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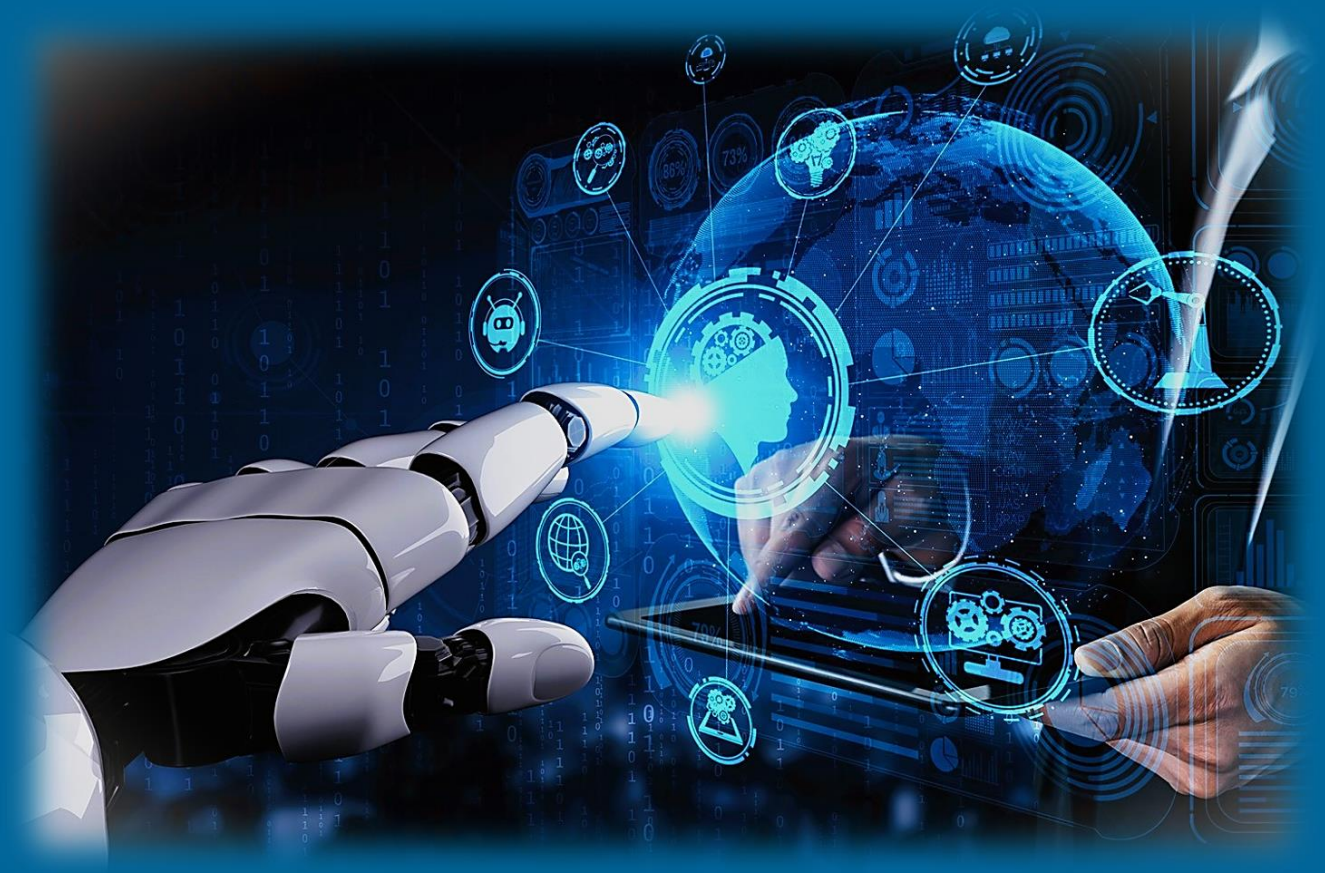
BİLGE INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY RESEARCH

Year / Yıl 2024 | Volume / Cilt 8 | Number / Sayı 1

p-ISSN: 2651-401X
e-ISSN: 2651-4028



BİLGE INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY RESEARCH

VOLUME:8 / ISSUE:1 / YEAR:2024
ISSN:2651-401X / e-ISSN:2651-4028

Press:
Kutbilge Association of Academicians
Distribution, Sales, Publisher;
Certificate No: 42086
32040, Isparta, Türkiye

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VOLUME:8 / ISSUE:1 / YEAR:2024
ISSN:2651-401X / e-ISSN:2651-4028

A peer reviewed international journal, published biannually by Kutbilge Association of Academicians.

The journal is indexed in **Index Copernicus, Crossref, AcademicKey, Araştırmax, CiteFactor, Eurasian Scientific Journal Index (ESJI), Infobase Index, ROAD, JIFACTOR, Rootindexing, Science Library Index, Cosmos Index, Directory of Research Journals Indexing (DRJI), International Institute of Organized Research (I2OR), Journal Factor, Google Scholar, Researchbib, Scientific Indexing Service (SIS), ISI (International Scientific Indexing), IPIndexing, ASOS Indeks, ScienceGate, Scilit, IJI Factor** and **ACAR Indeks.**

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VOLUME:8 / ISSUE:1 / YEAR:2024
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Research Articles

Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2024, Volume: 8, Issue: 1, 1-8
Received: 04.09.2023; Accepted: 16.01.2024
DOI: 10.30516/bilgesci.1354831

Investigation of Dynamic Behavior of Piled Pier Structure

Münire FINDIK*[1]

Abstract: In regions with advanced global trading, sea-borne transportation is preferred. Currently, most of the transportation volume is transported by means of sea transportation. Therefore ports are an important aspect of the transportation network. In our country, studies about offshore structure design has been increasing in the recent 15 years. Primarily, technical approaches and durability for designing offshore structures were prominent. However, recently, performance continuity has been one of the primary concerns. During their service life, static and dynamic loading is applied to these structures. The system should be analysed under static and dynamic loading with accurate parameter selection for a proper behavior estimation of the structure under seismic activity. For this purpose, in order to study the dynamic behaviour of a pier structure, changes in the parameters have been examined. For parameters; 600, 800 and 1000 mm pile diameter, 4D and 8D placement gap has been considered. Analysis has been carried out using SAP2000 finite element model program. Limit displacement values and structural capacity of the structure have been determined using pushover analyses. Based on the determined limit values, dynamic condition performance is evaluated using time-history analysis The maximum base shear force values obtained in the pile system for the seismic data and ground condition defined in the displacement-controlled analyzes did not exceed the linear limit. When the pile system behavior was evaluated in terms of seismic performance, the ground provided sufficient rigidity to the pile system for 80 cm diameter and 4D pile spacing. As a result, in all models, it has been observed that the first deformation interacts with the foundation system on the pile and in the first one meter section where it comes into contact with the ground, buckling and plastic deformation begins in the piles and this height intensifies as the ratio of the pile embedded in the ground increases.

Keywords: Pier Structure, soil- structures interactions, pile foundations, dynamic analysis

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Citation: Fındık, M. (2024). Investigation of Dynamic Behavior of Piled Pier Structure. Bilge International Journal of Science and Technology Research, 8(1): 1-8.

1. INTRODUCTION

The increasing needs of the countries and technological advancements have increased the demand for imports and exports. With it's economic advantages, high volume transportation capacity, and reach to more regions, Seaborne transportation is preferred for the most volume on the globe. With the need for energy increasing worldwide, structures like; oil searching and extracting platforms, drilling platforms, wind and sun energy facilities, and mooring structures that are designed with the superstructure, substructure to carry the platform and the foundation are gaining importance day by day (Chandrasekaran., 2015; Zhang et al., 2017; Yan et al., 2018). For our country, being a peninsula, the need for modern ports and docks has become prominent. Carrier systems for offshore structures like single pile, multiple pile, and framework type systems that can be produced with different materials are subjected to; wind, wave, currents, seismic activity (Pérez-collazo et al., 2015; Randolph and Gourvenec, 2017). In addition, under wave, wind and seismic activity effects, these structures need to safely maintain their performance level. For offshore structure design, soil bearing capacity, subsidence criteria, and seismic resistance should be considered. Because Turkey is in a seismically active region, the investigation of seismic behaviour of the offshore structures is important. Generally, offshore structures prefer piled foundation design to safely transfer the superstructure and lateral loads, wind, wave, and mooring, to the soil.

For the vertical load from the platform that makes up the superstructure, it is possible to create substructure systems safely with simple mathematical approaches. However, a commonly accepted design model is unavailable for lateral loaded piles. One of the main reasons for this is that when such piles are subjected to displacement, they display a

three-dimensional soil-structure interaction throughout their surface area (Zhang et al., 2017b).

In recent years, it was observed that damage occurred on most of the offshore structures is caused by instability of the seabed soil. In order to determine the design criteria and damage levels, the behaviour being affected by some parameters should be foreseen. Due to the nonhomogenous, nonlinear and anisotropic behaviour of soils, the system's complex sections, and the inertial and kinematical interaction of the structural elements with the soil, an analytical solution is generally impossible. Therefore, for such problems, numerical solutions are mostly preferred.

Kishida and Takwaki (2010), evaluated detailed seismic reactions for piled foundation systems with a three-dimensional finite element model. They observed that the seismic response of a piled foundation system is connected to the pile cap moment of inertia that is dependent on the kinematic effect.

