and activation of microorganisms, especially protozoa and cellulolytic bacteria (Sızmaz et al, 2017). In studies related to boron, it has been determined that this trace element is used in both human foods and animal feeds in the form of boric acid and/or its salts (Lavinia et al., 2014).

It is stated that boron reserves in Türkiye constitute 73% of the world's total reserves. There is Ca in the structure of (50.8% B_2O_2, $Ca_2B_6O_{11}.5H_2O$) colemanite, Ca and Na in the structure of (43% B_2O_3, $NaCaB_5O_9.8H_2O$) ulexite, and Na in the structure of (48%, $Na_2(OH)_2.8H_2O$) etibor-48 from the boron sources (Anonymous, 2022).

There are no studies on the use of boron on meat quality and fatty acid properties in ruminant feeding. Boron sources especially such as colemanite, ulexite and etibor-48 mostly found in Türkiye have never been used in animal nutrition. Based on the idea that supplementation of boron sources may be a factor affecting the expression of trace elements in the body, it is thought that these B sources can be used as a mineral additive in ruminant feeding, and may affect meat quality and fatty acids, and may be a mineral additive in ruminant nutrition.

For this purpose; in this study was investigated the effect on meat quality and fatty acid composition of adding some boron sources (colemanite, ulexite and etibor-48) to the low Ca and P-containing diets of Akkaraman lambs weaned at the age of 2.5 months.

2. Materials and Methods

2.1. Materials

Fifty Akkaraman single, male lambs weaned at the 2.5 months-old were used as animal material in the study. Concentrated feed containing 17.56% HP and 2600 Kcal/kg ME and meadow hay were used as feed material. Boron sources; colemanite (50.8% B_2O_2, $Ca_2B_6O_{11}.5H_2O$), ulexite (43% B_2O_3, $NaCaB_5O_9.8H_2O$) and etibor-48 (48%, $Na_2(OH)_2.8H_2O$) were obtained from Eti Maden Operations in Türkiye.

2.2. Methods

A standard and low-level Ca and P-containing basal diet was prepared in the first phase of the experiment. Each of the boron sources (colemanite, ulexite and etibor-48) was added 90 ppm/kg (NRC, 2007) to the low Ca and P containing lamb diet, taking into account their purity, and 5 treatments groups 1- with standard Ca and P content, 2- with low Ca content and P content, 3- low Ca and P content +colemanite, 4- low Ca and P content +ulexite, 5- low Ca and P content + etibor-48) were formed. The concentrated feed was prepared as Crude protein (CP) content of 17.12% and metabolisable energy (ME) 2720 kcal/ Dry matter (DM) (Sarıçiçek and Yıldırım, 2021). The roughage was kept free in front of the animal, and concentrated feed was given in two meals. The roughage/concentrated feed ratio was 60/40. The daily need was determined taking into account the level specified in NRC (1985).

50 male Akkaraman single lambs weaned at 2.5 months-old were divided into 5 groups with equal weight (approximately 22 kg) and 10 lambs in each group, and the lambs were fattened in individual cages for 90 days. At the end of 90 days, a total of 30 animals, randomly 6 heads from each group, were slaughtered. LT muscle (longissimus thoracis) was separated between 12-13 ribs to determine meat quality, the separated meat was vacuum packed and kept for 5 days at 4ºC in a refrigerator. Then, nutrient analysis (dry matter, protein, fat, ash, Ca, P, Mg, boron in ash), pH, meat juiciness (water holding capacity: WHC and cooking loss: CL), fatty acids (saturated and unsaturated), color determination (brightness, redness, yellowness), and texture (flexibility, hardness, warner blatzer shear force; WBSF) in meats were examined.

2.3. Analysis

Dry matter, protein, fat, and ash analysis of meat were made according to AOAC (1990), mineral substance contents (Ca, P, Mg, and B) in the ash were determined by plasma emission spectroscopy (CAP) method (ICP/6500 system sequential analysis Perkin-Elmer, Norwork, CT) in ICP.

The first pH value was determined in the meat taken from the right LT muscle as soon as the animals were slaughtered, and the final pH value was determined in the same meat that was rested for 24 hours. pH values were measured with a digital, portable pH meter (Orion 210A pH-meter and Orion 9106 glass electrode). The glass probe of the pH meter was placed directly in the center of the samples after calibrating with standard buffers pH 4.00 and pH 7.00.

The juiciness of the meat was estimated by two methods: water holding capacity (WHC) and cooking loss (CL). Meat samples for WHC were sliced into 1 cm thick, 4 cm^2 diameter steak, wrapped in gauze and placed between pre-weighed Wattman 18 papers, then a 2-2.5 kg weight was placed on it for 5 minutes and calculated according to the formula below (equation 1).

WHC= first weight of meat - final weight of meat/first weight of meat) x 100 (Grau and Hamm, 1953) (1)

The samples for CL, were cut into 65 g blocks and placed in polyethylene bags and kept in a thermostatically controlled water bath set for 1 hour at 75°C, then the samples were cooled in cold water for 30 minutes, the meats removed from the bag were dried with a dry towel and weighed. CL was calculated according to the formula below (equation 2).

CL= (weight of raw meat - weight of cooked meat) / weight of raw meat x 100 (Franko et al., 2011) (2)

Analysis of fatty acid methyl esters from the extracted lipid was made according to Anonymous (1987). Composition of fatty acids determined using Shimadzu brand gas chromatography (Model GC-2010, Japan) with flame ionization detector (FID) and with a DB-23 column (60 m x 0.25 mm I.D, 0.25 µm). Fatty acids were identified by comparing based on their arrival time of the standard 37-component FAME mixture (Supelco 37 Components FAME Mixture, Cat. No. 18919-1AMP, Bellefonte PA, USA).

Determination of color in meat; Lightness (L*), redness (a*) and yellowness coordinate (b*) Chroma (C*), and hue angle (H°) values were determined by Konica Minolta CR400 instrument. The chromameter was calibrated on a white calibration plate (Y = 87.1, x = 0.3158, y = 0.3225) before measuring color. The measuring head was adjusted to illumination C with 2° standard observer and 8 mm aperture.

The texture analysis of the meats (TA-HD Plus Texture Analyzer, UK) was determined by the compression test using a 5 N load cell. Hardness, flexibility and WBSF values were determined in the compression test. 3 sub-samples of 1x1 cm parallel to the direction of the muscle fibers were prepared from each cooked meat sample and WBSF was measured.

2.4. Statistical Analysis

The data obtained according to the criteria discussed at the end of the whole study were analyzed using SPSS 25.0 (IBM, Chicago, IL, USA) and Origin 2021b software (OriginLab, Northampton, MA, USA). Analysis of variance was performed to evaluate the differences between the means and then analyzed with the Duncan multiple comparison test (SAS, 2008). The least significant differences were determined as $P < 0.05$.

3. Results

3.1. Nutrient Content of Meats

The nutrient contents of the meat samples taken from the LT muscle are given in Table 1.

Table 1. Nutrient content of meats

Groups and Parameters	S	L	C	U	E
DM, %	26.88±0.478	25.42±0.576	26.98±0.483	26.73±0.247	25.67±0.269
Protein, %	20.56±0.001a	1.08±0.006a	20.96±0.001a	19.88±0.003ab	19.79±0.002ab
Fat, %	3.96±0.313	3.72±0.239	3.95±0.200	4.38±0.278	4.38±0.232
Ash, %	1.61±0.144a	0.94±0.017c	1.60±0.160a	1.58±0.132a	1.22±0.073b
Ca, mg/100g	5.94±0.238a	3.58±0.264b	5.71±0,249a	5.56±0,236a	3.53±0,228b
P, mg/100g	17.99±0.086a	15.48±0,035b	17.37±0.027a	15.55±0.038b	15.42±0.081b
Mg, mg/100g	19.78±0.057b	22.84±0.040a	22.27±0.042a	23.92±0.010a	23.11±0.092a
B, mg/100g	0.01±0.001	0.01±0.002	0.01±0.001	0.01±0.001	0.01±0.002

S= standart (Ca, P), L= low (Ca, P), C= low (Ca, P) + colemanite, U= low (Ca, P) + ulexite, E= low (Ca, P) + etibor-48, DM= dry Matter, Ca= calcium, P= phosphorus, Mg= magnesium, B= boron.

[a, b, c]There is a significant difference between the means shown with different letters on the same line, (P<0.05).

There was no statistically significant difference between the groups for dry matter (DM) and fat content of meat. The protein content was highest in C and S groups, and the lowest in L group, and the difference between them and the L group was significant ($P < 0.05$), however, the difference between E and U and L was not statistically significant. The lowest ash content of the meats was determined in L group, followed by E group. The difference between these two groups and the other groups was significant ($P < 0.05$), there was no significant difference between S and C and U groups. When the mineral contents of the meats were examined, the lowest Ca were determined L and E groups. Significant differences were found between these and other groups ($P < 0.05$). No significant difference was determined between S control group and boron groups. The highest P content was determined in S and C groups. While there was no significant difference between these groups, the difference between these groups and the others was significant ($P < 0.05$). While S group had lowest Mg content ($P < 0.05$) compared to all other groups, no significant difference was found between the other groups. B content of meat was the same in all groups. No significant differences were found between the treatment groups.

3.2. Meat pH Water Holding Capacity and Cooking Loss Value,

The pH at 0 h and 24 h after slaughtering, water holding capacity (WHC) and cooking loss (CL) values of the meats after the first and 24 hours are given in Table 2. As seen in Table 2, the initial pH_0 value of the meats varied between 6.60 and 7.04, and the pH_{24} value varied between 5.61-5.83. There was no significant difference between the groups in terms of pH_0 and pH_{24}. Addition of boron to the ration had no effect on the pH values of the meat compared to the S control group.

The WHC varied between 16.67-21.67%. In terms of WHC, S and C groups were significantly higher ($P < 0.05$) compared to the other groups. There were no significant differences between both S with C and L with U, E. CL values in Akkaraman lamb's meat varied from 27.38 to 32.96%. There was no significant difference between the groups in terms of CL. But C and U were numerically lower.

Table 2. pH, water holding capacity and cooking loss values of meats

Groups and Parameters	S	L	C	U	E
pH_0	6.77±0.223	7.04±0.164	6.76±0.108	6.60±0.111	6.77±0.053
pH_{24}	5.67±0.032	5.83±0.023	5.65±0.023	5.61±0.033	5.67±0.025
WHC, %	21.67±0.103[a]	16.67±0.133[b]	20.54±0.169[a]	16.74±0.131[b]	17.12±0.141[b]
CL, %	32.96±2.434	31.06±1.776	27.38±1.963	30.16±2.468	31.05±2.638

S= standart (Ca, P), L= low (Ca, P), C= low (Ca, P) + colemanite, U= low (Ca, P) + ulexite, E= low (Ca, P) + etibor-48, WHC= water holding capacity (%), CL= cooking loss (%).

[a, b, c]There is a significant difference between the means shown with different letters on the same line, ($P<0.05$).

3.3. Color Characteristics of Meat

Color analysis were made with a color measuring device in the meat taken from the LT muscle and results are shown in Table.3. The a* value, which is an indicator of redness in the meat of Akkaraman lambs, ranged from 8.77 to 10.47. The highest a* value compared to the other groups was determined in the U group, and the numerical difference between the other groups was not significant. There was no difference between the groups in terms of the yellow color indicator "b*", chroma "c*" and lightness expression "L" values in meats. The b* and L* values in the boron groups were numerically higher than S group. However, while Hue angle value, which indicates the color intensity, was highest in C, E and L groups, there was no significant difference between these groups. H° was lowest in U group. While the difference between U and S groups was not significant, a significant difference was found between the other groups ($P < 0.05$).

Table. 3. Color characteristics of meat

Groups and Parameters	S	L	C	U	E
L*	30.78±1.383	33.61±1.069	33.45±1.350	31.94±1.049	35.02±0.816
a*	9.45±0.398[b]	9.37±0.318[b]	8.77±0.206[b]	10.47±0.181[a]	9.42±0.213[b]
b*	7.36±0.369	7.84±0.232	7.66±0.341	7.65±0.257	7.95±0.241
C*	11.99±0.472	12.22±0.314	11.66±0.314	12.98±0.151	12.33±0.216
H°	7.81±0.035[ab]	8.43±0.033[a]	8.74±0.036[a]	7.37±0.031[b]	8.52±0.037[a]

S= standart (Ca, P), L= low (Ca, P), C= low (Ca, P) + colemanite, U= low (Ca, P) + ulexite, E= low (Ca, P) + etibor-48, L*= lightness, a*= redness, b*= yellowness, C*= chroma, H°= hue angle.

[a, b, c]There is a significant difference between the means shown with different letters on the same line, ($P<0.05$).

3.4. Textural Characteristics of Meat

After the cooking loss was determined in the meat samples taken from the LT muscle, the texture analysis of the meat was performed. Data on flexibility, hardness and WBS cutting force of meats are given in Table 4. The flexibility of the meat of the trial lambs are between 54.91-79.02 N, the hardness measured with a 1 mm probe is between 6.36-10.91 N; WBSF varied between 27.92-35.91.

The use of different boron sources in the ration did not have a significant effect on the flexibility, hardness and cutting force properties of the meats. However, while the addition of C increased the flexibility, the addition of C and U increased the hardness numerically, the addition of C, U and E caused a decrease in WBSF compared to the control groups.

3.5. Fatty acid content of meats

In the material extracted from meat samples taken from LT muscle, 37 fatty acids were examined in gas chromatography, and fatty acids found at very low levels were not taken into consideration. Data on fatty acids of meats are given in Table 5. Caproic acid ranged between 0.11-0.39 g/100g, caproic acid was found to be significantly higher than the others in group E ($P < 0.05$), but there was no significant difference between the other groups. Stearic acid was found to be significantly higher

in L group compared to all groups ($P < 0.05$), no significant difference was found between boron groups and S group. Cis-8,11,14-eicosatrienoic acid was high in U, E and S groups, and the lowest in L group, followed by C group. While the difference between these two groups was significant ($P < 0.05$), the difference between these groups and other groups was also significant ($P < 0.05$). While oleic acid was higher in C group (41.56 g/100g), Linoleic acid was lower in L group (3.57 g/100g) compared to all groups.

Table 4. Texture characteristics of meat

Groups and Parameters		S	L	C	U	E
Flexibility, N		58.53±81.541	78.32±30.378	79.02±34.860	54.91±28.303	72.96±70.475
Hardness, N (1mm)		6.36±23.806	7.58±21.277	10.17±17.858	10.91±17.014	7.45±17.746
Warner	Shear Force, N	19.84±64.388	20.90±76.179	19.58±33,122	18.06±23.352	17.69±36,686
Blatzer	Work shear, N	35.91±89.538	31.97±77.138	29.73±68.184	28.19±60.583	27.92±108.539

S= standart (Ca, P), L= low (Ca, P), C= low (Ca, P) + colemanite, U= low (Ca, P) + ulexite, E= low (Ca, P) + etibor-48, N= Newton.

Table 5. Fatty acids content of meat (g/100g)

	S	L	C	U	E
Caproic acid	0.22±0.024^{b}	0.11±0.025^{b}	0.13±0.051^{b}	0.20±0.024^{b}	0.39±0.074^{a}
Capric acid	0.17±0.006	0.17±0.013	0.18±0.008	0.16±0.010	0.15±0.005
Lauric acid	0.18±0.020	0.15±0.013	0.18±0.021	0.16±0.017	0.20±0.037
Myristic acid	2.40±0.140	2.37±0.102	2.54±0.105	2.27±0.116	2.42±0.241
Myristoleic acid	0.16±0.093	0.05±0.005	0.07±0.003	0.06±0.004	0.06±0.007
Pentadecanoic acid	0.36±0.028	0.36±0.030	0.30±0.026	0.30±0.015	0.33±0.018
Palmitic acid	23.77±0.288	24.48±0.70	24.71±0.733	24.28±0.938	23.31±0.994
Palmitoleic acid	1.28±0.042	1.08±0.058	1.39±0.079	1.30±0.058	1.31±0.107
Heptadecanoic acid	1.52±0.091	1.55±0.132	1.18±0.094	1.20±0.106	1.33±0.099
cis-10 heptadecanoic acid	0.76±0.046	0.70±0.052	0.72±0.043	0.71±0.037	0.72±0.026
Stearic acid	18.57±1.084^{b}	22.53±1.327^{a}	18.45±0.747^{b}	18.31±0.925^{b}	19.42±1.346^{ab}
Elaidic acid	1.54±0.117	1.33±0.429	0.79±0.254	1.41±0.91	1.40±0.167
Oleic acid (C18:1n9t)	40.42±1.243	38.97±0.647	41.56±0.908	38.99±0.996	39.55±0.721
Linolelaidic acid	0.13±0.030	0.08±0.012	0.07±0.07	0.10±0.049	0.10±0.029
Linoleic acid	4.63±0.541	3.57±0.197	4.30±0.321	5.73±0.877	5.08±0.435
Arachidic acid	0.12±0.014	0.15±0.020	0.12±0.019	0.12±0.013	0.13±0.010
gama-linoleic acid	0.11±0.009	0.08±0.004	0.10±0.008	0.10±0.010	0.10±0.006
cis-11-eicosenoic acid	0.15±0.011	0.13±0.005	0.14±0.009	0.15±0.010	0.14±0.007
Heneicosanoic acid	0.46±0.027	0.42±0.029	0.44±0.031	0.51±0.063	0.48±0.033
cis-11,14-eicosadienoic acid	0.07±0.004	0.07±0.006	0.06±0.003	0.07±0.003	0.07±0.007
cis-8,11,14-eicosatrienoic acid	0.32±0.007^{a}	0.18±0.013^{c}	0.29±0.006^{b}	0.39±0.007^{a}	0.38±0.004^{a}
Tricosanoic acid	2.42±0.52	1.26±0.144	2.13±0.30	3.00±0.690	2.57±0.409
cis-5,8,11,14,17-eicosapentaenoic acid	0.21±0.033	0.11±0.007	0.16±0.033	0.24±0.049	0.21±0.024
cis-4,7,10,13,16,19-docosahexaenoic acid	0.11±0.020	0.05±0.005	0.09±0.018	0.12±0.024	0.10±0.014

S= standart (Ca, P), L= low (Ca, P), C= low (Ca, P) + colemanite, U= low (Ca, P) + ulexite, E= low (Ca, P) + etibor-48.

$^{a, b, c}$There is a significant difference between the means shown with different letters on the same line, ($P<0.05$).

4. Discussion

4.1. Nutrient Content of Meats

The DM, protein, fat, and ash content of Akkaraman lamb meat in study varied between 25.42-26.98%, 19.08-20.96%, 3.72-4.38% and 0.94-1.61%, respectively. Since there were no studies on boron sources in lambs, the findings were compared with the results of other studies on lamb meat quality. Jacques et al. (2016) determined the water, protein and fat values in Dorset male lambs meat fed with concentrated feed respectively was 75, 21.5 and 3.2%. These values were like the results of Akkaraman lamb meat in this study. Likewise, the information obtained in water, protein, and ash of Akkaraman lamb meat in our study was compatible with Camacho et al. (2015), who determined the water (72.70%), protein (21.86%) and ash (0.92%) contents of 25 kg Canarian lamb as meat. Similar results were obtained by Oliveira et al. (2019) for water (73.54%), protein (20.20%) and ash (0.99%) in Santa Ines lamb meat, but the result for fat (5.16%) was found to be higher than the current study. The reason of these differences may be due to the different breeds, ages, production systems and nutritional management of the animals.

In our study, Ca in meat varied between 3.53-5.94 mg/100g, the lowest Ca content was found in L and E groups, it was highest in S groups and C and U groups. The presence of Ca in structure of colemanite and ulexite may have caused an increase in the Ca content of the meat. Kaić et al. (2016) determined as 5.03 mg/100g Ca concentration in LT muscle of Lika Pramenka lambs. This result is similar to the results of the other groups in our study, except for L group. Phosphorus content ranged between 15.42-17.99 mg/100g and was highest in S and C groups, and was similar in L, E and U groups. Mg levels changed between 19.78-23.92 mg/100g and the lowest was determined in S control group. Similar findings were reported by Bellofet al. (2006), who determined as 16.06 mg/100g P content in German Merino lamb in the growing period, but Mg content was found to be higher than the results of our study. On the contrary, The Ca (7.05 mg/100g) content determined by Lima et al. (2013) for lamb meat was higher than our study, and Mg content (19.24 mg/100g) was similar to result of our study.

Mostert and Hoffman (2007) reported that the mineral content of meat can be affected by various factors such as mineral concentration in the diet, hormones, age, species, and region. According to Lin et al. (1988), it is possible that changes in lamb feeds affected mineral content in animal meat. The B level of meat was the same in all boron added treatment groups. Bharti et al. (2007) stated that boron is not stored in soft tissues but excreted in the urine. This view supports the results of the research. There is boron at the level of 0.015-0.6 (mg/kg fresh tissue) in the soft tissues and fluids of humans and animals (Nielsen, 1997). The B level determined in meat in this study is consistent with the literature report.

4.2. Meat pH, Water Holding Capacity and Cooking Loss Value,

In the present study, pH_0 of Akkaraman lamb meat was between 6.60-7.04, and after 24 hours the pH value changed between 5.61-5.83. Since there was no study on meat quality with the Boron sources studied, Akkaraman lamb meat quality criteria were compared with the results of different lamb meats. Similar findings were reported by McGeehin et al. (2001), who the pH values of male lambs meat taken from LT at 0 and 24 hours found as 6.37-6.91, 5.31-5.76, respectively. Likewise, Tejeda et al. (2008) similarly found pH 24 (5.83-5.60) for merino male lambs, while Díaz et al. (2002) also similarly found pH 24 values (5.51 and 5.71) of Talaverana lambs slaughtered at 24 and 28 kg carcass weight.

In the current study, WHC was determined the highest in S (21.67%) and C (20.54%) groups, while L and other B groups (16-17%) had lower WHC. Boron addition caused an increase in WHC compared to L group. List et al. (2011) determined 18.48% for WHC in Spanish Ternasco type lambs weaned at the 45 days-old and fed with feed containing 18% CP and 11.5 MJ ME, and this results is lower than the findings in this study. In the present study, the CL of meat varied between 27.38 and 32.96%. CL decreased numerically in all groups, especially in the C group compared to the S group. Since there was no study on CL with boron sources in lambs, the results were compared with studies on meat quality of lambs. This result was higher than the findings of Sheridan et al. (2003), who in Meat Merino lambs meat fattened for 28 and 56 days found as 33.93% and 39.62% of CL, respectively. Likewise, the CL values (35.90, 35.49, 36.37 and 35.23%) determined in the meat of growing crossbred male lambs (¾ chios. ¼ ossimi and ½ chios. ½ ossimi) by Abd El-aal and Suliman (2008) were higher than the results of this study. This difference may be due to the age of the animal, the cooking time of the meat, the temperature, and the feeding difference. As the age or physiological maturity of the lambs' progress, it is necessary to cook the meat longer, which leads to more cooking loss (Russo et al., 2003). During the cooking, meat greatly loses the water present in its structure, which leads to a decrease in the tenderness and flavor of the meat. Moreover, if the cooking temperature rises above 70 °C, cooking losses in meat increase (George-Evins et al., 2004).

4.3. Color Characteristics of Meat

The L* value, which is the indicator of lightness, is ranged 30.78-35.02 this study. This result was lower than the report of Akcay et al. (2014) in Bafra lambs (39.55), but the L value (31-40) determined by Şirin (2009) in Kıvırcık lambs was found to be similar to this study. The a* (11.5) and L* (41.3) values determined by Jacques et al. (2016) in Dorset male lambs were higher than the current study, the b* (5.3) value was lower than the study, and the C* value (12.7) was similar to the study. The values of a* (19.59), b* (6.08) L* (43.89), C* (20.61) and H° (20.61) determined in 25 kg Canarian lambs in

study of Camacho et al. (2015), are higher than results of study. L* value in this study was similar (33.2) to the report for Texel crossbred male lambs (5-8 months old) of Johnson et al. (2005) but the a* value was lower than report (13.8).

In this study, a* value, which is an indicator of redness in the meat of Akkaraman lambs, ranged between 8.77 - 10.47. Adding U to the ration caused an increase in a* value. Akcay et al. (2014) determined a* value as 18.37 in Bafra lambs and Şirin (2009) determined between 17-21 in Kıvırcık lambs, these results are considerably higher than the value determined for Akkaraman lambs.

b* value, which is an indicator of yellowness in meat, ranged between 7.36-7.95 in this study. These results, similar with the b* value (4-7) determined in Kıvırcık lambs by Şirin (2009), and b* (7) value determined in Bafra lambs by Akçay et al. (2014).

In a study conducted in Merinos, the L*, a* and b* values (respectively; 43.68-43.39, 13.27-12.73, 8.73-9.13) found in male lambs for slaughter weight of 24 kg and 29 kg were calculated higher than the current study (Tejeda et al., 2008). These differences between the results may be atributed to race and age differences, as well as the structure of the feed fed to the animal and the difference in feeding. According to Priola et al. (2001) the fattening method, slaughtering practices, carcass preservation, rigor mortis temperature, and meat pH may also affect these values. No study has been found on the effect of B sources on the color characteristics of lamb meat. But it is clear that boron sources provided a numerical increase in a*, L* and C* values in this study. The color of the meat is affected by the age, sex, muscle fiber, ration used, roughage-concentrated and roughage types, glycogen concentration in the muscle, cooling rate, oxidation rate, and pH level (Sarıçiçek, 2007; Suman et al., 2014).

4.4. Textural Features of Meat

In the present study, Flexibility ranged from 54.91 to 78.32 N. A numerical decrease was observed with the addition of Ulexide to diet. While the addition of boron sources caused an increase in hardness and a decrease in WBSF. However, no study was found on B sources to support the result in lambs' meat.

Many researchers have determined the WBSF value of different breeds of lamb higher than the results in this study (Liste et al., 2011; Camacho et al., 2015; Thorkelsson et al., 2019). In addition, boron was not used in the studies by researchers. According to Shorthose et al. (1986) ≤5 kg (~49 N) WBSF threshold can be interpreted as the meat obtained from slaughter systems is acceptably tender. In this study, the WBSF value remained below 49 N, therefore, it can be considered sensitive.

WBSF values were determined by researchers for lambs slightly older than 4 months (17.2 N and 18.3 N) and lambs over 7 months old (27.6 N) are similar to the study (Sañudo et al., 2003, Berge et al., 2003).

The difference between research results also depends on other factors such as race, processing before and during slaughter, age, breed, type of muscle used, pH, cooking temperature, and cooking time, as reported in previous studies (Behrends et al., 2009). It is seen that addition of B sources to the ration caused a decrease in WSBF compared to the control groups in this research.

5. Conclusion

When all findings of our study are evaluated again, the addition of colemanite, ulexite and etibor-48 to the diets of Akkaraman male lambs weaned at the age of 2.5 months, containing low Ca and P, resulted in an improvement in some parameters related to meat quality. While the addition of C to the ration causes an increase in protein, P and Ca and oleic acid content of the meat, the addition of C and U causes an increase in Ca content, but numerically lowered the WBS value.

Linoleic acid was high in all groups except L group. All boron sources increased the Mg content, the addition of U improved the a* value of the meat, while the addition of all the boron sources improved the b* and c* value of the meat. The addition of C and E increased the H° value of the meat. The addition of U and E showed an improvement in cis-8,11,14-eicosatrienoic acid content compared to the L group. Numerical increases in linoleic acid, tricosanoic acid and palmitoleic acid levels were determined in boron groups. Thus, the use of colemanite, ulexide and etibor-48 in the ration in this study caused an improvement in the quality of Akkaraman lamb meat, but it would be beneficial and need to conduct other studies in ruminants.

Author Contributions

B.Z.S (% 100) designed of study initiated the research. B.Z.S (% 50) and B.Y. (% 50) collected data, analyzed and interpreted the data. B.Z.S (% 100) wrote the manuscript. B.Z.S (% 100) supervised the research, suggested the research methods, structured the paper and edited the manuscript. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest

Ethical Consideration

This study was approved by Ankara University Animal Experiments Ethics Committee (Decision No. 2013-4-13) protocol, which complies with the international guidelines on the use of animals in scientific research procedures.

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THE EFFECT ON FERTILITY OF USING DIFFERENT DOSES OF PMSG IN ANATOLIAN MERINO SHEEP

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Abstract: The objective of this study was to obtain the appropriate dose of PMSG hormone to use in the heat synchronization method during the breeding season of Anatolian Merino sheep. The animal material consisted of 100 heads of Anatolian Merino sheep. Choronogest® sponges containing 20 mg of fluorogestone acetate were applied to all sheep which lasted for 12 days. Then the sponges were removed, and the animals were divided into two groups with equal numbers (n=50). The first group received 300 IU PMSG (application I, n=50) and the second group received 600 IU PMSG (application II, n=50) intramuscularly. Sheep were mated at 96h following the PMSG application. Lambing rate was 94% in the treatment I group and 96% in the treatment II group (P<0.01). Single and twin birth rates were higher in the application II group than in the application I group (P<0.01). The infertility rate was found to be lower in the treatment II group than in the treatment I group (P<0.01). As a result, using 600 IU PMSG hormone in the application of estrus synchronization during the breeding season in sheep ensures better fertility results.

Keywords: Anatolian merino, Heat syncronization, PMSG, Fertility

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1. Introduction

Sheep are among the first domesticated herbivores in human history (Demirsoy, 1989). Sheep breeding is one of the agricultural production branches on which all nations have worked throughout history. For centuries, sheep have been a versatile source of animal production and have an important role in Turkish economy (Gökçen, 2014).

In the sustainability of animal husbandry, it is necessary to keep the fertility rate at the highest level to protect and spread the high-yielding genotypes (Alçam, 2010). Fertility is increased through genetic and environmental breeding. Fertility in sheep, as in other farm animals, is affected by environmental factors such as care, feeding, season, age, live weight, and diseases, as well as the species, race, herd, and individual (Aşkın, 1982; Kaymakçı, 2012; Sen et al., 2021).

Reproduction is accepted as the physiological basis of all animal products. To maximize reproductive potential in sheep breeding; particularly, fertility breeding achieves the goals of increasing the number of lambings per unit time, increasing twinning, and, on the other hand, reaching two lambings per year or three lambings in two years (Eliçin et al. 1986).

Biotechnological methods such as artificial insemination, embryo and semen freezing, heat aggregation, embryo transfer, in vitro embryo production, offspring of the desired sex, increasing the twin rate, cloning, and transgenic animal production technologies are being used to increase animal yield and obtain high-yielding offspring (Emsen and Koşum, 2009; Yılmazer, 2015; Sen and Kuran, 2008).

It is possible to increase sheep fertility and produce lamb meat at any time by stimulating ovarian activity both during and outside of the breeding season by using various exogenous hormones (Aşkın, 1982; Sen et al., 2016). It is critical to support estrus with these hormonal processes in order to supply animal products derived from sheep during market demand periods.

Ovarian activity and plasma concentrations of reproductive hormones are the primary indicators of reproductive performance in sheep (Hafez, 1993). However, an animal's reproductive performance is measured by the rates of estrus, mating, pregnancy, and the number of lambs born following heat synchronization.

While prostaglandins and progestagens are commonly used to regulate estrus, gonadotropins are used to stimulate the ovarian function and provide superovulation (Soydan and Sen, 2013; Sen and Onder, 2016). Gonadotropins are classified into two types: follicle stimulating and ovulatory. The primary gonadotropins for stimulating function is FSH (follicle stimulating hormone) and eCG or PMSG (pregnant mare homone). However, the doses of hormones used in such applications are also of great importance (Jainudeen and Hafez 1987).

The purpose of this study was to determine the effects of

specific doses of PMSG hormone on fertility, which is widely used in heat synchronization applications in sheep.

2. Material and Methods

2.1. Experiment Materials

The animal material of this experiment was 100 heads of yearling Anatolian Merino sheep. All sheep in the experiment were subjected to similar environmental, rearing and feeding conditions under standard breeding conditions, endo- and ectoparasite protection practices were applied initially. Choronogest® sponge containing 20 mg of FGA (Fluorogestone acetate) was applied in the breeding season, individually and lasted for 12 days. The sheep used in the study were split into 2 groups of equal size. The groups received 300 IU (Treatment 1) and 600 IU PMSG (Treatment II) intramuscularly and mating was carried out for 96 hours subsequently. Birth records (single, twin or strile) for each group of animals were collected during the breeding season.

The equations below were used to determine fertility levels in the experimental groups (equations 1, 2, 3, 4, 5, 6 and 4);

Lambing rate: (ewes lambed/ ewes mated) × 100 (1)

Single birth rate: (single-born lambs/ewes born) × 100 (2)

Twins birth rate: (twin-born / ewes born) × 100 (3)

Dead birth rate: (dead-born/ ewes born) × 100 (4)

Infertility rate: infertile ewes/ ewes exposed × 100 (5)

Fecundity: lambs born/ ewes mated (6)

Litter size: lambs born/ number of lambing ewes (7)

2.2. Statistical Analyzes

The percentage data obtained within the scope of the study was transformed. The Minitab 13.0 program was used to perform an analysis of variance on the progeny yield results. Tukey's multiple comparison test was used to compare the means.

3. Results

Table 1 summarizes the fertility results of the study. The lambing rate in the treatment II group was higher than that in the treatment I group ($P<0.01$). However, it was observed that the twin rate was higher in the treatment II group ($P<0.01$). Furthermore, it was determined that the dead birth rate was higher in the treatment II group ($P<0.01$). As a result, 600 IU PMSG applications increased the amount of lamb obtained in the present study. Therefore, it was demonstrated that sheep received 600 IU PMSG to achieve estrus synchronization during the breeding season yielded significantly improved results for fertility.

4. Discussion

Our findings are comparable with the results of Koyuncu et al. (2001) who reported that injection of 700 IU PMSG improved lambing rate, twins' rate, fecundity and litter size. Besides, Koyuncu et al. (2000) reported that the injection of 700 IU PMSG reduced the rate of infertility. In the present study, increasing dose of PMSG resulted in a decrease in infertility rates. According to these findings, we can speculate that a certain increase in the PMSG dose to achieve estrus synchronization may improve fertility in Anatolian Merino ewes. The reason for this improvement is that the PMSG hormone directly stimulates gonadotropin secretion without interfering with melatonin synthesis, allowing sheep to begin reproduction. Emrelli et al. (2003) reported an 87.5% birth rate in Merino sheep when estrus was synchronized using 500 IU PMSG hormone outside the breeding season, whereas we obtained higher results for twins' birth. This improvement in our study could be attributed to the fact that it was conducted during the breeding season. During the breeding season, sheep respond much better to the estrus synchronization program. Depending on the circumstances, the fertility results may improve.

Table 1. Fertility results in various doses of PMSG

Fertility parameters	Treatment 1	Treatment II
Lambing rate (%)	94[a]	96[b]
Infertility rate (%)	6[a]	4[b]
Single lambing (%)	70[a]	56[b]
Twins lambing (%)	22[a]	36[b]
Stillbirth Rate (%)	2[a]	4[b]
Litter Size	1.14[a]	1.28[b]
Fecundity	1.21[a]	1.33[b]

[a,b] The differences between the means shown with different letters in the same line were found to be very significant ($P<0.01$).

4. Conclusion

As a result, 600 IU PMSG application resulted in better fertility in estrus synchronization during the breeding season in Anatolian Merino ewes. Multiple births are particularly important in sheep breeding when the income from lamb sales is considered. Higher-dose PMSG

application has been observed to increase lamb yield by promoting multiple births. Depending on the circumstances, such applications may be used to increase the income from sheep breeding. The amount of income obtained from the increase in lamb yield by using this method is far greater than the costs of doing so. It was concluded that using 600 IU PMSG in estrus synchronization applications during the breeding season increased lamb yield and improved farm income.

Author Contributions

All task made by E.Ş. (100%) data acquisition and analysis, writing up, submission and revision. The author reviewed and approved final version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

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Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The experimental procedures were approved by the Local Animal Care and Ethics Committee of Ahi Evran University, Kırşehir, Türkiye, ensuring compliance with directive 86/609/EEC for animal experiments (Date: March 17, 2020, Approve number: 2020/8).