Yüksel and Orhan (2013), have given insights about seismic risks of port structures and consequent possible losses. Yasser (2012), have examined the effect of local and spherical scouring near the offshore structures and bridge footings on the behaviour of lateral loaded piles. Especially in sandy soil, spherical scouring has increased the pile lateral displacement and bending moments. Therefore, the lateral load-carrying capacity of the piles has decreased. Due to the pile-soil system having nonlinear responses, the effect of scouring has been detected to be more significant with higher lateral loads. Kadıoğlu (2015), modelled piled pier model located on İzmit gulf and performed nonlinear push analysis. By changing angles of the inclined steel piles and comparing them to vertical piles, observed while there is a 14-times difference between their displacements, there were 1,5 times axial force and 1,8 times bending moment differences. It is suggested that inclined and vertical piles should be investigated with nonlinear dynamic analysis in time-history. Topsoy (2016), observed that the base shear force of vertically placed lateral loaded piled pier structure is proportional with R coefficient. It has been mentioned that soil and wind loads are also effective on pile edge forces like seismic forces. Panchaland et al. (2018), have investigated the group behaviour of pile groups that have placement gaps between 2D-6D and different numbers of piles, under seismic loads. Through numerical and experimental analyses it was determined that axial force on the pile increased as the pile length increased. However, it was observed that for the increase in the pile length, shear force and bending moment have decreased for all different gap conditions. Studies in the literature have shown that numerical methods give results that are closer to reality rather than analytical and empirical methods. Therefore, this study has carried out pushover and time-history dynamic analyses of piled offshore structure. In the context of this study, three different pile diameters; 60, 80, 100 cm, and 4D-8D two placement gaps have been utilised.

2. MATERIAL AND METHOD

This study investigates the dynamic behavior of a pier structure with a foundation with circular steel piles. For this purpose, 6 numerical models are created. For the model creation, pile diameters and placement gaps have been considered parameters. DLH (2008) defined pile gaps based on pile diameter and stated that pile gaps can be accepted between 3D-8D. Pile gaps relative to pile diameters have been selected in 4D and 8D. Pile diameters and pile wall thicknesses are as follows: D600x12 mm, D800x12 mm and D1000x12 mm. While 3 pile diameters were selected, 1 pile wall thickness has been considered. Piles are modeled frame members. The steel tube section material grade is S275. Parameters that determine the pile placement is presented in Figure 1. Here; L refers to platform length, V refers to platform width, L1 and L2 refers to pile gaps in x direction, V1 and V2 refer to pile gaps in the y direction. Pile gaps determine the plan placement of the pier foundation. Created model namings and dimensional parameters are presented in Table 1.

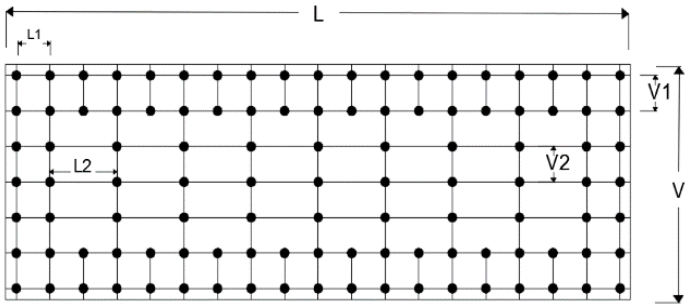


Figure 1. Plan placement of the piles used in numerical models

Pile count is varies throughout the models as can be seen in Table 1. In this context, M1 M2, M3, M4, M5 and M6 models have 128, 68, 99, 48,78 and 44 piles respectively. A pile cap that surrounds the piles from all directions with an 80 cm thick pile cap beam and 40 cm thick cap slab is defined and the superstructure is completed. The pile cap beam and slab are modelled shell element. Beam and slab material is defined as reinforced concrete. The concrete grade is selected C40 as indicated in the code (DLH, 2008). Pile lengths were assumed a constant value and accordingly with the inclined seabed, the buried depth has been defined as a parameter.

Table 1. Pile Diameters and Gaps

Model Number	Pile Diameter (mm)	Pile Spacing	L (m)	L1 (m)	L2 (m)	V (m)	V1 (m)	V2 (m)	Pile Count
M1	D600	4D	59,60	2,4	4,8	21,2	2,4	4,8	128
M2	D600	8D	59,60	4,8	9,6	21,2	2,4	4,8	68
M3	D800	4D	59,60	3,2	6,4	21,2	3,2	3,2	99
M4	D800	8D	59,60	6,4	12,8	21,2	3,2	6,4	48
M5	D1000	4D	58,0	4,0	8,0	22,0	4,0	4,0	78
M6	D1000	8D	58,0	4,0	16,0	22,0	4,0	4,0	44

Soil layers the piles are connected to are considered medium-density sand on coarse clay. For the defined soil layers, all the piles within the 26m are located in medium-dense sand. Soil-structure-pile interaction is investigated in this study, pile behaviour is investigated using the lateral bedding coefficient method (Feng et all, 2017; Yeter et all, 2019). Pile-soil interaction is defined in the numerical models using the spring coefficient. For the calculation of the spring coefficient, cohesionless medium-dense sand parameters and parameters for calculating bedding coefficients are presented in Table 2 and Table 3.

Table 2. Design parameters for cohesionless soil (Polat, 2008).

Soil	**E_s (kN/m^2)**	**ϕ**	**Y_d (kN/m^3)**	**v**
Medium-dense Sand	30000	35°	19	0,3

E_s:Modulus of Elasticity (Young's modulus)

ϕ:Effective shear resistance angle

Y_d: Soil saturated weight per unit of volume

v= Poisson ratio.

In sands, lateral bedding coefficient is proportional with the depth. In these soils, Kh (bedding coefficient, kN/m3) value is calculated using Equation 1 (Polat, 2008).

$$Kh = nh*(z/B) \quad (1)$$

In equation 1, nh refers to a coefficient related to soil density, z is depth (m), and B is pile diameter (m).

Table 3. Nh Values in Cohesionless Soils (DLH 2008)

Soil Stiffness	**On YASS nh (kN/m^3)**	**Under YASS nh (kN/m^3)**
Loose	2200	1300
Medium Density	6600	4400
Density	18000	11000

For structures that extend to sea from the shore, buried depth is heterogeneous due to the inclined seabed (Erkan et all, 2014). Pile buried depth varies between 9-15 m in numerical models. Springs representing bedding coefficients are defined in every 1 m interval in the Sap2000 program (CSI, SAP 2000). In numerical models, pile systems are defined with spring coefficients that are lateral boundary conditions in the x and y.

Three-dimensional views from the numerical models of the pier structure modelled for 4D and 8D arrangement are presented in Figure 2. Plan arrangement is of relevance to pile diameters and placement gaps.

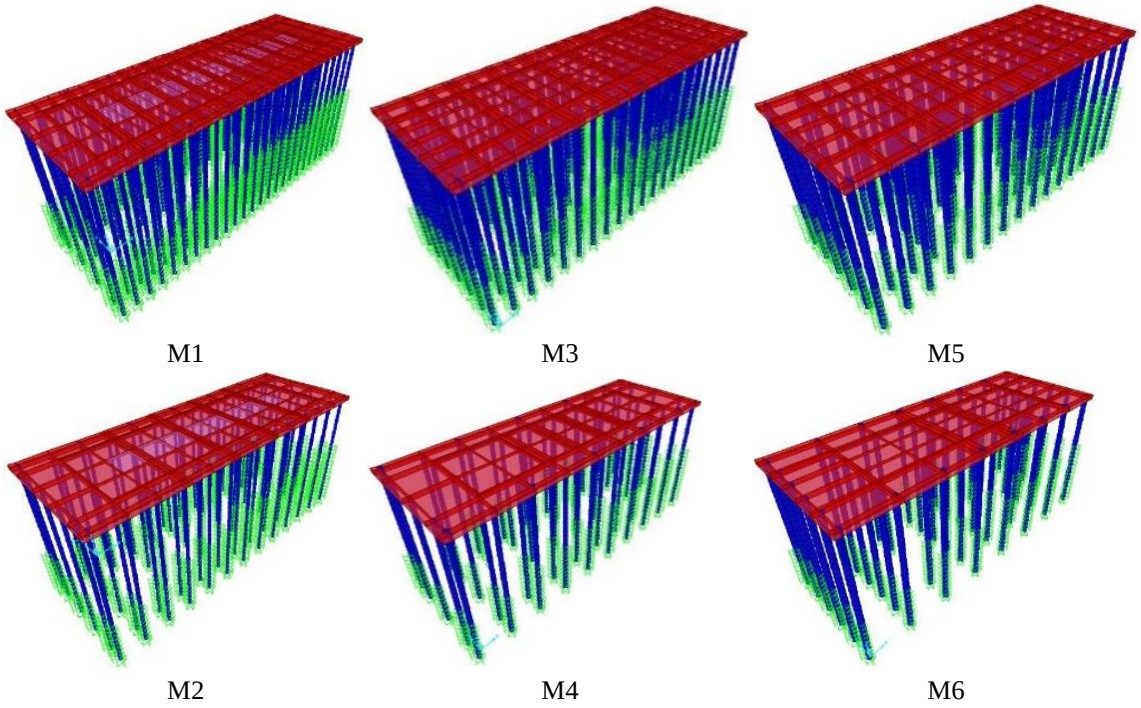


Figure 2. General views of the numerical models

Structural capacity is displayed by a pushover curve. In the study, displacement-controlled pushover analysis is carried out. In nonlinear pushover analysis, nonlinear material behaviour, plastic hinges, plastic rotation, distributed plastic behaviour model, strain values, performance point and structure performance is calculated automatically. For this performance analysis, "hinge" definitions are made on the piles. Internal forces caused by the affecting loads and section properties have determined the hinges. Therefore, columns are analyzed for "PMM" shear force and moment.

After performing a nonlinear static pushover analysis of the structure under dead weight, earthquake input for DD1 seismic condition is defined for X direction in the Sap2000 program under the time history tab. For a new analysis, nonlinear time history analysis is defined with Add New Loadcase. Kocaeli earthquake record is presented in Figure 3. In the figure, x axis refers to time while y axis refers to acceleration and x direction earthquake record is given with DDX.

Previously, pushover analysis was completed and hinges were defined. For nonlinear analysis, direct iteration method and initial condition have been selected. Spring coefficients that were used for soil representation are also used in the time history dynamic analysis.

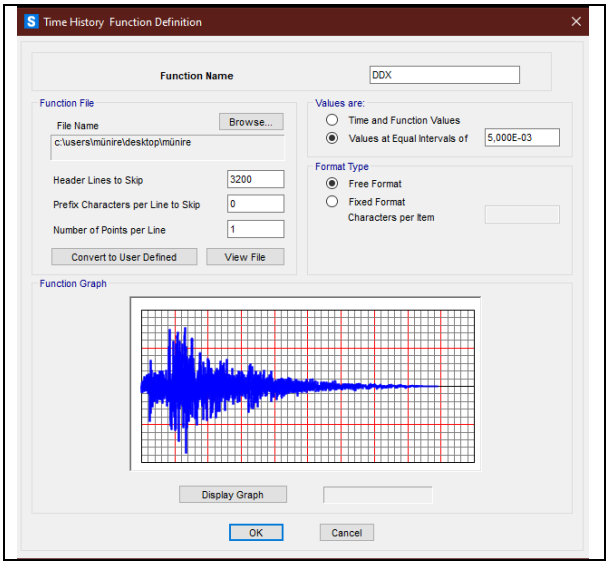


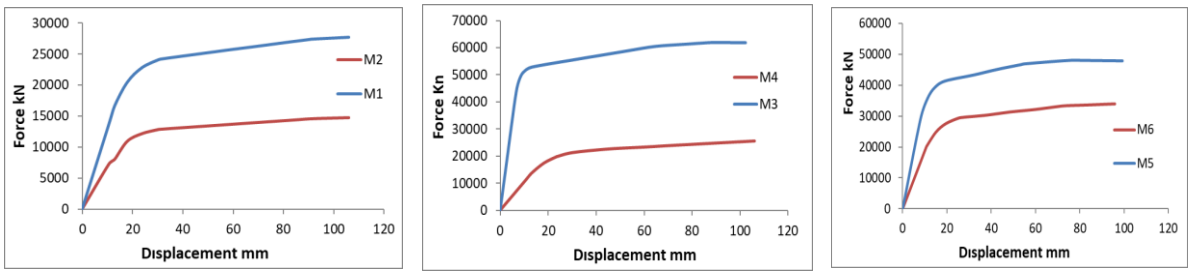
Figure 3. Earthquake record visual

3. DISCUSSION AND CONCLUSIONS

In this study, a performance analysis of a pile foundation system used in a pier-type offshore structure has been carried out. In this context, 600 mm, 800 mm 1000 mm diameter piles for 4D and 8D arrangement plan are numerically analysed. From the numerical analysis, seismic performance of the models under lateral load is obtained. Analyses are carried out using Sap2000 V.22 finite element model program.

For the performance evaluation of the piles, axial force, moment and shear force reactions are used. In this context, plastic hinges are defined on the piles at PM2M3 (Axial

force, Moment in 2 direction, Moment in 3 direction), V2 (2-direction shear force) and V3 (3-direction shear force), and load-displacement graphs are obtained from the analysis. In the definition, 2 and 3 directions refer to horizontal directions. Load-displacement graphs of the models are given separately based on the diameters in Figure 4.



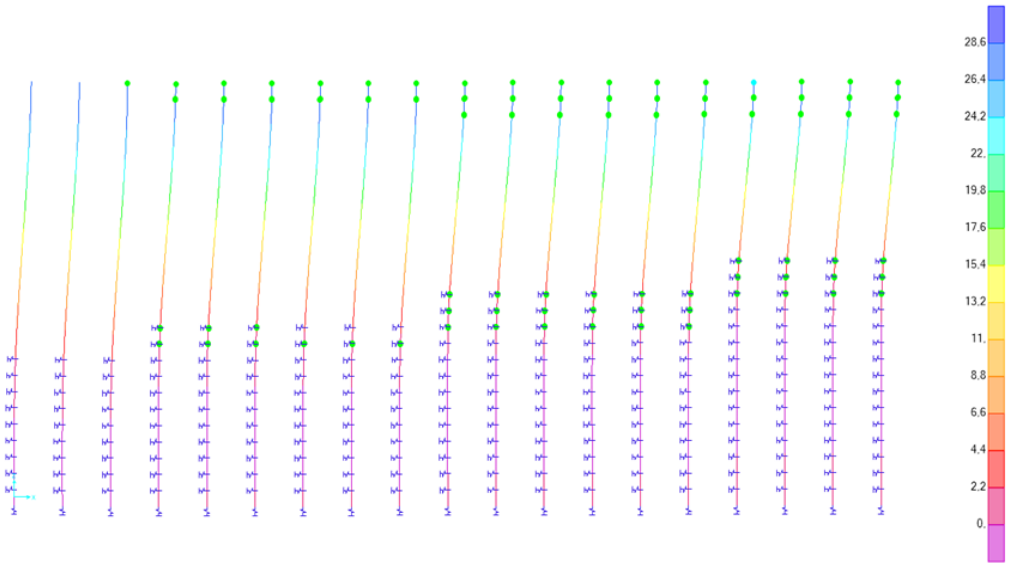
a) **b)** **c)**

Figure 4. Performance comparison of 4D and 8D arrangements a) 600 mm pile system behaviour, b) 800 mm pile system behaviour, c) 1000 mm pile system behaviour

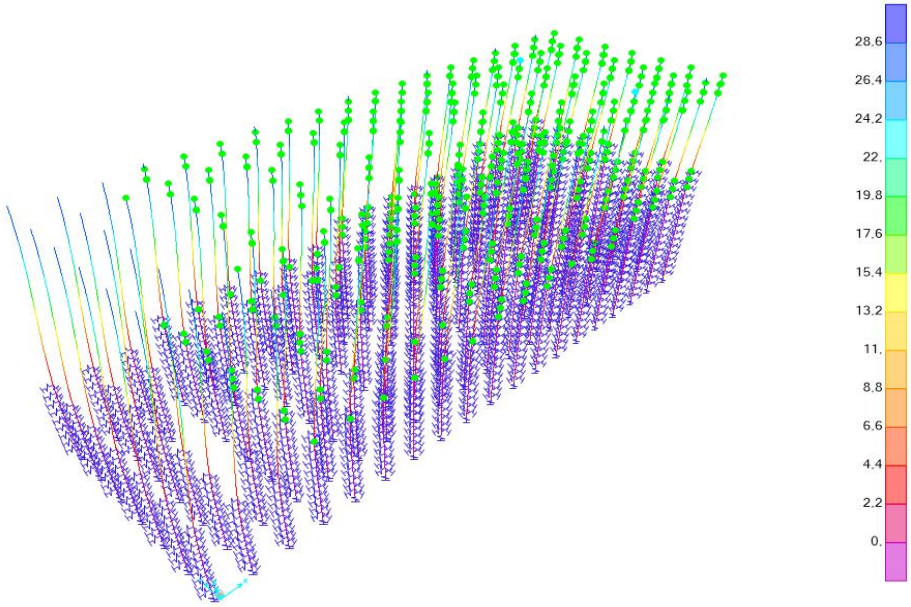
Capacity curves obtained from the analysis results are a results of pile behaviour integrated in the system. In this context, all the systems having sufficient stiffness and ductility can be seen. From the figure, it can be deduced that the 800 mm diameter condition has the highest performance level. For different pile gap conditions, 600 mm and 800 mm diameter models resulted with high efficiency. Performance levels of 600 mm diameter for 4D arrangement is approximately 2,5 times compared to 8D arrangement. The performance levels for 800 mm diameter for 4D to 8D conditions is approximately 3 times. For 1000 mm diameter, this value is approximately 1,25 times. M3 model, the model with 800 mm diameter and 4D arrangement, has given the most effective result from performance points for linear limit and 100 mm displacement mark.

For all pile diameters, in 4D arrangement models M1, M3 and M5, nonlinear dynamic analysis is carried out using scaled accelerogram data. In order to determine if the

displacements in the x direction has exceeded the linear limit, firstly, a nonlinear pushover analysis is carried out. Capacity curves given in Figure 4 shows linear and nonlinear limit (failure) conditions. The deformed condition for the M3 model is presented in Figure 5. From the figures, it can be seen that the plastic deformations have begun with the buckling level. The first deformation has occurred at the interaction zone of the upper foundation, piles and the soil and in the first metre of the piles, buckling and plastic deformation started. Plastic deformations are detected to be higher in places where the pile-soil interaction is higher, piles with more buried depth (i.e. places of the pier closer to shore). Springs representing the soil around the pile and hinge points are shown in the 3 dimensional visual of the pier structure (Figure 5). In addition, plastic deformations on the section and 3 dimensional visuals are presented for displacement.



a)



b)

Figure 5. Displacement scale visual of deformed pier a) Pier section, b)3D Visual

The base shear force and displacement graph obtained from the dynamic analysis results are presented in Figure 6.