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Research Article
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THE EFFECT OF PROGESTIN SOURCE ON SOME REPRODUCTIVE PERFORMANCE IN AKKARAMAN EWES

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Abstract: This study was conducted to determine the effect of progestin source on some reproductive performance, lamb birth weight and lamb mortality rate in Akkaraman ewes. A total of 40 Akkaraman sheep breed with similar body weight (51.3 ± 1.5 kg) and at least two parturitions were used as experimental animals. In all ewes, the intramuscular injection of $PGF_{2\alpha}$ (2.5 mg) was administered 96 hours before estrus synchronization to obtain corpus luteum regression. The ewes were allocated randomly into two groups according to body weight and ages. Estrus of ewes in the first group (n=20) were synchronized with an intra-vaginal CIDR device containing 0.30 g of natural progesterone. Estrus of ewes in the second group (n=20) were synchronized with an intra-vaginal sponge containing 30 mg flugestone acetate (FGA). CIDR and sponge were withdrawn following 12 days and 600 IU PMSG were injected intramuscularly. After 24 hours from injections, all ewes were introduced to Akkaraman rams and ewes in estrus were recorded. There were no significant differences between natural and synthetic progesterone applications in terms of estrus rate, gestation rate and duration of gestation in Akkaraman ewes. Additionally, lamb birth weight and lamb mortality rates were similar in both experimental groups. However, the application of CIDR increased the total and multiple lamb birth rates of Akkaraman ewes ($P<0.05$). These results show that the application of CIDR device with PMSG may increase the success rate in lamb production in Akkaraman ewes.

Keywords: Estrus synchronization, Natural progesterone, Flugestone acetate, Lamb production, Akkaraman

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1. Introduction

Reproduction is the physiological basis of all animal production (Jainudeen and Hafez, 1987). The reproductive performance of sheep is essential for productivity in lamb production (Jainudeen and Hafez, 1987). To realize the reproductive potential of sheep at an optimal level; is based on the improvement of fertility, which can be listed as increasing the number of lambing per unit time, increasing twinning, and reaching two lambing per year or three lambing in two years (Elinç et al., 1986). Reproduction in animal husbandry directly determines the economic level of production, as it provides the continuation of the species and is the source of various yields (Aşkın, 1982; Aksoy et al., 2018; Aksoy et al., 2019; Kızılaslan et al., 2021). For these reasons, it is essential to control the reproduction processes to increase reproductive performance.

Although obtaining semen from selected male animals in livestock and keeping it for a long time allows controlled breeding to a large extent, methods that will detect estrus provide a tremendous advantage for the effective and timely use of artificial insemination (Kaymakçı, 2006). Considering the growing dimensions of animal production, almost no approach to standard breeding methods includes an understanding that will allow insemination at the optimum time for reproduction. In this case, the control of the estrus cycle using exogenous hormones and thus the estimation of the time when the animals' estrus starts to appear is a more logical and satisfying method (Wheaton et al., 1993; Moeini et al. 2007).

In traditional sheep breeding, rams together with the sheep throughout the year, and the physiological stages of sheep may occur in a wide variety in mating season (Sen et al., 2016). Some animals may exhibit estrus, some dry, and some may be in late gestation or lactation. Due to these different physiological structures, the needs of the sheep also differ. Therefore, it is very difficult to synchronization of estrus or births in traditional sheep breeding. This situation causes the irregular use of resources in the enterprise and may adversely affect the economic profit and level (Edea et al., 2012). With exogenous hormone applications, it is possible to collect the animals' estrus, create simultaneous births, and lamb outside the breeding season (Wheaton et al., 1993).

Many estrus synchronization methods are applied in sheep, and the most commonly used protocols are progesterone-based ones (Şen and Onder, 2016; Gül and Keskin, 2010). The success rate of estrus synchronization in sheep is affected by many factors such as the hormone used in the applications, the dose of the hormone, the application time, animal age, breed, and breeding season

(Hashemi et al., 2005; Gül et al., 2020). The main reason is that the individual responses of ewes to estrus synchronization applications are different. In general, synthetic precursor hormones are used in estrus synchronization in sheep, and the effects of these hormones may differ (Kutluca, 2009; Gül and Erdoğan, 2021). Therefore, it is crucial to determine the response of ewes to applications containing natural hormones to assess the effects of estrus synchronization applications on reproductive performance.

In the last 30 years, many studies have been conducted to determine the effects of estrus synchronization applications on the reproductive performance characteristics of sheep. Akkaraman sheep breed is widely raised in the Middle Anatolian region and is one of Türkiye's crucial domestic gene resources. However, there is a shortage of information on estrus synchronization efficiency and reproductive performance in this breed induced by hormonal treatments during the breeding season. Therefore, this study aims to determine the effects of natural and synthetic progesterone applications for estrus synchronization on some reproductive performance characteristics of Akkaraman ewes.

2. Materials and Methods

The study was conducted within sheep's normal seasonal breeding cycle in Türkiye (September to March). A total of 40 adult Akkaraman ewes, which had at least two parturitions, ranging from 2 to 3 years of age and similar body weight (51.3 ± 1.5 kg) were used as experimental material. In all ewes, the intramuscular injection of $PGF_{2\alpha}$ (2.5 mg) was administered 96 hours before estrus synchronization to obtain corpus luteum regression. Ewes were randomly divided into two equal groups considering their body weight and ages. The estrus of the ewes in the first group (body weight=51.2±1.4; n=20) was synchronized by using the CIDR vaginal implant device containing natural progesterone (0.30 g progesterone). The estrus of the ewes in the second group (body weight=51.5± 1.7; n=20) were synchronized using an intravaginal sponge containing synthetic progesterone (30 mg flugestone acetate; FGA). The CIDRs and sponge were left in the vagina for 12 days. Following the withdrawal of the CIDR or sponge, 600 IU pregnant mare serum (PMSG) was injected intramuscularly simultaneously. The ewes were introduced to the Akkaraman ram 48 hours after the injection by group mating, and ewes that showed estrus and mated were recorded during two weeks. The ewes that did not exhibited estrus and did not mate were excluded from the experiment.

The estrus rates of ewes were calculated by the ratio of the number of ewes in estrus to the number of ewes in the experiment. Birth type (single or twin), sex and birth weight of lambs were determined within 12 hours after lambing. The gestation rate (the number of pregnant ewes/number of ewes in the experiment × 100), gestation period, lambing rate (the number of lambs born / the number of ewes in the experiment × 100), twin birth rate (the number of twins born / the number of ewes in the experiment × 100), birth period (starting and ending of births) and the lamb mortality rate until weaning (the number of lambs died until weaning/number of lambs born × 100) were also determined in both experimental groups (Tamer and Sirin, 2021).

2.1. Statistical Analysis

Data related to lamb birth weight, lambing period, and gestation period were analyzed using a completely randomized design by the General Linear Model procedure of the SPSS package program. Duncan's test showed significant differences between means, and results were computed as mean ± SE. Statistical significance was considered at $P<0.05$. CIDR or sponge drop rate, estrus rate, gestation rate, lambing rate, twin birth rate, and singleton birth rate were analyzed by chi-square ($\chi 2$) test. Kruskall-Wallis H test was performed to analyze the effect of lamb birth weight on survival status until weaning.

3. Results

Some reproductive characteristics of Akkaraman ewes, which are synchronized with CIDR or sponge applications, are presented in Table 1.

Table 1. Some reproductive characteristics of Akkaraman ewes, which are synchronized with CIDR or sponge applications

Traits (%)	CIDR (n=20)	Sponge (n=20)
Lost rate*	0/20 (0.0)	2/20 (10.0)
Estrus rate	16/20 (80.0)	15/20 (75.0)
Gestation rate	15/16 (94.0)	14/15 (93.0)
Lambing rate*	22/20 (110.0)[a]	16/18 (89.0)[b]
Twin lambing rate*	7/15 (47.0)[a]	2/14 (14.3)[b]
Singleton lambing rate*	8/15 (53.0)[b]	12/14 (86.0)[a]
Lamb mortality rate	3/22 (14.0)	2/16 (13.0)

*Means in rows are significantly different at $P < 0.05$.

In the current study, during the estrus synchronization application (12 days), the CIDR device never fell, while sponge lost was observed in 10% of the ewes that were treated with synthetic progesterone sponge (P<0.05). While no difference was observed between the experimental groups in terms of the estrus and gestation rates, it was determined that the rate of total (110.0% and 89.0%) and twin lambing (47.0% and 14.3%) in sheep treated with CIDR were higher than in sheep treated with the sponge (P<0.05). In addition, the rate of singletons (53.0% and 86.0%) was found to be lower in sheep treated with CIDR than in sheep treated with the sponge (P<0.05). There were no significant differences in terms of the lambs' mortality rate until the weaning age between experimental groups.

Lambing interval and gestation period of Akkaraman ewes, which is synchronized with CIDR or sponge applications, are presented in Figures 1 and 2, respectively. In the present study, no difference was found between the CIDR and sponge applications groups regarding lambing interval (CIDR; 18 days and sponge; 20 days) and gestation period (CIDR; 146 sponge; 149 days).

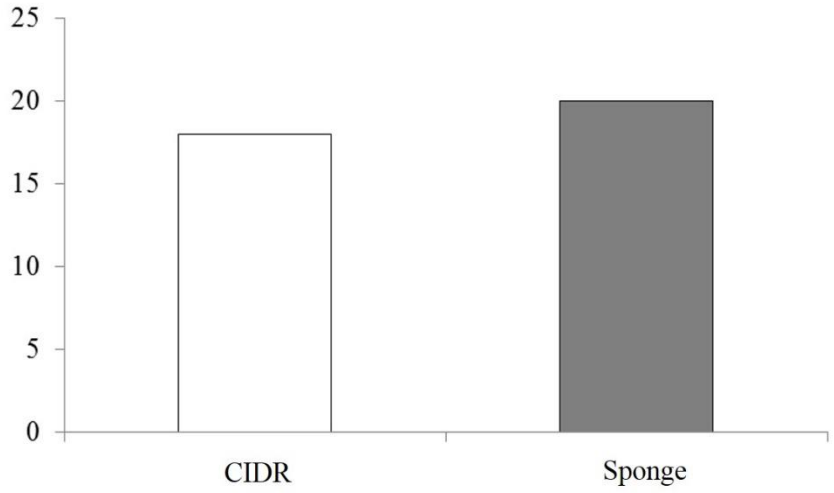

Figure 1. Lambing interval of Akkaraman ewes, which is synchronized with CIDR or sponge applications.

Lamb birth weights of Akkaraman ewes, which is synchronized with CIDR or sponge applications, are presented in Figure 3. There were no significant differences in terms of lamb birth weights between the experimental groups. The mean lamb birth weight in the CIDR treated group was 4.8±0.14 kg, and the average lamb birth weight in the sponge treated group was 4.6±0.13 kg.

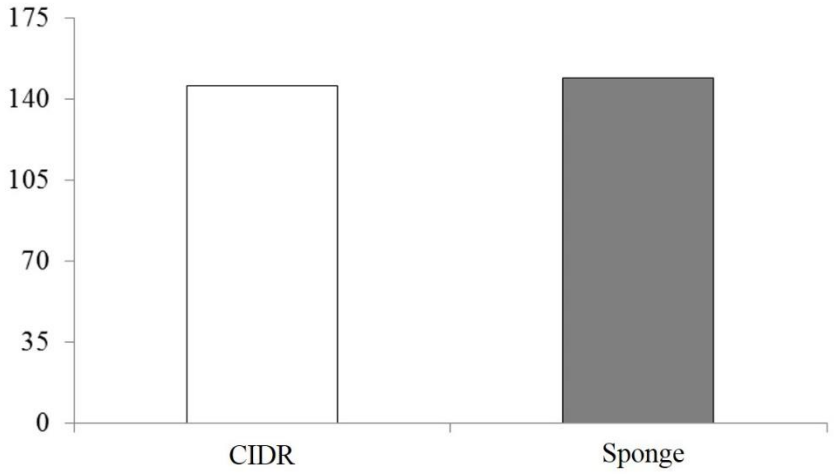

Figure 2. Gestation period of Akkaraman ewes, which is synchronized with CIDR or sponge applications.

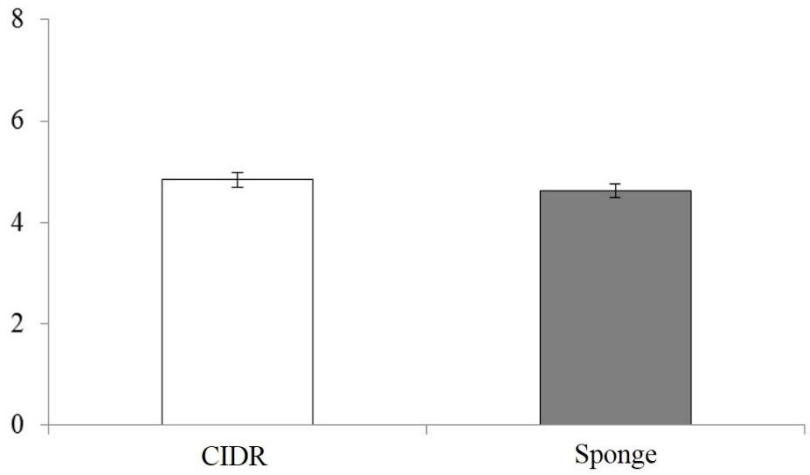

Figure 3. Lamb birth weights of Akkaraman ewes, which is synchronized with CIDR or sponge applications.

4. Discussion

In the current study, it was observed that the use of a CIDR device containing natural progesterone in estrus synchronization increased the total lambing rate and twinning rate compared to the intravaginal sponge containing synthetic progesterone. There was no difference between the treatments in terms of estrus rate, gestation rate, lamb birth weight and the lamb mortality rate weaning age.

In the previous studies with the use of CIDR and sponge in sheep, techniques employed in inserting and factors such as texture and consistency could influence CIDR or sponge retention in the vagina (Alifakiotist et al., 1982; Romano, 1996). Aşkın (1982) reported that intravaginal sponge loss was 6.34% in Anatolian Merinos ewes and 1.32% in Akkaraman ewes. On the contrary, Moeini et al. (2007) and Romano (1996) reported that no CIDR or intravaginal sponge were lost during estrus synchronization period. Previous studies reported that a higher number of CIDR drop in ewes (Welch et al., 1984; Rhodes and Nathanielsz, 1988). There was no loss during the synchronization period in the CIDR in the current study, while experienced a 10% loss in the intravaginal sponge. Although this result is higher than previous studies results, this difference may be due to breed or the number of ewes used in the experiments.

Başaran and Dellal (1997) reported that the application of synthetic progesterone (40 mg FGA) impregnated sponge and PMSG (500 I.U.) provided 97% of estrus in Akkaraman sheep. Aşkın (1982) reported the application of synthetic progesterone and different doses of PMSG in Anatolian Merino and Akkaraman ewes' estrus synchronization at rates of 98.12% and 97.89%, respectively. Karakuş and Aşkın (2007) investigated the effect of estrus synchronization with synthetic progesterone (40 mg FGA) impregnated sponges on fertility in Anatolian Merino and Malya sheep breeds and they were observed 100% estrus after synchronization in both breeds. In the current study, estrus was induced in 80.0% and 77.3% of the Akkaraman ewes treated with CIDR and sponge, respectively. This result indicated that natural and synthetic progesterone application and fixed-dose PMSG applications did not affect the rate of estrus in the synchronization of estrus in Akkaraman ewes. In previous studies during breeding or out of breeding season similar results observed (Hashemi et al., 2006; Moeini et al., 2007). The current study results were lower than the studies of Başaran and Dellal (1997) and Aşkın (1982) in the same breed. This difference may be due to the number of animals used in the experiments or the regional conditions.

Although many factors such as type of intravaginal device, hormone dose, breed, age and season affect the fertility of sheep with synchronized estrus (Wheaton et al., 1993). The current study observed that natural and synthetic progesterone and fixed-dose PMSG applications did not affect the gestation rate in the synchronization of estrus in Akkaraman sheep. This agrees with results of previous studies (Luther et al., 2006; Moeini et al. 2007). Yaralı and Karaca (2004) reported that the average gestation rate was 59% as a result of using different PMSG doses together with synthetic progesterone sponge in estrus synchronization of Kıvırcık ewes. Bekyürek (1994) showed using the sponge containing 60 mg MAP and 500 I.U. PMSG injection caused a 70% gestation rate. Kaçar et al. (2008) reported a 50% gestation rate in Tuj sheep due to vaginal sponge application (containing 40 mg cronolone) and 600 I.U. PMSG injections. Smith (1988) determined the gestation rates in sheep whose estrus was synchronized using CIDR and PMSG, covering different seasons, as 94% for the winter period, 24% for the spring period, and 11% for the summer, and 63% for the autumn period. The present study results were higher than the studies mentioned above. These differences may be due to the animal breed used, the different physiological responses of the breeds to the treatments, or the breeding season.

Total lambing rate in sheep flocs is one of the most important indicators of farming profitability. Moreover, the total lambing rate following estrus synchronization applications shows successful degree of the applications.

Previous studies showed that different sources of progesteron application (MAP; 45.0%, fluorogestone acetate; 41.5%, CIDR; 57.9%, and progesterone sponge; 39.5%) to estrus synchronization influence lambing rate (Fukui et al., 1999). Aşkın (1982) reported that intravaginal sponge containing synthetic progesterone (40 mg FGA) and 200, 400, and 600 I.U. of PMSG applications caused 150.76%, 178.57%, and 197.14% in Anatolian Merinos ewes, and 135.71%, 178.26%, and 170.83% in Akkaramans ewes a total lambing rate, respectively. Ozcan et al. (1994), reported that sponge (containing 40 mg chronolone) and 500 I.U. PMSG application caused 51.1% lambing. Başaran and Dellal (1997) reported that the lambing rate was 170.0% after the application of sponge (40 mg FGA) and PMSG (500 I.U.) in Akkaraman sheep. Koyuncu et al. (2001) reported the lambing rate as 94.87% and 96.66%, respectively, after applying an intravaginal sponge (containing 40 mg FGA) 500 or 700 I.U. PMSG in Kıvırcık sheep. Daşkın (2001) reported that applying synthetic progesterone (30 mg FGA) and 500 mg PMSG during the breeding season in Akkaraman sheep caused 92.30% lambing. Kutluca (2009), at the end of the mating season in Morkaraman sheep, as a result of natural and synthetic progesterone (CIDR, Crestar, Natural progesterone, Cronolone, and MAP) applications, lambing rates were determined by CIDR; 74%, Cronolone; 61%, natural progesterone; 56%, MAP; 33% and 10% for crestar. It should be pointed out that in various studies, reproductive parameters may be calculated differently which should be taken into consideration when results are compared. For example, the number of lambs born per ewe lambed and the number of lambs born per ewe in estrus were accounted for in calculating fecundity and prolificacy, respectively. In the current study, PMSG injection and natural progesterone application were more successful than synthetic progesterone application in terms of lambing rate in synchronization of estrus in Akkaraman sheep. The present study results are in agreement with the studies mentioned above.

PMSG is widely used to increase ovarian activity and litter size in progesterone-based estrus synchronizations in small ruminants. However, in estrus synchronization applications, progesterone source can be effective on PMSG activity or multiple births (Wheaton et al., 1993; Romano, 1996; Moeini et al. 2007). Aşkın (1982) reported that sponges (containing 40 mg FGA) and different doses of PMSG caused 52.3% singleton, 44.62% twin, and 3.08% triplets in 200 I.U. PMSG, 27.14% singleton, 68.57% twin; 2.86% triplets and 1.43% quadruplets in 400 I.U. PMSG, 15.71% singleton, 71.43% twins and 12.86% triplets in 400 I.U. PMSG were born in Anatolian Merinos. The same author observed that sponge (containing 40 mg FGA) and different doses of PMSG caused 94.29% singleton and 5.71% twin in 200 I.U. PMSG, 30.43% singleton, 60.87% twin, and 8.70% triplet in 600 I.U. PMSG, 33.33% singleton, 62.50% twin, and 4.17% triplet in 80 I.U. PMSG application. Ozcan et al. (1994) reported that sponge (containing 40 mg chronolone) and 500 I.U. PMSG application caused 61.7% twins, 6.4% triplets, and 0.5% singletons in the Awassi sheep breed. Daşkın (2001) observed that sponge (30 mg FGA) and 500 I.U. PMSG application caused 58.34% singleton and 41.66% twins born in Akkaraman ewes. Karakuş and Aşkın (2007) reported that sponge (40 mg FGA) application caused 56.14% singleton, 36.84% twin, and 7.02% triplet birth in Anatolian Merino ewes, and also 54.84% single, 35.48% twin and 9.68% triplet birth in Malya ewes. The current study observed that PMSG injection and natural progesterone application in the synchronization of estrus in Akkaraman sheep causes twinning at a higher rate than PMSG injection together with synthetic progesterone application. The present study results are in agreement with some of the studies, but different from some of the studies. These differences may be caused by the breed, the different physiological responses of the breeds to the treatments, the breeding season, or the nutritional conditions.

Previous studies indicated that different sources of progesterone application (CIDR; 10.5%, cronolone; 7.9 %, and natural progesterone; 11.6%) to estrus synchronization did not influence lamb's mortality rate until weaning (Kutluca, 2009). Berhan and Van Arendonk (2006) reported breed affected the lamb mortality rate until weaning following in the vaginal sponge (containing 40 mg of FGA) application (Horro breed 13% and Menz breed 27%). Basaran et al. (1996) reported that the lamb mortality rate until weaning in the vaginal sponge (containing 40 mg of FGA) treated France x Akkaraman crosses was 17.65% and 7.14%, respectively, and the lamb mortality rate in Border Leicester x Akkaraman crosses 6.67% and 5.88%, respectively. In the current study, CDIR and sponge application and fixed-dose PMSG applications did not affect the lamb mortality rate until weaning in Akkaraman sheep. The present study results are in agreement with the studies mentioned above.

Godfrey et al. (1997) reported that CIDR applied St. Croix White, Barbados Blackbelly, and Florida ewes had a shorter gestation period than the ewes in the control group. Ülker et al. (2004) reported that sponge (containing 40 mg medroxyprogesterone acetate; MAP) and 600 I.U. PMSG application did not affect the gestation duration between Karakaş and Norduz sheep breeds. Zarkawi (2001) reported that sponge (containing 40 MAP) and 600 I.U. PMSG applied Awassi sheep had 150.7 days of gestation duration, but control ewes had 52.5 days. Timurkan (2005) reported that the gestation duration in estrus synchronized Hamdani ewes with the sponge containing 40 mg FGA and 500 IU PMSG and non-synchronized ewes was 152 and 160 days, respectively. In the current study, CDIR and sponge application and fixed-dose PMSG applications did not affect the gestation duration of Akkaraman sheep. The present study results are mainly similar to the studies mentioned above.

Aşkın (1982) reported intravaginal sponge (containing 40 mg FGA) and PMSG application caused 91.08% of

Anatolian Merinos and 92.37% of Akkaraman sheep to give birth within one week. Başaran (1995) reported that lambing was completed by 92.08% within nine days in Awassi ewes, which were applied intravaginal sponge (containing 60 mg MAP) and PMSG, and by 97.45% within 30 days in the control group. Similarly, Başaran and Dellal (1997) reported that the lambing was completed within ten days in Akkaraman ewes treated with progesterone and PMSG while finished in 45 days in the control group. In the current study, CDIR and sponge application and fixed-dose PMSG applications did not affect lambing duration in Akkaraman ewes. The present study results were higher than the studies mentioned above, and these differences may be due to the animal breed used, the different physiological responses of the breeds to the treatments, or the breeding season.

Kutluca (2009) reported that lambs' birth weight wasn't affected by the applications of natural and synthetic progesterone (CIDR; 4.84 kg, cronolone; 4.96 kg and natural progesterone; 5.25 kg) for estrus synchronization in Morkaraman sheep breed at the end of the breeding season. Moreover, Kutluca (2005) reported the birth weights of lambs born from Awassi and Morkaraman ewes, treated with sponges containing synthetic progesterone, as 3.2 and 4.20 kg, respectively. Ülker et al. (2004) determined that lamb birth weights were 4.61 kg in Karakaş and Norduz sheep, resulting from sponge application (containing 40 mg MAP) for estrus synchronizing. In the current study, it was observed that natural and synthetic progesterone application and fixed-dose PMSG applications did not affect the live weights of the lambs born in the synchronization of the estrus of Akkaraman sheep. The present study results are mainly similar to the studies mentioned above.

5. Conclusion

As a result, it has been revealed that CIDR (natural) and sponge (synthetic) sourced progesterone application can be used in combination with PMSG for estrus synchronization in Akkaraman ewes, but the application of CIDR device containing natural progesterone together with PMSG increases the success in twin rate. In addition, the absence of losses in the CIDR device containing natural progesterone during the application makes this application more preferable in Akkaraman sheep breed.

Author Contributions

D.G. (25%), B.B. (25%) and U.Ş. (50%) design of study. D.G. (25%), B.B. (25%) and U.Ş. (50%) data acquisition and analysis. D.G. (25%), B.B. (25%) and U.Ş. (50%) writing up. D.G. (50%), B.B. (25%) and U.Ş. (25%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The experimental procedures were approved by the Local Animal Care and Ethics Committee of Ahi Evran University, Kirsehir, Türkiye, ensuring compliance with directive 86/609/EEC for animal experiments (Date: February 23, 2016, Approve number: 2016/4).

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Research Article
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DETERMINATION OF THE EFFECT OF DIFFERENT NITROGEN DOSES ON THE YIELD, YIELD COMPONENTS AND CHLOROPHYLL CONTENTS OF BREAD WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS USING ARTIFICIAL NEURAL NETWORK

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Abstract: This research investigates an artificial neural network for predicting the chlorophyll concentration index and the effect of different nitrogen doses on the yield, yield components of Bread Wheat (*Triticum aestivum* L.). Plants were fertilized with 5, 10, 15, 20, and 25 kg da^{-1} nitrogen doses. The chlorophyll concentration index of each leaf was measured using a SPAD meter. The coefficient of determination values was found to be 0.99. In artificial neural network modeling, chlorophyll concentration values were estimated with SPAD readings. Artificial neural network modeling successfully described the relationship between actual chlorophyll concentration index values and predicted chlorophyll concentration index values. Agronomic parameters plant height (110.66-92.73 cm), the number of spikes per square meter (461.01-355.50), the number of seeds per spike (43.88-23.83), the weight of seed per spike (2.07-0.91 g), hectoliter weight, thousand-grain weight (43.10-35.89 g), grain yield (638.76-343.06 kg.da^{-1}), protein contents (11.16-8.34 %), the value of sedimentation (19.40-11.94) were found statistically important.

Keywords: Artificial neural network, Chlorophyll, Breat wheat, Yield

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1. Introduction

In recent years, studies as to the expression of the plant growth in agriculture by mathematical models have been carried out extensively. The relationship between plant growth and yield and the changes in plant growth under the influence of environmental conditions (such as light, air, soil temperature, etc.) are aimed to be expressed by plant growth models (crop model). The development of information technologies and their applicability to all areas demonstrate the availability of computer-assisted models. In this study, chlorophyll content in the plant was estimated by using computer assisted machine learning methods. From this perspective, this study serves as an interdisciplinary study that will create a hardware and theoretical infrastructure for other studies in the field of precision agriculture practices (Odabas et al., 2009).

Wheat sprouts include 90 of 102 natural minerals, along with 20 types of amino acids and hundreds of different enzymes. When consumed fresh, it regulates metabolism, increases heart function, normalizes blood pressure, lowers cholesterol and cleanses the intestines. Wheatgrass juice is a substance that has a stronger detox effect than carrot juice, vegetable juices and other juices. In the light of this information, it is our objective to determine how different doses of nitrogen applied to wheat varieties change the content of chlorophyll (Akgun and Topal, 2006). For this purpose, in our study conducted using different nitrogen doses, chlorophyll measurements were carried out from the seed germination, these measurements were recorded and the content of chlorophyll was determined using artificial neural networks (Temizel et al., 2014).

The model is a simplified picture of reality and reveals the characteristics of the system being studied. The model can also be defined as an explanation of the main components of a system using mathematical and/or statistical formulas (Dhillion et al., 2002). A model that accurately and adequately represents a real system contains the characteristics of the system studied in the model. We do not expect that a model contains all the

features of the system being studied, because in this case it becomes the system itself, which is impossible (Freeman and Skapura, 1991). Another feature of models is that they are accurate and adequate (Gao et al., 2000). After the establishment of a model, validation and verification tests should be applied to the model. If the model passes these tests successfully, the model created is considered to accurately and adequately represent the system being studied. A model can be used for a variety of purposes (Hoareau and Dasilva, 1999). For this reason, the modeling process should begin with the determination of the purpose that will essentially be considered in the model to be established. The goals in this study determine for what purpose the entire model which makes up the characteristics of the model will be used, and what should be expected of this model. If a model consists of a certain number of sub-models, the goals of these sub-models must also be determined. Models are potentially powerful means but must be used within a strict discipline (Kocabas, 1995).

Recent developments in model studies on plants have revealed the importance of structural and functional components of the plant canopy. The leaves and other photosynthetic organs on the plant serve as collectors of the solar energy and gas exchangers. The trunk and branches are arranged in such a way that these exchange surfaces are effectively formed by radiative conversions (Monteith, 1996). All processes of growth and development in plants result in yield. In recent years, intensive research has been carried out to fully clarify the impact of environmental conditions on the growth and yield of plants. These studies have comprised an important basis for understanding the factors affecting crop photosynthesis, which are the cause of most of the changes in yield (Uzun et al., 1998). In developed countries in terms of agriculture, intensive studies have been performed in recent years on the expression of plant growth via mathematical models. Therefore, the impact of environmental conditions (light, air, soil temperature, etc.) and changes in plant growth have been tried to be expressed through plant growth models (Crop model).

2. Material and Methods

The research was carried out for 2 years in the experimental field of the Faculty of Agriculture at Ordu University. 11 different wheat varieties (Es-26, Alpu 2001, Soyer 02, Mesut, Altay 2000, Mufitbey, Nacibey, Harmankaya 99, Sultan 95, Yunus and Sönmez 2001) were used as materials and 5 different nitrogen doses (5, 10, 15, 20 and 25 kg da^{-1}) were applied. Regarding agronomic properties; plant height, the number of spike per square meter, the number of seed per spike, the weight of seed per spike, hectoliterweight, thousand grain weight, grain yield, protein contents, the value of sedimentation, leaf area and chlorophyll contents were analyzed.

In the study, all analyses were combined for 2 years and carried out using SAS-JMP 11.0 package program by the factorial arrangements in randomized complete block design. Some quality parameters of the study (protein content, Zeleny sedimentation value and hectoliter weight) were analyzed through NIT in the Department of Field Crops at the Faculty of Agriculture of Dicle University. For regression and correlation analysis, the SPSS package program was used. Artificial neural networks were modeled with MATLAB.

Chlorophyll is found in significant amounts, especially in leaves. For this reason, the development of leaves, leaf area and therefore the amount of chlorophyll are important. Chlorophyll analyses of leaf samples taken at certain intervals during the development period of the plant were carried out. This measurement was made with the chlorophyll meter (SPAD-502 chlorophyll meter) device. This saves time for other operations.

2.1. Artificial Neural Network

This network consists of three layers: the input layer, the output layer, and at least a hidden layer. The hidden layer and the number of nodes in the hidden layer can be changed (Haykin, 1994). Increasing number of nodes increases the network's ability to remember, but also extends the learning time (Gomm and Yu, 2000). The output signals of neurons in a layer are passed on to the next layer as inputs via weights (Hagan and Menhaj, 1994). The input layer transmits the inputs it receives from the external inputs to units at the hidden layer without changing; the inputs are processed at the hidden layer and at the output layer, determining the output of the network (Kermani et al., 2005). In Figure 1, the MLNN (Multi Layered Neural Network) structure consisting of H hidden layers, N inputs, M outputs and L neuron were given. Activation functions that can be used in these networks are given in Figure 2 (Hagan and Menhaj, 1994).

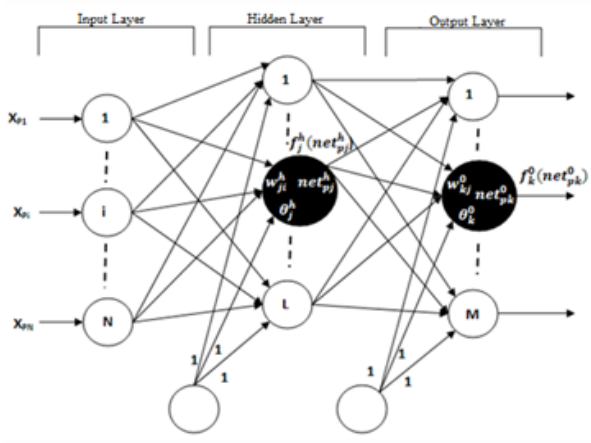

Figure 1. Example of multi layered neural network.

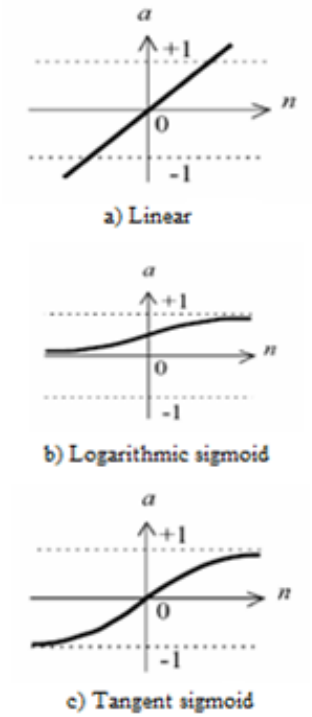

Figure 2. Activation functions.

An algorithm commonly used in the training of artificial neural networks is the Back Propagation Algorithm. The back propagation learning method is an optimization process based on reducing the system error, as in the rule which is known as the delta learning rule or least squares method. Because it also performs the process of reducing this error through changing weights, in other words, it propagates the output error back; it is called "Back Propagation". Back propagation consists of feed-forward and back propagation. In Feed-Forward, the output of the network is calculated. In back propagation, weights are changed (Bogdan et al., 1999).

When back propagation learning is used, the weights of the hidden layer are arranged using errors in the output layer. This process repeats in the same format till the first hidden layer. In this way, errors are propagated back by making weight arrangements of the related layer (layer by layer), and these operations are repeated until the total error is minimized (Singh et al., 2005). According to the network structure given in Figure 1, the output of artificial neuron J belonging to layer h is defined in Equation 1.

$$net_{pj}^{h} = \sum_{i=1}^{N} w_{ji}^{h}\, x_{pi} + \theta_{j}^{h} \quad (1)$$

In this equation w_{ji}^{h} i. from the input unit h. hidden layer j. θ_{j}^{h} is defined as the threshold value of layer h for j units. It is obtained by passing the net input through the activation function, i_{pj}^{h} being hidden layer unit outputs (Equation 2, 3).

$$i_{pj}^{h} = f_{j}^{h}(net_{pj}^{h}) \quad (2)$$

$$net_{pk}^{0} = \sum_{i=1}^{N} w_{kj}^{0}\, x_{pj} + \theta_{k}^{0} \quad (3)$$

Equation 4 is obtained for k output units:

$$o_{pk}^{o} = f_{k}^{0}(net_{pk}^{0}) \quad (4)$$

For the training vector *p* of a feed forward network *n*. The performance function of recursion is given by Equation 5. Here the epk error is the number of all neurons in the output layer of the *M* network.

$$E_{p}(n) = \frac{1}{2}\sum_{j=1}^{M} e_{k}^{2}(n) \quad (5)$$

As y_{pk} requested output, opk network output, the epk error is given by Equation 6.

$$e_{pk}(n) = y_{pk}(n) - o_{pk}(n) \quad (6)$$

In case some error values are negative, the total squared error is calculated to find the total error of the network. The purpose of the training is to minimize the total squared error value (E), also called (the total) error energy, by arranging the network parameters. One of the training algorithms used for this purpose, the backward propagation algorithm, is based on reducing the error by changing weights in the backward calculation phase (Neruda and Kudova, 2005). For this purpose, it calculates the negative derivative of the error energy for weights, multiples it with the leaning parameter and adds it to the previous weights. This equality is obtained by calculating the partial derivative, since the derivative of the error is in question for weights.