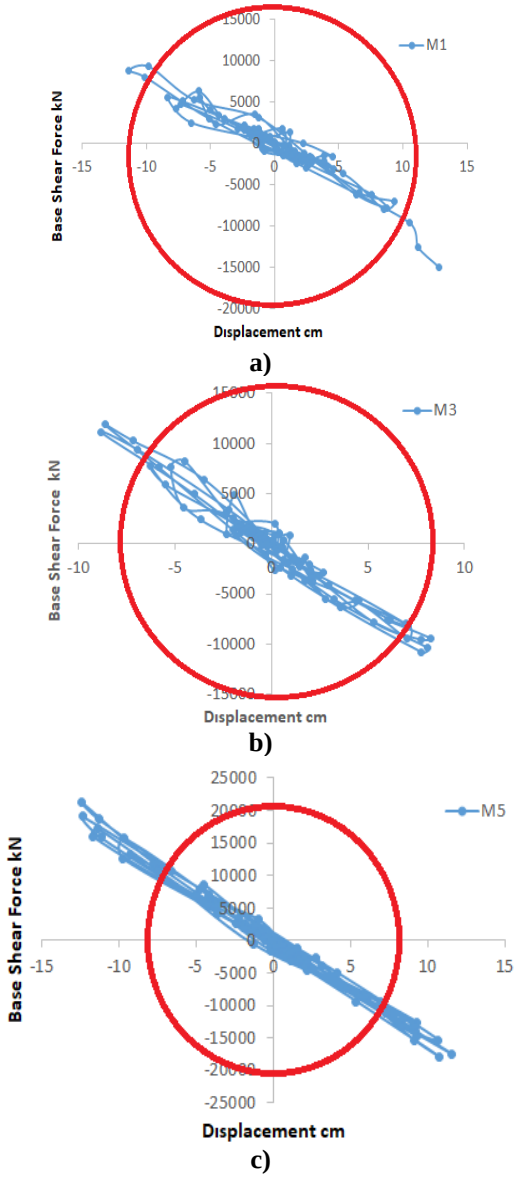


Figure 6. Time history nonlinear analysis graph a) 600 mm Diameter 4D arrangement, b) 800 mm diameter 4D arrangement, c) 1000 mm diameter 4D arrangement

For the static pushover results, the red circle represents the linear capacity of the structure in the x direction and is presented in Figure 6. Base shear force and displacements are examined and for the accelerogram, with this capacity considered, most of the system remaining in the linear zone can be seen. 600 mm diameter piled system flexibility is higher. Therefore displacement capacity and the linear limit value were higher than other two diameters.

When the load is applied, it will not be distributed equally on the pile system. For the pile group consideration, pile group carrying capacity can be determined with a reduction factor. Depending on their position in the group, load-carrying capacity differs (Walsh, 2005). Piles on the front end meet the forces first and bear the most of it, the stresses caused by this and the stress entrance zones overlapping soften the soil in the zone. The Softening effect on the soil decreases the loads piles can carry. This effect is applied to the piles depending on their position on the plan, thus a reduction coefficient was used. Therefore, piles are placed in 4D spacing, while piles in the inner regions are placed in 8D spacing. It was detected that pile buried depth differentiates the buckling length. Buckling length is related to pile slenderness. To restrain this parameter, either pile buckling length should be decreased or pile diameter should be increased. With the pile diameter increase, the system can tolerate the slenderness. 600 mm diameter pile has a more wavy behaviour while 800 mm and 1000 mm piles pose a more integrated behaviour. Deviation in the displacement behaviour of the piles are related to their buried depths. If the pile buried depths were the same, it would be expected from load-displacement curves from the linear behaviour to shift within the same boundaries. However, this structure is a pier and the effect of the natural slope formation by the shore results with piles that have different effective lengths. Therefore, piles in the system have different capacity and displacement values. For this reason, the damping of the building system behaviour was not formed by a cyclic effect in the same line but by a wavy displacement.

4. RESULTS

In this study; a performance evaluation of a piled pier foundation system with different pile diameters and pile spacings is carried out. Used pile diameters are; 600 mm, 800 mm and 1000 mm. Used pile spacings are, 4D and 8D. In total 6 models are created. Dimensional parameters of the numerical models are determined by pile diameters and analysed using Sap 2000 finite element model program. In the program, pushover analysis is carried out and capacity curves are obtained. After, nonlinear time history analysis using a scaled seismogram data is carried out. Obtained values are presented comparatively.

In this context;

- From the capacity curves, it was detected that all of the models have sufficient stiffness and ductility.
- From the 600,800 ve 1000 mm diameter conditions, the highest performance level was obtained from 800 mm diameter with a 4D spacing condition, which is the model M3.
- For the defined earthquake model and soil properties, pile system maximum base shear force values have not exceeded the linear limit. For 800 mm diameter and 4D arrangement, soil has provided sufficient stiffness for piled system behaviour under seismic effect,
- It was detected that plastic deformations on the piles have started with the buckling level. The first deformation has occurred at the interaction zone of the upper foundation, piles and the soil and in the first metre of the piles and this height concentrates as the buried depth of the pile increases
- Dynamic behaviour of the pier structure is affected by slenderness and correspondingly buckling shape change. As the pile diameter increases, system stiffness and load-carrying capacity of the system also increases accordingly.

- Buried depths of the piles varying has affected the buckling length of the piles, therefore 600 mm, 800 mm and 1000 mm diameter systems have shown different behaviour.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization, Investigation, Material and Methodology, Supervision, Visualization, Writing-Original Draft, Writing-review & Editing ;M.F.. Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Chandrasekaran, S. (2015). Dynamic Analysis And Design Of Offshore Structures. New Delhi: Springer India,

CSI, SAP2000 V.20. Integrated Finite Element Analysis and Design of Structures Basic Analysis Reference Manual,ComputersandStructuresInc., Berkeley, CA, USA.

DLH, (2008). T.R. Ministry of Transport, Earthquake Technical Regulation on Coastal and Port Structures, Railways and Airports Construction.

Erkan, Ş., Doran, B., Selçuk, M. E., Berilgen, M. M., Yüksel, Y. (2014). Numerical Analysis of Pier Piles, 8th Coastal Engineers Symposium

Feng, S., Liu, X., Cui, H. (2017). Dynamical Response Of An Elastic Supporting Pile Embedded In Saturated Soil Under Horizontal Vibration, Civil Engineering Journal-Stavebni Obzor", 2, 99-113

Kadıoğlu, Z. (2015). Nonlinear Behavior of Piled Coastal Structures and Design Based on Deformation, Gebze Technical University, Master's Thesis, p. 83, Gebze.

Kishida A.,Takewaki I. (2010). Response Spectrum Method for Kinematic Soil-Pile Interaction Analysis Advances in Structural Engineering Vol. No. 13 one

Panchaland, K.M., Rangari, M.S. (2018). Dynamic Analysis Of Laterally Loaded Piles In Group, International Journal Of Engineering Science Invention (IJESI), 2319 – 6726

Pérez-Collazo, C., Greaves, D., Iglesias, G.,A. (2015). Review Of Combined Wave And Offshore Wind Energy. Renewable And Sustainable Energy Reviews, 42, 141-153, 2015.

Polat, S.S. (2008). DeformationBasedSeismic Design of Pile Supported Marine Facilities, Boğaziçi University, Kandilli Observatory and Earthquake Research Institute, Doctoral Thesis, p. 195, Istanbul.

Randolph, M., Gourvenec, S. (2017). Offshore geotechnical engineering. CRC press

Topsoy, E. (2016). Design, Repair and Strengthening of Coastal Docking Structures, Istanbul Technical University, Master's Thesis, p. 179, Istanbul.

Yan, H.K., Wang, N., Wu, N., Lin, W. (2018). Maritime Construction Site Selection from the Perspective of Ecological Protection: the Relationship Between the Dalian Offshore Airport and Spotted Seals (Phoca Largha) in China Based on the Noise Pollution, Ocean and Coastal Management, Vol. pp. 152, 145–153

Yasser E.M. (2012). Effect of Local and Global Scour on Lateral Response of Single Piles in Different Soil Conditions. Engineering, 04(06), 297–306. doi:10.4236/eng.2012.46039.

Yeter, B., Garbatov, Y., Soares, C. G. (2019). "Uncertainty Analysis Of Soil-Pile Interactions Of Monopile Offshore Wind Turbine Support Structures", Applied Ocean Research, 82, 74-88

Yüksel Y., Orhan K. (2013). Seismic Risk and Damage Types in Ports, 1st National Port Congress, Dokuz Eylül University, Izmir.

Zhang, B. L., Han, Q. L., Zhang, X. M. (2017). Recent Advances In Vibration Control Of Offshore Platforms, Nonlinear Dynamics, 89(2), 755-771, 2017.