The back propagation method which involves trial and error remains slow in solving many problems, despite improvements in changing the learning rate, using momentum, and in scaling variables. One of the most effective algorithms for training weights of an artificial neural network is the Levenberg-Marquardt (LM) algorithm, which includes standard optimization techniques (Neruda and Kudov, 2005).

3. Results and Discussion

3.1. The Number of Spikes per Square-meter

In the experiment, a very significant (P<0.01) statistical difference was found in terms of the analyzed varieties and in terms of the treatment, treatment x variety interaction and the number of spikes per square meter. Variant analysis results regarding the number of spikes per square meter are offered in Table 1 while mean values and materiality group is available in Table 2. As can be seen in Table 2, the number of spikes per square was measured from the Soyer 02 variety (461.01) as the highest whereas the number of spikes per square was measured from the Yunus cultivar (355.50) as the lowest.

Table 1. Variance analysis results of the agronomic properties

S.O.V.	df	NSSM	Y	TGW	SWS	PH	SSN	PC	SV	HW
Block	2	ns	ns	ns	ns	ns	ns	ns	ns	4.96**
Cultivar	10	6.74**	13.71**	26.65**	63.65**	16.42**	38.40**	53.08**	44.17**	11.41**
Application	4	6.66**	21.94**	1.43	25.12**	70.15**	7.35**	51.96**	90.58**	10.93**
Cultivar x Application	40	2.64**	2.58**	4.09**	3.07**	8.84**	4.86**	4.94**	10.12**	7.59**
Error	108									
Total	164									
% CV		13.87	18.59	4.66	11.17		10.93	4.41	8.58	2.05

S.O.V.= source of variance, df= degree of freedom, NSSM= number of spike per square meter, Y= yield, TGW= thousand grain weight, SWS= seed weight per spike, PH= plant height, SSN= seed per spike number, PC= protein content, SV= sedimentation value, HW= hectoliter weight, **= significant at 0.01 level.

Table 2. Mean values and significance groups of the number of spikes per square meter

Cultivar /Application	5	10	15	20	25	Mean
Soyer 02	400.00 g-n	517.83 abc	405.17 f-n	441.83 c-j	540.21 ab	461.01 A
Müfitbey	475.00 b-g	394.09 g-n	374.53 ı-o	408.66 f-n	593.33 a	449.12 AB
Nacibey	358.11 j-o	387.50 g-o	503.00 a-e	506.66 a-d	457.67 b-ı	442.58 AB
ES-26	390.00 g-o	416.00 d-j	462.66 b-ı	389.36 g-o	496.00 b-f	430.80 AB
Sultan-95	445.55 c-j	390.55 g-o	386.67 g-o	466.33 b-h	408.07 f-n	419.43 BC
Harmankaya 99	365.00 j-o	381.51 h-o	472.50 b-h	421.66 d-k	420.66 d-l	412.27 BCD
Sönmez - 2001	404.69 g-n	430.00 c-j	321.66 nop	355.00 j-o	413.00 e-m	384.87 CDE
Alpu – 2001	257.55 p	373.33 ı-o	383.33 h-o	435.00 c-j	433.33 c-j	376.50 DE
Altay 2000	330.00 l-p	373.97 ı-o	337.83 k-p	442.16 c-j	357.50 j-o	368.29 E
Mesut	354.69 j-o	362.57 j-o	372.50 ı-o	338.03 k-p	383.50 h-o	36.26 E
Yunus	300.00 op	318.66 nop	444.25 c-j	391.42 g-n	323.16 m-p	355.50 E
Mean	370.96 C	395.09 BC	405.82 B	417.83 AB	438.76 A	405.69

Cultivar LSD= 40.75, Application LSD= 27.47, Interaction of Cultivar x Application LSD= 91.13.

Cekic (2007) states that the most important effect of the drought in wheat is the reduction of the number of spike per square meter and a 14.3% decrease occurs as the result of the drought. On the other hand, Onder (2007) reports that varieties effectively protecting the viability of tillering under dry conditions rather than only varieties of appearance of much tillering are suitable for use and there is a very significant negative relationship between the number of spike per square meter and the number of grains per spike under dry conditions while there is no relationship between the number of spike per square meter and grain yield under wet conditions.

Yilmaz and Simsek (2012) found that the effect of different nitrogen doses that they used in their study on the number of spikes per square meter in wheat was statistically significant and reported that the number of spikes per square meter increased with the increased nitrogen doses. Cifci and Dogan (2013) reported that the highest number of spikes per square meter is obtained from 1 per 20 kg doze of nitrogen with a total of 823.19 while the lowest number of spike per square meter is obtained from parcels to which no nitrogen is applied and in higher doses of nitrogen than 1per 20 kg, the number of spike per square meter has showen decrease. Atar and Akman (2014) reported that the effect of nitrogen doses on the number of spikes per square meter is positive and that while 514.3 spikes were found in parcels to which nitrogen is not applied, 561.1 spikes were detected in 15 kg of Nitrogen doses. It is believed that the effect of nitrogen on increasing plant development is also effective in creating a spike. Asif et al. (2019) in a study of pot in which they investigated the effects of nitrogen and zinc on the bread wheat found out that the effect of nitrogen on the number of spikes is very significant because as the nitrogen dose increased, the number of spikes increased as well.

In order to obtain a higher yield from wheat, it is necessary to use genotypes with a high number of fertile spikes per square meter (Ozturk and Akten, 1999). Donmez (2002) reported the mean of fertile spikes per square meter in a study with 25 varieties of bread wheat is 242.8-597.5. Similarly, Kaydan and Yagmur (2008) reported the mean of fertile spikes per square meter in their study with 16 varieties of bread wheat is 265.25-412.25. The values stated are similar to our findings. The change in the number of fertile spikes and fertile flowers according to genotypes represents the source of the difference in the number of seeds (Bayram et al., 2017). How many fertile tillers the plant can feed with the photosynthesis products as a result of photosynthesis is of great importance in terms of the grain yield. For this reason, it is very important to know the contribution rates to the grain yield according to the appearance order of fertile tillers in the plant (Destro et al., 2001).

3.2. Yield (kg, da^{-1})

In the experiment, a very significant (P<0.01) statistical difference was found in terms of yield of varieties, treatment and variety x treatment interaction. Results of variant analysis of yield values are shown in Table 1 while mean values and materiality level is given in Table 3. As can be seen in Table 3, the highest yield was measured from the Nacibey variety (638.76) and the lowest yield was measured from the Mufitbey variety (343.06). Ozen and Akman (2015) reported that yield values are as 427-638.5 kg da^{-1} in their study of different wheat varieties. Coskun and Oktem (2003) reported that the effects of nitrogen doses on the grain yield and yield components are significant, and a significant increase in grain yield occurs with an increase in the nitrogen dose. Haque et al. (2017) reported that the effect of applied nitrogen doses on the wheat yield is significant and that the lowest yield value is obtained from parcels to which nitrogen is not applied, and that yield values increase with the increasing nitrogen doses.

Grain yield occurs as a result of the combined effects of environmental factors and genetic potential. At the beginning of the factors limiting wheat yield are varieties, and there is a decrease in the yields of varieties that are still grown (Ozen and Akman, 2015). Yield in wheat is significantly affected by the genotype and purity of the variety used as well as its adaptation to the region (Naneli et al., 2015). A previous study on this subject showed that grain yield on the basis of wheat differs according to the variety used, cultivation techniques and environmental conditions of the region (Dokuyucu and Akkaya, 1999; Mut et al., 2007).

3.3. Thousand Grain Weight (g)

In the experiment, a very significant (P<0.01) statistical difference was found in terms of varieties analyzed, variety x treatment interaction and thousand grain weight. No statistical differences were found in terms of the treatment. Results of variance analysis as to the values of thousand grain weight are shown in Table 1 while mean values and materiality group are available in Table 4. As can be seen in Table 4, the highest thousand grain weight was measured from the Harmankaya 99 variety (43.10), and the lowest thousand grain weight was measured from the Sultan-95 variety (35.89).

In the selection of the plant material; seed size, germination speed and power, emergence rate, homogeneity quotient, the development of the first plant in a strong way, resistance to unfavorable conditions are the desired characteristics in terms of the efficient cultivation (Kara and Akman, 2007; Asif et al., 2019).

Table 3. Mean values and significance groups for yield

Cultivar/Application	5	10	15	20	25	Mean
Soyer-02	393.33 m-t	656.00 a-d	505.90 e-n	533.47 c-m	533.75 c-m	524.61 BCD
Müfitbey	276.44 stu	245.90 tu	277.49 stu	309.61 q-u	605.87 b-g	340.06 E
Nacibey	569.78 b-ı	507.41 e-n	666.36 abc	755.06 a	695.18 ab	638.76 A
ES-26	450.00 h-r	587.11 b-h	493.57 e-o	445.37 h-r	550.08 b-l	505.22 CD
Sultan-95	411.74 k-s	302.64 r-u	328.52 p-u	556.84 b-k	405.68 l-s	401.08 E
Harmankaya-99	416.66 j-s	581.4 c-m	695.82 ab	689.21 ab	613.57 a-f	590.68 AB
Sönmez-2001	224.12 u	444.09 h-r	389.37 m-t	511.17 d-n	423.99 ı-s	398.55 E
Alpu-2001	344.69 o-u	502.52 e-n	483.00 e-o	629.26 a-e	666.03 abc	525.10 BCD
Altay 2000	430.00 ı-r	458.36 g-q	469.07 f	666.87 abc	756.94 a	556.24 BC
Mesut	394.96 m-s	511.87 d-n	422.84 ı-s	456.28 h-q	681.27 abc	493.44 CD
Yunus	380.00 n-t	428.29 ı-r	560.74 b-j	478.06 f-o	492.62 e-o	467.94 D
Mean	390.15 C	471.17 B	481.15 B	548.29 A	584.09 A	494.97

Cultivar LSD= 66.60, Application LSD= 44.90, Interaction of Cultivar x Application LSD= 148.93.

Table 4. Mean values and significance groups of thousand grain weight

Cultivar/Application	5	10	15	20	25	Mean
Soyer 02	36.66 o-s	39.79 f-n	41.82 c-g	38.69 ı-p	40.46 e-l	39.48 C
Müfitbey	38.17 k-q	38.23 k-q	37.67 l-r	38.26 k-q	37.82 k-q	38.03 DE
Nacibey	40.58 e-l	43.00 b-e	38.71 h-p	40.26 e-n	41.80 c-g	40.87 B
ES-26	36.00 p-s	37.61 l-r	34.69 rst	35.54 q-t	36.21 o-s	36.01 F
Sultan-95	40.12 e-n	33.91 st	38.15 k-q	34.28 st	33.01 t	35.89 F
Harmankaya 99	41.66 c-ı	43.75 a-d	44.25 a-d	44.57 abc	41.30 d-j	43.10 A
Sönmez - 2001	38.27 k-q	42.92 b-e	42.68 b-f	46.31 a	38.95 g-p	41.83 AB
Alpu – 2001	37.45 m-r	39.19 g-o	38.53 j-q	36.68 o-s	36.34 o-s	37.64 E
Altay 2000	38.00 k-q	40.37 e-m	42.42 b-f	44.14 a-d	41.93 c-g	41.37 B
Mesut	44.10 a-d	37.28 n-r	41.68 c-h	41.28 d-j	45.36 ab	41.94 AB
Yunus	38.00 k-q	40.70 e-k	39.18 g-o	37.89 k-q	40.17 e-n	39.19 CD
Mean	39.00	39.70	39.98	39.81	39.39	39.57

Cultivar LSD= 1.33, Interaction of Cultivar x Application LSD= 2.98.

Components of grain yield in wheat are grain weight and number of grains per unit area and increasing grain yield depend on the increasing grain weight. In terms of milling, it is desirable that the thousand grain weight is high. As grain size increases, the protein content, bran and ash content decreases. On the other hand, hectoliter weight, thousand grain weight and flour yield increase (Gaines et al., 1997). Naneli et al., (2015) reported that the difference among thousand grain weight in different bread wheat varieties is significant at the level of 1%. Altuntas and Akgun (2016) reported the effect of different nitrogen doses on the mean thousand grain weight in wheat are insignificant and they also obtained a thousand grain weight of 38.89 g in 8 kg da^{-1} application and 39.89 g in 14 kg da^{-1} application. Mert et al. (2003) reported a thousand grain weight between 34.53-38.67 g in wheat varieties with the application of different nitrogen doses. According to them the effect of nitrogen doses on a thousand grain weight was insignificant. However, the effect of varieties was reported as significant. El-Temsah (2017) in a study with 3 different nitrogen doses in wheat (60-80-100 kg of nitrogen) reported that thousand grain weight is respectively as 42.14-46.29-47.55 g according to the doses. The effect of the nitrogen on the thousand grain weight is statistically significant and the grain weight has increased with the increasing nitrogen doses.

3.4. Seed Weight/Spike

In the experiment, a very significant (P<0.01) statistical difference was found in terms of the analyzed varieties, treatment, x variety treatment interaction and seed weight. Variance analysis results for seed weight values are available in Table 1. Besides, mean values and materiality groups are shown in Table 5. As can be observed in Table 5, the highest seed weight was measured from Harmankaya99 and Altay (2000) variety (2.07), the lowest seed weight was measured from Mufitbey variety (0.91).

Ozturk and Akten (1999) reported that with increasing seed weight and spike number per unit area, grain yield also increases and that for a high yield, an increase in the spike number per unit area is more effective than an increase in seed weight. Yildirim et al. (2005) reported that the seed number is higher in varieties having a long spike length and they recommend varieties with a large number of seed numbers to achieve a high grain yield. Dokuyucu and Akkaya (1999) reported that seed weight between is 1.50-1.97 g. Hussain et al. (2006) reported that the effect of nitrogen doses (0-50-100-150-200 kg ha^{-1}) on seed weight (2.8-3.2-3.3-3.2-3.5 g) is significant.

3.5. Plant Height (cm)

In the experiment, a very significant (P<0.01) statistical difference was found in terms of varieties studied, treatment and variety x treatment interaction and the plant height. Results of variance analysis for plant height values are shown in Table 1. Mean values and materiality groups are, on the other hand are available in Table 6. As shown in Table 6, the highest plant height was measured from the Nacibey variety (110.66) and the lowest plant height was measured from the Alpu-2001 variety (92.73).

In cereals, the plant height is affected by the factors such as the genetic potential of the variety, high fertilizer levels (especially nitrogen) and low light (Hussain et al., 2006; Yürür et al., 1987; Genctan and Saglam, 1987; Kun, 1988). Mut et al. (2005) found in their study on different wheat varieties and ecotypes that the plant height was between 66.9-98.8 cm according to the mean of locations, and the difference within genotypes was statistically very significant.

In their study with different varieties and different nitrogen doses in wheat, Oncan Sumer et al. (2010) found that the plant height is between 65.1-109.8 cm and reported that the effect of the fertilizer-variety interaction and fertilizer and variety factors are significant in terms of the plant height.

Ozseven and Bayram, (2005) in their study investigating the effects of nitrogen doses (6-12-18-24 kg da^{-1}) in different wheat varieties, showed that increasing nitrogen doses have an increasing effect on the plant height in wheat. They also reported that this happens in the form of a continuous increase according to varieties up to a certain point, then of a decrease.

Table 5. Mean values and significance groups of seed weight per spike

Cultivar/Application	5	10	15	20	25	Mean
Soyer 02	1.30 $^{r-u}$	1.59 $^{j-q}$	1.53 $^{l-s}$	1.48 $^{m-t}$	2.08 $^{c-f}$	1.59 ^{CD}
Müfitbey	0.70 ^{z}	0.76 ^{yz}	0.93 ^{wx}	0.92 ^{xyz}	1.24 $^{s-v}$	0.91 ^{F}
Nacibey	1.88 $^{f-j}$	1.72 $^{g-n}$	1.72 $^{g-x}$	2.00 $^{d-g}$	2.18 $^{b-e}$	1.90 ^{B}
ES-26	1.00 $^{v-z}$	1.25 $^{r-v}$	1.31 $^{q-u}$	1.43 $^{n-t}$	1.37 $^{p-t}$	1.27 ^{E}
Sultan-95	1.29 $^{r-v}$	1.02 $^{u-y}$	1.06 $^{u-x}$	1.50 $^{l-t}$	1.28 $^{r-v}$	1.23 ^{E}
Harmankaya 99	1.60 $^{j-q}$	1.91 $^{e-i}$	2.21 $^{a-d}$	2.34 ^{abc}	2.32 ^{abc}	2.07 ^{A}
Sönmez - 2001	1.21 $^{t-w}$	1.54 $^{k-r}$	1.69 $^{h-o}$	1.73 $^{g-m}$	1.41 $^{o-t}$	1.51 ^{D}
Alpu – 2001	1.49 $^{l-t}$	1.71 $^{g-n}$	1.78 $^{g-l}$	1.87 $^{f-j}$	1.53 $^{l-s}$	1.67 ^{C}
Altay 2000	1.70 $^{h-o}$	1.82 $^{f-k}$	1.97 $^{d-h}$	2.50 ^{a}	2.38 ^{ab}	2.07 ^{A}
Mesut	1.86 $^{f-j}$	1.88 $^{f-j}$	1.73 $^{g-m}$	1.97 $^{d-h}$	2.39 ^{ab}	1.96 ^{AB}
Yunus	1.50 $^{l-t}$	1.91 $^{e-ı}$	1.66 $^{ı-p}$	1.54 $^{k-r}$	1.72 $^{g-n}$	1.66 ^{C}
Mean	1.41 ^{C}	1.55 ^{B}	1.60 ^{B}	1.75 ^{A}	1.81 ^{A}	1.62

Cultivar LSD= 0.13, Application LSD= 0.08, Interaction of Cultivar x Application LSD= 0.29.

Table 6. Mean values and significance groups of plant height

Cultivar/Application	5	10	15	20	25	Mean
Soyer 02	102.33 h-p	108.33 f-l	104.66 g-o	110.66 d-h	119.66 b-e	109.13 A
Müfitbey	88.14 q-w	92.00 p-t	109.53 d-j	102.30 h-p	128.15 ab	104.02 B
Nacibey	99.33 i-q	103.66 g-o	123.33 abc	109.00 e-j	118.00 b-f	110.66 A
ES-26	79.00 v-y	104.33 g-o	96.00 m-s	107.00 f-m	131.00 a	103.46 B
Sultan-95	88.00 q-w	77.33 wxy	110.00 d-ı	114.66 c-g	85.00 s-x	95.00 C
Harmankaya 99	91.00 q-u	80.33 u-x	76.00 xy	103.00 h-p	97.66 k-r	89.60 D
Sönmez - 2001	73.00 y	82.00 t-y	108.66 e-k	82.66 t-y	128.33 ab	94.93 C
Alpu – 2001	82.00 t-y	77.33 wxy	98.66 j-r	108.33 f-l	97.33 l-r	92.73 CD
Altay 2000	106.66 g-n	95.00 o-s	95.66 n-s	131.33 a	120.66 a-d	109.86 A
Mesut	103.33 h-o	97.66 k-r	103.00 h-p	90.00 q-v	122.00 abc	103.20 B
Yunus	110.00 d-ı	73.33 y	94.00 o-s	104.66 g-o	120.66 a-d	100.53 B
Mean	92.98 D	90.12 D	101.77 C	105.78 B	115.31 A	101.19

Cultivar LSD= 5.03, Application LSD= 3.39, Interaction of Cultivar x Application LSD= 11.25.

In varieties where the plant height constantly increases, the amount of increase usually decreases after nitrogen dose of 12 kg da^{-1}. Hussain et al. (2006) reported that the effect of 5 different doses of nitrogen (0-50-100-150-200 kg ha^{-1}) on the plant height in wheat (65.6-76.3-82-81.1-82.2 cm) is significant, with the increase of nitrogen doses, plant height increases, and plant height values obtained from 100, 150 and 200 kg ha^{-1} doses take place in the same statistical group.

3.6. Seed/Spike Number

In the experiment, a very significant (P<0.01) statistical difference was found in terms of the number of the varieties, treatment and variety x treatment interaction and seed number. Variance analysis results of seed number values are presented in Table 1. Mean values and materiality groups are, however, offered in Table 7. As can be seen in Table 7, the highest seed number was measured from the Mesut variety (43.88), and the lowest seed number spike was measured from the Mufitbey variety (23.83).

Ozen and Akman, (2015) reported that the seed number is between 22 and 46. Tunca, (2012) on the other hand, reported that the seed number is between 12.5 and 31. Caglar et al. (2006) reported that it is between 19.9 and 30.4.

3.7. Protein Content

A very significant (p<0.01) statistical difference was found in terms of analyzed varieties in the study, treatment and variety x treatment interaction and protein contents of varieties. Results of variance analysis of protein content values are available Table in 1. In addition, mean values and materiality groups are given in Table 8. As can be seen in Table 8, the highest protein content was measured from the Mufitbey variety (11.16%) and the lowest protein content was measured from the ES-26 variety (8.34%).

The protein content of wheat is very important in bread making, because flours with high-protein wheat are important in bread making that is high in volume, that can make more water absorption and that is better stored (Schofield, 1994). It is reported that the protein content in wheat varies depending on factors such as climate, variety, amount of nitrogen fertilizer applied, time of application, soil fertility, plantation date (Unal, 2002) Cereals are evaluated as very low (9% and below), medium (11.6%-13.5%), high (13.5%-15.5%) and extra high (17.5% and above) according to the protein content (Williams et al., 1988). Mou and Kronstad (2002) noted that there are statistically significant relationships between protein content, long grain filling and, low grain filling periods in wheat, and early flowering rate.

Table 7. Mean values and significance groups for the number of seed per spike

Cultivar/Application	5	10	15	20	25	Mean
Soyer 02	25.00 s-x	32.00 l-r	35.66 g-n	27.66 q-v	35.66 g-n	31.20 DE
Müfitbey	19.50 x	23.98 t-x	22.93 u-x	29.01 o-t	23.74 t-x	23.83 F
Nacibey	37.33 e-m	37.00 e-m	26.33 r-w	39.33 d-j	40.66 b-h	36.13 C
ES-26	21.33 wx	29.33 o-t	29.00 o-u	28.86 p-u	22.77 vwx	26.26 F
Sultan-95	24.00 t-x	25.33 s-x	32.50 k-q	34.00 ı-p	34.66 h-p	30.10 E
Harmankaya 99	30.66 n-s	42.00 a-f	40.00 c-i	37.00 e-m	46.66 ab	39.26 B
Sönmez - 2001	43.66 a-d	38.00 d-l	37.33 e-m	39.33 d-j	33.00 k-q	38.26 BC
Alpu – 2001	28.66 p-v	31.66 m-r	43.00 a-e	35.00 h-o	28.66 p-v	33.40 D
Altay 2000	36.33 f-n	27.66 q-v	41.66 a-g	38.00 d-l	37.66 d-m	36.26 C
Mesut	45.66 abc	41.33 a-g	46.66 ab	47.33 a	38.43 d-k	43.88 A
Yunus	35.00 h-o	46.33 ab	41.33 a-g	37.00 e-m	33.33 j-q	38.60 BC
Mean	31.56 C	34.05 B	36.03 A	35.68 AB	34.11 B	34.28

Cultivar LSD= 2.71, Application LSD= 1.83, Interaction of Cultivar x Application LSD= 6.07.

Table 8. Mean values and significance groups of protein content

Cultivar /Application	5	10	15	20	25	Mean
Soyer 02	8.60 m-r	8.70 k-q	9.27 f-l	9.55 c-h	10.01 b-d	9.23 BC
Müfitbey	12.00 a	11.50 a	9.71 c-g	10.63 b	12.00 a	11.16 A
Nacibey	9.21 g-m	9.34 e-k	9.02 h-o	8.83 ı-p	10.10 bc	9.30 BC
ES-26	7.53 u	7.84 s-u	8.04 r-u	8.45 n-t	9.88 c-f	8.34 G
Sultan-95	8.96 h-p	8.50 o-r	8.35 p-t	8.50 o-r	9.69 c-g	8.80 DEF
Harmankaya 99	9.16 g-n	9.40 d-j	9.80 c-g	9.42 d-ı	9.40 d-j	9.43 B
Sönmez - 2001	7.59 u	8.67 l-r	8.77 j-p	9.21 g-m	9.90 c-f	8.82 DE
Alpu – 2001	7.77 s-u	8.10 q-u	8.50 o-r	8.81 ı-p	9.43 d-ı	8.52 FG
Altay 2000	8.40 o-s	8.75 j-q	9.01 h-o	9.95 c-e	10.10 bc	9.24 BC
Mesut	8.81 ı-p	8.50 o-r	8.96 h-p	8.93 h-p	10.01 b-d	9.04 CD
Yunus	7.63 u	7.74 tu	9.49 c-h	8.68 l-r	9.25 f-m	8.56 EFG
Mean	8.69 D	8.82 CD	8.99 BC	9.18 B	9.98 A	9.13

Cultivar LSD= 0.14, Application LSD= 0.19, Interaction of Cultivar x Application LSD= 0.65.

Acer (2004) reported in a study that according to the two-year results of different nitrogen applications, the protein content in wheat in the first year was obtained from 12.90 kg da^{-1} nitrogen dose as well as from %12.548 kg da^{-1} nitrogen dose. It was also obtained from 12.194 kg da^{-1} nitrogen dose in the second year, from 14.7312 g.da^{-1} nitrogen dose, from 14.328 kg da^{-1} nitrogen dose and from 13.494 kg da^{-1} nitrogen dose. Haque et al. (2017) reported that the effect of different nitrogen doses applied to wheat (0-60-120-180 kg da^{-1}) on the protein content is significant and that the protein content increases as doses increase (10.64-11.23-12.45-13.41%).

Oncan-Sumer et al. (2010) reported that the protein content increases in increased nitrogen doses in wheat varieties where they applied different doses of nitrogen. Although the protein content is determined by the genotype and the environment, the effect of the nitrogen fertilizer application on the protein content is greater (Triboi et al., 2000)

3.8. Sedimentation Value

A very significant (P<0.01) statistical difference was found in terms of analyzed varieties, treatment and variety x treatment interaction and sedimentation value. Results of variance analysis of sedimentation values are presented in Table 1. Mean values and materiality groups are, however, given in Table 9. As can be seen in Table 9, the highest sedimentation value was measured from the Nacibey cultivar (19.40) and the lowest sedimentation value was measured from the ES-26 cultivar (11.94).

Sedimentation value is a quality criterion that determines the quality of the protein contained in the grain and has a high degree of heritability. It gives information about the bread value of wheat and it is desired to be high (Kocak et al. 1992). Mini SDS sedimentation values in bread wheat are analyzed as weak (10 ml and below), medium (10-12 ml) and strong (13 ml and above) (Pena et al., 1990). Kinaci and Kinaci (2004) reported that wheat has a mean sediment value of 30.2 ml while Aydin et al. (2005) reported it is between 26.3 ml and 54.5 ml. Koc and Akgun (2018) explained that the sedimentation value varies depending on the genotype but is also affected by environmental factors.

3.9. Hectoliter Weight

A very significant (P<0.01) statistical difference was found in terms of analyzed varieties, treatment and variety x treatment interaction and hectoliter weight of varieties. Results of variance analysis of hectoliter weight values are shown in Table 1. Mean values and materiality groups are also given in Table 10.

Table 9. Mean values and significance groups of sedimentation (mm) value

Cultivar /Application	5	10	15	20	25	Mean
Soyer 02	14.06 h-n	14.29 h-m	18.57 c-e	20.00 b-d	22.10 ab	17.80 B
Müfitbey	16.00 f-h	13.00 k-q	14.41 h-m	18.54 c-e	13.50 ı-o	15.09 D
Nacibey	17.21 e-g	23.19 a	20.15 bc	17.21 f-h	20.47 bc	19.40 A
ES-26	8.80 u	9.05 tu	11.24 p-s	11.53 o-s	19.09 c-e	11.94 F
Sultan-95	11.00 q-t	10.50 r-u	11.50 o-s	11.50 o-s	18.73 c-e	12.64 F
Harmankaya 99	12.92 k-q	12.50 l-s	14.50 h-l	14.90 h-k	14.50 h-l	13.86 E
Sönmez - 2001	10.41 s-u	15.87 f-h	14.95 h-k	15.34 g-j	13.50 i-o	14.01 E
Alpu – 2001	13.19 j-p	15.48 g-ı	17.85 d-f	17.23 e-g	21.49 ab	17.05 BC
Altay 2000	12.06 n-s	14.58 h-l	17.24 e-g	18.50 c-e	21.37 ab	16.75 C
Mesut	12.28 m-s	12.61 l-r	14.32 h-m	14.22 h-m	19.96 b-d	14.68 DE
Yunus	12.03 n-s	13.16 k-p	23.36 a	17.95 d-f	18.61 c-e	17.02 BC
Mean	12.72 D	14.02 C	16.19 B	15.97 B	18.48 A	15.47

Cultivar LSD= 0.96, Applicaiton LSD= 0.64, Interaction of Cultivar x Application LSD= 2.15.

Table 10. Mean values and significance groups of hectoliter weight (kg)

Cultivar /Application	5	10	15	20	25	Mean
Soyer 02	74.00 d-k	74.14 d-j	66.87 t	74.32 d-j	78.37 ab	73.54 BC
Müfitbey	72.66 g-o	69.50 rs	69.77 q-s	67.84 st	76.00 b-d	71.15 EF
Nacibey	73.00 f-n	72.60 g-o	70.48 o-r	71.44 l-r	69.55 rs	71.41 EF
ES-26	71.00 m-r	72.30 h-p	70.04 p-s	73.75 d-l	73.24 e-m	72.06 DE
Sultan-95	71.00 m-r	70.00 p-s	72.50 h-o	74.50 d-ı	65.98 t	70.79 F
Harmankaya 99	72.00 j-q	70.50 o-r	72.50 h-o	70.26 n-r	71.50 l-r	71.42 EF
Sönmez - 2001	75.17 d-f	78.93 a	69.90 p-s	74.20 d-j	75.00 d-g	74.64 A
Alpu – 2001	74.62 d-h	74.03 d-j	72.18 ı-q	71.59 k-r	72.00 j-q	72.88 CD
Altay 2000	72.06 j-q	72.53 h-o	73.50 e-l	74.50 d-ı	77.86 b-c	74.09 AB
Mesut	74.22 d-j	71.26 l-r	71.11 l-r	71.11 l-r	71.21 l-r	71.78 EF
Yunus	72.00 j-q	75.62 c-e	72.24 h-p	73.00 f-n	74.09 d-j	73.39 BC
Mean	72.88 A	72.85 A	71.00 B	72.44 A	73.16 A	72.46

As can be observed in Table 10, the highest hectoliter weight was measured from the Sönmez-2001 variety (44.64 kg) and the lowest hectoliter weight was measured from the Sultan-95 variety (70.79 kg).

The hectoliter weight in wheat varies depending on factors such as variety, environmental conditions, cultural practices, lodging, disease and damage (Sener et al., 1997; Atli et al., 1999). The shape, density, size and homogeneity of the grain are also important properties that affect the hectoliter weight. Since the hectoliter weight in wheat and flour yield are positively related (Ozkaya and Kahveci, 1990) it is also important in breeding as a selection criterion due to its being the subject of milling and trade. Cifci and Dogan (2013) determined hectoliter weights of varieties as 75.94 and 76.84 kg, while they observed that nitrogen doses do not effect on the hectoliter weight and the mean values range from 75.90 to 76.91 kg.

3.10. Artificial Neural Network

A database was created with the chlorophyll values obtained within the scope of the project. Determination of the targeted chlorophyll content with an artificial neural network was completed with this project study. At this point, it is the first step in which an interdisciplinary collaboration is carried out in the project area. In addition, studies of obtaining some information from the image (for example, determination of chlorophyll concentration index, plant growth, yield estimates) with the help of computer have come to conclusion.

In this study, 70% of the data set was used as training data. Output error (MSE) and maximum iteration value were found to be 0.001 and 1000 epoch, respectively. The number of hidden layers and the number of neurons in the hidden layer were determined by the training error method. In this study, MSE and R^2 value determine the performance of the artificial neural network. These criteria are calculated according to the Equations 7 and 8.

$$MSE = \frac{\sum_{i=1}^{N}\left(y_{ai} - y_{pi}\right)^2}{N-1} \quad (7)$$

$$R^2 = \frac{\left(\sum_{i=1}^{N}\left(y_{ai} - \bar{y}_a\right)\left(y_{pi} - \bar{y}_p\right)\right)}{\sum_{i=1}^{N}\left(y_{ai} - \bar{y}_a\right)^2\left(y_{pi} - \bar{y}_p\right)^2} \quad (8)$$

R^2 performance values obtained as a result of the data analysis via artificial neural networks are offered below (Figure 3). Considering the graphs of the training, testing, validation and all values of the artificial neural network, it is seen that the network performs 99% calculations with high accuracy.

Looking at the performance graph of the network (Figure 4), the artificial neural network width performance was also shown in 933 epoch.

A linear relationship was found between SPAD readings and leaf chlorophyll (Chl), and R2 value was taken into account in the evaluation of this relationship. The best relationship between Chl and SPAD is shown in the figure below (Figure 5).

The linear equation obtained from these graphs and the R2 value for chlorophyll (y = 1.81x + 5.166) and the R2 value as 98.1% were found as a result of the training of SPAD values with ANN. The high R2 value indicates the accuracy of the values measured with the SPAD meter and its usability with ANN in predicting the chlorophyll content in the plant.

4. Conclusion

In this study, bread wheat varieties which can be grown in Ordu Province, Türkiye have been tested. In the experiment, differences of these varieties in terms of yield and yield components were determined in Ordu Province. The experiment focused particularly on the chlorophyll content. ANN has been shown to predict leaf chlorophyll concentration index. The use of an artificial neural network has been shown as an alternative solution to determine the chlorophyll concentration index instead of expensive optical based devices. This method is a non-destructive approach and simple, rapid, and accurate estimation of SPAD values. In addition, nowadays increasingly importance of computer-aided smart farming systems was highlighted.

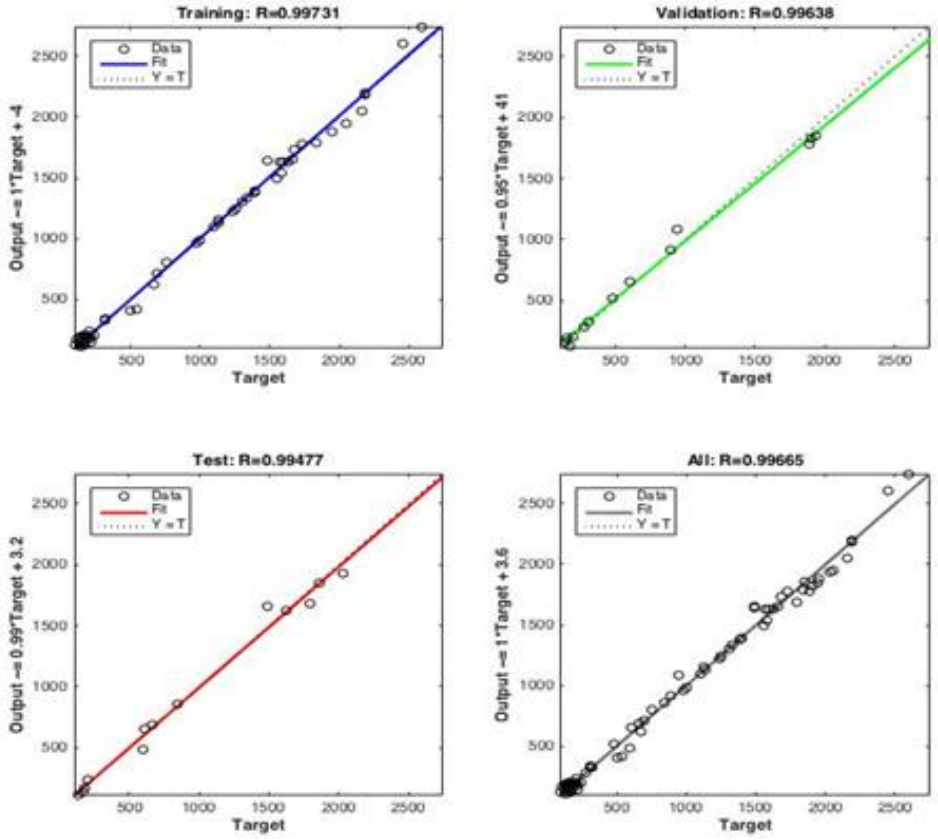

Figure 3. R^2 performance values.

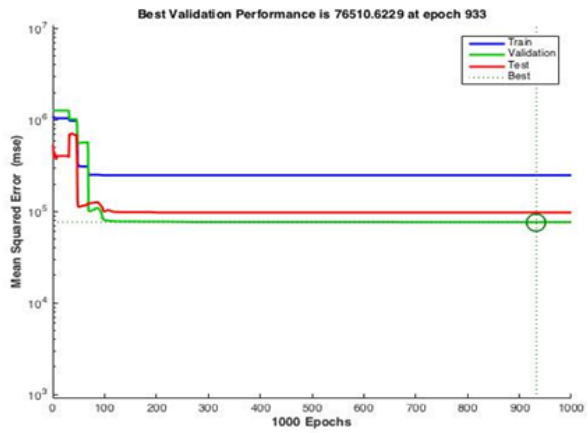

Figure 4. Network performance.

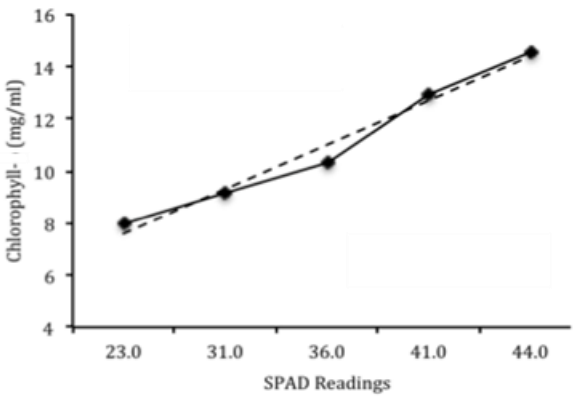

Figure 5. Relationship between SPAD value and chlorophyll.

Author Contributions

F.Ö. (25%), G.K. (15%), E.R. (15%), F.Öz. (15%), R.O. (15%) and M.S.O. (15%) design of study. F.Ö. (25%), G.K. (15%), E.R. (15%), F.Öz. (15%), R.O. (15%) and M.S.O. (15%) data acquisition and analysis. F.Ö. (25%), G.K. (15%), E.R. (15%), F.Öz. (15%), R.O. (15%) and M.S.O. (15%) writing up. F.Ö. (25%), G.K. (15%), E.R. (15%), F.Öz. (15%), R.O. (15%) and M.S.O. (15%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Research Article
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THE EFFICACY OF HOT WATER TREATMENTS AGAINST *FUSARIUM FUJIKUROI*: THE FUNGAL AGENT OF BAKANAE DISEASE

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Abstract: Rice is one of the most grown agricultural products in the world. It is the most preferred food item in the Turkish diet. One of the most important fungal diseases of rice is Bakanae disease. It is a seed-borne and complex disease caused by the *Fusarium* species. *Fusarium fujikuroi* is the most virulent and widespread species. The excessive use of fungicides has raised concerns such as a decrease in the fungicide sensitivity of *F. fujikuroi* in the world. For this reason, alternative methods are being investigated to control the disease. In this study, the therapeutic effect of hot water treatment on contaminated seeds was investigated. Trials were carried out under *in vitro* and *in vivo* conditions, to determine the effects of hot water treatment on the germination rate of rice seeds. Hot water treatments at 55 °C and 57 °C were the most effective treatments against pathogen. However, pathogen was not inhibited at 50 °C. The lowest disease severity was determined at 57 °C (2.5%) and this was followed by hot water treatments at 55 °C (22.33%) and 52 °C (77.30%) *in vivo* tests, respectively. No disease symptoms were observed in the negative control plants. According to evaluations, the disease incidence decreased when treatment temperature was increased, resulting in a slightly reduced germination rate.

Keywords: Rice, Bakanae disease, *Fusarium fujikuroi*, Hot water treatment, Seed germination

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1. Introduction

Rice (*Oryza sativa* L.), whose center of origin is estimated to be India and China in Southeast Asia, is an important cereal of the Poaceae family (Crawford and Shen, 1998; Gnanamanickam, 2009). It is one of the oldest cultivated plants that can germinate in water and its roots can utilize oxygen in the water. It has an important role in human nutrition with its protein, carbohydrate, minerals, and vitamins in the world (Çevik, 2011). Rice is one of the most grown agricultural products in the world with a cultivation area of 167.132.623 ha and a production amount of 782.000.147 tons. Countries such as China, India, and Vietnam supply a significant part of the rice produced in the world (FAO, 2020). It is cultivated on 130.042.4 ha and total rice production is 940.000 tons in Türkiye. The rice is preferred to be cultivated by Turkish farmers due to its high yield (8218.8 kg ha^{-1}), thus the rice growing area in Türkiye increases (TUIK, 2020).

Rice is one of the most preferred staple food of Turkish society, so it is imported at increasing rates since our domestic rice production is not adequate. In most regions in Türkiye, rice is cultivated in fields where other crops cannot grow and therefore alternative farming cannot be done. The crop pattern of rice is not changed and disease agents continue their vitality in the soil. Rice cultivation is carried out in the same field for at least five years in a row, even 20-25 years. Therefore, disease, pests, and weeds increase over time and these biotic factors cause significant yield losses in rice production (Sade et al., 2011). Plant residues and soil may also affect the spreading of pathogens. Previous studies indicated that the agents of disease can survive for months in rice seed and also plant residues and soil (Mishra et al., 1989; Dodan et al., 1994; Sunder and Satyavir, 1997, Manandhar, 1999).

One of the most important biotic factors affecting rice yield is fungal disease. It has been reported that Bakanae disease causes yield losses of up to 70% in epidemic areas (Hajra et al., 1994; Singh et al., 1996; Batsa and Manandhar, 1997). The disease has been identified as the most destructive disease of rice, causing significant economic yield and quality losses in most of the countries where rice production has been done in the past 20 years (Khan et al., 2000; Carter et al., 2008; Zainudin et al., 2008; Karov et al., 2009; Rabbi and Ali, 2011; Jeon et al., 2013; Gupta et al., 2014; Kim et al., 2015; Chen et al., 2016).

Bakanae disease is caused by *Fusarium* species within the

Gibberella fujikuroi species complex. Studies have reported that *F. fujikuroi* Nirenberg, *F. proliferatum* (Matsushima) Nirenberg, *F. sacchari* Buttler, *F. subglutinans* (Reinking) Wollenworth, *F. verticillioides* (Sacc.) Nirenberg) and *F. andiyazi* Marasas are the most virulent species (Amoah et al., 1995; Desjardins et al., 2000; Zainudin et al., 2008; Amatulli et al., 2010, Pra et al., 2010; Choi et al., 2018; Eğerci et al., 2020a, Eğerci et al., 2020b). The previous studies reported that *F. fujikuroi* is the most virulent and widespread species among these species complex and that gibberellin produced *F. fujikuroi* increases disease severity (Desjardins et al., 2000; Malonek et al., 2005; Zainudin et al., 2008; Amatulli et al., 2010; Ora et al., 2011; Bashyal et al., 2016, Eğerci et al., 2020a).

The disease is a seed-borne pathogen and it develops systemically within the rice plant. The symptoms of the disease may emerge within about a month after sowing (Webster, 2004). The seed either does not germinate at all or then most of the seeds turn brown and rot with their sprout. The most common symptoms are thin, yellowish-green and elongated seedlings, chlorotic leaves, root, and crown rot. In addition, early ripening can cause symptoms such as empty husk (glumes) and white ear formation (Copçu and Karaca, 1983; Desjardins et al., 2000; Webster, 2004; Zainudin et al., 2008, Eğerci et al., 2020a). All of these symptoms are described as Bakanae syndrome.

Bakanae disease caused by *F. fujikuroi* is a widespread disease that causes yield and product losses particularly in Balıkesir and Çanakkale provinces of Türkiye (Eğerci et al., 2020a). The most of the rice farmers in Türkiye separate some of their product grown as the seed which they use in the next production year, the disease continues to increase year by year causing serious yield losses because the disease is seed-borne. Moreover, *F. fujikuroi* has a high degree of genetic variation and the risk of creating an epidemic has increased with the spread of the disease in recent years. Seed treatments with chemical fungicides are the most common control practice for this disease in the world. However, the excessive use of fungicides has raised concerns such as a decrease in the fungicide sensitivity of pathogens. Previous studies have reported that the increasing occurrence of resistant strains of *F. fujikuroi* to fungicides is posing a new risk. For this reason, alternative methods of control are being investigated in the world to manage the disease (Kim et al., 2010; Matic et al., 2017).

To prevent this disease by producing permanent solutions has become important. For these purposes, the therapeutic effect of different temperatures of hot water on infected seeds was investigated in this study. To evaluate the effect of hot water treatment on the disease, *in vitro* and *in vivo* experiments were carried out to determine the effects of these treatments on the germination rate of rice seeds.

The objectives of the the study were to (i) investigate the therapeutic effect of different temperatures of hot water on infected seeds and (ii) determine the effects of these treatments on the germination rate of rice seeds.

2. Materials and Methods

2.1. Preparation of the Inoculum

F. fujikuroi isolate which had been identified previously with morphological and molecular methods (Acc. no: MN091843) and determined to have high virulence and is widely common in the region was used in the study (Eğerci et al., 2020a). The PCR amplification of the TEF-1α gene region was carried out by using EF1/EF2 primers described by O'Donnell et al. (1998). The fungal isolate was incubated on PDA medium for 7-10 days in the incubator at 25 ± 2 °C. The isolate of *F. fujikuroi* grown on PDA was flooded by adding 5 ml of sterile distilled water containing 1-2 drops of Tween 80 per liter. The resulting suspension was sifted through a double layer of cheesecloth to remove mycelium, spore count was performed under the microscope (Olympus, Stream 2.1) and a spore suspension with a density of $1x10^6$ spores/ml was prepared (Egerci et al., 2020b).

2.2. Hot water Treatment of Seeds

The seeds of the Baldo rice variety, which is known to be susceptible to the disease, were sterilized with 1% sodium hypochlorite for 45 seconds. The seeds were soaked in sterilized distilled water for 24 hours and then in spore suspension of $1x10^6$ spore/ml density for 48 hours. Inoculated seeds were kept in hot water at 50 °C, 52 °C, 55 °C and 57 °C for 15 minutes in the hot water bath and allowed to dry on filter paper. The seeds used for negative (healthy) control were kept in sterile distilled water for 72 hours. Isolation of pathogen from treated and nontreated seeds was carried out under *in vitro* conditions with 5 replications and by placing 5 seeds per PDA plates. The plates were allowed to incubate at 24 ± 2 °C for 7 days in the incubator under 12 hours light / dark conditions.

Pot tests were also conducted under *in vivo* conditions to determine the effect of hot water treatment of infected seeds. The experiments were carried out in plastic pots (22×14×9 cm) containing sterilized soil which was autoclaved twice; plastic pots were kept in a growth chamber at 24 °C and 80% relative humidity, with 12 hours of light. The experiment was laid out in randomized design with 5 replications. Ten seeds were planted in each 5 plots. Irrigation was performed 3 times a day and 3 g ammonium sulphate was given to each tray at the 15^{th}-day interval. After sowing the seeds, the disease evaluation was made on the 30th day by using the Ooi (2002) disease scale (Table 1). The plants' tissues were used for the fungal reisolation as to Koch's postulates, after scoring. Statistical analyses were performed using the SPSS 16 (IBM Corp., Armonk) software package. Data were scored and analyzed by analysis of variance (ANOVA) implemented in SPSS to determine the significant variation within and among the treatments. Data means were treated using the least significant difference test at P =0.05 level.

2.3. Seed Germination Test

Germination rates of rice seeds treated to hot water were determined under *in vitro* and *in vivo* conditions. *In vitro* tests were performed using the Blotter method. According to the ISTA (International Rules for Seed Testing) rules, a filter paper moistened with sterile distilled water was placed in each petri dish and 100 seeds were placed on it, and the rice seeds were covered with another moistened filter paper. The tests were carried out with 4 replications. The germination rates were calculated at the end of the 7th day by leaving the seeds to germinate in the incubator at 24 ± 2 °C and 12 hours of lighting.

In vivo tests were arranged in randomized design and carried out in a growth chamber at 24-26 °C and 80% relative humidity, 12 hours of lighting conditions. Experiments of the *in vivo* tests were carried out in plastic pots containing sterilized soil. The disease evaluation was carried out when the plants were 2 mm tall on the soil surface. After the seed germination rates (%) were determined, statistical analyses were performed using the SPSS 16 (IBM Corp., Armonk) software package (equation 1). Data were analyzed by analysis of variance (ANOVA) implemented in SPSS to determine the significant variation within and among the treatments. Data means were treated using the least significant difference test at P =0.05 level.

$$\text{Germination rate} = \frac{(\text{number of germinated} * 100)}{\text{total number of planted seeds}} \quad (1)$$

Table 1. Symptoms of rice plants were scored basing on disease scale 0–4

Disease scale	Disease symptoms
0	Healthy and uninfected plants (no external symptoms)
1	Normal growth but leaves beginning to show yellowish–green
2	Abnormal growth, elongated, thin and yellowish-green leaves; seedlings also shorter or taller than normal
3	Abnormal growth, elongated; chlorotic, thin and brownish leaves; seedlings also shorter or taller than normal
4	Seedlings with fungal mass on the surface of infected plants or died

3. Results

3.1. Hot Water Treatment of Seeds

Isolation of pathogen from treated and nontreated seeds were transferred to PDA plates and incubated at 24 ± 2 °C for 7 days in incubator under 12 hours of light/dark conditions. The results from seeds treated with hot water are summarized in Table 2. The highest disease severity rate of 100% was observed on positive control and the treatments at 50 °C and 52 °C. This was followed by the treatments at 55 °C (36%) and 57 °C (8%). There was no mycelial development in the negative control seeds (healthy). Statistical analysis showed that there was a difference between treatments (Table 2).

Table 2. The disease severity on seeds treated with different hot water temperatures

Hot water treatments (°C)	Disease severity (%)
50	100.0 a
52	100.0 a
55	36.00 b
57	8.00 c
Positive Control	100.0 a

*There is no statistical difference between the values indicated by the same letter (P=0.05).

Pot trials were carried out under *in vivo* conditions to evaluate the effect of hot water treatments on disease development and evaluation of which were made on the 30th day according to the Ooi (2002) disease scale. The disease severity (%) obtained in pot tests from seeds treated with hot water is given in Table 3.

According to the results of *in vivo* tests, the highest disease severity was determined in the treatment at 50 °C (85.45%). This was followed by hot water treatments at 52 °C (77.30%), 55 °C (22.33%) and 57 °C (2.5%), respectively (Table 3). The disease symptoms were observed within 30 days of rice seed sowing and accordingly, typical symptoms of Bakanae diseases were observed on seedlings. *F. fujikuroi* caused pale green to yellowing of foliage, chlorotic leaves, and necrotic roots on plants. No disease symptoms were observed in the negative control plants. The statistical difference was found between the treatments (Figure 1).

Table 3. The disease severity (%) on seeds treated with hot water *in vivo* tests

Hot water treatments (°C)	Disease severity (%)
50	85.45 a
52	77.30 b
55	22.33 c
57	2.50 d
Positive Control	85.50 a

*There is no statistical difference between the values indicated by the same letter (P= 0.05).

3.2. Seed Germination Test

The germination rates of rice seeds subjected to hot water treatment were determined by the Blotter method and pot tests under *in vivo* conditions. In the Blotter method, germination rates were calculated at the end of

the 7th day. In pot tests, the evaluation was carried out, when the plants on the soil surface were 2 mm tall.

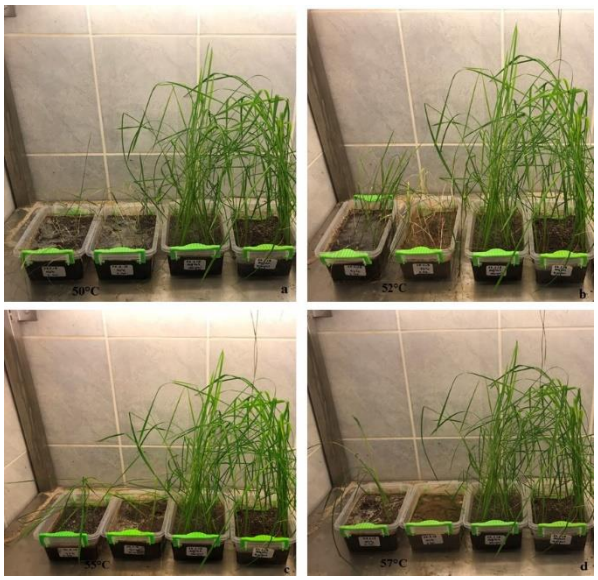

Figure 1. The efficacy of hot water treatments on infected seeds and compared control (healthy) plants a) Comparison of hot water application at 50 °C with control (healthy) plants, b) Comparison of hot water application at 52 °C with control (healthy) plants, c) Comparison of hot water application at 55 °C with control (healthy) plants, d) Comparison of hot water application at 57 °C with control (healthy) plants

In Table 4, the seed germination rates (%) obtained in the Blotter method and pot trials are given. As a result of the Blotter method, the highest seed germination rate of 86.25% was observed in the hot water treatments at 50 °C, followed by 52 °C (75%), 55 °C (40%) and 57 °C (9%), respectively (Table 4). There was a statistical difference between the treatments. In pot trial tests, the highest germination rate was observed in hot water treatments at 50 °C (74%). This was followed by 52 °C (64%), 55 °C (36%) and 57 °C (8%), respectively. There was no statistical difference between the 50 °C and 52 °C treatments. In the blotter and pot trial tests, seed germination percentages decreased as the temperature increased.

4. Discussion

Rice is one of the most important agricultural products with rich nutrient contents in the world. Due to the high amount of average yield of rice production in Türkiye, rice producers have increasingly preferred to cultivate rice in recent years, thus an increase in rice cultivation areas. It has been reported in the world that Bakanae disease causes significant yield and product losses in areas where the epidemic occurs (Singh et al., 1996; Batsa and Manandhar, 1997). In Türkiye, the situation of this disease is very serious. Eğerci et al. (2020a) reported that *F. fujikuroi* is widespread in Balıkesir and Çanakkale provinces of Türkiye. The low-level usage of certified seed in rice production, separation of part of the harvested product as seeds for subsequent production, and use of rice crops from abroad as seeds play an important role in the spread of this disease in Türkiye. The management of Bakanae disease is difficult due to the genetic mutation potential of *Fusarium* spp. and also an epidemic character of the disease (Serefica and Cruz, 2009). For this reason, alternative methods (hot water applications, resistant varieties, etc.) are being investigated in the world to control the disease.

Table 4. The seed germination rates (%) in the blotter method and pot trials

Hot water treatments (°C)	The seed germination rates (%)	
	Blotter Method	Pot Trials
50	86.25 b	74.00 c
52	75.00 c	64.00 c
55	40.00 d	36.00 d
57	9.00 e	8.00 e
Negative Control	95.75 a	100.0 a
Positive Control (Contaminated)	85.75 b	98.00 b

*There is no statistical difference between the values indicated by the same letter (P = 0.05).

In this study, the therapeutic effect of hot water treatments which is an alternative control method was investigated in the 'Baldo' rice variety, which is known to be sensitive to Bakanae disease. According to the studies conducted under *in vitro* and *in vivo* conditions were evaluated, it was determined that the most ineffective hot water treatments at 50 °C and 52 °C. Disease severity rates were determined at almost the same rate as the positive control. The disease severity values are significantly decreased in the treatments of hot water at 55 °C and 57 °C, on the other hand the seed germination with the lowest rates was determined. Miyasaka et al. (2000) reported that the disease was decreased in seeds kept for 15 minutes in hot water application at 60-62 °C, but seed germination decreased, too. Fukuda et al. (2013), determined the effect of hot water treatment on the germination of rice seeds by treating seeds of the "Bekoaoba" variety for 10 minutes at 50-60 °C. They found that the germination rate decreased significantly, but the disease was prevented. In a different study, the seeds showing the signs of rice root rot disease were treated for 15 minutes with hot water at 57 °C, and it was

determined that seed infection was significantly prevented. No negative effect on seed germination was reported (Wolf, 2002).

In previous studies, it was observed that both disease outbreak rate and seed germination rates decreased when temperatures increased. Rocha (1984) determined the effect of hot water treatments on the quality of rice seeds belonging to different varieties in this study. The treatments done at 52 °C for 15 and 30 minutes and at 57 °C for 15 minutes did not have any negative effects on the seed quality. In the application performed at 57 °C for 30 minutes, it was determined that the quality of the seeds was diminished. Likewise, Permana et al. (2017), reported that among Japanese varieties 'Koshihikari' rice variety was the most resistant to hot water treatments and that even at 70 °C. The seed germination was observed in this rice variety at a rate of 76%, whereas hot water treatment reduced the germination rate in other varieties. They suggested that it may be due to the difference in sensitivity to temperature and tolerance levels among varieties.

In conclusion, the effectiveness of different temperatures of hot water against Bakanae disease in rice seeds was investigated in this study. Tests were carried out in order to evaluate the effect of hot water treatment on the disease, and also to determine the effects of these applications on the germination rate of treated rice seeds. It was determined that hot water application significantly reduced the disease rate, but the temperature negatively affected seed germination. The results obtained from hot water applications are light for future studies.

Author Contributions

Y.E. (50 %) and P.K.T. (50 %) conceived and designed the study. Y.E. (100 %) performed the trials. Y.E. (50 %) and A.U.M. (50 %) analyzed the data and wrote the manuscript. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Research Article
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THE EFFECTS OF PARTIALLY SLATTED FLOOR DESIGNS ON SOME EARLY BEHAVIORAL TRAITS IN BROILER CHICKS

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Abstract: This study was carried out to determine the effects of different levels of slatted floor applications on some early behavioral characteristics of broiler chickens. In this study, male-female mixed 600 fast-growing broiler chicks (Ross308) were used. The experiment consisted of five treatment groups with 120 chicks each (7 chicks/m^2): fully littered, fully slatted, ½ littered+½ slatted, 1/3 littered+2/3 slatted, 2/3 littered+1/3 slatted. In the study, the feeding, drinking, resting, aggregation, other behaviors, and slatted floor preferences of the chicks were evaluated three times a day (at 9.00, 13.00, and 17.00 h) at 2, 5, 9 and 11 days of age. Each behavioral trait was expressed as a percentage of the total number of chicks showing the relevant behavior at the pen level. Different floor designs significantly affected the chicks' feeding, resting, aggregation behavior, and preference for being on the slatted floor ($P < 0.05$). Feeding behavior was higher in chicks reared on the fully slatted floor than in the others ($P < 0.001$). The percentage of chicks showing resting behavior was highest in the 2/3 littered+1/3 slatted floor application ($P = 0.001$). The 64.42% of the chicks reared in 2/3 slatted, 47.53% of those reared in ½ slatted, and 36.38% of those raised in 1/3 slatted preferred the use of the slatted floor. The percentage of chicks showing feeding behavior was highest at 5 (16.12%) and 2 d-old (15.73%) ($P = 0.001$). Resting behavior was highest at 2 (78.72%), 5 (76.89%), and 9 (72.82%) days of age ($P < 0.001$). In conclusion, this study revealed that different floor designs affect some behavioral characteristics in the early chick period. Since it is known that early rearing conditions affect later performance in broilers, slatted floor systems with higher feeding behavior can be an effective tool for better performance in broiler production.

Keywords: Broiler chick, Slatted floor, Litter, Behavior, Feeding, Resting

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1. Introduction

The rapid increase in the world population brings with it the need for adequate and balanced nutrition. At least 1/3 of the daily protein needs of humans in a quality and balanced diet should be of animal origin (Öztürk et al., 2013). Poultry products have an important place in meeting this demand. In recent years, with the increasing level of awareness on balanced and adequate nutrition, efforts to obtain more yield per unit area have accelerated. In commercial conditions, chicken meat production is commonly carried out in closed poultry houses on litter material. It is very important to keep the litter in the desired quality in these poultry houses, and dermatitis occurring on the foot pads, hock joints and breast meat are frequently encountered in animals due to poorly managed litter quality (Özbek, 2021). Alternative floor systems have come to the fore as a solution to the problems related to animal health and welfare that arise when litter management is not sufficient in broiler production (Çavuşoğlu and Petek, 2019). Although cage and grill floor applications have been known for many years in broiler production, they have not become widespread due to their negative effects on welfare, foot-leg health and behavioral characteristics (Zhao et al., 2009; Shields and Grager, 2013). It has started to be used again in chicken meat production due to the technological improvements made on the cage and slatted floor material. However, the restricted movement of animals in the cage system still causes concerns in terms of animal welfare. Therefore, it is thought that the partially or fully slatted floor system can be used in commercial production (Özbek, 2021). At this point, the search for alternative production systems has begun in order to eliminate some of the negative effects of intensive production in broiler breeding and laying hen farming, taking into account the physiological needs and natural behavior of animals, and appropriate housing, care, management, feeding and health protection. Since broilers spend their life-time on the litter material, the type and characteristics of the litter used are important (Sarıca and Erensayın, 2018). In traditional broiler production, littered, cage and slatted rearing systems are used. Although each of these systems has positive and negative aspects (Ghanima et al., 2020), the littered system is the most common, despite some disadvantages (Aviagen, 2018; Altan and Bayraktar, 2018; Çavuşoğlu et

al., 2018; Sarıca and Erensoy, 2020). Numerous studies have been conducted to examine the performance and welfare characteristics of broilers reared using different litter, cage and wire materials (Petek et al., 2015; Kaukonen et al., 2017; Chuppava et al., 2018; Çavuşoğlu and Petek, 2019). However, there is a lack of studies evaluating plastic slatted and littered floor systems together. This study was carried out to determine the effects of different levels of floor design practices on some early behavioral characteristics of broiler chickens. Thus, it is aimed to minimize the negativities arising from both systems by combining the littered and slatted systems and to reveal the advantages of these systems by using them together.

2. Material and Methods

This study was carried out in Ondokuz Mayıs University, Faculty of Agriculture, Research and Application Farm, Poultry Production and Research Unit. In the study; five treatment groups were used: fully littered, fully slatted, ½ littered + ½ slatted, 1/3 littered + 2/3 slatted, 2/3 littered + 1/3 slatted. Five replicates (pens) were used for each treatment with a net floor area of 3.50 m2. A total 24 chicks (7 chicks/m^2) were placed in each pen. As the slatted system, plastic material produced at a height of 10 cm from the ground and with grid spacing of 1.5 cm was used. In the slatted system, in order to facilitate the movements of 0-7 d-old chicks, it was covered with a plastic sheet with 1x1 cm spacing on the slatted floor and this cover was removed at the end of the first week. On the other hand, 8-10 cm thick wood shavings were used in the groups in which the litter was used. As animal material, 600 fast growing broiler chicks (Ross308) at daily age mixed male-female were used. Initial weights were taken and 120 day-old chicks were randomly distributed to each treatment group. In the first week, 2 chick feeders and 3 chick drinkers were used in each pen. At the end of the first week, a tube feeder with a capacity of 15 kg and a drinker line with 5 nipples were used in each pen. In the house where the study was carried out, automatic heating and ventilation was carried out, and the temperature at the litter level, which was 33-34 °C on the first 3 days, and was gradually reduced to 28-29 °C until the age of 11 days. The lighting program was applied 24 hours for the first 3 days and 20 hours of light and 4 hours of darkness in the following days. Feeds were obtained from a commercial feed factory and broiler chick starter feed (23.0% CP and 3000 Kcal/kg) was used. Standard broiler rearing procedures were applied to chickens in all treatment groups. In the study, the feeding, drinking, resting, other behaviors, aggregation behaviors, and slatted floor preferences were evaluated three times a day (at 9.00, 13.00, and 17.00) of the chicks in each treatment group at the 2, 5, 9 and 11 days of age. Each behavioral trait was expressed as a percentage of the total number of chicks showing the relevant behavior at the pen level.

One-way analysis of variance was applied in the statistical analysis of behavioral traits. Since the percentage behavior data did not show normal distribution, statistical analysis was performed by applying arcsin square root transformation. However, actual averages were used to interpret the traits. In cases where the significance level between the means was $P<0.05$, multiple comparisons were performed with the Tukey test and SPSS 21.0 package program was used for statistical analysis.

3. Results and Discussion

The effects of floor design applications at different levels on some behavioral characteristics of broiler chicks are given in Table 1. Different floor designs significantly affected the chicks' eating, resting, aggregation behavior and the preference of being on the slatted floor ($P < 0.05$). Percentage of chicks showing feeding behavior was found higher in fully slatted system than in the other treatment groups ($F = 11.201$; $P < 0.001$), consistent with the fact that broilers housed on partially or fully slatted floors had no or limited access to the litter, leading their foraging behavior to the feeder rather than the litter (Chuppava et al., 2018). The highest level of resting behavior (77.47%) was observed in chicks reared on 1/3 slatted floor, while the lowest (68.66%) in chicks reared on fully slatted ($F = 5.393$; $P=0.001$). Since the chicks do not have the opportunity to show behaviors such as pecking, foraging and dust bathing in the fully slatted floor system, the absence of these behaviors may have led the chicks to more feeding behavior (Blokhuis, 1989; Chuppava et al., 2018). The feed consumption values that we evaluated within the scope of our other study also support this prediction by revealing higher feed intake in chicks reared on slatted floor system.

We can associate the percentage of chicks with resting behavior with the lowest (68.66%) and the highest (77.47%) feeding behavior on the fully slatted floor system. Because there is no litter material on the slatted floor, pecking behavior occurs during feeding (Blokhuis, 1989). Considering that the high feeding behavior is associated with the pecking behavior, we can say that the percentage of feeding behavior on the slatted floor increases. Aggregation score was higher in chicks reared on a fully littered (0.66) and 1/3 slatted system (0.56) than other groups ($F = 2.981$; $P=0.024$). This indicates that the chicks in the littered floor are more in a group during rest. However, we do not think that this is an indicator of fear behavior because the treatments did not affect the percentage of chicks exhibiting aggregation (fear) behavior. 64.42% of the chicks reared in the 2/3 slatted, 47.53% of the chicks reared in the ½ slatted and 36.38% of the 1/3 slatted chicks preferred the use of slats ($F = 52.833$; $P < 0.001$). This seems likely to be related to the slatted floor area per chick, as preference increased as slatted floor area increased and vice versa. It was determined that the different level of slatted floor designs did not have a significant effect on drinking and other behavioral characteristics.

The effects of age (2, 5, 9 and 11 days old) on some behavioral characteristics of broiler chicks are given in Table 2. The effects of age periods on behavioral traits of broiler chicks were significant (P < 0.01), consistent with Weeks et al. (2000), Bokkers and Koene (2003) and Giersberg et al. (2020). The percentage of chicks showing feeding behavior was highest at 5 (16.12%) and 2 d old (15.73%), and lowest at 9 d old (10.71%) (F = 6.295; P = 0.001). Our study results were inconsistent with Giersberg et al. (2020), who reported that feeding behavior increased from d-old to 12 days of age. In a previous study, the effect of pecking behavior on social behavior at an early age was evaluated, and as a result, the effect of pecking behavior on social behavior was found to be significant (Brown and Kiely, 1974). Considering the effect of pecking behavior on social behavior, it is thought that pecking behavior is replaced by feeding behavior and feeding behavior may increase in day-old chicks. In this study, drinking behavior was highest at 9 (14.81%) and 11 d old (9.90%), and lowest at 2 (4.70%) and 5 (6.95%) days of age (F = 18.811; P < 0.001). However, Giersberg et al. (2020) reported that drinking behavior is independent of age. Studies have shown that water intake generally increases at higher environmental temperatures (May and Lott, 1992). Another study investigated the effect of heat stress on feed and water intake and revealed that heat stress causes chicks to consume less feed and drink more water (Saeed et al., 2019) also explained this increased water intake as helping to lower body temperature in chicks. In our study, the effect of different floor designs on drinking behavior was found insignificant, and we can consider that the lowest water drinking behavior at 2 (4.70%) and 5 (6.95%) days of age, that is, at 2 and 5 days of age, is an indication that the temperature of the house is in suitable conditions. Resting behavior is observed to have the highest percentage of behavior at 2 (78.72%), 5 (76.89%) and 9 (72.82%) d old (F = 14,832; P < 0.001). Results from a study show that, if given the opportunity, d-old chicks spend a significant amount of time resting, which means rest is very important for them (Malleau et al., 2007) and these results show that most of the first 14 days in the early period are based on rest alone. Although it shows that it is important, it also shows that the chicks do not need a lot of time to perform other behaviors such as drinking. Consistent with Giersberg et al. (2020), the percentage of chicks showing resting behavior (sitting and lying) had the highest at 2 (78.72%), 5 (76.89%) and 9 (72.82%) d-old and decreased with advancing age. When we examine the other behavioral characteristics, it is seen that it has the highest percentage of behavior at the 11 d-old and the lowest at 2, 5 and 9 d old (F = 181.602; P < 0.001). The fact that the resting behavior was high at 2 (78.72%), 5 (76.89%) and 9 (72.82%) d old may have contributed to the low percentage of other behaviors. It was determined that the highest aggregation was observed on the 2 d-old (18.35%), and the lowest was observed at 11 d-old (2.56%). In the aggregation score, the highest value occurred at 2 d-old (0.84), while the lowest value occurred at 11 d-old (0.16). We can explain the fact that the aggregation score is highest at 2 d old and the lowest at 11 d-old, with the aggregation behavior. As stated in Giersberg et al. (2020), recognizing environmental stimuli with advancing age in broiler chicks causes a gradual decrease in fear behavior, which also supports our findings. We expected the chicks to get used to the slatted floor and use it more with advancing age, which partially happened. Although the use of slatted floor increased by 9 d-old, it surprisingly decreased by up to 40% at 11 days of age.

Table 1. The effects of partially slatted floor designs on some behavioral traits in broiler chicks (n = 600 chicks)

Behaviors	Fully slatted	$^2/_3$ slatted + $^1/_3$ littered	$^1/_2$ slatted + $^1/_2$ littered	$^1/_3$ slatted + $^2/_3$ littered	Fully littered	SEM	Treatment effect
Feeding, %	20.60a	14.04b	12.35b	11.65b	10.75b	1.002	F = 11.201, P<0.001
Drinking, %	7.10	9.99	9.53	8.00	10.85	0.960	F = 2.234, P=0.073
Resting, %	68.66b	71.88b	75.19ab	77.47a	74.30ab	1.496	F = 5.393, P=0.001
Others, %	3.60	3.98	2.89	2.84	4.20	0.557	F = 1.622, P=0.177
Aggregation, %	8.79	7.75	6.42	10.36	13.57	1.840	F = 2.442, P=0.053
Aggregation score [1]	0.48ab	0.35b	0.31b	0.56a	0.66a	0.082	F = 2.981, P=0.024
Slatted floor preference, %	-	64.42a	47.53b	36.38c	-	1.872	F = 52.833, P<0.001

[1]Aggregation score was determined by the scoring method on a 0-3 scale. 0= no aggregation (0-3 chicks), 1= little aggregation (4-6 chicks), 2= moderate aggregation (7-9 chicks); 3= high aggregation (10 chicks and above).

[a-c]The means shown with different letters on the same row differ from each other at the P<0.05 significance level according to the Bonferonni multiple comparison test. SEM= standard error of the mean.

The behavior of the chicks in the early period is an important indicator of adaptation to their environment. Behavior is one of the important indicators in the evaluation of animal welfare and provides us with comprehensive information about the emotional state and health of animals (De Jong et al., 2016), Observed behaviors help in regulating the indoor environmental conditions and the needs of the animal (Dawkins, 2003). Since all the chicks were reared under the same conditions in the data we obtained in the study, different levels of floor treatments, which were variable between groups, helped us to reveal the differences in behavioral characteristics.

4. Conclusions

This study revealed that different levels of slatted floor treatments affect some behavioral characteristics in the early chick period. Chicks reared on the slats showed less time to rest and more feeding behavior. The percentage of chicks showing resting and aggregation behavior decreased with advancing age, while the percentage of other behaviors increased. Since it is known that early rearing conditions affect later performance in broilers, slatted floor systems with higher feeding behavior can be an effective tool for better performance in broiler production.