Zhang, Q., Zhou X.L., Wang, J.H., Guo, J.J. (2017b). Wave-Induced Seabed Response around an Offshore Pile Foundation Platform", Ocean Engineering, Vol. 130, pp. 567–582

Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2024, Volume: 8, Issue: 1, 9-13
Received: 05.10.2023; Accepted: 13.03.2024
DOI: 10.30516/bilgesci.1370407

The Role of Slope As An Environmental Variable In Plant Biodiversity Change In Aegean Rangelands by SHE Analysis: The Case of Çakmar Rangeland

Emre Kara*[1], Mustafa Sürmen

Abstract: Rangelands, which have rich plant and animal biodiversity, are very important as a source of roughage for livestock. Rangeland vegetation patterns vary considerably under the influence of environmental factors. Indicator factors need to be identified and analyzed in order to manage conservation and utilization objectives. In rangelands of the Aegean region, the slope factor can greatly affect the rangeland plant biodiversity in areas close to the base rangeland. In order to investigate the spatial distribution and species variation in plant biodiversity caused by slope, 6 rangeland sites with different slopes were sampled in Koçarlı region (Aydın / Türkiye). Sampling was carried out using the transect method in the spring 2017 based on field observations. Following sampling, indicator species and species distribution and abundance were determined. Alpha biodiversity indices were used to determine the change in species biodiversity by SHE analysis. SHE analysis tests the relationship between S (species richness), H (Shannon-Wiener diversity index) and E (equality). This method aims to examine the contribution of the number of species and the concept of equity in the context of diversity. According to the information obtained as a result of the analysis, it was seen that the increase in slope may cause a decrease in species biodiversity. More species diversity was found in rangelands with low base and slope. Factors such as erosion and water transport affect the canopy and species abundance in high slope rangelands. However, other factors such as grazing intensity can reverse this situation. For this purpose, it has been determined that slope is an important environmental variable when preparing management plans based on grazing capacity and grazing animal species in vegetation studies.

Keywords: alpha biodiversity, rangeland vegetation, Shannon-Wiener index, rangeland ecology.

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Citation: Kara, E., Sürmen, M. (2024). The role of slope as an environmental variable in plant biodiversity change in Aegean rangelands by SHE analysis: The case of Çakmar rangeland. Bilge International Journal of Science and Technology Research, 8(1): 9-13.

1. INTRODUCTION

Rangelands play a crucial role in Türkiye's ecosystem and economy. Mismanagement practices have led to a significant loss of original vegetation in Turkish rangelands (Sürmen & Kara, 2022). Suitable range management changes are recommended to conserve natural resources in highland rangelands (Koç et al., 2020).. Rangelands, covering 22.9 million hectares in Türkiye, historically support livestock production through seasonal grazing (Yetişgin & Aydemir, 2019). These rangelands are vital for animal husbandry in the eastern Anatolia region due to the challenging topography and short growing season (Koç et al., 2021). Additionally, the use of commercial feeds for animal farming is common throughout Türkiye, impacting the utilization of rangelands (Mohammadi et al., 2021).Environmental variables are also under the influence of this situation. One of the most important of these environmental variables is slope. The relationship between slope and rangeland dynamics is a critical aspect of understanding the ecological and environmental processes in these ecosystems. Research by Koç et al. (2020) highlights the impact of elevation, slope aspect, and the degree of slope on the vegetation structure, composition, and productivity of rangelands in the Palandöken Mountains, Erzurum, Türkiye. This study emphasizes the influence of topographic factors, including slope, on rangeland deterioration due to heavy grazing, providing valuable insights into the specific mechanisms driving rangeland changes in this region.

The negative effect of slope on animal distribution disrupts homogeneous grazing in rangeland sites. Overgrazing in certain areas is exacerbated by environmental factors such as slope (Han et. al., 2008; Zeng et. al. 2014). This situation leads to a decrease in the canopy ratio in plant cover, decreased heterogeneity in botanical species composition and negative effects in terms of abundance. The Aegean Region is very important for the livestock sector with its 802,882 ha rangeland area (Demiroğlu and Özkan, 2017). Although these rangelands are generally subject to overgrazing, slope as an environmental variable affects the type and density of livestocks. In addition to the vegetation effect of the slope, erosion may cause the removal of useful soil cover. In order to prevent erosion and to maintain the active growth of vegetation, a certain amount of biomass must be left. The amount of biomass required to maintain active growth in short rangelands should be approximately 400 kg ha $^{-1}$(Molinar et. al., 2001).

Biodiversity; species diversity, structural diversity and functional diversity is defined in three different ways while in studies on forest ecosystems focusing mainly on plant species diversity (Negiz et. al., 2017). The current status of rangeland biodiversity is significantly changed by the reduction in habitat, land-use changes, loss of species, unplanned fire, overgrazing, climate change and the invasion of non-native species (Gemechu and Dalle, 2023).
The relationship between species richness and evenness is a much-unresolved issue in ecology (Tuomisto, 2012). Some researchers have argued that there is a strong relationship between species richness and evenness, while others have argued they are completely independent. The species richness- evenness relationship seems to be quite contradictory between theoretical and empirical perspectives (Su, 2018).

Shannon (H) and Simpson (E) indices are widely used in rangelands to measure species/type richness. In addition to these indices, diversity indices such as Margalef (D), Berger-Parker Dominance, Mclntosh D, Brillouin D, Fisher α and Q Statistic are also used. Apart from these, taxonomic species diversity indices have also been developed to assess taxonomic species diversity. This is preferred because it determines structural and functional diversity. (Özkan, 2012; Yazgı and Yılmaz, 2017).

It has been reported that the calculation of Alpha and Beta diversity formulas can be used as a tool to evaluate rangeland plant biodiversity and that changes that may occur over time can be controlled by identifying areas that need to be protected in intensively grazed areas (Gülsoy and Özkan, 2008).

Within the scope of this study, changes in rangeland sites with different slopes were examined together with this information by using this analysis method.

2. MATERIAL AND METHOD

The experiment was conducted in the spring season of 2017 in Çakmar / Koçarlı rangelands located in Büyük Menderes Basin. In the experiment, rangeland sites with 6 different slopes in approximately 55 ha rangeland area were determined as environmental variables. The Modified Wheel Point method with loop was used in vegetation measurements (Koç and Çakal, 2004). The measurements were carried out during the flowering period of indicator species in the rangeland, in five different part wtih 100 m line of the slopes.

The slope of the rangeland were classified as almost flat (0-1%), slightly sloping (2-5%), moderately sloping (6-11%), steep sloping (12-19%), very steep sloping (20-29%) and problematic (>30%). (İspirli et. al., 2016). Among these, summer asphodel (*Asphodelus aestivus*) (Figure 1.). was the most abundant species in every site. *Bromus tectorum* was the most abundant species only in the lowest slope area (.).. The average soil coverage rates of the slopes are 73%, 73%, 70%, 65%, 63% and 48% respectively. Along with the measurements, species richness and abundance were determined with the help of alpha species diversity analysis.

The analysis of biodiversity in rangeland studies often involves the assessment of various factors such as species richness, ecosystem services, and livestock production. emphasized the importance of landscape-scale features and practices, such as hedgerows, in enhancing biodiversity and ecosystem services in agroforestry systems (Torralba et al., 2016). Similarly, utilized a model to assess the impact of livestock production on rangeland biodiversity, highlighting the significance of understanding the effects of food demand and livestock production on future biodiversity (Alkemade et al., 2012).

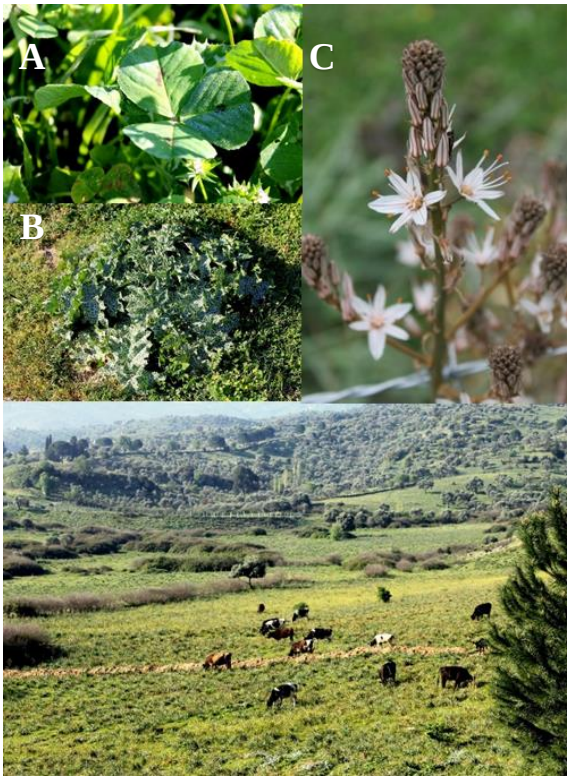


Figure 1. Çakmar rangelands (A: *Medicago arabica*; B: *Silybum marianum*; C: *Asphodelus* sp.)

Traditional alpha diversity indices include species richness, species diversity and equality. It is stated that SHE analysis is performed to see all of these expressions together. SHE analysis (S=Species Richness, H=Shannon-Wiener index,

E=equity, balance) is the graphical expression of H, E, ln(E) and ln(E)/ln(S). Formulas related to the terms used in SHE analysis are given below (Özkan, 2016).

$$S = \sum_{i}^{s} S_i$$

$$P_i = x_i / \sum_i^s x_i$$

$$H = -\sum p_i \ln p_i$$

$$E = e^H / S$$

In this formula, S is the number of species; Pi is the ratio of the percent cover value of species i to the sum of the percent cover values of all species; ln is the natural logarithm. It is calculated for each species in H's formula and is obtained by dividing the abundance value of a species in the sample area by the total abundance value of the species (N). Evenness takes a value between 0 and 1, with 1 representing full evenness. The higher the Shannon-Weiner value, the closer the number of individuals of species in this region is to each other than in other communities. (Kılınç et. al., 2006; Sürmen et. al., 2020).