Table 2. The effects of age and treatment x age interaction on some behavioral traits in broiler chicks (n = 600 chicks)

Behaviors	2 d old	5 d old	9 d old	11 d old	SEM	Age effect	Treatment x age effect
Feeding, %	15.73a	16.12a	10.71b	12.94ab	0.897	F=6.295, P=0.001	P=0.197
Drinking, %	4.70b	6.95b	14.81a	9.90a	0.960	F=18.811, P<0.001	P=0.860
Resting, %	78.72a	76.89a	72.82a	65.58b	1.338	F=14.832, P<0.001	P=0.230
Others, %	0.85bc	0.04c	1.66b	11.58a	0.498	F=181.602, P<0.001	P=0.007
Aggregation, %	18.35a	10.37b	6.23bc	2.56c	1.645	F=17.662, P<0.001	P=0.384
Aggregation score [1]	0.84a	0.57ab	0.33bc	0.16c	0.074	F=15.870, P<0.001	P=0.141
Slatted floor preference, %	40.49b	54.54a	62.77a	39.98b	2.162	F=23.492, P<0.001	P=0.494

[1]Aggregation score was determined by the scoring method on a 0-3 scale. 0= no aggregation (0-3 chicks), 1= little aggregation (4-6 chicks), 2= moderate aggregation (7-9 chicks); 3= high aggregation (10 chicks and above).

[a-c]The means shown with different letters on the same row differ from each other at the P<0.05 significance level according to the Bonferonni multiple comparison test. SEM= standard error of the mean.

Author Contributions

H.Ç. (50%) collected data and conducted the experiment, (100%) wrote and (50%) edited manuscript. M.S. (100%) initiated the research idea, developed the project and supervised the research. K.E. (100%) suggested the research methods, analyzed the data, (50%) edited the manuscript and (100%) interpreted the results. R.A. (50%) collected data and conducted the experiment. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Ethical approval was obtained from the Ondokuz Mayıs University Ethical Committee for Experimental Animals according to the decision dated 29.04.2021 and numbered 2021/25.

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Research Article
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3-D CLASSIFICATION OF AGRICULTURAL AREAS OF TURKEY USING MAMMALIAN LIVESTOCK EXISTENCE

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Abstract: Animal production is valuable importance for human being and countries in terms of both economic and human nutrition. To increase the value of benefits from the livestock sector, there are many attempts to make policies. In this study, 26 different agricultural areas of Turkey according to their agricultural properties were clustered by using mammalian livestock existence such as cattle, water buffalo, sheep, goat and horse. For this aim 3-D clustering was applied using R software with FactoMineR and factoextra packages. The results showed that the number of 26 agricultural areas were clustered in four clusters. TR83 area including Samsun, Tokat, Çorum and Amasya cities was formed in a cluster lonely. The second cluster included agricultural areas of TRA2, TRC2 and TRB2 that these areas consist of the cities Ağrı, Kars, Iğdır, Ardahan, Şanlıurfa, Diyarbakır, Van, Muş, Bitlis and Hakkari. TRC3, TR62 and TR61 agricultural areas formed the third cluster including the cities of Mardin, Batman, Şırnak, Siirt, Adana, Mersin, Antalya, Isparta and Burdur. The other agricultural areas were included in the fourth cluster. These results are also important for traders' financial and human capital and trading practices such as the use of brokers and regular suppliers and customers had varying effects on margins and costs of animal trade. It is also amenable to public policy to improve the market environment and marketing efficiency.

Keywords: Mammalian, Livestock, Türkiye, Agricultural areas, 3-D clustering

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1. Introduction

Mammalian livestock has been important components of rural life and human nutrition and also still play a substantial role in the livelihood of farmers. In Turkey, animal production plays an important role in the livestock sector because of the country's geography and climate, as well as social, cultural, and economic structures (Şen et al., 2021a). Although Turkey is among the leading countries in the world in terms of goat and sheep assets, almost all of the small ruminant population consists of local breeds with low yield potential, but good adaptation to different climatic conditions (Şen et al., 2021b). Also, water buffalo and horse breeding are valuable for the Turkey economy.

In Turkey small ruminant population is about 57.5 million head consisting of 45.2 million head of sheep and 12.3 million head of goat. Turkey's cattle population is about 17.9 million head, the water buffalo population is 66215 and the horse population is 83718 according to TUIK (2021). Turkey has 26 different agricultural areas according to their agricultural properties (Table 1).

Among these areas animal existences can be so different to produce policies. This imbalance brings some hardness for policy making for agricultural areas (Önder, 2019; Tirink et al., 2019).

In this study, it was aimed for 3-D clustering the agricultural areas according to mammalian livestock existence for producing information useful for policy makers.

2. Material and Methods

The data was taken from the Turkish Statistical Institute (TUIK, 2021) for the year 2021. To classify the agricultural areas, a hierarchical clustering algorithm was used with the nearest neighborhood method with Euclidean distance. Hierarchical clustering analysis according to the factor scores was derived from principal component analysis. Tree-based hierarchical clustering of individuals to define clusters of similar populations according to interested traits was strongly suggested. A dendrogram is structured where the root corresponds to a cluster containing all data points and the leaves correspond to the n input data points.

Each internal node of the dendrogram corresponds to a cluster of the data points in its sub-tree. The clusters (internal nodes) become more refined as the nodes are lower in the tree. The goal is to construct the tree so that the clusters deeper in the tree contain points that are

relatively more similar. All analysis was executed using R software with *FactoMineR* and *factoextra* packages (Şen et al., 2021a).

Table 1. Agricultural areas of Türkiye

Area Code	Cities
TR62	Adana, Mersin
TR51	Ankara
TR61	Antalya, Isparta, Burdur
TR32	Aydın, Denizli, Muğla
TRA2	Ağrı, Kars, Iğdır, Ardahan
TR22	Balıkesir, Çanakkale
TR41	Bursa, Eskişehir, Bilecik
TRA1	Erzurum, Erzincan, Bayburt
TRC1	Gaziantep, Adıyaman, Kilis
TR63	Hatay, Kahramanmaraş, Osmaniye
TR82	Kastamonu, Çankırı, Sinop
TR72	Kayseri, Sivas, Yozgat
TR42	Kocaeli, Sakarya, Düzce, Bolu, Yalova
TR52	Konya, Karaman
TR71	Kırıkkale, Aksaray, Niğde, Nevşehir, Kırşehir
TRB1	Malatya, Elazığ, Bingöl, Tunceli
TR33	Manisa, Afyonkarahisar, Kütahya, Uşak
TRC3	Mardin, Batman, Şırnak, Siirt
TR83	Samsun, Tokat, Çorum, Amasya
TR21	Tekirdağ, Edirne, Kırklareli
TR90	Trabzon, Ordu, Giresun, Rize, Artvin, Gümüşhane
TRB2	Van, Muş, Bitlis, Hakkari
TR81	Zonguldak, Karabük, Bartın
TR10	İstanbul
TR31	İzmir
TRC2	Şanlıurfa, Diyarbakır

3. Results and Discussion

When the cluster analysis results were examined (Figure 1), the number of 26 agricultural areas were clustered in four clusters. TR83 area including Samsun, Tokat, Çorum and Amasya cities was formed in a cluster lonely. The second cluster included agricultural areas of TRA2, TRC2 and TRB2 that these areas consist of the cities Ağrı, Kars, Iğdır, Ardahan, Şanlıurfa, Diyarbakır, Van, Muş, Bitlis and Hakkari. TRC3, TR62 and TR61 agricultural areas formed the third cluster including the cities of Mardin, Batman, Şırnak, Siirt, Adana, Mersin, Antalya, Isparta and Burdur. The other agricultural areas were included in the fourth cluster. According to PCA results 80.4% of the total variance was explained.

These results indicated that different policies can be used for the mammalian livestock sector for Samsun, Tokat, Çorum and Amasya cities including in the TR83 agricultural area. For this area the featured by the existence of water buffalo (Atasever, 2022). For TRA2, TRC2 and TRB2 small ruminants based policies can be conducted (Ertaş, 2019). Mardin, Batman, Şırnak, Siirt, Adana, Mersin, Antalya, Isparta and Burdur cities included in TRC3, TR62 and TR61 agricultural areas can be suitable to make policies on small ruminant especially on goats (İşler and Ünlü Ören, 2021; Tarhan, 2021). The fourth cluster including the other 19 agricultural areas had a higher cattle number (URL1).

These findings can be used to make animal production related policies by the policy makers. Even though the country has 26 different agricultural areas, these areas can be evaluated in four clusters to make improvements and deciding for mammalian livestock.

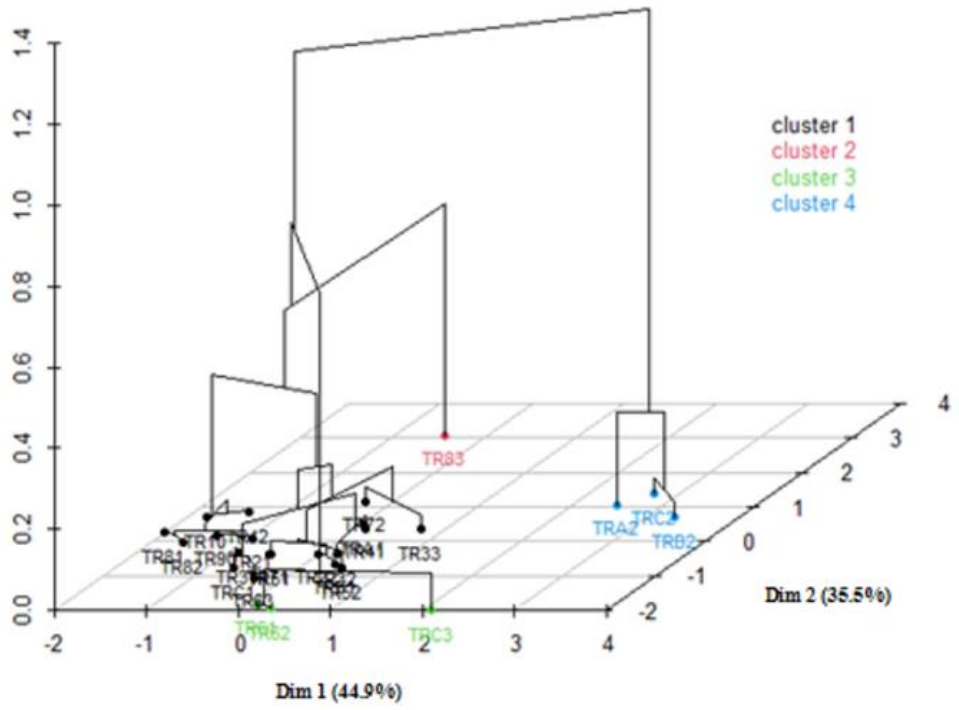

Figure 1. Hierarchical clustering on the factor map.

4. Conclusion

According to the results it can be offered for policy makers that the four clusters had different needs of policies. For these four clusters pastures may be improved, and heath protection should be differentially planned for these agricultural areas. Animal associations should give special importance to these agricultural areas. These results are also important for traders' financial and human capital and trading practices such as the use of brokers and regular suppliers and customers had varying effects on margins and costs of animal trade. It is also amenable to public policy to improve the market environment and marketing efficiency.

Author Contributions

H.M.Y (100%) the data collected. B.K. (100%) data analysis. H.Ö. (40%) and C.T. (60%) the manuscript writing up. H.Ö. (40%) and C.T. (60%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans. The used data was taken from Turkish Statistical Institute.

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A COMPARATIVE RESEARCH ON DATA ANALYSIS WITH FACTORIAL ANOVA, LOGISTIC REGRESSION AND CHAID CLASSIFICATION TREE METHODS

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Abstract: When the data structure is large and complex, the extraction of information hidden within the data is called data mining. In the context of data mining, there are numerous methods developed for statistical data analysis. When these methods are classified as conventional-classical methods and current methods, factorial ANOVA (FANOVA) and Logistic Regression (LR) methods are shown as conventional methods, while decision trees called Classification Tree (CT) and Regression Tree (RT) can be shown as current methods. The method to be used in statistical data analysis is directly related to the researcher's hypothesis (i.e. purpose) and variable type. Therefore, the choice of data analysis method is important. In this regard, studies in which methods are examined comparatively are guiding. In this study, a dataset on which inferences could be made by ANOVA, LR, and CT methods was analyzed. With this dataset, the relationship between the birth type (single-twin) as dependent variable and the yield year and maternal age as independent variables in an Awassi sheep flock was examined. The findings of each method were interpreted in its own specific way. The methods were compared in terms of explaining the similarities and differences of the information they presented and the relationship between dependent and independent variables.It was concluded that each method offered different inferences based on purpose and perspective. It is believed that it is the right approach for researchers to determine the data analysis method appropriate to their goals by taking into account the data structure.

Keywords: ANOVA, Binary logistic regression, Classification tree algorithm, Awassi sheep, Birth type

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1. Introduction

Data constitutes the raw material of scientific research. Data can be obtained under controlled conditions through experimental studies as well as it consists of information formed in their natural environment and collected in the relevant data center. In experimental research, data is obtained by simulating the actual event. Obtaining data in this way is difficult, but analysis processes are easy.

After the data obtained depending on their actual occurrence is collected in the relevant centers, these data can reach a large data size in terms of volume, diversity, and rate of occurrence. These data can also be in a complex structure consisting of a large number of dependent and independent variables. An important part of scientific research today is comprised of the extraction of hidden information in this large and/or complex data. With a more clear expression, a dependent variable being studied is formed in a complex structure as a result of the effects of a large number of independent variables (factors). By examining the effect(s) of an independent variable(s) on the dependent variable, the researcher may aim to determine the significance, magnitude, or direction of these effects.

There are many statistical methods developed to extract information hidden in complex data. Statistical methods used for this purpose are generally called data mining.

There are many methods in data mining. These methods are widely used in fields such as economics, health, education and agriculture. The best known of these methods are Naïve Bayesian Classifiers (NBC), Artificial Neural Networks (ANN), k-Nearest Neighbor Approach, Support Vector Machines (SVM), and Decision Trees (Alev Çetin and Mikail, 2016). The use of these methods in animal husbandry was discussed by Alev Çetin and Mikail (2016) in a comprehensive review study and examples of studies in this field were presented.

The decision tree method, one of the data mining methods, contains a large number of algorithms. The major types of these algorithms are as follows: CHAID

(chi-squared Automatic Interaction Detector), Exhaustive CHAID, CART (Classification and Regression Trees) SLIQ (Supervised Learning in Quest), MARS (Multivariate Adaptive Regression Splines), SPRINT (Scalable Parallelizable Induction of Decision Trees), and QUEST (Quick, Unbiased, Efficient Statistical Tree) (Vupa Çilengiroglu and Yavuz, 2020).

To be able to make inferences from a data set based on the purpose of the research, appropriate statistical methods are used. In some cases, a data set can be analyzed with different methods for the same purpose. The most important issue here is whether the data set is in accordance with the assumptions (data volume, variable type, normality, etc.) that the statistical method to be used for its analysis considers necessary.

Another issue in choosing the appropriate analysis is the accuracy of the information produced by the method. Choosing the appropriate statistical method for the current dataset is also related to the researcher's "statistical literacy". To shed light for researchers, studies that comparatively examine the information produced by statistical methods applied to the same dataset and their reliability and the selection criteria of the correct statistical method have been conducted. Şata and Çakan (2018) examined Logistic Regression (LR) and CHAID methods comparatively in educational sciences, while Vupa Çilengiroğlu and Yavuz (2020) examined LR and CART methods comparatively on life satisfaction data. Kurt *et al*. (2008) comparatively examined Logistic Regression, Classification Trees (CT), Regression Tree (RT), and Artificial Neural Networks (ANN) methods on coronary artery diseases, while Kuyucu, (2012) comparatively examined Logistic Regression Analysis, ANNs, CART classification, and Regression Tree methods in the medical field. Kacar and Karakoc (2020) examined LR, CT, and RT methods comparatively on housing prices. CART, CHAID, and Exhaustive CHAID decision tree algorithms were studied comparatively on animal husbandry data by Tatlıyer (2020).

One of the methods of multi-factor data analysis is factorial (multi-factor) analysis of variance (FANOVA). Although the use of this method in the analysis of complex data is older than other methods, it is still widely used today. This method is used if there are effects of a large number of factors, whose subgroup numbers are equal or different, on the dependent variable (Bek and Efe, 1989; Yıldız and Bircan, 1994). Because the method is old and its use is widespread, it is a method that is better known by researchers in terms of interpreting analysis inferences. Whereas there are studies more commonly examining LR, CT, RT, or ANN algorithms comparatively in the literature, studies comparing the factorial ANOVA (FANOVA) method with the above-mentioned methods have not been encountered. FANOVA method is a more conventional and classical method than other methods. Although the inferences of the FANOVA method differ in one dimension from other methods, it also offers similar inferences. Therefore, it would be useful to compare the FANOVA method with the current methods.

The objectives of this study are: 1) interpreting the inferences produced by FANOVA, LR, and CT methods specific to all three algorithms, 2) discussing information produced by the methods in terms of their similarities, differences, or superiority by examining the inferences of the methods comparatively.

2. Material and Methods

2.1. Animal Materials

The data of the study belongs to an Awassi flock in Ceylanpinar Agricultural Enterprise located in Sanliurfa province in Türkiye. Data were obtained from the yield and breeding records kept in the flock between 2006 and 2010. In this study, all available records regarding the sex, year of yield, type of birth and maternal age (dam age) of Awassi lambs were used. Data material of the study consisted of data about 5454 head sheep that gave birth between 2006 and 2010. Mating of sheep in the enterprise is held in June and births begin in November and are completed by the end of that year. Therefore, the year in which pregnancy is provided and the yield year usually occur in the same year. The conclusion of birth as twins is a desirable condition in sheep flocks. Giving birth twin is a result of the genetic structures of the animals in addition to environmental factors such as care-feeding, pasture status, and climate in the flock during the year of pregnancy. The inheritance level of the property of being twin as birth type is low (Notter, 2008; Vatankhah and Talebi, 2008; Cottle et al., 2016); it occurs as a result of environmental factors.

2.2. Statistical Analysis

In this study, the birth type (single or twin) was considered as a dependent variable, the yield year and the maternal age as independent variables. In the enterprise from which the dataset of the study was taken, the yield year and the maternal age factors, which are thought to be effective on the birth type, were archived. Some other factors, such as the season in which pregnancy is achieved and the genetic groups formed in the flock, can be considered effective on twin births. But since they were not archived, these factors could not be studied in the data analysis. The data was analyzed by three different data analysis methods. These were FANOVA, LR and CT methods. Since the dependent (predicted) variable (birth type) is denoted by Y, and the independent (predictor) variables (yield year and maternal age) are denoted respectively by X1 and X2, the functional relationship between the dependent variable and the independent variable is written with the matrix form as follows (Equation 1):

$$Y=X\beta+\varepsilon \quad (1)$$

Where; Y is the vector of the dependent variable, X is the fixed effect matrix, β is the coefficient matrix of fixed effects, ε is the independent error vector.

2.3. Factorial ANOVA Method

In variance analysis, the dependent variable *Y* must be continuous and the independent variables *X* (factor) must be discrete. But variables exhibiting binomial distribution fit the normal distribution assumption if the volume of data (*n*) is too large (Yıldız et al., 2020). In this case, the analysis of the data can be done using the ANOVA approach, which prioritizes the normality assumption. In this context, the biometric model used in data analysis for the FANOVA method in this study is written as follows (in this model, the linear biometric model was used), (Equation 2):

$$Y_{ijk} = \mu + a_i + b_j + ab_{(ij)} + e_{ijk} \quad (2)$$

where;

Y: Observation vector of birth type

μ: Population mean of the birth type

a_i: Fixed effects of levels belonging to the variable of yield year

b_j: Fixed effects of levels belonging to the variable of maternal age

$ab_{(ij)}$: Interaction effect of yield year and maternal age

e_{ijk}: Random residuals (random error); e_{ijk} ~N; $(0,\sigma^2_e)$.

2.4. Chi-Square Independence Test and Logistic Regression (LR) Method

Chi-Square independence test is preliminary test for LR analysis. The independent variables found to be significant according to chi-square independence test are included in the LR analysis model. The analysis process related to Chi-Square independence test was explained by Yıldız *et al*. (2020).

Simple and multiple linear regression methods give accurate results under the assumptions that the dependent variable and independent variables are continuous variables with normal distribution, the independent variables are measured without error, and the error term of dependent variable is $e \sim N(0,\sigma^2)$ (Özdamar, 1999). If the dependent variable is discrete, the appropriate data analysis method for the relationship between the independent variables (can be discrete or continuous) and the dependent variables is logistic regression. If the dependent variable has two results (binomial), the method is called "Binary Logistic Regression"

When a binary dependent variable is denoted by Y_i=(0,1) and the independent variables by $X = (X_1, X_2, \ldots X_p)$, the regression model of the binary variable is written as (Equation 3):

$$Y_i = \beta_0 + \beta_1 X_i \quad (3)$$

Here, since the Y_i categorical dependent variable shows the Bernoulli distribution, the expected value of *Y* is $0 \le E(Y_i) = \pi \le 1$; when the logit transformation of it is applied, the final model for the binary logistic regression is as follows (Bircan, 2004; Vupa Çilengiroğlu and Yavuz, 2020), (Equation 4):

$$\pi(x_i) = \frac{\exp(\beta_0 + \beta_1 X_i)}{1 + \exp(\beta_0 + \beta_1 X_i)} \quad (4)$$

or

$$\pi(x_i) = [1 + \exp(-\beta_0 - \beta_1 X]^{-1}$$

In the logistic regression model, the estimation of the coefficients of the variables is obtained using the "maximum likelihood" method. The significance of these estimated coefficients is determined by the "G statistic" or the "Wald test" (Çokluk, 2010).

Exp(β) values included in the logistic regression summary tables are exponential logistic regression coefficients. This value is also the Odds ratio (OR) calculated for each variable. For OR, $0 < OR < \infty$ can be written and it is the ratio of two values to each other, such as "occurrence rate" and "non-occurrence rate". In other words, OR logarithm does not take a negative value (Çokluk, 2010; Vupa Çilengiroğlu and Yavuz, 2020).

OR is a metric measurement that bases on a level for each of the discrete variables, and by accepting this level "1", it refers to other levels as a multiple of this.

In logistic regression analysis, the model fit reported with the measures of the Cox and Snell R^2 statistics, the Nagelkerke R^2 statistics, the Hosmer and Lemeshow test, and the overall χ^2 test results. The Cox and Snell R^2 statistics and the Nagelkerke R^2 statistics tend to take small values. Therefore, reporting these R^2 values is not recommended (Alpar, 2011; Şahin, 2017). This R^2 values between 0.20 and 0.40 indicates that the accuracy of the model is high (Şenel and Alatlı, 2014). If the probability value of the Hosmer and Lemeshow test is P>0.05, it is an indication that the model is fit.

In LR analysis, the dependent variable is natural-class or must be turned into the natural-class position. Independent variables can be discrete or continuous. This analysis method requires to have large sample sizes (at least 15; ideal 20 and above) in each subgroup of each independent variable. There are no other assumptions that restrict the method other than these two assumptions. Therefore, LR is a much preferred data analysis method in the analysis of the relationship between the dependent variable and the independent variables. Another important reason why the method is preferred is that LR also does not require the relationship between the independent and dependent variables to be linear. The functional relationship can be exponential or polynomial. LR can produce non-linear models by assuming that there is a logit relationship between dependent and independent variables. LR analysis is a useful method that performs logarithmic transformations to bring the relationship to a linear form by preserving the nonlinear relationship in cases where the relationship between the dependent and independent variables is nonlinear (Şata and Çakan, 2018).

A detailed explanation of the statistics produced by this

method was made by Çokluk (2010), while other reasons for choosing the method were explained by Çokluk (2010), Şahin (2017) and Şata and Çakan (2018). Analysis findings that should be reported in studies using LR analysis were summarized in a review study conducted by Şenel and Alatlı (2014). For further information on LR analysis, these sources can be referred.

2.5. Decision Tree and CHAID algorithm

In the context of data, another method applied for determining and analyzing the relationship structure between dependent and independent variables is the decision tree. The aim of decision trees is to estimate the outcome values of datasets by developing a model based on data mining (Güner, 2014). Multiple regression and LR analyses are considered classical methods in relationship analyses (Gacar and Karakoç, 2020). The decision tree method is an up-to-date method and has been widely used in data analysis in recent years.

The structure of decision trees is similar to the natural tree structure, that is, it is in the form of roots, branches and leaves. Decision trees begin with the root, which covers all observations in the dataset, and are divided into branches that divide the data into subgroups. In the tree structure, separated from the root to the branches, each knuckle is named "node" (Pehlivan, 2006; Gaçar and Karakoç, 2020). The test process for each divided node is performed, and the branching process continues consecutively to the last node. After the separation process is finished, inferences are made based on the ratios belonging to the divided nodes and the categories within the last branch (group).

In the decision tree method, heterogeneous datasets are divided into homogeneous subgroups depending on the dependent variable. According to Dangeti (2017), the separation process is carried out by examining values such as entropy, Chi-Square, variance reduction criterion, and homogeneity structure in nodes (Özgür and Doğanay Erdoğan, 2020). Using these techniques, homogeneity measurements are carried out from the root node to the terminal nodes. The resulting values on the terminal node are the values estimated for the dependent variable. A large number of algorithms are used to create a decision tree. The main ones of these are CHAID, exhaust CHAID, CART, SLIQ, MARS, SPRINT and QUEST algorithms. In decision tree algorithms, the method is called a Classification Tree (CT) if the dependent variable is discrete, and a Regression Tree (RT) if the dependent variable is continuous (Breimann et al., 1984; Özkan, 2012; Koç, 2016; Eyduran et al., 2016).

Because the dependent variable discussed in this study is discrete, the decision tree created will be the classification tree. It is also reported that the CHAID algorithm works better in discrete data (Şata and Çakan, 2018). In this direction, the CHAID algorithm was selected to create the classification tree.

In this research, the results of the analysis in all three methods will be interpreted separately. In addition, the following considerations will be examined in relation to all three methods:

a) The information they provide and the level of model-specific fit
b) Significance states of the independent variables
c) Compatibility of similar statistics offered by methods
d) Information specific to the methods (i.e. inferences found in one method not found in the other method).

3. Results and Discussion

The relationship between the independent variables (yield year and maternal age) and the dependent variable (birth type) was analyzed by FANOVA, LR, and CT methods, and the findings are summarized below.

3.1. Results of the Factorial ANOVA

If the number of observations is too large, the variables in the binomial (binary) property show a normal distribution. The analysis of variance (ANOVA) results belonging to yield year and maternal age variables, which are thought to have an effect on the binary birth type (single and twin) variable, and the interaction of these two variables are summarized in Table 1.

According to the results of factorial (multi-factor) analysis of variance (FANOVA), the effect of yield year and maternal age on birth type (single or twin) was found to be significant ($P<0.001$) (Table 1). In other words, in terms of birth type, there were statistical differences between birth years and maternal ages. Also in terms of birth type, the interaction of yield year and maternal age was not statistically significant ($P=0.071$). However, the observed probability was very small. This indicates that there may be differences between some ages in terms of birth years in further analysis. Since the aim of this study was to compare analysis methods (FANOVA, LR and CT), no further evaluation related to the interaction was performed. It was satisfied with the evaluation of the main variables.

Table 1. ANOVA results related to the birth type

Source	df	Mean of squares	F	P	η^2	Power
Yield year	4	6.215	32.149	<0.001	0.023	1.000
Maternal age	3	3.220	16.657	<0.001	0.009	1.000
Yield year x Maternal age	12	0.320	1.653	=0.071	0.004	0.862
Error	5434	0.193				

R^2= 0.895 (Adjusted R^2= 0.895).

Duncan's multiple comparison test was used to determine the statistically significant factors' subgroups one of which is different from the other. When multiple comparison test results were evaluated in terms of yield year (Table 2), it was determined that the highest twin birth rate occurred in 2010, and it was followed by 2008. While the lowest twin birth rate occurred in 2009, the difference between 2006 and 2007 was not statistically significant. When the confidence interval is evaluated, the twin birth rate can go down to 25%, or up to 45% in the 95% confidence interval. Also when confidence limits for years are evaluated, while the upper and lower limits for similar years (2006-2007) overlap, the limits for different years are separate. For example, when evaluating the years 2007 and 2008, it is seen that the upper limit of 2007 (1.30) is statistically different from the lower limit of the following 2008 year (1.33). A similar situation is also observed when other years are evaluated. In research studies, confidence interval findings present additional information about between what values the point estimate (mean or ratio) will be.

When evaluating the twin birth rate in terms of maternal age, this rate is the lowest in 3-year-old dams. The twin birth rate increased with age and occurred at the highest rate (about 34%) in 6-year-old dams. However, in terms of dams aged 4, 5, and 6, the differences between twin birth rates were not statistically significant. In these age groups, twin births occurred at rates close to each other (between 23% and 32%). When confidence limits were evaluated, the upper limit value of 3-year-old dams (1.25) was found to be different from the lower limit value of 4-year-old dams (1.28). However, in the statistically similar age groups of 4, 5 and 6, the lower and upper limits did not differ from each other. This suggests that the difference in age groups is not significant. In summary, it is necessary to express that presentation of confidence interval values as well as point estimates (mean, ratio, etc.) makes a significant contribution to statistical inferences. Therefore, confidence interval values should also be presented as findings.

3.2. Chi-Square and LR Results

Dataset this study meets the assumptions of binary LR analysis one-on-one. The dependent variable (birth type) is binomial variable, single and twin. In LR, independent variables can consist of a combination of discrete and continuous variables. Here the independent variables are discrete. The LR method is sample size-sensitive, and it is necessary to have at least 15 (ideal 20 and above) observations in subgroups of each factor variable. The volume of observations in this study is quite large. If the independent variable or variables are categorical, performing Chi-Square (χ^2) independence analysis as a preliminary analysis of LR can be helpful in creating the LR model. Chi-Square analysis results values for yield year and birth type are presented in Table 4, and Chi-Square analysis results for maternal age and birth type are presented in Table 5.

Table 2. Birth type statistics by years

Yield Year	Mean	SEM	CI: 95%	
			Lower	Upper
2006	1.25[c]	0.017	1.22	1.28
2007	1.27[c]	0.016	1.24	1.30
2008	1.36[b]	0.016	1.33	1.39
2009	1.19[d]	0.016	1.16	1.22
2010	1.42[a]	0.016	1.39	1.45

[a, b]Means marked with different letters are different with an error of P<0.05.

Table 3. Birth type statistics by maternal age

Maternal Age	Mean	SEM	CI: 95%	
			Lower	Upper
3	1.23[b]	0.009	1.21	1.25
4	1.30[a]	0.012	1.28	1.32
5	1.32[a]	0.015	1.29	1.35
6	1.34[a]	0.020	1.30	1.38

[a, b]Means marked with different letters are different with an error of P<0.05.

Table 4. Distribution of birth type by yield years, and χ^2 test results

Birth Type	N=5454	Yield Year					Total
		2006	2007	2008	2009	2010	
Single	n	856	745	719	853	761	3934
	%	76.7	73.7	66.1	83.6	62.4	72.1
Twin	n	260	266	368	167	459	1520
	%	23.3	26.3	33.9	16.4	37.6	27.9

Pearson Chi-square test results χ^2 =159.999; df=4; P<0.001; Actual P=6.43 x10^{-33} R^2=0.167.

Table 5. Distribution of birth type by maternal age, and χ^2 test results

Birth Type	N=5454	Maternal age (dam age)				Total
		3	4	5	6	
Single	n	1950	1024	631	329	3934
	%	76.8	68.4	68.4	66.2	72.1
Twin	n	589	472	291	168	1520
	%	23.2%	31.6	31.6	33.8	27.9

Pearson Chi-square test results χ^2 =52.604; df=3; P<0.001; Actual P=2.23 x10^{-11} , R^2 =0.098.

Chi-Square independence test results for the relationship between the birth type variable and both independent variables were found significant (p<0.001). When the actual probability value (P) is evaluated, it is seen that the relationship between the yield year and twin birth and the measure of this relationship (R^2) is higher. According to the results of Chi-Square analysis, the R^2 value for the yield year and birth type correlation is 0.167, and for the maternal age and birth type correlation is 0.098. According to these findings, each factor can be involved in the LR model and their effects are expected to be significant.

3.3. Binary Logistic Regression Analysis Results

The LR analysis results of the relationship between dependent variables and independent variables examined in the dataset handled as defined in the method section are summarized in Table 6. In the presentation of the findings, the criteria proposed by Şenel and Balatlı (2014) were taken into account.

Table 6. Binary Logistic regression analysis results

Factors	β	SEM	Wald	df	P	Exp (β)	For EXP(β) CI 95%	
							Lower	Upper
Yield year (a)			153.940	4	<0.001			
2007	0.152	0.102	2.242	1	=0.134	1.165	0.954	1.422
2008	0.520	0.096	29.229	1	<0.001	1.683	1.393	2.032
2009	-0.433	0.111	15.180	1	<0.001	0.649	0.522	0.806
2010	0.710	0.094	57.619	1	<0.001	2.034	1.693	2.443
Maternal age(b)			53.907	3	<0.001			
4	0.386	0.074	27.251	1	<0.001	1.472	1.273	1.701
5	0.476	0.087	30.059	1	<0.001	1.610	1.358	1.909
6	0.570	0.108	27.905	1	<0.001	1.769	1.431	2.186
Constant	-1.446	.081	321.584	1	<0.001	0.235		

(a) Reference year: 2006; (b) Reference maternal age (3 year old); 2LL= 6239.051; Cox and Snell R^2 =0.039; Nagelkerke R^2 =0.056; $\chi^2(7)$= 209.488 and P<0.001; Hosmer and Lemeshow test: P=0.124.

When the results of LR analysis were examined in the dataset, the effects of both yield year and maternal age on the dependent variable (birth type) were found to be significant (P<0.001), as in the FANOVA method.

In terms of yield year, the twin-birth rates of 2006 are not different from 2007 but different from other years. When β and Exp(β) coefficients are examined, it is observed that 2009 was the year that reduced the twin-birth rates. In addition, the highest twin birth rate occurred in 2010. The twin birth rate in 2010 is nearly double that of 2006 (Exp(β)=2.034). These findings in the LR analysis are quite similar to the findings obtained by the FANOVA method.

When the findings related to maternal age are evaluated, it is observed that the effects of old ages (4, 5, 6) on twin-birth rates are positive. Increase in twin birth rate regularly increased with increasing age, and the rate of twin birth in 6 older mothers was about 1.8 times more than 3 years-old mothers (Exp(β)=1.769). These findings related to maternal age are also consistent with the findings of FANOVA.

In binary LR analysis, the results related to the model fit and related to what extent the model explains the variation in the dependent variable should be evaluated first. In this context, when Cox & Snell and Nagelkerke "pseudo" R^2 statistics for model performance (Şenel and Alatlı, 2014) are evaluated, it is observed that both values are quite small (0.039 and 0.056, respectively). Şenel and Alatlı (2014) report that for good model performance, these statistics must be between the ranges of 0.20 and 0.40. Another criterion that expresses to what extent the model explains the variation in the dependent variable in LR analysis is the Hosmer and Lemeshow statistics. This statistic is a probability value, and P> 0.05 indicates a good model fit (Çokluk, 2010). Here, since P=0.124 for the Hosmer and Lemeshow test statistic, it can be said that the model fit is good.

3.4. Classification Tree Results

The results of this method are presented as figure. In the dataset studied by the classification tree method, root, branch and leaf formation and nodes belonging to

homogeneous subgroups formed depending on birth type are shown in Figure 1. As seen in Figure 1, the effects of both studied factors on the birth type (twin born rate) are significant (P<0.001); while yield year is the first-degree effective factor, the maternal age is the second-degree effective factor. According to the CHAID algorithm, the data are summarized under 10 homogeneous subgroups (nodes) in terms of the twin-birth rate. According to age factor, 2007 and older years (here 2006 and 2007) were collected in the same node. The years of 2008, 2009 and 2010 are homogeneous within themselves in terms of years and heterogeneous groups between them. 2007 ended as the last node. However, other years were divided into two branches depending on maternal age. In terms of maternal age, these branches are in the form of 3-year-old dams and dams older than 3-years-old. The significance status of the examined independent variables (yield year and maternal age), and the differences belonging to subgroups of these factors respectively show similarity to the results of the FANOVA and LR Analyses.