3. RESULTS

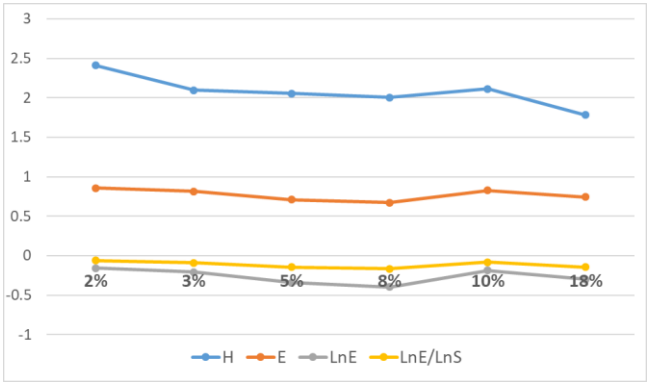
Following the vegetation measurements, negative findings were obtained in species diversity and abundance due to heavy grazing in rangeland areas. Intensive grazing continues almost all months of the year, especially in the rangeland area. In the rangeland area where the research was conducted, it was observed that species diversity and soil coverage rate were high in the slope, which is the determinant environmental factor, especially in the sites with a lower percentage of slope. In the rangeland site with a slope of 2%, the highest species diversity was detected with 13 species, while the species diversity decreased to 8 when the slope reached 18%. (Table 1;2.).

When the SHE analysis results were evaluated, the lowest H value among the 6 slopes was obtained from 18% slope. In addition, the lowest E value was observed at 8% slope, while the highest values in terms of LnE/LnS were determined at 2%, 3% and 10% slopes. According to these results, the rangeland area with 2% slope showed this result due to its higher species diversity, while 3% and 10% slopes showed more advantageous results due to their regular distribution although species richness was less than 3% and 5%. (Table 1;2.).

Table 1. Calculation results for SHE analysis terms of Çakmar rangeland sites

Slopes	S	H	E	LnE	LnE/LnS
%2	13	2,40972	0,85622	-0,15522	-0,06051
%3	10	2,09592	0,81329	-0,20665	-0,08975
%5	11	2,05610	0,71049	-0,34179	-0,14253
%8	11	2,00257	0,67346	-0,39532	-0,16486
%10	10	2,11450	0,82854	-0,18808	-0,08168
%18	8	1,78129	0,74219	-0,29814	-0,14337

Table 2. Graphical Representation of Outputs of SHE Analysis Terms According to Rangeland Slopes



Water retention decreases with the increase in slope in rangeland areas. This situation causes the soil to dry out faster and the area covered with soil decreases. In addition, grazing can affect this situation. Although the area where the experiment was conducted does not have a high rate of rangeland area, slope was one of the main environmental factors that could affect plant species and diversity.

4. DISCUSSION AND CONCLUSIONS

Rangeland plant communities are formed in a long process with the effects of soil, topography and climate factors. Therefore, the vegetation of each rangelands are unique.

In plant succesion process, the change may be in the number of species that make up the vegetation, the proportion of each species in the botanical composition or the proportion of vegetation covering the soil. The direction of this change may be in a more desirable or productive direction, or in an undesirable or less productive direction (Blanchet et. al., 2003).

The data obtained from the experiment showed that the increase in slope has a negative impact on biodiversity. Although the area covered by soil is high, the increase in slope due to erosion affects soil depth and species diversity (İspirli et. al., 2016). This situation was observed in the study. Especially the decrease in SHE analysis results was more pronounced with the increase in slope.

It is stated that it leaves the soil surface unprotected against erosion, reduces the carbon and nitrogen storage capacity of the soil by reducing plant root mass (Han et. al., 2008). Kenneth et. al. (2009) pointed out the inverse relationship between soil coverage and erosion.

Slope in rangeland areas not only affects species diversity but also has a negative effect on yield. Severoğlu and Güllap (2020) stated that there may be a decrease in forage yield and quality after high level of slope. The fact that the increased surface flow due to the increasing slope negatively affects the moisture balance and the transportation of the nutrients by erosion can be effective in reducing the forage allowance.

It was also reported by Şentürk et al. (2019) that landforms formed by different degrees of slope have significant effects on plant species diversity.
Rangeland slope sites have been an important environmental factor in determining grazing management. In the study conducted according to SHE biodiversity analysis in six different rangeland sites, highest biodiversity was observed in the bottom rangeland area with 2% slope. As the slope percentage increases, the negative effects experienced and to be experienced will affect the plant biodiversity and the area covered with soil in the rangeland. Although the slope percentages in the experiment had lower percentages than the average slopes in Türkiye, factors such as duration of illumination, grazing time of animals, and rainfall revealed that slope may be important in plant biodiversity. Research has shown that the slope of rangelands plays a significant role in determining the utilization rate and forage quality of the area Kara (2020) and affects the vegetation dynamics (Gebremedhn et al., 2023). Specifically, high-altitude sites and slopes facing east and southwest should be given priority in rangeland rehabilitation studies due to heavy grazing pressure versus low forage production (Kara, 2020). Moreover, the unique buffering capacity of forest and rangeland ecosystems across mountainous regions helps in slope stability and enhances ecological integrity (Dhyani et al., 2022).

The slope, in conjunction with grazing practices, can have a significant impact on biodiversity conservation in rangelands. Moreover, the amount and temporal distribution of precipitation received, which can be influenced by the slope, are critical for regrowth and plant production on rangelands (Koç, 2001). Additionally, diversified vegetation types on rangelands promote multiple soil-based ecosystem services, highlighting the interconnectedness of slope, vegetation, and soil health (Waterhouse, 2023).

Taking into account different environmental factors and examining their effects on biodiversity in the long term will lead to the adoption of rangeland management policies that will adapt to the changing climate with global climate change. In addition, it was seen how important the slope can be in terms of the number of animals and animal preference according to the grazing capacity.

Acknowledgements
I would like to thank the lecturers who organized the scientific event titled "Biodiversity Measurement Processes: Inventory, Data Transfer and Computational Techniques" (TUBITAK-2229 Scientist Support Programme - Project No. 1059B291600793). This study was presented as a poster abstract at Ecology 2018 (Kastamonu / Türkiye) symposium.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: E.K.; Investigation: E.K. ; M.S.; Material and Methodology: E.K; M.S., Visualization: E.K.; Writing-Original Draft: E.K., M.S.; Writing-review & Editing: E.K., M.S.; All authors have read and agreed to the published version of the manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Alkemade, R., Reid, R., Berg, M., Leeuw, J., Jeuken, M. (2012). Assessing the impacts of livestock production on biodiversity in rangeland ecosystems. Proceedings of the National Academy of Sciences, 110(52), 20900-20905. https://doi.org/10.1073/pnas.1011013108

Dhyani, S., Sivadas, D., Basu, O., Karki, M. (2022). Ecosystem health and risk assessments for high conservation value mountain ecosystems of south asia: a necessity to guide conservation policies. Anthropocene Science, 1(1), 211-225. https://doi.org/10.1007/s44177-022-00010-8

Gebremedhn, H., Sintayehu, D., Tuffa, S., Tesfay, Y., Adam, D. (2023). Traditional grazing management practices affect vegetation dynamics in the somali pastoral ecosystem of ethiopia.. https://doi.org/10.22541/au.167955328.89297988/v1

Gemechu, T., Dalle, G. (2023). Rangeland Biodiversity: Status, Challenges and Opportunities Review. Journal of Rangeland Science, 13(3), 1-9.

Gülsoy, S., Özkan, K. (2008). Tür Çeşitliliğinin Ekolojik Açıdan Önemi ve Kullanılan Bazı İndisler. Süleyman Demirel Üniversitesi Orman Fakültesi Dergisi Seri: A, Sayı: 1, Yıl: 2008, ISSN: 1302-7085, 168-178

Han, J. G., Zhang, Y. J., Wang, C. J., Bai, W. M., Wang, Y. R., Han, G. D., Li, L. H. (2008). Rangeland degradation and restoration management in China. The Rangeland Journal, 30(2), 233-239.

İspirli, K., Alay, F., Uzun, F., Çankaya, N. (2016). Doğal meralardaki vejetasyon örtüsü ve yapısı üzerine otlatma ve topografyanın etkisi. Türkiye Tarımsal Araştırmalar Dergisi, 3(1), 14-22.

Kara, A. (2020). Determination of the factors affecting utilization rate of eastern anatolian semiarid public rangelands in Türkiye. International Journal of Agriculture Environment and Food Sciences, 4(1), 119-129. https://doi.org/10.31015/jaefs.2020.1.14

Kenneth, E. S., Pierson, F. B., Robichaud, P. R. (2009). Effect of plant species composition on rangeland hydrology and erosion. In 62nd Society for Range Management Annual Meeting, Paper (No. 07-9).

Kılınç, M., Kutbay, H. G., Yalçın, E., Bilgin, A. (2006). Bitki ekolojisi ve bitki sosyolojisi uygulamaları. Palme Yayıncılık, Ankara.

Koç, A. (2001). Autumn and spring drought periods affect vegetation on high elevation rangelands of Türkiye.

Journal of Range Management, 54(5), 622. https://doi.org/10.2307/4003594

Koç, A., Çakal, Ş. (2004). Comparison of some rangeland canopy coverage methods. In International Soil Congress Natural Resource Management for Sustainable Development (Vol. 7, No. 10, pp. 41-45).