The characteristic that this method offers differently from other methods and is not found in other methods is that homogeneous groups in terms of twin birth rate and the lowest and highest twin birth rate groups are determined. The total twin-birth rate in the flock is 27.9%. In 2009, the twin-birth rate was lowest with 12.3% in 3-year old dams (node 7). The highest twin-birth rate was in dams aged 4 and older (4, 5, and 6) in 2010 with 44.2%.

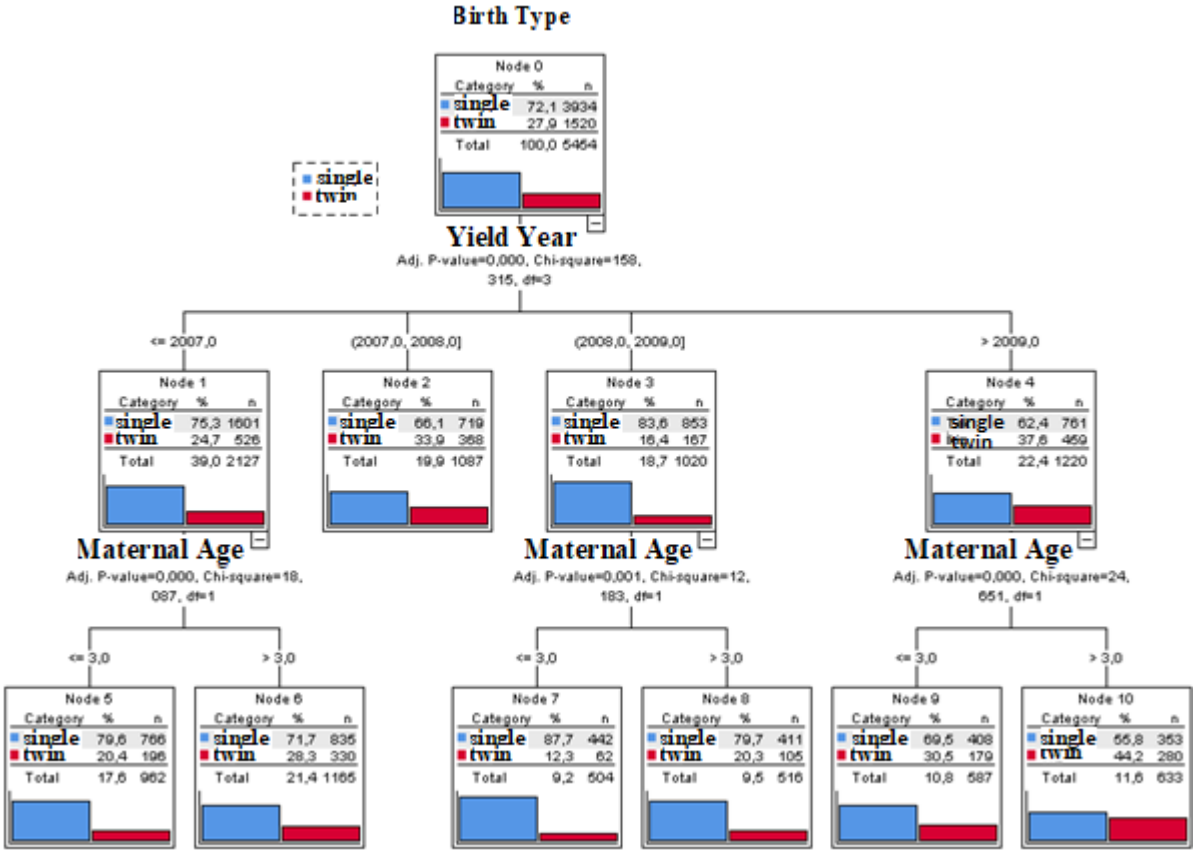

Figure 1. Classification Tree of factors that affect the birth type.

3.5. Comparative Study of the Methods

To be able to compare the studied methods, the information presented by the models is summarized in Table 7. In all three methods, the significance (P) of factors and the effect orders are similar. The most effective variable in all three methods is yield year, while the second is maternal age.

When the different inferences of the methods are evaluated, it is seen that FANOVA additionally offers information about the interaction of factors. This information is not presented directly by the other methods. However, when the definition of interaction is taken into account, interaction can be inferred from the CT structure. In the CT structure, although the maternal age in all other years except 2008 is grouped as 3 years and other ages, the termination of the node in this year can be interpreted as the interaction of maternal age and the yield year.

The LR method does not offer an inter-factor interaction information. However, based on a level defined for each factor, it presents the values, which the dependent variable will receive at other levels, as a layer of this level. This inference is not directly presented in other methods. The CHAID CT algorithm classifies different groups and presents homogeneous subgroups as more descriptive. In addition to a visual design, it also offers significance results and subgroup statistics.

In FANOVA and LR methods, confidence intervals of point

estimates can also be shown. Classification trees do not offer an inference in this direction.

FANOVA and LR methods provide R^2 statistics. However, the CT algorithm does not report these statistics. In this research, R^2 statistic is 0.895 for FANOVA. Cox and Snell R^2 is 0.039 and Nagelkerke R^2 is 0.056, for LR. The FANOVA and LR models are not comparable in the magnitude of the R^2 statistic. FANOVA R^2 statistics and LR R^2 statistics are evaluated on their own. While the FANOVA R^2 statistic is very close to one, Cox and Snell R^2 and Nagelkerke R^2 statistics are well below the 0.20 to 0.40 range.

In the literature, any study comparing the ANOVA method and the LR and CT methods was not encountered. The results of some studies examining LR and CT methods are summarized below in terms of the method proposing.

In their study in which the different forms of LR analysis and CT methods are examined comparatively, Vupa Çilengiroğlu and Yavuz (2018) reported that the method explaining the dependent variable best was the LR c=0.4 form. In a study in which they studied CHAID analysis and LR analysis comparatively, Şata and Çakan (2020) considered the use of CHAID analysis more appropriate in classification studies because CHAID analysis gave more detailed and understandable results than logistic regression analysis and explained the common effect between independent variables.

In this study, when the inferences of the methods are evaluated generally, it is seen that the results of all three methods are similar in terms of the significance of the independent variables, their significance order, and explaining the dependent variable. In addition, each method has its own inferences that are unique (and not in other methods). In this context, while FANOVA classifies interaction between factors, the LR method classifies layer values belonging to other subgroups based on one level of the factor. The CT, on the other hand, classifies data by presenting homogeneous groups at the last ends (nodes).

Table 7. Summary results of the models

Factors	sd	Factorial ANOVA		Binary Logistic Regression		Classification tree
		Test Statistic F	P	Wald Test Statistic	P	FR
Yield Year	4	32.149	$1.6E^{-26}$ [a]	153.940	$2.91E^{-32}$	1st Factor
Maternal age	3	16.657	$9.0\ E^{-11}$	53.907	$1.17E^{-11}$	2nd Factor
Interaction	12	1.653	=0.071			
Model	→	2318.460	≅0.0 (df=20)	209.448	$1.12\ E^{-41}$ (df=7)	-
Measures model fit		R^2=0.895[b]		R^2 =0.039[c] R^2 =0.056[d]	P=0.124[e]	

[a]=1.6 x 10^{-26}; [b]= very close to zero; [c]= Cox and Snell R^2=0.039; [d]= Nagelkerke R^2=0.056 ; [e]= Hosmer and Lemeshov probability P=0.124; Note: [b] vs [c] and [d] are not comparable, FR= factor ranking.

4. Conclusion

In conditions where the independent variables are discrete or continuous, but the dependent variable is binomial (binary), the appropriate method that analyzes the relationship between variables in a data structure of sufficient size is the binary LR method. If the number of observations is too large, binomial variables can also be analyzed with ANOVA. If the dependent variable is categorical, and the aim of the researcher is to summarize the data in homogeneous subgroups in the independent variable in terms of the dependent variable, the appropriate method is the classification tree. In this study, a dataset on which inferences could be made by all three methods was analyzed. In this context, the relationship between the dependent variable (birth type-single or twin) and the independent variables (yield year and maternal age) in an Awassi sheep flock was examined.

When the methods were examined comparatively, the following conclusions were reached:

1) In all three methods, the significance (P) of factors and the effect orders are similar. In research, the yield year is more effective in all three methods, while the second is the maternal age.
2) When evaluating the different inferences of the methods, it is seen that FANOVA additionally offers information on the interaction of factors.
3) However, taking into account the definition of interaction, the researcher can obtain information about the existence of the interaction from the CT structure.
4) Based on a level defined for each factor, LR presents the values, which the dependent variable will receive at other levels, as a layer of this level. This inference is not directly presented in other methods.
5) The CHAID CT algorithm classifies different groups and presents homogeneous subgroups as more descriptive. In addition to a visual design, it also offers significance results and subgroup statistics.
6) In FANOVA and LR methods, confidence intervals of point estimates can also be presented. Classification trees do not offer an inference in this direction.
7) R^2 statistics, which is a measure for the explanation level of a dependent variable by independent variables, are presented in the FANOVA and LR methods, but the CT algorithm does not report these statistics. However, FANOVA R^2 statistics and LR R^2 statistics (Cox -Snell R^2 and Nagelkerke R^2 statistics)

are not comparable in terms of fit of FANOVA and LR models.

Author Contributions

A.K. (50%) and I.Y. (50%) designed the study and collected the data, critically reviewed. Ö.A. (100%) analyzed the data and wrote the article. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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STRUCTURAL FEATURES OF SHEEP FARMS IN ORDU PROVINCE

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Abstract: This study was carried out to determine the structural characteristics of sheep breeding, herd owners, and general characteristics of sheep breeding enterprises in Ordu province by survey method. There are 1883 sheep farms in Ordu, and it has been determined that in 1116 (59.27%) of these farms, the number of sheep is below 100 heads, the number of animals per farm, in general, varies between 1-3242 heads, and the average number of animals per farm is 102 heads. The study data were obtained from 76 farms selected according to the stratified sampling method among 100 animals or more farms. Of the 76 breeders surveyed, 59.21% graduated from primary school, 14.47% from secondary school, 22.36% from high school and 3.95 bachelor degrees. It has been determined that the level of education does not make a difference in production, management, and the attempt to do additional work (χ^2=9.666, P=0.139). In the study, 26.3% of the herd owners are engaged in sheep breeding as a source of livelihood, while the remaining 73.7% are involved in sheep breeding together with other business areas. In conclusion, it was determined that the level of education, experience, age, and additional work status of the breeders were not effective on the size of the flock, the number of lambs obtained per ewe, lamb survivability and business management in sheep farms. As a result, for sheep farms to reach a more profitable, innovative and sustainable situation in Ordu province, those with less than 100 sheep per farm should be encouraged to increase the number of animals and the problem of finding a shepherd should be resolved.

Keywords: Farmer, Education level, Sheep farm, Herd size, Management

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1. Introduction

The characteristics of the herd owner such as education, health, age, partially gender, economic situation, whether she is open to innovations, and turning risks into opportunities are critical in animal husbandry. Because these characteristics are among the factors affecting the size of the enterprise to be established, the production amount of the enterprise, the production quality, competitiveness of the enterprise with other enterprises and the sustainability of the enterprise. It is an unpleasant fact that the owners of small ruminant herds in general are inferior to other livestock breeders in terms of the mentioned characteristics above. However, this should not be absolutely understood as the fact that sheep-goat breeders are not intelligent and their grasping skills are low.

Sheep breeding is carried out in the form of family businesses throughout Turkey and with the participation of all members of the family. The manager of the business is usually the dominant male (father). In addition, it is seen that there has been an increase in the number of agricultural enterprises dominated by women in recent years. In the research, it is reported that women-dominated enterprises are more sensitive to the environment, and they focus on organic production and sustainability of enterprises (Unay-Gairhard and Bojnec, 2021).

Family businesses in agricultural production are the basic building blocks of the society; which are generally located on small lands; with very low capital and investment, making significant contributions to both their own livelihoods and the country's economy by making vegetable and animal production by making use of their own family workforce (Mujica and Riveros, 2021). It is a necessity for such families to deal with both plant and animal production in many parts of the world (Olaizola et al., 2015). Plant and animal production of the family in rural areas to meet their own needs is essential in terms of socio-economic, socio-political, and food security, such as preventing migration from the countryside to the city bringing small areas into the economy. On the other hand, for producers with minimal resources, sheep are among the most ideal and beneficial animals, having a very significant impact on their income, social status, and even the local environment (such as assessing the vegetation among the hazelnut trees and fertilizing the soil (Park and Deller, 2021). Questionnaires are valuable studies that can provide valuable clues in defining and solving problems if they are well planned, selecting suitable subjects in the target, and obtaining the correct answers. Since sheep breeding has an important place in rural settlement, employment,

animal husbandry, and the country's economy, but it is not in the desired position, policies and programs are carried out to strengthen its structure by authorities. For this reason, this study was carried out to determine the general characteristics of herd owners and enterprises dealing with small cattle breeding in Ordu and contribute to the achievement of sheep breeding to the position it deserves.

2. Materials and Methods

2.1. Identification of Businesses in the Study

Sheep farms are divided into four groups (100-175, 176-250, 251-350, and those with more than 351 animals) according to the number of animals they have. The number of farms (sample size) used in the study was determined according to the stratified sampling method (Işık, 2006) from 1883 sheep breeding enterprises (Anonim, 2022). The study was carried out as a face-to-face survey study in 76 of 1883 sheep farms in Ordu province in Türkiye and aimed to reveal the owners and enterprises engaged in sheep breeding. This survey study did not include businesses or owners with less than 100 sheep.

In our survey study, when it was determined that the sheep herding enterprises had other agricultural and commercial activities, we grouped the enterprises according to the activities they carried out to demonstrate the success in sheep breeding and formed three groups. We named only those who do animal husbandry as the 1st group, those who do animal husbandry and hazelnut production, which is common in the region, as the 2nd group, and the enterprises engaged in both animal husbandry hazelnut production and commercial activities as 3rd group.

2.2. The Ability of the Herd Owner to Manage Her Business

This concept can be expressed as the ability of the herd owner to manage the animals on his farm in a way that makes a reasonable profit. The size of the flock, the number of male and female animals in the herd, and the number of lambs obtained from ewes to join rams were used.

2.3. Sensitivity of the Herd Owner to Animal Health and Welfare

In assessing the sensitivity of herd owners about animal welfare and health, whether there is a vaccination schedule, the precautions they take to protect animal health, their sensitivity in litter management and shelter construction was evaluated using square test.

2.4. Statistical Analysis

In the study, the evaluations of the effects on animal production depending on factors such as the education level of the herd owner, age, work experience, activities other than animal husbandry, and family support were made with the SPSS package program (Ver.21) one-way analysis of variance. Four of the farmers participating in the survey were women and gender was excluded from being considered a factor since it could not be determined to affect the characteristics emphasized. While evaluating the effects of factors such as the education level of the breeders, their occupations other than animal husbandry, and family assistance on animal production, GLM was used, and the mentioned factors were taken as constant. The age and work experience of the farm owners were taken as covariates. Values were given as mean ± SEM (standard error of mean). Differences between means, expressed as percentages, were performed by χ square analysis.

3. Results

The sheep holdings in Ordu in terms of animal assets and the animal assets of the enterprises participating in the survey are shown in Table 1 and Table 2, respectively. It has been determined that the number of sheep per farm in Ordu province varies between 1 and 3242 heads, and the rate of farms with less than 100 sheep is 59.27%. Among the farms with less than 100 animals, 7.2% have five or fewer animals. The relations between the business owners' qualifications and the farm's production planning (number of the ewe joined to ram, the number of lambs produced), health protection practices, and activities other than animal husbandry are given in Table 3-5.

The information given by the breeders participating in the survey about both their businesses and their characteristics was evaluated. It was determined that 45 farm owners who participated in the survey were primary school graduates, 11 secondary school graduates, 17 high school graduates, and three undergraduate graduates. The education level of the farm owners did not make a statistical difference in the number of animals on the farms. The average age of the business owners participating in the survey is 48.6 (min 22, max 75), and their work experience is 30.8 (min 2, max 40) years.

3.1. The Relationship between the Education Level of the Owner of the Business, Production and Animal Welfare

The management style that the education level of the herd owner will reveal in processing management may show differences from other businesses with animal welfare, health, profitability, work discipline, and order. These differences are seen in practices such as the preparation and implementation of vaccination programs, which indicate the importance given to animal welfare and health, the planning of shelters, birthing compartments, quarantine compartments, ventilation systems, and litter management.

The educational status of the enterprises participating in the research, the total number of animals owned by the enterprises, and the number of lambs obtained per ram sheep are shown in the Table 3. It has been determined that women's active business responsibility ratio in sheep enterprises is around 3.95%, and their support is about 80.26% in male-run businesses. According to the

education level of business owners, the support rate of spouses to each other is 82.22% for primary school graduates, 81.82% for secondary school graduates, 70.59% for high school graduates, and 100.00% for university graduates. It was determined that family support was ineffective in herd size and management.

It was determined that 81.6% of the breeders made and implemented the necessary vaccination programs and made the arrangements needed in the shelters for the welfare and health of the animals. When the education level, experience, gender, additional work status of the breeders, and the importance they give to herd health (vaccination) and animal welfare (additional service departments such as animal shelter, lamb, sick animals, maternity departments) are examined, it has been determined that there is no difference in terms of the mentioned factors. This study determined that 24.32% of the enterprises participating in the research were engaged in animal husbandry, 58.11% in sheep breeding and hazelnut production, and 17.37% in animal husbandry hazelnut production and other commercial activities.

Table 1. Distribution of sheep farms and the number of sheep in farms by districts throughout Ordu province (Anonim, 2022).

County	n	Number of farms Less than 5 & 100		Total numbers of Sheep on farm	Number of animals per farm (Min- Max)	Mean
Akkuş	93	1	55	9.626	4-417	104
Altınordu	261	3	161	27.882	3-3242	107
Aybastı	206	4	109	26.691	1-1297	130
Çamaş	11	0	8	789	10-186	72
Çatalpınar	17	1	7	1.652	2-191	97
Çaybaşı	20	0	14	2.163	10-374	108
Fatsa	147	15	88	13.961	1-642	95
Gölköy	111	1	63	11.221	3-493	101
Gülyalı	32	1	18	4.297	1-769	134
Gürgentepe	56	5	43	2.977	1-219	53
İkizce	25	0	12	3.251	11-365	130
Kabadüz	57	2	35	5.394	1-598	95
Kabataş	93	4	51	9.945	1-424	107
Korgan	72	3	50	5.081	1-219	71
Kumru	160	8	102	14.514	1-662	91
Mesudiye	111	2	57	14.822	1-861	134
Perşembe	131	15	85	9.096	1-406	69
Ulubey	127	7	69	13.855	1-594	109
Ünye	153	8	89	13.996	1-433	92
Total	1883	80	1116	191.213	7-1500	103.62

Table 2. The surveyed districts, businesses, the number of sheep and the ratio of the surveyed businesses to the total (Anonim, 2022)

County	n	Sheep (Min-Max)	Number of ewe	Ram	FN/TF
Altınordu	21	110-1500	5228	194	27.63
Aybastı	18	120-1000	4388	162	23.68
Gölköy	4	135-400	905	30	5.26
Mesudiye	7	110-400	2020	76	9.21
Fatsa	8	105-200	1200	56	10.53
İkizce	1	120	120	6	1.32
Kabataş	*3*	130-132	392	15	3.94
Gürgentepe	1	150	150	5	1.32
Ulubey	6	130-250	988	36	7.89
Kabadüz	2	175-200	375	15	2,63
Gülyalı	1	170	170	7	1.32
Kumru	2	155-180	335	12	2.63
Perşembe	1	220	220	9	1.32
Ünye	1	250	200	10	1.32
Total	76	100-1500	16.691	633	100.00

FN= farm number, TF= total farm

Table 3. The effect of the education level and workload of the farm owner on the size of the herd and lamb yield per ewe.

Education Level	Business	Herd Size	Number of lambs per ewe
Primary (45)	Overall (45)	208.9±20.83	0.95±0.026
	SF (14)	234.8±60.46	0.97±0.039
	SF+H (26)	199.2±14.54	0.95±0.034
	SF+HP+OA (5)	183.0±45.27	0.85±0.117
Secondary (11)	Overall (11)	175.7±15.91	0.94±0.058
	SF (1)	105.0	0.70±0.000
	SF+HP (8)	173.5±10.42	0.98±0.072
	SF+HP+OA (2)	220.0±8.00	0.91±0.044
High School (17)	Overall (17)	275.2±80.24	0.97±0.026
	SF (4)	190.0±41.03	1.03±0.037
	SF+HP (9)	163.3±17.04	0.94±0.026
	SF+HP+OA (4)	612.0±304.80	0.97±0.091
University (3)	Overall (3)	267.7±72.56	0.88±0.191
	SF (1)	123.0± 0.000	1.08±0.000
	SF+HP+OA (2)	340.0±10.00	0.78±0.283

SF= sheep farm, HP= hazelnut production, OA= other activities

Table 4. The relationship between the working status of herd owners in different business branches and their educational status

	Education level (n-%)				
Business type	Primary	Secondary	High	University	Overall*
SF	14 (31.1)	1 (9.1)	4 (23.5)	1 (33.3)	20 (26.3)
SF - HP	26 (57.8)	8 (72.7)	9 (52.9)	0	53 (56.6)
SF – HP - OA	5 (11.1)	2 (18.2)	4 (23.5)	2 (66.7)	3 (17.1)
Overall	45 (100)	11 (100)	17 (100)	3 (100)	76 (100)

SF= sheep farm, HP= hazelnut production, OA= other activities

*The status of herd owners to do different jobs is independent of their education level (χ^2=9.666 P=0.139).

Table 5. Herd management and productivity status according to the work distribution of herd owners

Business type	Average Herd Size	Number of ewes Joined to ram	Lambs yield	Number per 100 ewes joined to ram
SF (18)	221.2±47.92	164.8±28.22	152.2±18.39	92.7±3.55
SF + HP (43)	186.5±9.85	144.1±6.77	137.4±6.68	95.1±2.34
SF + HP + OA (13)	335.5±102.62	229.9±59,59	199.7±42.11	87.0±4.44

SF= sheep farm, HP= hazelnut production, OA= other activities

4. Discussion

Sheep breeding is an essential source of meeting the red meat needs in Turkey. It is necessary to determine the current situation of this meat production source in each region where it is grown and make plans from the obtained data for the ideal use of existing resources. In the vast majority (59.27%) of the sheep and goat farms in Ordu, the number of animals is less than 100 heads (Table 1). This situation poses a risk regarding the sustainability of enterprises, continuity, and production stability. Improving and maintaining the existing structure in terms of business and animal assets will be beneficial in terms of preventing the dominance of large enterprises in animal production and maintaining production and price stability. The main factors affecting the enterprise's profitability in animal production can be listed as the number of animals, the amount of product obtained from the unit animal, the input costs, and the sales value of the product. There are two options for increasing production and profit: increasing the number of animals or increasing the yield per unit animal. Increasing the number of animals in rural conditions will be a result-oriented approach in a short time (Düzgüneş et al., 2012).

Production in sheep breeding in Ordu is based on pasture. The number of animals in the surveyed farms varies between 110 and 1500 heads, and the owners' education, experience, age, and different work status do not make any difference in herd management and productivity (Table 2). Traditional production methods continue regardless of the herd owners' education (Table 3), gender, experience, age, and additional work (Table 5). Equipping the traditions with information about herd management and influential factors in productivity

increase will contribute to the farm's sustainability and the herd owner's welfare.

Among sheep breeders, the rate of working in other jobs is high (73.7%, Table 4). This situation makes it difficult to develop sustainable and effective policies in sheep breeding because a slight risk in the sector strengthens the possibility of turning to other businesses.

The health and welfare of animals raised in animal production are becoming increasingly important. Animals' exposure to adverse effects of the environment outside the comfort zone causes negative effects such as low productivity and diseases (Marcone et al., 2022). In providing ideal conditions for animals, sometimes negligence may occur due to lack of awareness or knowledge and sometimes due to economic inadequacies. Considering that sheep breeders are more intertwined with nature and more connected to nature than other livestock sectors, and their economic opportunities are more limited, it is understood that they produce under challenging conditions. Although it is strange to prioritize the animal's welfare that the producer raises without taking into account her own welfare, it is seen that herd owners of all education levels try to provide the best conditions for their animals in this study.

It has been determined that the relationship between the education level of the farm owner, his approach to innovations, animal welfare and production level is not at the expected level. There is no difference in herd management between breeders with low and high education levels has been evaluated as an indicator of not getting rid of traditions and not being open to innovations.

Turkey's sheep population can be easily doubled, primarily by solving the problems enterprises face with less than 100 heads in production, encouraging them to raise more animals and train the breeders. Considering the age of business owners, the number of sheep in the business, the number of the companies that are content with only animal husbandry, the number of people sharing the income of the business, family support, business planning, it is predicted that the employment rate in rural areas will decrease in the future and this sector will go further back.

5. Conclusion

The results presented in this study are based on the comments obtained from examining the official records of sheep holdings and the statistical evaluation of the information provided by the breeders participating in the survey. The results of present study indicated that sheep breeders in Ordu province mostly carry out hazelnut production and animal production together, some breeders participate in production with a small number of animals in a way that can be considered as a hobby because they cannot break with their traditions. It has been determined that there are very few female herd owners among the breeders, the average age of the herd owners is around 49, and the younger generations are not very enthusiastic about sheep breeding. As a result, it is thought that maintaining the current production level by improving it, or at least maintaining it as such, will be beneficial for both the rural population and the country's economy. It has been determined that the relationship between the education level of the farm owner, his approach to innovations, animal welfare and production level is not at the expected level. Turkey's sheep population could easily be doubled, primarily by solving the problems faced by farms with fewer than 100 animals in production and by encouraging breeders to raise more animals. Plans should be developed to improve social and cultural conditions that will make life in rural areas attractive, maintain employment, and increase contributions to the family and national economy. It is hoped that these improvements will effectively increase productivity in farm production, ensure sustainability, and gain the attraction of younger generations

Author Contributions

A.A. (100%) taken data, structured the paper and wrote the manuscript. M.A.Ç. (100%); initiated the research idea, suggested the research methods, supervised the research, analyzed, interpreted the data, and edited the manuscript. All authors reviewed and approved final version of the manuscript.

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Ethical Consideration

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The experimental procedures were approved by the Local Social and Human Sciences Research Ethics Committee of Ondokuz Mayıs University (Approve number: 2022/169).

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Research Article
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COMPARATIVE EVALUATION OF SALICYLIC ACID (SA) AND 2,4-DICHLORO-6-{(E)-[(3METHOXYPHENYL)IMINO]METHYL} PHENOL (DPMP) ON GROWTH AND SALT STRESS TOLERANCE IN FORAGE PEA (PISUM SATIVUM SSP. ARVENSE L.)

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Abstract: Alleviation of salt stress is becoming one of the urgent needs of agricultural production. Even though enhancement of tolerance levels with genetic variation is a common approach, exogenous applications of various compounds are a newly emerging field. Here, the effects of two different plant elicitors, salicylic acid (SA) and 2,4-dichloro-6-{(E)-[(3methoxyphenyl)imino]methyl} phenol (DPMP) on growth and stress tolerance levels of forage pea (*Pisum sativum* ssp. *arvense* L.) were evaluated. Plants were exposed to salt stress (100 mM) in addition to DPMP, SA, or DMSO (Solvent) foliar spraying. The results revealed contrasting effects for each elicitor. Under non-stressed conditions, DPMP applied plants had higher values in plant height, shoot dry weight (SDW), and taproot length, while SA applied plants had significantly higher shoot fresh weight (SFW), and DMSO applied plants had higher values in root fresh (RFW) and dry (RDW) weights, and root/shoot ratios. When we evaluated stress tolerance index (STI) levels, DPMP applied plants had higher STI values in SFW, SDW, RFW, and RDW. DPMP improved STI and biomass allocation better than SA and DMSO. These elicitors may have significant potential in abiotic stress tolerance, in addition to their well-known biotic stress eliciting roles. There is a need for further research to define appropriate doses and application times.

Keywords: Elicitor, Forage pea, Root development, Salicylic acid, Salt stress

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1. Introduction

Salt stress affects approximately 6% of the world's agricultural areas and it is becoming one of the most urgent limitations in agriculture (Yang and Guo, 2018; Acosta-Motos et al., 2020). When plants face salinity, they show common osmotic stress symptoms, and productivity gets declined or is completely prevented, based on salt accumulation, duration, and the tolerance level of the plant (Yang and Guo, 2018; Liang et al., 2018; Grozeva et al., 2019). The source of the salt stress could be irrigation water or accumulation of salt in the soil may lead to excessive salt stress for plants. Plant roots are exposed to salinity in the first place and the induction of stress signals from roots warn stomata to open less frequently. The stress signals minimize photosynthetic activities eventually. In addition to these initial salt stress responses, ion toxicity, as well as osmotic stress, occurs as secondary stresses (Yang and Guo, 2018; Liang et al., 2018). There have been a significant number of publications to understand plants' responses to salt stress (Singh et al., 2021). The effects of genotype on stress tolerance (Li et al., 2020; Acosta-Motos et al., 2020; Zhang et al., 2021), and its molecular mechanism (Mullan and Barrett-Lennard, 2010; Cornacchione and Suarez, 2017; Amoah et al., 2020; Li et al., 2020) were evaluated with in-depth observations.

Forage pea (*Pisum sativum* ssp. *arvense* L.) is a member of the Legume family. It is mostly used for fresh or dry herbage production and it has a significant role in soil nitrogen sustainability (Ateş and Tekeli, 2017; Çaçan et al., 2019). Legume forage crops have a soil nitrogen recovery advantage compared to forage crops from the Poaceae family due to nitrogen fixation ability (Den Herder et al., 2010). Genetic diversity (Demirkol and Yılmaz, 2019), common root trait diversity (Acikbas et al., 2022), yield and yield components (Uzun et al., 2012; Ateş and Tekeli, 2017; Tan and Kadıoğlu, 2018), and PEG induced osmotic stress (Bektas, 2022) responses of forage pea were previously reported. In a study conducted by (Demirkol et al., 2019) germination characteristics of forage pea were also evaluated. According to their results, 90 mM was the tolerance threshold level for the genotypes tested. A similar study (Grozeva et al., 2019) reported significantly reduced shoot and root biomass compared to less affected plant height in three different pea cultivars.

Plant elicitors are synthetic or organic compounds that aim to induce plants' response to abiotic or biotic stress factors (Bektas and Eulgem, 2015). Even though relatively new, they are mostly tested against biotic stress factors (Bektas and Eulgem, 2015; Tripathi et al., 2019), and some for abiotic stresses (Tripathi et al., 2019; Palmer et al., 2019; Koo et al., 2020; Ahmad et al., 2021). Salicylic acid (SA) is a phytohormone and one of the most important regulatory compounds of the plant immune systems. Research showed that the exogenous application of SA is also important for abiotic stress response, in addition to biotic stress, and has been used extensively in various applications (Larqué-Saavedra and Martin-Mex 2007; Koo et al., 2020). Also, some research provides information about the promising role of Salicylic acid (SA) as a plant growth regulator and the enhancement of plant adaptation to different stress conditions (Li et al., 2013; Samota et al., 2017; Wani et al., 2016; Zhao et al., 2017). Increasing evidence has shown that exogenous application of SA can improve plant tolerance to salinity. Some research showed its promising role in salt stress including; reduced salt stress by improving photosynthesis and growth in mustard (Nazar et al., 2015) alleviation of salt stress by enhancing antioxidant systems (Zhang et al., 2014), increasing enzymatic and non-enzymatic pathways (Shamili et al., 2021). In addition to SA, some studies also demonstrated the activity of other compounds to increase plant adaptation to abiotic stresses. For example; pretreatment with β-aminobutyric acid (BABA) increases salt stress tolerance in rapeseed (Mahmud et al., 2020) and barley (Mostek et al., 2016). Chitosan application increased the salt-adaptive factors in stevia (Gerami et al., 2020), and coating seeds with chitosan improved growth performance under salinity stress (Peykani and Sepehr, 2018). All of the above reports provide strong insight that plant defense elicitors may have the potential to increase abiotic stress tolerance and reduce the severity of stress factors including salt stress. Recently 2, 4-dichloro-6-{(E)-[(3-methoxyphenyl) imino] methyl} phenol (DPMP) is described as an analog of SA and promising synthetic elicitor. Its activity against some pathogens including oomycetes *Hyaloperonospora arabidopsidis* (*Hpa*) (Bektas et al., 2016) and bacterial pathogens*; Pseudomonas syringae* pv. *tomato* (*Pst*) and *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*) were revealed (Bektas et al., 2016; Bektas, 2021). In our previous study, we also showed its activity against PEG-induced osmotic stress (Bektas, 2022). However, there were no studies evaluating DPMPs role against salt stress and comparison of its activity with a well know defense-related phytohormone, SA. The effects of SA and DPMP, as well as their mode of action under salt stress, have not been revealed. Therefore, we aimed to comparatively evaluate the effects of SA and DPMP on seedling above and below-ground growth and development as well as their effects on stress tolerance index values under controlled conditions.

2. Material and Methods

2.1. Plant Material and Growth Conditions

Forage pea (*Pisum sativum* ssp. *arvense* L.) was selected as a model organism to investigate the possible roles of two plant elicitors, salicylic acid (SA) and 2,4-dichloro-6-{(E)-[(3methoxyphenyl)imino]methyl} phenol (DPMP), under salt-stressed and non-stressed conditions on the plant above- and below-ground growth and stress tolerance indexes. DMSO (Sigma Aldrich GMBH) was used as the solvent for DPMP and considered the control. Experiments were conducted under controlled conditions in the Department of Agricultural Biotechnology, Siirt University, Siirt, Türkiye (37°58'13.20"N - 41°50'43.80"E). The study was conducted following a modified cigar-roll method (Hohn and Bektas, 2020; Acikbas et al., 2021) according to randomized complete blocks design (RCBD) with three replications and ten plants per replication. During experiments, mean temperature and relative humidity ranged between 25-27°C and 60-70%, respectively, with 12/12 h day and night periods. Three different subsets (DPMP, SA, and DMSO) were prepared following Bektas and Eulgem (2015 and Bektas et al. (2016). There were a total of six different treatment groups, DPMP_control (10 µM DPMP), SA_control (100 µM SA), DMSO_control (0.2% DMSO), and DPMP_NaCl (10 µM DPMP+100 mM NaCl), SA_NaCl (100 µM SA++100 mM NaCl), and DMSO_ NaCl (0.2% DMSO+100 mM NaCl). SA was ordered from Sigma-Aldrich Chemie GmbH, Germany, and DPMP was kindly obtained from Prof. Dr. Thomas Eulgem, University of California, Riverside, USA.

An adequate number of seeds for the "Gap pembesi" cultivar were surface sterilized with 70% ethyl alcohol (C_2H_5OH) and 5% sodium hypochlorite (NACIO) for 5 minutes each and rinsed under running water. It is followed by placing seeds of similar size on germination papers (60 x 40 cm) as 10 seeds per paper and covered with a second paper layer (Hohn and Bektas, 2020). Each set is rolled and placed in large beakers filled with distilled water or saline solution (100 mM). After seedlings emerged, plant elicitors were applied as a foliar spray on the 7^{th} and 10^{th} days after the initial establishment of the experiments on May 20^{th}, 2020.

2.2. Data Collection and Image Analysis

The experiments were completed on the 15^{th} day when the roots of the %50 plants reached 40 cm depth. Germination papers were taken out of beakers and placed on the bench, and root images were collected using a portable hand-held scanner (Iscan Color Mini Portable Scanner) at 300 DPI resolution. Above and below-ground fresh and dry weights were measured using a precision scale (Weight lab instruments). Image analysis was performed to collect root length using ImageJ software (Rueden et al., 2017). Stress tolerance indices were calculated according to Moursi et al. (2020). The effects of SA and DPMP were evaluated by comparing their effects on above and below-ground traits listed in

Table 1. Analysis of variance (ANOVA) and variance groupings (TUKEY's Honest Significant Difference (HSD) test) was calculated using the Statistix software package (Analytical Software; Tallahassee, FL, USA).

Table 1. Seedling above- and below-ground growth-related traits and stress tolerance index traits evaluated under controlled conditions on forage pea

Trait name	Abbreviation-Calculation
Plant height	PH
Taproot length	TapRL
Shoot fresh weight	SFW
Root fresh weight	RFW
Shoot dry weight	SDW
Root dry weight	RDW
Plant height/Taproot length ratio	PH/RL
Shoot fresh weight/root fresh weight ratio	RFW/SFW
Shoot dry weight/root dry weight ratio	RDW/SDW
Stress tolerance index	
Stress tolerance index	STI
Reduction of PH	PH_Control–PH_NaCl
Reduction of SFW	SFW_Control–SFW_NaCl
Reduction of SDW	SDW_Control–SDW_NaCl
Reduction of RFW	RFW_Control–RFW_NaCl
Reduction of RDW	RDW_Control–RDW_NaCl
TapRL_STI	(TapRL_NaCl/TapRL_Control)*100
PH_STI	(PH_NaCl/PH_Control)*100
RFW_STI	(RFW_NaCl/RFW_Control)*100
SFW_STI	(SFW_NaCl/SFW_Control)*100
RDW_STI	(RDW_NaCl/RDW_Control)*100
SDW_STI	SDW_NaCl/SDW_Control)*100

3. Results

3.1. Root-Shoot Growth and Seedling Vigor

The effects and comparative performances of DPMP and SA were evaluated under salt-stressed and non-stressed conditions. Plant growth indicators including PH, SFW, RFW, SDW, RDW, RFW/SFW, RDW/SDW, and TapRL were evaluated. According to the results, there were significant differences between treatments for all the above traits except TapRL. At the non-stressed conditions, DPMP had significantly ($p<0.05$) higher values in PH, SDW and TapRL compared to SA and DMSO (Figure 1a, d, h). On the other hand, SFW was significantly higher in SA compared to DPMP and DMSO (Figure 1b). Finally, DMSO caused significantly higher values in RFW, RDW, RFW/SFW ratio, and RDW/SDW ratio (Figure 1c, e, f, and g). These results suggest different levels of effects in each chemical compared to one another.

Plant height (PH) was the highest (24.93 cm) in DPMP application, followed by SA (23.76 cm) and the least was in DMSO (21.80 cm). Under salt stress, all PH values were reduced to about one-third of the non-stressed conditions between 6.90 cm (DPMP) and 6.64 cm (SA) (Figure 1a). Shoot fresh weight (SFW) was the highest in SA with 0.73 g, and lowest in DPMP with 0.54 g in the non-stressed, while it was the opposite in the salt-stressed conditions with DPMP having the highest and SA having the lowest SFW values (Figure 1b). Root fresh weight (RFW) was the highest (0.68 g) in DMSO (no elicitor applied, only solvent) and lowest (0.50 g) in DPMP under non-stressed, while the highest in DPMP (0.16 g) and lowest in SA (0.11 g) under salt-stressed conditions (Figure 1c). Shoot dry weight (SDW) was the highest in DPMP application (0.10 g) and lowest in DMSO (0.08 g) under non-stressed conditions and the same ranking was observed under salt-stressed conditions (Figure 1d). RDW was found to be not significant within each treatment (non-stressed and salt-stressed), the ranking under non-stress was DMSO, DPMP, and SA, while it was ordered as DPMP, SA, and DMSO, respectively, under salt-stressed conditions (Figure 1e). Plant carbon allocation patterns can be estimated by the ratios of root and shoot biomass using fresh or dry weights. The highest RFW/SFW ratio was obtained in DMSO, followed by DPMP and SA under non-stressed and salt-stressed conditions (Figure 1f). When we evaluated RDW/SDW ratios, DMSO_NaCl was followed by DMSO_control, DPMP_NaCl, DPMP_control, SA_control, and SA_NaCl, respectively (Figure 1g). Our last growth-related trait was Taproot length (TapRL). Even though the longest TapRL was obtained in DPMP under non-stressed conditions it was not significantly different from DMSO and SA under non-stressed conditions (Figure 2). Similar outcomes were obtained in salt-stressed conditions (Figure 1h).

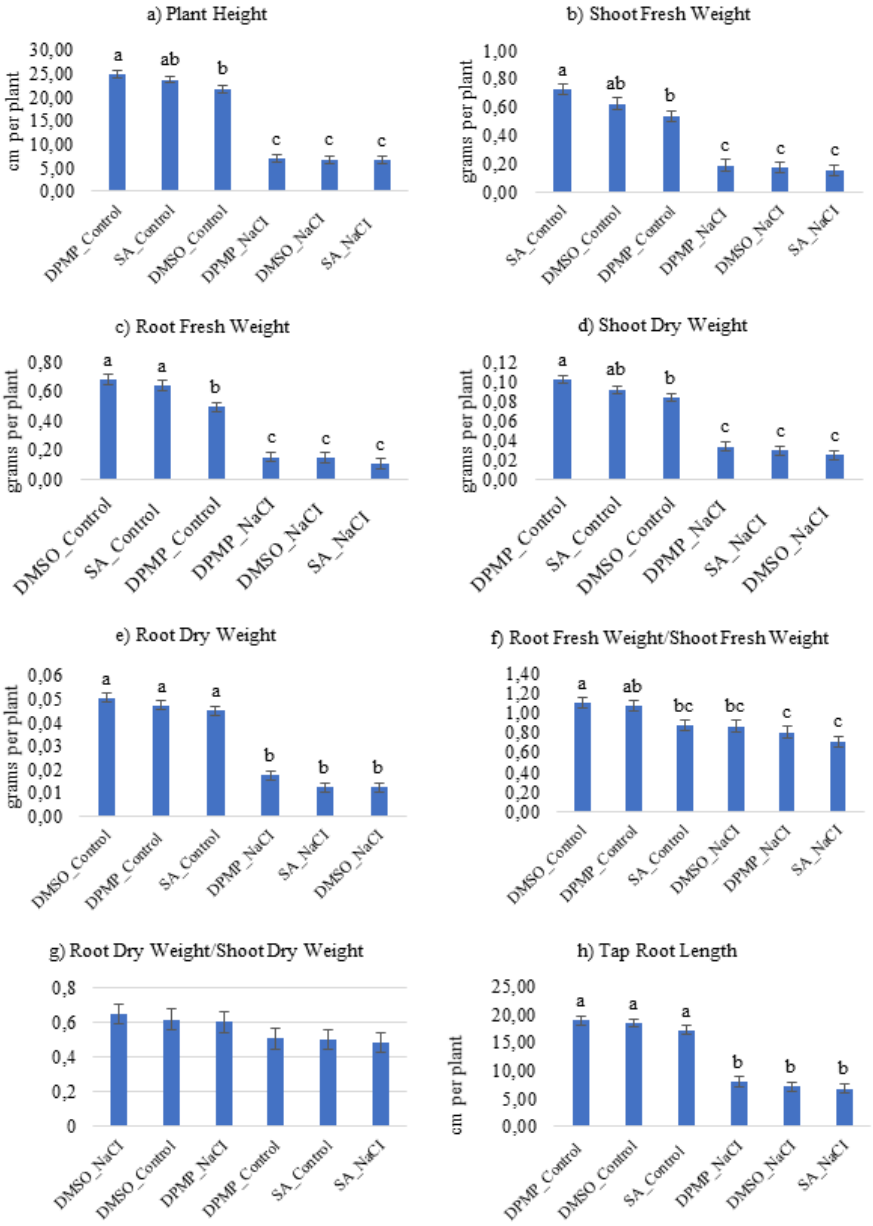

Figure 1. Mean values for plant height, shoot fresh and dry weight, root fresh and dry weight, root/shoot fresh and dry ratios, and taproot length. For each trait, means followed by different letters are significantly different at a $p < 0.05$ level according to the TUKEYs honest significant difference (HSD) test.

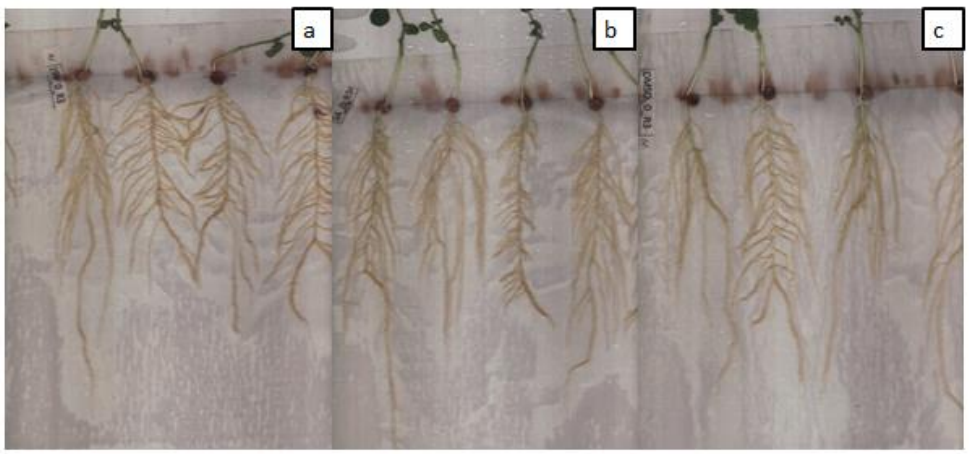

Figure 2. The TapRL was obtained under a) DPMP non-stressed conditions, b) SA non-stressed conditions, and c) DMSO non-stressed conditions.

3.2. Relative Efficiency of DPMP and SA Against Salt Stress

To evaluate the effectiveness of DPMP, we tested it against a well-known plant elicitor, SA, under non-stressed and salt-stressed conditions. This provided a better comparison efficiency. The values for each morphological trait under non-stressed conditions were compared with its values under salt-stressed conditions. The % decrease values and STIs for PH, SFW, SDW, RFW, RDW, and TapRL were calculated. According to the results, the lowest decrease in the values of SFW, SDW, RFW, and RDW were obtained in DPMP with 63.99, 65.56, 66.88, and 62.79% decrease compared to non-stressed trials of the same traits. For the PH, DMSO had the lowest decrease ratio with 68.91% and for the TapRL, SA had the lowest decrease ratio with 46.62%. These values were used to compute stress tolerance index (STI) values. In line with the percent decrease ratios, DPMP had the highest STI on SFW (36.00), SDW (34.44), RFW (33.12), and RDW (37.21). While DMSO had the highest STI in PH (31.09) and SA had the highest STI value in TapRL (53.38) (Table 2).

4. Discussion

Salt stress is one of the most destructive abiotic stress factors, following drought stress (Liang et al., 2018; Yang and Guo, 2018)**.** It is affecting more than %6 of the global agricultural land and this rate is increasing with wrong or excessive irrigation as well as fertilizer applications (Yang and Guo, 2018). One of the ways to cope with salt and other osmotic stresses is to select/breed new varieties. There is a significant genetic diversity available in the wild or domesticated gene pools (Shabala et al., 2016; Liang et al., 2018; Kumar et al., 2021) for the improvement of abiotic stress tolerance in crops. However, replacing all cultivars with the tolerant/resistant ones in the world is almost impossible. There is a need for enhancing the growth potential and stress tolerance efficiencies of the currently grown cultivars. A relatively new approach, plant elicitors, or enhancers, started to gain interest due to their practicality and reduced or minimal effects on the environment. Salicylic acid (SA), γ- aminobutyric acid (GABA), β-aminobutyric acid (BABA), and Acibenzolar-S-methyl (ASM) are the well-known plant elicitors, while newly identified organic or synthetic substances are introduced as possible elicitors against biotic or abiotic stress factors or just to enhance plant growth and development (Bektas and Eulgem, 2015). DPMP is one of those new chemicals that has proven effective against several pathogens (Bektas et al., 2016; Bektas, 2021). DPMP is reported to be effective against *Pst,* while its activity on plant growth and stress tolerance levels is not well defined. Here, we evaluated DPMP by comparing its role with phytohormone SA in crop growth under non-stressed and salt-stressed conditions.

Table 2. Stress tolerance index (STI) values for plant height (PH), shoot fresh weight (SFW), shoot dry weight (SFW), root fresh weight (SFW), root dry weight (SFW), and taproot length (TapRL) evaluated salt-stressed compared non-stress conditions. The results are presented as % decrease (non-stress -salt stress) and STI values

Trait	Control vs. NaCl	Value Decrease	% Decrease	STI
	DPMP	18.06	72.46	27.54
PH	DMSO	15	68.91	31.09
	SA	17.15	72.19	27.81
	DPMP	0.35	63.99	36
SFW	DMSO	0.45	72	28
	SA	0.57	78.02	21.98
	DPMP	0.07	65.56	34.44
SDW	DMSO	0.06	69.41	30.59
	SA	0.061	66.45	33.55
	DPMP	0.33	66.88	33.12
RFW	DMSO	0.53	77.34	22.66
	SA	0.53	81.73	18.27
	DPMP	0.03	62.79	37.21
RDW	DMSO	0.04	75.39	24.61
	SA	0.03	71.78	28.22
	DPMP	9.5	47.41	52.59
TapRL	DMSO	11.32	158.59	38.68
	SA	9.21	46.62	53.38

4.1. Effects of DPMP and SA on Plant Growth and Development

According to the results on above and below-ground morphological traits, DPMP, SA, and DMSO (only solvent) had differing effects on seedling growth. DPMP applied plants had higher values in SDW, PH, and TapRL (Not significant). These results suggest that DPMP has a comparable, even higher effect on plant growth than SA. The role of SA on plant growth, development, and stress tolerance (abiotic or biotic) enhancement is well known and documented by multiple reports (Filgueiras et al., 2019; Tripathi et al., 2019; Koo et al., 2020). So, its role in growth is no surprise, while there were no reports on the effect of DPMP on plant growth, except in our previous report (Bektas, 2022). According to (Koo et al., 2020) SA acts as a plant hormone and regulates, plant immunity, growth, and development. It has crosstalk with absisic acid, ethylene, jasmonic acid, and auxin. With these roles, SA can be considered a key element in plants. Even though there is not much knowledge on the role of DPMP on plant hormones and regulation, it may be listed as analogous to SA (Bektas et al., 2016).

4.2. Comparative Evaluation of DPMP and SA on Salt Stress Tolerance Index (STI)

Stress tolerance index (STI) calculation is a way to evaluate the effects of genotypic differences or applied substances on plant growth under stressed conditions. Here, the STI was calculated according to Moursi et al. (2020) with slight modifications. Accordingly, DPMP applied plants had higher biomass allocation and STI values compared to SA or DMSO applied plants under salt-stressed conditions. As previously reported (Filgueiras et al., 2019; Koo et al., 2020; Ahmad et al., 2021) SA has well-known effects as a plant growth enhancer. Here, SA applied plants had longer TapRL under salt-stressed conditions, compared to DPMP and DMSO. On the other hand, DMSO caused taller plant stature (PH) compared to other chemicals. DPMP applied plants had higher biomass and STI values under salt-stressed conditions, compared to SA and DMSO. According to the results of the current experiment, DPMP helps plants to cope with the negative effects of salt stress. Its mode of action and specific hormonal effects are yet to be identified. DPMPs' role against *Pst* is confirmed by morphological and molecular observations (Bektas et al., 2016). It induced SA-related defense genes and reduced the disease severity of *Hyaloperonospora arabidopsidis, Pst,* and *Cmm*. The results of the current study provided preliminary evidence for the positive effects of DPMP on salt stress tolerance. It seems to enhance biomass production potential under the currently applied salt dose. However, there is a need for defining optimal doses, application frequencies, and effective application procedures. We are currently working on identifying its role on other abiotic and biotic stress factors as well as on other crops. Synthetic or organic compounds may provide new insights into plant stress tolerance improvement and growth enhancement.

Author Contributions

N.Ö. organized (100%), analyzed, and interpreted the data (100%) and wrote the original manuscript (70%). Y.B. initiated the research idea (100%), supervised the research (100%), suggested the research methods (100%), structured the paper (100%), and edited the manuscript (30%). All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Review
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THERAPEUTIC SOURCE: PLANTS

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Abstract: Plants are used to cure many diseases due to their therapeutic properties. The history of phytotherapeutic applications using plants for treatment goes back thousands of years. The reason plants have been used for treatment for so long is that they have produced secondary compounds with thousands of different structures that have therapeutic properties. Many of the secondary metabolites produced by plants have been converted into drugs through rational phytotherapeutic applications. The demand for herbal medicines is increasing day by day all over the world because the synthetic drugs used for treatment have serious side effects, are not sufficiently effective and there are diseases for which there is no cure yet. In our country there are almost 13 thousand plant taxa, and very few of these plants are used for medicinal purposes. In fact, thousands of plants and tens of thousands of secondary compounds that can be used for treatment are waiting to be discovered. The discovery of new, effective and safe herbal medicines is a remarkable field of research today, and the discovery of effective and safe alternative medicines will bring great benefits to human health.

Keywords: Phytotherapy, Secondary compounds, Drug discovery

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1. Introduction

Plants have been used to treat many diseases for thousands of years (Samuelsson, 2004). The first records of treatment with herbs were found on clay tablets in the Mesopotamian civilization in 2600 BC. Hittite inscriptions, Egyptian papyrus and ancient books give local names of plants with healing properties and information about their use (Gurib-Fakim, 2006). The use of plants to treat and/or prevent various diseases was first defined as phytotherapy by the French physician Henri Leclerc (1870-1955) (Schulz and Tyler, 1998; Sarker and Nahar, 2018).

Phytotherapeutic applications were used in traditional ways until the 19th century.

Plants with therapeutic properties; consumed by society for centuries either directly nutrients (cranberry, Echinacea, hot herbs, garlic, Ginkgo biloba, etc.) or the lowland mix people in Mexico *Hyptis verticillata* (Lamiaceae) as used plant ground and made into an ointment with alcohol skin infections and was used to treat wounds or consumed as a tea to cure gastrointestinal disorders (Heinrich, 2001). This use of plants for therapeutic purposes is known as traditional phytotherapy, and traditional phytotherapy is a medical practice favored by people both today and in the past. Especially in developing countries, 80% of the population benefits from herbal products for therapeutic purposes. In some countries in regions such as Asia, Africa and the Middle East, this percentage is as high as 95%. The World Health Organization predicts that treatment with herbs will increase worldwide in the coming years (Ozçelik and Toprak, 2015; Heinrich et al., 2017).

In addition to the traditional use of plants for treatment, the discovery of drugs from plants is also an important area of research today. Obtaining new drugs from medicinal plants is one of the most important goals of rational phytotherapy. Rational (rational) phytotherapy applies an evidence-based approach that includes a set of scientific criteria for treatment efficacy and safety, in contrast to traditional phytotherapy, which is based on long-standing experiences and traditions (Fürst and Zündorf, 2015; Colalto, 2018). Rational phytotherapy could be applied with the development of distillation technology in the 19th century, and many drug discoveries were made with rational phytotherapy applications in the 19th century. The chemical structure of some of the discovered drugs is shown in Figure 1.

One of the first drugs identified in this century is the morphine alkaloid, discovered in 1804 by the German pharmacist FW SertuWrner in the poppy plant (*Papaver somniferum*, Papaveraceae) and used today as a very potent analgesic (Heinrich et al., 2017). Another drug discovered in the 19th century is the substance quinine, which was extracted from the bark of the tree (*Cinchona succirubra*, Rubiacea) in 1820 by French scientists Pierre Joseph Pelletier and Joseph Bienaime Caventou (Sappington, 1844). Quinine is the active ingredient in anti-malarial drugs such as quinacrine and chloroquine, which are currently used to treat malaria (Permin et al., 2016).

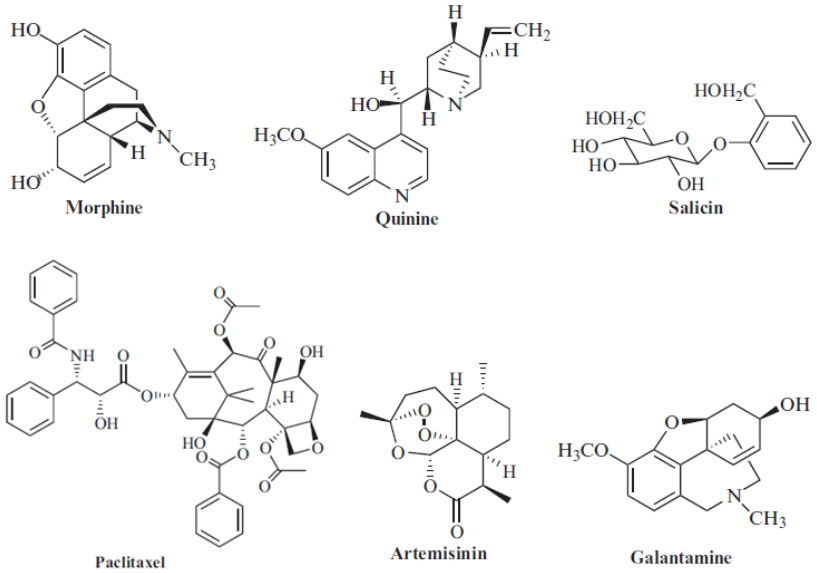

Figure1. Examples of drugs derived from therapeutic plants.

Salicin, a type of phenolic glycoside extracted from the bark of the willow tree (*Salix* spp., Salicaceae) by Johannes Buchner in Germany, was also discovered as a result of rational studies on phytotherapy. Also in 1899, the metabolite of salicin was used to make aspirin (Maclagan, 1876). Rational phytotherapeutic practices continued throughout the 20th century, and many drugs were discovered. The anticancer agent paclitaxel, the antimalarial agent artemisinin, and the antidementive galanthamine are just a few (Heinrich and Teoh, 2004; Cragg and Newman, 2005; Heinrich, 2010).

The reason plants are used in both drug discovery and traditional treatment is because of the secondary metabolites they produce. When plants are exposed to stressors such as pest attack, drought, and salt stress, they produce hundreds of thousands of different types of secondary metabolites to protect themselves. Secondary metabolites are a very important source for the production of drugs because of their therapeutic properties. Numerous drugs used in the treatment of many diseases such as cancer, neurodegenerative diseases, diabetes, heart diseases, muscle, and bone diseases have been produced from plant secondary metabolites (Heinrich et al., 2017). This review introduces researchers to the therapeutic potential of plants and also shows how important plants are for drug discovery.

2. What Do Plants Owe Their Therapeutic Properties?

For centuries, people have used plants not only to meet their food needs, but also for religious and cultural rituals, for hunting, for warfare, and to treat disease. The vast majority of plant chemicals used for purposes other than nutrition are secondary metabolites, which are biosynthetically produced from plant primary metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in plant growth, development, or reproduction.

Secondary metabolites are active biomolecules that protect the plant from abiotic (drought, radiation, salinity, etc.) and biotic stresses (such as bacterial, viral, and fungal infections, and attacks by nematodes, mammals, and other animals) and regulate the mechanisms of flowering, oil production, pollination, and pigment production in the plant. These bioactive molecules can be classified into 4 groups according to their chemical structure: phenolic compounds, terpenes, alkaloids, and glycosides.

2.1. Phenolic Compounds

These compounds consist of at least one aromatic ring linked by one or more hydroxyl groups (Nicholson and Hammerschmidt, 1992). Phenolic compounds found in plants include secondary metabolites such as phenolic acids, coumarins, lignins, lignans, condensed and hydrolyzable tannins, phenylpropenes, and flavonoids (Soto-Vaca et al., 2012). The source of many drugs are phenolic compounds produced by plants.

The compound podophyllotoxin, used as a precursor for anticancer drugs and extracted from the roots of *Pelarganium peltatum* (Geraniaceae), is one of the phenolic compounds that inhibit tubulin polymerase, the enzyme required for the synthesis of tubulin, an important component of cell division (mitosis). Because of this property, the podophyllotoxin compound has been modified and transformed into two anticancer drugs, teniposide and etoposide (Heinrich et al., 2017). The phenolic compound aesculetin, isolated from the plant *Aesculus hippocastanum* (Sapindaceae), is used to treat capillary fragility (Coruh and Ozdogan, 2014; Heinrich et al., 2017). Scopoletin synthesized by *Solanum tuberosum* (Solanaceae) has antimicrobial properties and is used in the treatment of fungal infections (Gnonlonfin et al., 2012). The plant *Hieracium pilosella* (Asteraceae), also known as mouse ear, produces the compound

umbelliferone, which is used in veterinary medicine to treat brucellosis and has antibacterial activity (Mazimba, 2017). Khellin is a product of *Ammi visnaga* (Apiaceae) and has spasmolytic and vasodilator activity (Travaini et al., 2016). The phenolic compound dicoumarol from the species *Melilotus officinalis* (Fabaceae) is used alone or in combination with heparin as an anticoagulant for prophylaxis and treatment of blood clots and prevention of gangrene after frostbite (Hroboňová et al., 2018). Figure 2 shows the chemical structure of some phenolic compounds used as drugs.

2.2. Terpenes (Terpenoids)

Terpenes are the most structurally diverse class of secondary metabolites and include more than 40,000 compounds (Bohlmann and Keeling, 2008). In plants, terpenoids occur as photosynthetic pigment (phytol and carotenoids), electron transmitter (ubiquinone, plastoquinone), hormone (gibberellins, abscisic acid), and structural component of cell membranes (sterols). It also contributes to the formation of aromas in the plant (Heinrich et al., 2017).

Secondary metabolites belonging to many terpene classes have been used as raw materials for drugs. The compound gaminobutyric acid (GABA) derived from the valerian plant (*Valeriana officinalis*, Valerianaceae) is converted to gabapentin and used for its sedative effects in the treatment of epilepsy and neuropathic pain (Heinrich et al., 2017). One of the most important examples of pharmaceutical terpenes is the antimalarial drug artemisinin from sweet jelly (*Artemisia annua*, Asteraceae). The drug artemether, the ether of dihydroartemisinin, is used for the treatment of Plasmodium falciparum (Zhang et al., 2020).

Paclitaxel, an ancient antitumor agent, is an important component of this natural product class. Paclitaxel is found in the bark of the Pacific yew (*Taxus brevifolia*, Taxaceae), a slow-growing tree in the forests of northwestern Canada and the United States. Paclitaxel (trade name Taxol®), which has antitumor activity, was introduced into the U.S. drug market in 1993 for the treatment of ovarian cancer (Rana et al., 2017). Diosgenin is another terpene compound used in the pharmaceutical industry, which is extracted from weeds (*Dioscorea* sp.). It enables the formation of many important hormones such as testosterone (a male sex hormone) and estradiol (a female sex hormone) through a chemical process known as diosgenin marker degradation. The diosgenin compound converts to progesterone through the loss of a methyl group (CH_3) (Nazir et al., 2021). Figure 3 shows the conversion of the diosgenin compound to progesterone.

2.3 Alkaloids

Alkaloids; nitrogenous compounds derived from amino acids such as tyrosine, lysine, tryptophan, and aspartic acid (Loomis and Croteau, 1980). A large number of biologically active alkaloids have been isolated from plants. At the cellular level, the actions of alkaloids vary widely. Some act on the nervous system, others on protein synthesis, and still others on membrane transport and enzyme activities (Yao et al., 2004). About 12000 alkaloids are used as narcotics, drugs, and poisons because of their different biological activities (Hesse, 2002).

For example, plant alkaloids widely used in medicine include vincristine and vinblastine, which are derived from the plant *Catharanthus roseus* (Apocynaceae) and used as anticancer agents. Colchicine, derived from the autumn crocus (*Colchicum autumnale*, Colchicaceae) and used as a gout remedy, and morphine, used as an analgesic and isolated from the opium fluid in immature poppy capsules (*Papaver somniferum*, Papaveraceae), are other alkaloid drugs (Crozier et al., 2008). Papaverine, which occurs with morphine in opium fluid, is an antispasmodic agent and is also used to treat male impotence, and the drug verapamil was developed from papaverine (Siddiqui et al., 2014).

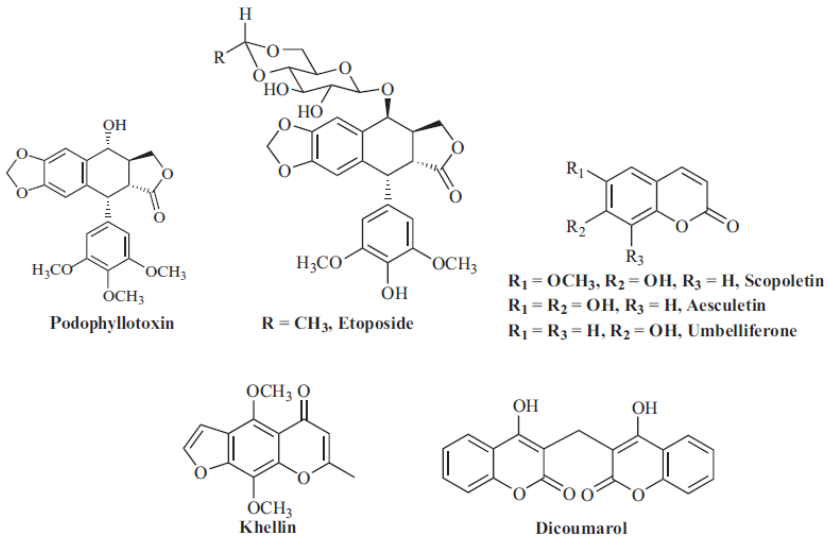

Figure 2. Chemical structure of some phenolic compounds used as drugs.

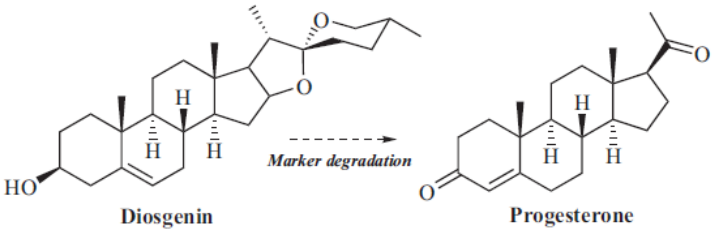

Figure 3. Conversion of the diosgenin compound to the hormone progesterone by the marker degradation.

Calabar bean (*Physostigma venenosum*, Fabaceae) is a plant that contains toxic alkaloids used to kill criminals. The toxic component of this species is physostigmine, an inhibitor of acetylcholinesterase that causes an increase in the activity of acetylcholine. This compound is of interest for the treatment of Alzheimer's disease, in which low concentrations of acetylcholine are observed in the brain (Batiha et al., 2020). Neostigmine and pyridostigmine, synthetic compounds prepared from physostigmine, are used to treat myasthenia gravis, a rare disease characterized by severe muscle weakness (Rumack, 1973). Pilocarpine, isolated from the jaborandi (*Pilocarpus jaborandi*, Rutaceae), a tree common in South America, is a cholinergic agent and is used to stimulate the muscarinic receptors of the eye in the treatment of glaucoma. In the eye, this compound and its derivatives (salts such as hydrochloride and nitrate) lower ocular pressure by providing pupillary constriction (miosis) and improved ocular outflow (Avancini et al., 2003). Figure 4 shows the chemical structure of some alkaloids used as drugs.

2.4. Glycosides

The term glycoside is a general term for a natural product that is chemically bound to a sugar. Many herbs contain cardioactive or cardiac glycosides that have a profound effect on heart rhythm. These glycosides are generally found in the genera *Convallaria*, *Nerium*, *Helleborus*, and *Digitalis*. *Digitalis purpurea*, a member of the plant family Scrophulariaceae, was widely used to treat heart disease until the 18th century because it contains the cardiac glycosides digoxin and digitoxin (Heinrich et al., 2017). Digoxin is the most commonly used cardiac glycoside in congestive heart failure (Figure 5) and is currently extracted from the related species *Digitalis lanata* (Withering, 2009).

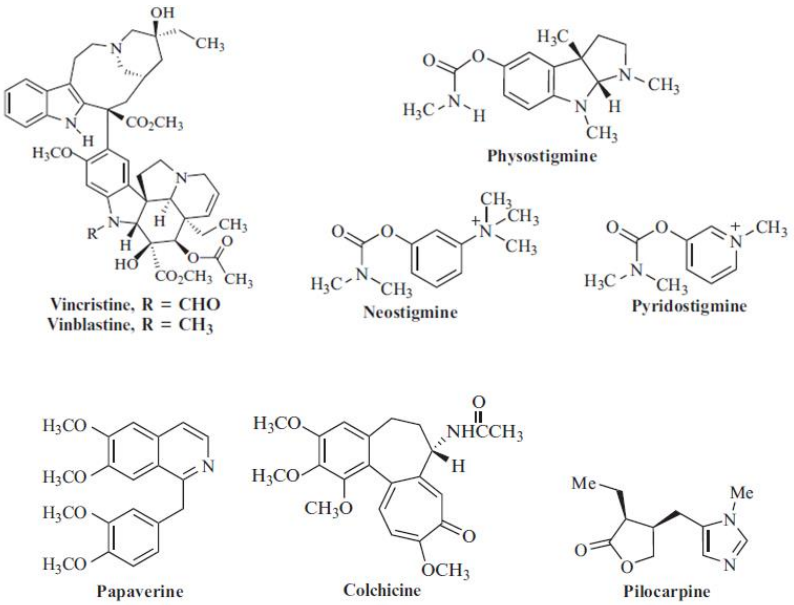

Figure 4. Chemical structure of some of the alkaloids converted into drugs.

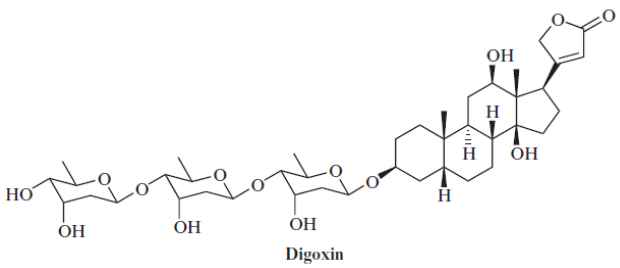

Figure 5. Chemical structure of digoxin glycoside.

The leaves of the senna plant, which is commonly used for constipation because of its laxative properties, contain the glycosides sennokote A and B. Senokot is a commercial product marketed as an anticonvulsant and contains these glycosides (Heinrich et al., 2017).

3. Role of Medicinal Plants in Drug Discovery

Although many drugs are used in modern medicine today, the discovery of new drugs is an important issue for life because existing drugs cannot effectively treat all known human ailments, many drugs have side effects, and there are still diseases today that are not treated with drugs (Hamburger and Hostettmann, 1991; Dar et al., 2017). Considering that antibiotic resistance is a major problem in the world and the incidence of many diseases such as cancer, heart disease, and neurodegenerative diseases that seriously affect people's lives is increasing every year, the need for effective, safe, and more economical medicines is important (McCord, 2000; Cars and Nordberg, 2005).

Although pharmaceutical companies are now interested in molecular modeling, combinatorial chemistry, and other synthetic chemistry techniques to produce new drugs, natural products and especially medicinal plants remain an important resource for new drugs and new drug precursors (Raskin et al., 2002; Kumar et al., 2015). Compared to chemical synthesis, the World Health Organization (WHO) advocates the inclusion of medicines from natural sources in national health programs because natural sources are much safer and more affordable than synthetic medicines (Ghosh et al., 2008; Dar et al., 2017). Plants, in particular, have long attracted the interest of researchers as they provide valuable raw material for drug discovery from natural sources (Samuelsson, 2004; Gurib-Fakim, 2006). About 270000 plant species have evolved over billions of years of evolution, and it is known that there are about 35,000 plant species used for the treatment of diseases, with only about 15% of the world's cultivated plant species being studied for their medicinal uses. Despite this low rate, 25% of the drugs used in modern medicine are of plant origin (Süntar, 2020).