Koç, A., Gullap, M., Sürmen, M., Erkovan, H. (2020). Changes in some vegetation properties of the rangelands of the palandöken mountains, erzurum, over two decades. Turkish Journal of Agriculture and Forestry, 44(6), 589-598. https://doi.org/10.3906/tar-1909-37

Koç, A., Gökkuş, A., Gullap, M., Erkovan, H., Sürmen, M. (2021). Changes in rangeland condition and health of Palandöken mountain rangelands over two decades. Turkish Journal of Range and Forage Science, 2(2), 37-43. https://doi.org/10.51801/turkjrfs.987396

Mohammadi, S., Keshavarzi, M., Kazemi, A., Berizi, E., Mohsenpour, M., Ghaffarian-Bahraman, A. (2021). Occurrence of aflatoxin m 1 in yogurt of five countries in west asia region: a systematic review and meta-analysis. Journal of Food Safety, 41(4). https://doi.org/10.1111/jfs.12897

Molinar, F., D. Galt, J. Holechek. (2001). Grazing management practices can be key to accumulating mulch– vegetation residue–which is beneficial for decreasing soil erosion, improving water infiltration and increasing forage production on rangelands. Rangelands 23 (4) 3-7.

Negiz, M. G., Kurt, E. Ö., Şentürk, Ö. (2017). Isparta-Yenişarbademli yöresi ormanlık alanlarında tür merkezli tıbbi ve aromatik bitki tür zenginliğinin hesabı üzerine örnek bir çalışma. Türkiye Ormancılık Dergisi, 18(4), 282-288.

Özkan, K. (2012). Taksonomik çeşitlilik indislerinin geleneksel çeşitlilik indisleri ile karşılaştırılması. SDÜ Orman Fakültesi Dergisi, 13, 107-112.

Özkan, K. (2016). Biyolojik Çeşitlilik Bileşenleri Biyolojik Çeşitlilik Bileşenleri (α, β ve γ) Nasıl Ölçülür? Süleyman Demirel Üniversitesi: Orman Fakültesi. Yayın No:98-Isparta, 2016.

Severoğlu, S., Gullap, M. (2020). Effects of animal grazing on favorable forage yield and quality of rangelands with different slope. Turkish Journal of Field Crops, 25(2), 168-173.

Su, Q. (2018). A relationship between species richness and evenness that depends on specific relative abundance distribution. PeerJ, 6, e4951.

Sürmen, M., Kara, E. (2022). Effects of suppression applications on summer asphodel (asphodelus aestivus brot.) density, botanical composition, forage yield and quality of aegean rangelands. Turkish Journal of Field Crops, 27(1), 61-70. https://doi.org/10.17557/tjfc.1051111

Sürmen, M., Yavuz, T., Sürmen, B., İmamoğlu, A. (2020). Samsun ili çayır ve meralarında bitki çeşitliliğinin orta dereceli tahribat hipotezine göre otlatma ve erozyon faktörleri ile test edilmesi. Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 6(3), 570-581.

Şentürk, Ö., Negiz, M. G., Gülsoy, S. (2019). Kızılçam meşcerelerinde alfa tür çeşitliliği–yetişme ortamı ilişkileri: Gölhisar yöresi örneği. Bilge International Journal of Science and Technology Research, 3(2), 178-188.

Topçu, G. D., Özkan, Ş. S. (2017). Türkiye ve Ege bölgesi çayır-mera alanları ile yem bitkileri tarımına genel bir bakış. ÇOMÜ Ziraat Fakültesi Dergisi, 5(1), 21-28.

Torralba, M., Fagerholm, N., Burgess, P., Moreno, G., Plieninger, T. (2016). Do european agroforestry systems enhance biodiversity and ecosystem services? A meta-analysis. Agriculture Ecosystems & Environment, 230, 150-161. https://doi.org/10.1016/j.agee.2016.06.002

Tuomisto, H. (2012). An updated consumer's guide to evenness and related indices. Oikos, 121(8), 1203-1218.

Waterhouse, H. (2023). Diversified vegetation types on rangelands promote multiple soil-based ecosystem services. Land Degradation and Development. https://doi.org/10.1002/ldr.4967

Yazgı, D., Yılmaz, K. T. (2017). Employing SHE analysis method for evaluating landscape diversity. Biological Diversity and Conservation, 10 (3), 65-72

Yetişgin, S., Aydemir, Ş. (2019). A preliminary study on grazing pattern of sheep transhumance in Türkiye. Turkish Journal of Agriculture - Food Science and Technology, 7(10), 1629-1634. https://doi.org/10.24925/turjaf.v7i10.1629-1634.2699

Zeng, X. H., Zhang, W. J., Song, Y. G., Shen, H. T. (2014). Slope aspect and slope position have effects on plant diversity and spatial distribution in the hilly region of Mount Taihang, North China. J Food Agric Environ, 12, 391-397.

Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2024, Volume: 8, Issue: 1, 14-18
Received: 02.11.2023; Accepted: 05.02.2024
DOI: 10.30516/bilgesci.1385144

Investigation of The Antioxidant Activity And Total Phenolic Substance of *Fomes fomentarius* And *Ganoderma applanatum* Mushrooms Showing Therapeutic Properties

Mertcan Karadeniz*[1], Temel Kan Bakır[2], Sabri Ünal[1]

Abstract: *Fomes fomentarius* (*Ffo*) and *Ganoderma applanatum* (*Gap*), which are mushroom species used in traditional medicine in Far East Asia, are widely found in our country and attract great attention with their antioxidant properties. In this study, the antioxidant activities of these mushrooms grown in our country were determined spectrophotometrically using by the DPPH method. The results were given as percent inhibition and also the IC_{50} values of the fungal samples were calculated using calibration equation for DPPH. IC_{50} values for *G. applanatum* and *F. fomentarius* were found to be 0.515 and 0.463 mg/mL, respectively. Additionally, total phenolic substances were analyzed by the Folin-Ciocalteu Method. Phenol content in methanolic extracts expressed in gallic acid equivalents (GAE) was found to be 8,447 and 10,300 mg/L for *G. applanatum* and *F. fomentarius*, respectively. In this study, both types of mushrooms were found valuable because they are rich in phenolic contents and antioxidant properties.

Keywords: *Fomes fomentarius* (*Ffo*), *Ganoderma applanatum* (*Gap*), Total phenolic substances, DPPH

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Citation: Karadeniz, M., Bakır, T. K., Ünal, S. (2024). Investigation of The Antioxidant Activity And Total Phenolic Substance of Fomes Fomentarius And Ganoderma Applanatum Mushrooms Showing Therapeutic Properties. Bilge International Journal of Science and Technology Research, 8(1), 14-18.

1. INTRODUCTION

Even though very few of the 2000 mushroom species belonging to the Basidiomycetes group of macrofungi can be considered food, they are one of the preferred ingredients of world cuisine with their unique tastes. In recent years, many studies have elucidated the medicinal properties of some useful wild edible mushroom species, and they can also be commercialized with their nutritional properties (Chang and Miles et al., 2008; Erbiai et al., 2021). Mushrooms show immunomodulatory, antitumor and antioxidant properties because they contain proteins, polysaccharides, terpenes, terpenoids and important antioxidants, including phenolic acids, glutathione and ergothioneine (Kalaras et al., 2017; Kozarski et al., 2015; Suarez-Arroyo et al., 2017; Li et al., 2019; Gonzalez-Palma et al., 2016). Recent research has shown that adding mushrooms to the daily diet increases the intake of important nutrients such as vitamin D, fiber and potassium (Fulgoni and Agarwal, 2021). Additionally, some mushroom species are known to have therapeutic effects on disorders such as neurodegenerative and cardiovascular diseases, cancer, inflammation and diabetes (Umeno et al., 2017; Rahman and Abdullah, 2015).

Fomes fomentarius, belonging to the family Polyporaceae, order Polyporales, is a large, inedible, woody fungus that causes rot in some deciduous tree species. This mushroom has been used since ancient times as a pain reliever and to treat rheumatism, hemorrhoids, bladder disorders, esophagus, stomach and uterine cancers (Umeno et al., 2017). Some studies have revealed that *F. fomentarius* exhibits antimicrobial properties against many viruses and bacteria that cause diseases in humans (Lee et al., 2004). In addition, it has been determined that *F. fomentarius*, like other fungi, contains valuable bioactive compounds with potential antioxidant, antitumor, immunomodulatory and anti-inflammatory activities, which are the subject of chemical and biological studies (Grienke et al., 2014; Kolundzic et al., 2016; Bojin et al., 2020). Additionally, *Ganoderma applanatum*, which is in the order Polyporales

and the Ganodermataceae family, is another type of fungus that causes wood rot in various trees. Species of the *Ganoderma* genus are used in traditional medicine in Asia, especially in China and Japan. As a result of the studies, extracts obtained from *Ganoderma* species have been shown to be beneficial against diseases that may be caused by various microorganisms such as bacteria and fungi in humans (Wasser, 2011). *G. applanatum*, like *F. fomentarius*, has been accepted as a valuable group of organisms due to its wide range of biological activities such as antioxidant, antitumor, immunomodulatory, anti-hepatotoxic, antibacterial and antiviral effects (Oviasogie et al., 2015).

The aim of this study is determined, evaluate and comparing these features with each other the antioxidant properties and total phenolic properties of *G. applanatum* and *F. fomentarius species* mushrooms collected from Kastamonu Province of the Western Black Sea Region. The data obtained for this purpose will be used to show the economic value of this class of mushroom species, which are not very valuable in the region, and to show some of their therapeutic properties.