One of these drugs is arteether (trade name Artemotil®), a potent antimalarial derived from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua* (Asteraceae), a plant used in traditional Chinese medicine (van Agtmael et al., 1999; Balunas and Kinghorn, 2005). Nitisinone (trade name Orfadin®) is a medicinal plant-derived drug that is active against the rare hereditary disease tyrosinemia (Frantz and Smith, 2003). Nitisinone was synthesized by modification of the herbicide mesotrione, which is produced from the compound leptospermone, a natural product of *Callistemon citrinus* (Myrtaceae) (Hall et al., 2001; Mitchell et al., 2001). Tiotropium (trade name Spiriva®) has recently been marketed in the United States for the treatment of chronic obstructive pulmonary disease (COPD) (Mundy and Kirkpatrick, 2004). Tiotropium is an inhaled anticholinergic bronchodilator based on ipratropium, an atropine derivative isolated from *Atropa belladonna* (Solanaceae) and other members of the Solanaceae family (Mundy and Kirkpatrick, 2004; Balunas and Kinghorn, 2005). Tiotropium has shown greater efficacy and longer-lasting effects compared with other available COPD medications (Mundy and Kirkpatrick, 2004).

Metformin, a derivative of natural products, is the main drug used in the treatment of type 2 diabetes mellitus (T2DM) and is prepared from the alkaloid aegiline, which is derived from the plant *Galega officinalis* (Fabaceae) (Bailey and Day, 2004). The structure of aegiline was confirmed by Barger and White in 1923. However, studies on diabetes in animals and humans have limited the use of aegiline due to the variety of therapeutic effects and short duration of action. As a result of the studies, the aegiline compound was modified and diguanidine compound was obtained by chemical synthesis. This compound prepared from aegiline was found to have significant blood glucose-lowering effect (Zhang et al., 2020). As a result of long-term, multicenter, large-scale randomized controlled clinical trials, diguanidine (trade name Metformin®) has become the drug of choice for the treatment of T2DM. Nearly eighty years after the discovery of aegiline, metformin was finally approved by the FDA for the treatment of T2DM in 1994. During the course of metformin's clinical use, other effects were discovered, including cardiovascular protection, antitumor activity, and blood glucose-lowering activity.

Today, metformin is also used to treat thyroid disorders, to treat polycystic ovary syndrome (PCOS), and to prevent bone fractures. The discovery of metformin is a good example of the discovery and development of drugs based on the therapeutic effect of natural products through structural modification and chemical synthesis (Zhang et al., 2020). Galantamine (trade name Reminyl®) is a natural product isolated from *Galanthus woronowii* (Amaryllidaceae) in Russia in the early 1950s (Heinrich and Teoh, 2004). Galantamine is an approved drug for the treatment of Alzheimer's disease because it slows the neurological degeneration process by inhibiting acetylcholinesterase (AChE) (Heinrich and Teoh, 2004). Vinflunine is a modification of vinblastine derived from the plant *Catharanthus roseus* (Apocynaceae) and is an anticancer agent with increased potency (Bonfil et al., 2002). Calanolide A is a natural dipyranocoumarin product isolated from *Calophyllum lanigerum var. austrocoriaceum* (Calophyllaceae) (Yu et al., 2003) and is an anti-HIV drug with a unique and specific mechanism of action as a non-nucleoside reverse transcriptase inhibitor (NNRTI) of type 1 HIV (Balunas and Kinghorn, 2005). All these examples and many more show how important plants are in drug discovery.

4. The Road to Medicine

Throughout history, herbal products have formed the basis of medicine, and even today most pharmaceutically and medically important compounds are derived from plant sources. There are a number of approaches that can be used to explore the potential for new medicines from plant sources, and all of these approaches are being used by large and small pharmaceutical companies to exploit the biological potential of plant products (Heinrich et al., 2017).

One of these approaches, the ethnobotanical approach, uses information about the use of a particular plant by an indigenous people to search for a drug precursor. In this case, observation of the use of a plant for a particular ailment, usually by a well-trained ethnobotanist, allows that plant to be collected and then tested for biological activity (Cox and Balick, 1994). In the chemotaxonomic approach, knowledge that a particular group of plants contains a particular class of natural products can be used to predict that taxonomically related plants might contain structurally similar compounds. This approach is particularly useful when the chemistry and biological activity of a compound are well defined and compounds with similar chemical structure are needed for further biological testing (Verpoorte, 1998). In the random approach, plants are collected regardless of knowledge of their chemical composition or biological activity. This approach is based on the availability of abundant plants in a given area. This approach is purely random, as random plant selection has a chance of providing access to extracts (and thus compounds) with biological activity (Katiyar et al., 2012). The knowledge-based approach uses a combination of ethnobotanical, chemotaxonomic, and random approaches, as well as a database containing all relevant information about a given plant species. The database is used to prioritize which plants to extract and screen for bioactivity. This approach is preferred by large organizations, such as pharmaceutical companies, interested in screening thousands or even hundreds of thousands of samples for bioactivity because avoiding repeated discovery of common or known drugs can reduce costs and save time (Patwardhan et al., 2004).

After determining the appropriate approach to search for plant drug sources, the first step is to collect the plant biomass. The collected biomass is then dried and extracted in a suitable organic solvent. The resulting extract is then analyzed to evaluate its biological activity (bioactivity). Screening or biological activity assessment is usually performed in two ways, depending on the number of extracts to be assessed (Katiyar et al., 2012; Heinrich et al., 2017). In low-throughput screening (LTS), a small number of extracts (from a single extract to hundreds of extracts) are analyzed in microplates or test tubes. This approach is widely used in academic laboratories where relatively few extracts are evaluated. High-throughput screening (HTS), on the other hand, typically involves thousands of extracts in multiwell microplates. This approach is preferred by the pharmaceutical industry.

This large-scale approach means that decisions about the status of an extract in the discovery process can be made quickly (Patwardhan et al., 2004; Katiyar et al., 2012; Heinrich et al., 2017). Extracts showing bioactivity as a result of analysis are fractionated using chromatographic techniques and biological activity is controlled at all stages until a pure active compound is obtained. The isolated bioactive product is subjected to a process known as cross-screening, and information is obtained on how selective the compound is, i.e., whether it is active in all assays or has specificity for a particular assay (Jachak and Saklani 2007; Heinrich et al., 2017). This is an important consideration because specificity is one of the criteria for selecting an agent for further development. In further biological evaluation, it will be necessary to determine the three-dimensional structure of the active molecule, as this will allow a search to determine whether the compound is novel, to which chemical class it belongs, and whether this type of compound has shown biological activity in the corresponding bioassay or another bioassay (Katiyar et al., 2012).

The precursor determined to have novel and potent biological activity is isolated in large quantities and it is decided whether the compound can be synthesized de novo or whether the chemical modification is required to improve biological activity. The precursor compound will undergo extensive in vivo studies for activity and toxicity research. First, preclinical experiments, known as animal experiments, are performed. A drug precursor will finally enter clinical trials after positive results from preclinical trials. This is the most comprehensive and most important evaluation stage of a drug candidate, as many drugs have failed at this stage due to toxicity or insufficient efficacy in humans. Successful completion of these trials usually results in the product being licensed, meaning that the compound is now a drug (Heinrich et al., 2017). Figure 6 schematics all processes to obtain the drug from therapeutic plants.

Given the complexity of the process described above, it is not surprising that many drugs of natural origin fail to enter the market. By some estimates, only 1 in 10,000 drugs are thought to actually enter the market. The process is very long and can take 12-15 years from the collection of the original biomass to the issuance of a new natural product-derived drug (Heinrich et al., 2017).

5. Conclusion

Plants have been used for centuries, both traditionally and commercially, as medicine to treat many diseases because they produce a variety of secondary metabolites with antibacterial, antiviral, antifungal, anticancer, antioxidant, antidiabetic, antimalarial, neuroprotective, and cardioprotective effects (Beppe et al., 2014; Afsheen et al., 2018; Governa et al., 2018; Reichling, 2018; Kamble and Gacche, 2019). The interest in exploring new drugs and compounds from plants is increasing day by day.

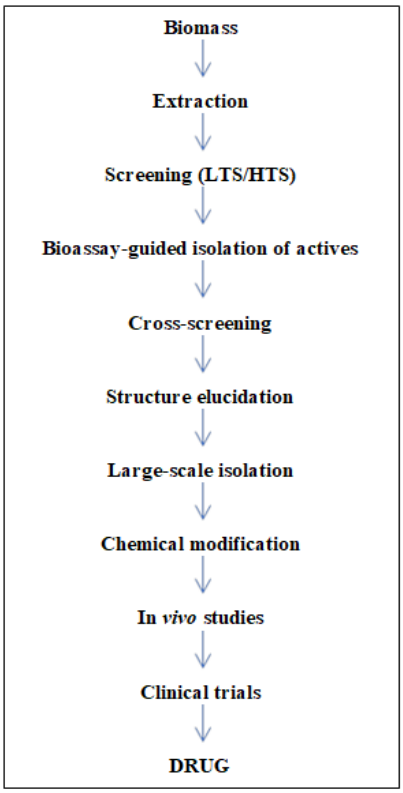

Figure 6. The process of obtaining drug from therapeutic plants.

Reasons for this include the fact that synthetic drugs used in the treatment of many diseases do not have the desired effect, cause serious side effects, some diseases cannot be treated until today, especially the negative effects of many chemotherapeutic drugs used in cancer on healthy cells and tissues, the positive attitude of Western countries towards natural medicines, and the increasing demand for herbal medicines (Klein et al., 2005).

There are 270000 tall stem plants in the world, and while humanity uses only about 70000 plants, the number of unused plants is 200000, and about 35000 of the used plants are used for therapeutic purposes. In our country, only 650 out of 13 thousand plant taxa have been defined as medicinal plants (Arslan, 2016). Considering both the world and our country, it would not be wrong to say that plants are a very rich source for discovering new medicines. If this wealth is properly utilised, safer and more effective drugs will take their place in the global pharmaceutical market.

Author Contributions

H.A. (100%) The idea of researching the article, obtaining the data, comments and writing of the article. E.Y. (100%) article edited. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Review
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AN OVERVIEW OF HAPLOID AND DOUBLE HAPLOID PRODUCTION METHODS IN WHEAT

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Abstract: For about a century, researchers have been working on haploidy approach. Progressively, they discovered the importance and usefulness of haploids in various research fields. On the other hand, it is suggested that climate change impacts on crop production, especially wheat, requires rapid and efficient methods of plant breeding to produce new cultivars with a sufficient level of biotic and abiotic tolerance to avoid significant production loss. Haploid plants are the source for producing homozygous pure lines and genetic variability for breeding programs. It reduces the time for producing pure and stable forms of new recombination by half in plant breeding. Furthermore, haploids are source for exclusive male plants generation, induction of mutations, stress resistance and tolerance cultivars, cytogenetic studies, and doubled haploid crops. This review presents a brief overview of the haploid wheat production methods and previous successful experiments on producing haploid wheat.

Keywords: Anther culture, Gynogenesis, Microspore culture, Wheat haploid

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1. Introduction

A plant with gametic chromosomal number is referred as haploid plant (from the Greek word *haplous*, which means single). They might arise naturally or as a result of different induction procedures (Watts et al., 2018). This phenomenon can occur spontaneously in the nature but it is rare or can be induced by *in vitro* or *in vivo* methods (Dwivedi et al., 2015). Haploid plants were discovered for the first time in 1922 by Blakeslee and his colleagues when studying *Datura stramonium*. In 1964, Guha and Maheshwari produced *in vitro Datura* haploid plants from anthers which increased the potential of haploidy in plant breeding. Many reports were published since then on many other plant species and for producing new cultivars. Many characteristics separate haploid plants from their diploid equivalents. Haploid plants have small size, narrow leaves, and relatively slow growth, which is partially due to their decreased cell size; in general, cell volume in plants is directly related to their ploidy level (Dunwell, 2010).

In wheat, haploid plants can be obtained from diploid or polyploid species. Haploid plants originated from *Triticum monococcum* L. (Einkorn wheat, diploid) have 2n = x = 7 chromosomes, whereas a haploid derived from a polyploid such as *Triticum turgidum* L., (durum wheat) (2n = 4x = 28; AABB) or *Triticum aestivum* L., (bread wheat) (2n = 6x = 42; AABBDD) called polyhaploid. Thus, their polyhaploid has 2n = 2x = 14 chromosomes with the genomic composition of AB, and 2n = 3x = 21 chromosomes with the genomic composition of ABD, for *Triticum turgidum* L. and *Triticum aestivum* L. respectively (Basu et al., 2011).

Haploid plants provide valuable information regarding recombination and genetic control of chromosomal pairing. Perhaps the most significant use of haploid plants was for crop improvement and doubled haploid production in plant breeding, which significantly shorten breeding cycles through the simultaneous genetic fixation at every locus within a single generational step (Kalinowska et al., 2019).

2. Methods of Haploid Plant Production

For many years, haploids have been presented for genetic studies. They were mostly obtained spontaneously from interspecific hybridization or by irradiated pollen, but usually only infrequently and in very small numbers. However, this situation changed after Guha and Maheshwari (1964) discovered that haploid plants could be obtained on a regular basis and in relatively large numbers by placing immature anthers of Datura into *in vitro* culture. The ability to produce haploid plants is a tremendous asset in genetic and plant

breeding studies.

Haploids can occur spontaneously or be induced by *in vivo* (chromosome elimination, intra specific hybridization, and inter specific hybridization) or *in vitro* (male or female gametes, or from unfertilized eggs). Currently, the main methods applicable for haploid production are androgenesis (cultured anthers or isolated microspores undergo embryogenesis/organogenesis directly or through intermediate callus), gynogenesis (cultured unfertilized isolated ovules, ovaries of flower buds, develop embryos from cells of the embryo sac), parthenogenesis (development of an embryo by pseudogamy, semigamy or apogamy), and wide hybridization followed by chromosome elimination from one of the parents. Generally, these methods require several main steps; starting with the induction of microspore derived embryos or calluses, then regenerate the plant either by embryogenesis (from embryos) or organogenesis (initiation and growth of shoots then root develop) and the final step is chromosome doubling of regenerated plants (Kim and Baenziger, 2005).

As doubled haploids become essential in plant breeding, considerable efforts to improve haploid embryogenesis and increase the frequency of recovery has been made. Puolimatka and Pauk (2000) explored different induction medium, induction period, and physical state of medium for improving spring wheat anther culture efficiency. For this purpose, two W 14 modified medium (double layer medium W14dl and Ficoll supplement W14f,), four different carbohydrates (sorbitol, maltose, trehalose, and cellobiose), and four glutamine concentrations (2.5, 5, 7.5, and or 10 mmol l^{-1}) were used for studying the androgenic response of two wheat cultivars. These were tested for induction durations of 6, 7, 8 and 12 weeks after isolation of the anthers. Based on the findings, embryo-like structures should be transferred to regeneration medium after 7 weeks of induction which was the optimum duration for obtaining maximum regeneration. However, medium supplemented with Ficoll, trehalose, cellobiose, maltose glutamine had no significant effect on studied parameters.

Xynias et al. (2001) cultured anthers of two spring wheat cultivars (Vergina and Acheloos) for haploid production. Spikes with microspore at mid to late uninucleate developmental stage were collected, half of these were pre-treated with cold (4 °C) for one week, then all samples were transferred to W14 induction medium and incubated at 28 °C and 32 °C. Experiment results suggested that genotype affects haploid production of spring wheat rather than temperature of incubation, also cold pre-treatment is not always required. In contrast, Sibi et al. (2001) claimed from findings of two seasons conducted experiment that most of the durum wheat genotypes studied had a significant interaction between genotype, induction medium and the duration of cold pre-treatment. They cultured unpollinated ovaries of six durum wheat genotypes (Sarif, Isly, Cham1, Jori, Oued, Cocorit, and Zénati) in two different induction media after cold pre-treatment at 4 °C in a cold chamber for 7, 11 or 15 days under two photoperiods (12 and 16 hours). Results showed that cold pre-treatment was necessary for tillers before anthesis for 7 or 15 days.

Zheng et al. (2001) used freshly isolated microspores as a method for producing wheat haploid and doubled haploid plants. In the experiment, six wheat cultivars (Pavon 76, WPB 926, Chris, Waldron, WED 202, and Calorwa) were grown at controlled greenhouse. From fresh tillers, microspores at mid to late uninucleate stage were isolated and then treated with four concentrations (0.06, 0.12, 0.18, and 0.30 mM) of inducer chemical solution (2-hydroxynicotinic acid, 2,3-pyridine carboxylic acid, 2,4-dihydroxy pyrimidine 5-carboxylic acid, 3-butanedione monoxime, benzotriazole-5-carboxylic acid, sulfanilamide, anthranilic acid, DL-histidine, benzotriazole, violuric acid monohydrate) for a short time. These chemicals were used to improve plant regeneration through triggering microspore embryogenesis and maintaining viability. The suggested method was easy and direct in determining the potential of the used chemicals for inducing embryogenesis. The method increased the survival rate of the fresh microspores when treated with 0.18 mM 2-hydroxynicotinic acid.

In durum wheat, genotypic variations in anther culture are well recognized and the medium for anther culture seems to be critical for haploid plant regeneration as Jauhar (2003) claimed. Furthermore, interactions between genotypes and growth conditions, as well as genotypes and medium, were also significant. Immature spikes were harvested and treated with cold, then sterilized and placed in water to prevent drying. Spikelets located at the middle of the collected spikes were inspected under light microscope to determine microspores developmental stage. Only those at mid uninucleate stage were cultured in different media at 25 °C. Comparing four different culturing media (BAC-1, BAD-I, BAD-3 and M-42), which were developed by previous scholars, showed that anther response was higher to the media BAC-1.

García-Llamas et al. (2004) examined the influence of different applications of hormone combinations and concentrations on caryopses, embryos and haploid production of durum wheat and maize intergeneric hybridization. Before flowering stage, tillers carrying emasculated spikes were cut off, sprayed with fungicide and insecticide, and placed in 40 g/l sucrose, 100 mg/l silver nitrate and 8 ml/l sulphurous acid solution then transferred to controlled growth chamber. Emasculated spikes were pollinated and placed in hormone containing medium for 48 hours. The hormone treatments were; (1) 100 mg l^{-1} 2,4-D (2) 5 mg^{-1} or 50 mg^{-1} dicamba (3) 95 mg^{-1} 2,4-D with 5 mg^{-1} dicamba (4) 50 mg^{-1} 2,4-D + 50 mg^{-1} dicamba. Later they were transferred to a hormone free medium, and caryopses number, embryos and haploid plants were counted. The authors stated that applying

dicamba with 2,4-D or only dicamba enhanced haploid plant production using the crosses of durum wheat with maize.

An enhanced wheat haploid production method using anther culture was suggested by Kim and Baenziger (2005) in which plants were grown from microspore derived embryos in a single medium and culture environment. For this reason, several experiments were carried out comparing different auxins, phenylacetic acid levels, various cytokinin types and different incubation conditions. In the first experiment, anthers were cultured in a modified C17 medium with five different auxins (9 mM 2,4-D, 9 mM dicamba, 8.3 mM picloram, 11.4 mM IAA, and 14.7 mM PAA). In the second experiment, anthers were cultured in a modified 85D12 basal medium with four different PAA concentrations (7.3, 14.7, 29.3, and 58.7 mM). Three types of cytokinin (kinetin 4.6 mM, zeatin 4.6 mM, and 6-benzylaminopurine 4.4 mM) were compared in experiment three. In experiment four, different incubation conditions were evaluated at 27°C and 30°C in the dark, and 30°C/12 hours dayligth. Finally, the haploid production protocol was improved by pre-treating the tillers at 4°C for 7 to 14 days, then placing the anthers on a modified 85D12 basal medium supplemented with zeatin and phenylacetic acid. The embryos from microspores were obtained in 2–3 weeks and after 3-4 weeks after culturing plants were produced.

Letarte et al. (2006) examined the effect of gum arabic (arabinogalactan protein) and Larcoll (arabinogalactan) to enhance wheat embryogenesis induction using microspore culture. The spikes containing anthers of two wheat varieties (Pavon 79 and Chris) were pre-treated at 4°C with cold mannitol (0.4 M), then microspores were isolated and culture in modified MS medium. The medium contained maltose (90 g l^{-1}), U2.5 amino acid mixture (355 mg l^{-1}), glutamine (975 mg l^{-1}), kinetin (0.5 mg l^{-1}), and PAA (2 g l^{-1}) with different concentrations of Larcoll or gum Arabic (1, 5, 10, 25, 50, or 100 mg l^{-1}). When the size of the embryo like structures were near 2 mm, they were placed on MMS5 medium containing maltose (30 g l^{-1}), U2.5 amino acid mixture (355 mg l^{-1}), GA3 (0.5 mg l^{-1}), kinetin (0.2 mg l^{-1}), PAA (0.5 mg l^{-1}), and $CuSO_4$ (10 mM) for differentiation. The researchers found positive effects of both compounds on the microspore viability and in embryo quantity and quality as well. Also, wheat plants from microspore cultures were generated by using gum arabic without ovaries presence.

Pratap et al. (2006) found that haploid induction of triticale x wheat hybrids and intergenotypic triticale was more efficient and economically valuable using chromosome elimination technique rather than anther culture. They conducted crosses under field conditions among 8 wheat cultivars (Raj 3702, HS 396, PW 565, VL 798, RL 14-1, VL 802, UP 2418, and HPW 42) with 10 triticale genotypes (TL 2919, ITSN 109, TL 2920, DT 126, ITSN 163, TL 2900, DT 123, ITSN 105 #58, ITSN 65, and DT 123,). The selection of the crossed cultivars was based on their parental diversity, yield traits, quality traits of grain, and resistance to disease. From each cross, main spike was selected and cut at the mid- to late uninucleate stage of the pollen development and pre-treated with cold at 4 ◦C for 48 hours before culturing. Then the excised anthers were cultured on Potato-II medium containing glutamine (0.5 mg l^{-1}) and supplemented with 2,4-D (2 mg l^{-1}) and kinetin (0.5 mg l^{-1}). The results indicated that the frequency of haploid embryo formation for the first-generation genotypes was 20.4% and 17.0% for triticale x wheat and triticale x triticale crosses respectively using chromosome elimination technique through crossing with maize. Furthermore, the frequencies of haploid plantlet regeneration were significantly higher, 42.7% and 49.4% for both triticale x wheat and triticale x triticale for the first-generation genotypes, while these values were 8.2 and 4.0% using anther culture method.

An efficient system for producing haploid/doubled haploid wheat using microspore culture without stress was reported by Shariatpanahi et al. (2006). In this system, tillers with late-unicellular to the pre-mitotic stage microspores were harvested and cold treatment was not applied to the tillers. The microspores were isolated using Shed microspore culture (SMC) (culturing in starvation medium), and the culture of freshly isolated microspores without stress (WM), and the same regeneration condition was used for both systems. A significant increase was recorded in the frequency of regeneration and green plants percentage using culture of freshly isolated microspores without stress (WM) method comparing to Shed microspore culture (SMC).

Ovary co-culture method was demonstrated by Broughton (2008) for Australian spring wheat as protocol for anther culture. Anthers at mid to late and late uninucleate microspores development stage of the two spring wheat varieties were incubated in liquid induction medium containing ovaries. The medium was supplemented with 0.5 mg l^{-1} Kinetin and 2 mg l^{-1} 2,4-D or 1 mg l^{-1} 6-Benzyladenine (BA) and 1 mg l^{-1} Indole-3-acetic acid (IAA). Results specified that using ovary co-culture method had a significant effect on the embryo-like structures and green plants production in Australian spring wheat varieties. Mean number raised from 7.6 to 50.1 and from 0.6 to 8.9 for embryo-like structures and green plants respectively in induction medium containing five ovaries. Furthermore, these findings lead to the development of a protocol for Australian spring wheat anther culture which is used in small scale breeding program.

Using butanol alcohol was claimed to enhance anther culture production in wheat. Soriano et al. (2008) tested the effect of *n*-butanol, sec-butanol and tert-butanol on microspore embryogenesis of two *Triticum aestivum* varieties in two experiments. After determining microspores development stage, pre-treated microspores at the mid to late uninucleate stage were inoculated in 2

ml liquid MS3M with 0.1% or 0.2% *n*-butanol (experiment 1), and with 0.2% tert-butanol, secbutanol or *n*-butanol (experiment 2). For each experiment, the number of responsive anthers, callus, embryo, green and albino plants per 100 anthers, and chromosome doubling percentage were recorded. Statistical analysis showed a strong genotypic and treatment effect on the responsive anthers number, divisions, embryos and green plants. Treatment with 0.1 and 0.2 % *n*-butanol triggers microspore division and embryogenesis. Thus, strong embryos and green plants can be produced.

Broughton (2011) reported similar effect of *n*-butanol with adding macronutrients and calcium to the mannitol pre-treatment medium, improving embryo and green plant production. In the experiment, anthers of Australian spring wheat cultivars were placed on two different medium; the first medium contained 182 g l^{-1} mannitol and 10 g l^{-1} agar, and the second medium contained 5.9 g l^{-1} CaCl2.2H2O, macronutrients, 182 g l^{-1} mannitol and 10 g l^{-1} agar. Then, anthers were divided between 0.2% *n*-butanol and without n-butanol (control) and both treatments were directly placed on the ovary conditioned in liquid induction medium plus ovaries. A significant increase in number of embryos and green plants was observed when combining *n*-butanol with calcium and macronutrient addition to the mannitol pre-treatment medium.

Sourour et al. (2011) implemented three experiments on different concentrations of $AgNO_3$, 2,4-D and their combination to originate an effective method of haploid production through intergeneric cross. They crossed two landraces and two durum wheat cultivars (as female parent) with a maize genotype under field grown conditions, and these pollinated spikes with maize pollens (male parent) were cultured in a solution supplemented with 8 ml l^{-1} of sulphurous acid and 40 g l^{-1} sucrose. Three different experiments were conducted on 10 spikes from each cross after 12, 14, 16, 18 and 20 days of pollination to examine the effects of applying $AgNO_3$ at different concentrations (0, 25, 50, 75, 100, 125, 150, 175 mg l^{-1}), 2,4-D (0, 25, 50, 75, 100, 125, 150, 175 mg l^{-1}), and a combination of 100 mg l^{-1} of 2,4-D + 75 mg l^{-1} of $AgNO_3$. The frequency of developed ovaries, formed embryos, and haploid plants were recorded for each experiment. The researcher claimed that the method is efficient for plant regeneration from durum wheat × maize crosses as 877 plants were regenerated and all obtained haploid plants were green. In addition, the results indicated that higher numbers of embryo and haploid plants were obtained from the combination of the two compounds.

Microbial contamination is a major concern in microspore culture which affects the success of the whole process. Asif et al. (2013a) examined contamination using two antibiotics, cefotaxime and vancomycin, to enhance anther culture during the induction phase. Spike of two wheat cultivars and one triticale cultivar were sterilized and from each, four ovaries placed in petri dishes with microspores at mid to late uninucleate microspore development stage. These microspores were treated with different concentrations of antibiotics (Van 100 $mg^{-1}l$, Van 500 $mg^{-1}l$, Cef 50 $mg^{-1}l$, Cef 100 $mg^{-1}l$, Van 100 $mg^{-1}l$ + Cef 50 $mg^{-1}l$, and Van 500 mg/l + Cef 100 mg/l). After incubating for 20-30 days at 28 ^{o}C at dark, microspore derived multicellular structures were observed and contamination signs were checked daily. Moreover, the number of embryos or embryolike structures, albino and green plants were recorded. Through fatty acid analysis and 16S ribosomal RNA sequences analysis, contamination with five bacteria species and yeast was recognized. The analyzed data showed that Cefotaxime at 50 and 100 mg/l besides preventing contamination also improved microspore culture, since the number of microspore derived embryo like structures and the ratio of green plants increased.

The importance and the role of antioxidants in green plant production frequency and albinism by isolated microspore culture was investigated by Asif et al. (2013b) on the embryogenesis or embryo development. For this purpose, two antioxidants with different concentrations were supplemented in the NPB99-10F induction medium. Plastid antioxidants treatments were glutathione, ascorbate and salicylic acid at 200 nM (1×) and 2 µM (10×), and mitochondrial antioxidants 10 mM proline, 10 nM MB, 100 µM NtBHA, 100 µM 2iP, and 100 µM of 2iP + 10 mM of proline + 10 nM MB + 100 µM NtBHA. Microspores from spikes of four spring wheat cultivars and one triticale cultivar were isolated and used in each experiment to study the effect of the two antioxidants supplemented in the induction medium. At 10 to 14 days, multicellular structures and embryos development was verified, and the determination of embryos or embryo like structures number was made after 3 to 4 weeks of isolation, green and albino plants number determination was made after the embryo germination. The study concluded that adding glutathione and proline to the culture medium increased the embryo and green plant formation.

The effect of cold pre-treatment and genotypic effect on microspore culture was researched by Khound et al. (2013). Anthers were dissected from spikes containing mid to late uninucleated stage microspores of three spring and three winter wheat cultivars and incubated at 25 ^{o}C - 28 ^{o}C for 4 - 5 days in dark, then for another 5 days at 4 ^{o}C for cold pre-treatment. Those microspores were incubated in the dark at 27-28˚C for 25 to 30 days after co culturing in induction medium (MMS4) containing ovaries (5–7). It was concluded that for both winter and spring wheat cultivars the number of multicellular structures, transferable embryos and green plants raised with cold pre-treatment in comparison to the control treatment.

Scagliusi (2014) setup a protocol for isolated microspore culture for Brazilian bread wheat genotypes suggesting that the method can be used in the Brazilian wheat breeding program. In the experiment, spikes of three

wheat genotypes were pre-treated with cold at 4 °C for 3 weeks in the dark, then obtained uninucleated microspores were cultured in petri dishes containing semi-liquid NPB 99 media, and finally, four ovaries were added to each petri dish. The evaluation of the embryo like structures were made on a daily base for gametic source confirmation, and green and albino plants number per genotype was recorded. The method recommended that induction medium along with ovary co-culture were essential to promote microspore culture. Furthermore, the influence of genotypic variation among wheat genotypes for microspore culture cannot be ignored.

Gupta et al. (2016) conducted various wheat x maize crosses to standardize a haploid production protocol. Five commercial wheat cultivars were used in wheat x maize haploid production and nine F1 lines were used for developing homozygous lines. In the crosses they carried out, spikes were pollinated after emasculation with fresh maize pollen after 3 to 4 days. Then, spikes were treated after 24, 48, 72 hours of pollination with 200 ppm 2,4-D to sustain embryo formation. Dissected embryos from caryopses were transferred to half strength MS medium supplemented with 40 g l^{-1} sucrose and solidified with 3g l^{-1} phytagel having pH 5.8, and treated with cold for 8 hours then incubated in the dark. After germination, regenerated plants were kept at 25˚C with 8-10 hours photoperiod. Finally, the developed haploids were placed for 30 days in hardening medium. The recorded data from the plants were the caryopses formation frequency, embryo formation frequency and plantlet regeneration frequency. Results indicated that in case of cultivars the range of caryopses formation frequency, embryo formation frequency, and plant regeneration frequency ranged between 25.9 to 51.4 %, 4.6 to 22.4 %, and 6.4 to 63.6 % respectively. For the F1 corn lines, the range was 21.4 to 60.5%, 1.3 to 21.2 % and 9.8 to 44.4 % for caryopses formation frequency, embryo formation frequency, and plant regeneration frequency respectively**.** In total 100 haploid plants were regenerated.

Lantos and Pauk (2016) compared anther culture of 10 winter wheat F1 combinations using two media in order to examine the genotypic effect and induction medium on the anther culture efficiency. Tillers were pre-treated for 2 weeks at 2 to 4 °C, anthers collected from tillers at early and mid uninucleated stages and cultured in W14mf and P4mf medium. Anthers within each medium were treated with heat shock (32 °C) for three days, and then incubated for 8 weeks at 28 °C. Every week, observations were made for the microspore derived embryo like structures and for embryo like structures. Green plantlets, albinos, and transplanted plantlets were measured for anther culture efficiency. The results indicated significant effect of the genotypes and induction medium on all measured parameters. Using W14mf medium resulted in increasing green plant regeneration compared to P4mf medium, 16.9% and 9.6% respectively. Comparing both media, the number of produced embryo like structures using P4mf medium was higher than W14mf medium, 48.84 and 28.14 embryo like structures per 100 anthers, respectively. Furthermore, green plantlets production using P4mf medium was 4.82 per 100 anthers which was higher than W14mf medium, 4.59 plantlets per 100 anthers.

In order to compare and determine the most efficient methods to produce durum wheat haploids, Slama-Ayed et al. (2019) started a study on two durum wheat cultivars, one landrace, and a maize genotype. They tested gynogenesis, isolated microspores culture, and intergeneric wheat x maize crosses for selecting the best haploid production method. In the isolated microspore culture method, tillers were pre-treated for 5 weeks at 4 °C then microspores were cultured in CHB3 medium, and before incubation at 24 °C immature ovaries were added to the culture. Then obtained embryos were transferred to growth regulators free MS medium and incubated, and regenerated plantlets number was recorded 2 weeks after transfer. For the gynogenesis method, tillers were pre-treated for 2 weeks at 4 °C and 1 to 1.5 mm ovaries were cultured for 5 to 6 weeks at 27 °C. Then calli were transferred to a differentiation medium. In the final method, three wheat cultivars were crossed with a maize genotype, and tillers with pollinated spikes were collected and cultured in 8 ml l^{-1} H_2SO_3, 40 g l^{-1} sucrose, 75 mg $^{-1}$l $AgNO_3$ and 100 mg l^{-1} 2,4-D. The grown embryos were cultured in B5 medium in growth chamber until germination. Based on the findings, it was suggested that induction, embryogenesis and microspore regeneration were the most important steps using isolated microspore culture method. Whereas for gynogenesis and interspecific crosses embryo or callus forming, and regeneration were critical. They claimed that gynogenesis as a method for producing durum wheat haploid was promising.

Wang et al. (2019) believed that the existing methods for microspore culture in wheat, especially winter types, are still insufficient as a routine application. Therefore, providing an effective procedure for microspore culture is essential. Through testing pre-treatments, maltose gradients, and histone deacetylase inhibitors there might be a chance to raise the frequency of microspore embryogenesis and improve the generation of fertile green plants. Findings of evaluating a number of wheat genotypes showed that spike cold pre-treatment for 21 and 28 days was optimum for spring and winter wheat respectively. Moreover, embryogenesis and/or green plant regeneration improved when trichostatin A was applied. Previously, Jiang et al. (2017) reported similar effect when using trichostatin A. In the experiment, different concentrations of trichostatin A (0, 0.1, 0.3, 0.5, and 10 μM) was compared on microspores culture of spring wheat. It was found that exposing microspores to 0.1μM of trichostatin A raised the yield of microspore-derived green haploid plants.

Orłowska et al. (2020) mentioned that regeneration of green plants from anther culture is affected by three

factors, the length of induction step, the concentration of silver nitrate, and the concentration of copper sulphate. They cultured anthers in a semi-solid induction medium with 2 mg l^{-1} 2,4-D and 0.5 mg l^{-1} 190–2 medium kinetin supplemented, and the regeneration medium (90–2 medium) supplemented with 0.5 mg l^{-1} NAA and 1.5 mg l^{-1} kinetin. For examining these three factors they used the Taguchi method in nine trials. They indicate that wheat prefers low concentration of copper sulphate (0.1µM) and high concentration of silver nitrate (60 µM) to produce higher number of green regenerants.

3. Conclusion

Studies have proved the importance of haploid wheat, and attempts to establish a simple haploid production procedure have been made in the past. In addition, the role of haploids in breeding programs for wheat improvement and double haploid production has been defined. However, additional research into the factors that influence the effectiveness of haploid production methods is needed. Studies on determining the optimum period for selecting ovaries and microspores is important, as well as studying cold pre-treatment duration and temperature. Adding supplemental compounds to the culturing medium, such as antibiotics or hormones, affects the plant regeneration. Finally, genotypic variation should be considered, since it has been confirmed that the generation of haploid plants is more genotype dependent than the incubation conditions for a certain technique.

Author Contributions

N.M.B. (%25), S.S. (%25), M.Y. (%25) and S.L. (%25) review and editing. N.M.B. (%25), S.S. (%25), M.Y. (%25) and S.L. (%25) original draft preparation. N.M.B. (%25), S.S. (%25), M.Y. (%25) and S.L. (%25) writing up. N.M.B. (%25), S.S. (%25), M.Y. (%25) and S.L. (%25) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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