2. MATERIAL AND METHOD

2.1. Preparation of mushroom samples

Taxonomy was determined of fresh *G. applanatum* collected from Taşköprü district of Kastamonu province and fresh *F. fomentarius* type mushrooms collected from Küre District was carried out at from Kastamonu University Faculty of Forestry Engineering. Deionized water was used in every stage of this study obtained from the Milli-Q system (18.2 MX/cm3, Human Power I Plus, Korea) Deionized water obtained from the Milli-Q system (18.2 The collected mushrooms were cut into small portions and dried under oven at 25°C for 48 hours Mushroom extracts were prepared according to standard protocol with minor modifications (Lee et al., 2004).

First, 2.5 g the dried and powdered mushroom was measured and added 20 ml methanol solution (80%) on sample. The extract mixture, which was kept at room temperature for 3 hours, was filtered through a filter crucible. 5 mL of methanol (80%) was added to the filtrate and then filtered again. The homogenate was centrifuged at 5000 rpm and 7500 rpm for 10 min (18°C), respectively, and the resulting supernatant was used for measurement.

2.2 Determination of Antioxidant Activity

DPPH radical scavenging method was used for antioxidant activity determinations. This method is based on monitoring the maximum absorbance of the purple solution of the DPPH (1,1-diphenyl-2-picryl hydrazyl) radical measured at 517 nm with decreasing color change (Bozdogan et al., 2018). In this study, absorbances were measured by spectrophotometer (SHIMADZU UVM-1240 UV-VIS, Shimadzu Corp., Kyoto, Japan). Antioxidant activities of two mushroom species were determined using the DPPH radical scavenging method and the results were calculated as percentage inhibition (%). For this, 30 µM concentration DPPH solution was used. Absorbance changes were measured in the concentration range of 1.66-6.66 mg/mL for each mushroom extract versus blank. DPPH radical damping percentage was calculated by the following formula:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Here, A_0 is the control absorbance for the blank and A_1 is the absorbance obtained for the fungal samples in solution. Additionally, IC_{50} (mg/mL) calculations were made, indicating the amount of antioxidant required to reduce the initial DPPH concentration by 50% (Frankel and Meyer, 2000).

2.3.Determination of Total Phenolic Substance

Total phenolic components of mushroom extracts were determined using Folin-Ciocalteu reagent as standard. Total phenolic components extracted from mushrooms by methanolic extraciton methods, were measured at 760 nm. To implement this method, , 4.5 mL of deionized water and 0.1 mL of Folin-Ciocalteu reagent were used. After 3 minutes, 0.3 mL Na_2CO_3 (2%) solution and 0.1 mL extract solution were added to the solution and stirred (Chandler and Dodds, 1983; Slinkard and Singleton, 1977).

In our total phenolic analysis, first the phenolic substances against the concentration of gallic acid were graphed. Using this calibration chart, the total phenolic concentrations of *G. applanatum* and *F. fomentarius* mushrooms were calculated as gallic acid equivalent. The equation found for gallic acid, which was used as a standard, was used to calculate the concentration of phenolic compounds:

Absorption = 0.0264 gallic acid (mg) + 0.0462 (R2 = 0.989)

2.4. Statistical Analysis

Standard deviation data among antioxidant concentration of mushrooms were calculated using descriptive statistical analysis with SPSS software for Windows version 13 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

Antioxidant activities of mushroom extracts prepared at different concentrations were determined. Accordingly, it was determined that the inhibition values of both mushroom samples increased with concentration (Figure 1). Inhibition values in the concentration range of 0.20-0.80 mg/mL were varied from 20.35-84.59% for *G. applanatum* and 23.88-82.18% for *F. fomentarius*. At all concentrations, *F. fomentarius* showed higher % inhibition than *G. applanatum* except at the highest concentration.

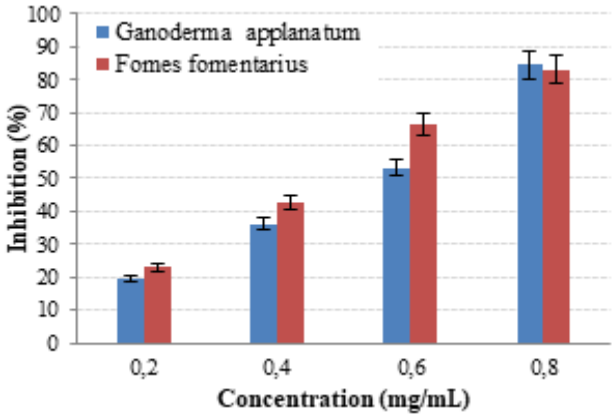

Figure 1. Inhibition (%) graph for *Ganoderma* and cultivated mushrooms at concentrations of 20-80 mg/mL. The calculated results are given as mean ± SEM (standard error of the mean).

IC_{50} and total phenolic substance values used to compare the antioxidant activities of both mushroom extracts are presented in Table 1. Accordingly, it was found to be 0.52±0.05 mg/mL and 0.46±0.04 mg/mL for *G. applanatum F. fomentarius*, respectively. Additionally, total phenolic substance values in gallic acid equivalents (GAE) were found to be between 8.45±0.12 mgGA/L and 10.30±0.13 mg GA/L, respectively. *G. applanatum* and *F. fomentarius* showed very close total phenolic contentto each other. Similarly, IC_{50} values were found to be close to each other. *F. fomentarius* with higher total phenolic concentration showed higher activity, as expected. These results showed that there was a strong correlation between negatively correlated IC_{50} values and total phenolic substance content, our results were shown similarity to previous studies (Gülçin et al., 2003).

Table 1. IC_{50} values and total phenolic substancesof *Ganoderma applanatum* (*Gap*) and *Fomes fomentarius* (Ffo) mushrooms

	Total phenolic substances (mgGA/L)	**IC_{50} (mg/mL)**
Ganoderma applanatum (Gap)	8.45±0.12	0.52±0.05
Fomes fomentarius (Ffo)	10.30±0.13	0.46±0.04

In the anticancer and antioxidant activity determination study conducted by Kolniak-Ostek et al. (2022) with *G. lucidum*, also known as the mushroom of immortality, the results showed that the characterization of compounds belonging to polyphenolic acids, flavonols, flavanols, flavones and stilbenes is compose of polyphenolic compounds (Kolniak-Ostek et al., 2022). *G. applanatum*, which is widely used in traditional Asian medicines, was previously optimized by liquid shake flask fermentation to investigate the media composition of the fungal strain and the antioxidant activity of exopolysaccharides. The results revealed that *G. applanatum* strain was able to scavenge exopolysaccharides, hydroxyl radicals, and superoxide anion radicals, which was positively associated with antioxidant activity (Zhong-Hua et al., 2015). A comparative study was carried out to elucidate the chemical properties between *G. applanatum* and *G. lucidum*, which belong to the Basidiomycota group, and the results showed that the total phenol and flavonoid content, betulinic acid and also antioxidant activity of *G. applanatum* measured by DPPH radical scavenging and FRAP methods were higher than the other, but they showed that *G. lucidum* had higher total polysaccharide and protein content (Mohammadifar et al., 2020). *Fomes* mushroom, many biological activities have been studied in recent years, has been used for medicinal purposes for centuries thanks to its active compounds. *F. fomentarius* antioxidant enzyme activity, enzymatic activity, antimicrobial activity, antifungal activity are existed by extracts or isolated compounds from fermentation broth, mycelia and fruit bodies (Aoki et al., 1993; Chen et al., 2008).

As a result of the information compiled by Elkhateeb et al. (2020), they stated that *F. fomentarius* and *Polyporus squamosus* have medical potential and can be used as a natural source for pharmacological research with their antioxidant and antimicrobial properties (Elkhateeb et al., 2020). These studies, in line with our data, have shown that *G. applanatum* and *F. fomentarius* species have medicinal values thanks to their effective antioxidant properties, which are in good correlation with the rich total phenolic substance amount.

4. CONCLUSIONS

DPPH radical scavenging activity and total phenolic substance contents were determined and each of features compared *G. applanatum* and *F. fomentarius* species mushrooms. Results: Although the *F. fomentarius* type mushroom showed a higher antioxidant activity than the *G. applanatum*, it was found that both mushroom species were valuable in terms of both their total phenolic content and antioxidant activities. This study will provide a resource for the use of both mushroom species in alternative medicine industry applications for therapeutic purposes, thanks to their antioxidant properties.

ACKNOWLEDGEMENTS

The summary of this study was presented as a summary text at the congress titled "XI. Turkish Edible Mushroom Congress" held at Samsun Metropolitan Municipality Art Center between 09-11 October 2019.

ETHICS COMMITTEE APPROVAL

N/A

PEER-REVIEW

Externally peer-reviewed.

AUTHOR CONTRIBUTIONS

Conceptualization: S.Ü., M.K, T.B.; Investigation: T.B.; Material and Methodology: M.K., T.B.; Supervision: T.B. ; Visualization: M.K.; Writing-Original Draft: T.B.; Writing-review & Editing: S.Ü., M.K, T.B.; Other: All authors have read and agreed to the published version of manuscript.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

FUNDING

The authors declared that this study has received no financial support.

REFERENCES

Aoki, M., Tan, M., Fukushima, A., Hieda, T., Kubo, S., Takabayashi, M., Ono, K., Mikami, Y. (1993). Antiviral Substances with Systemic Effects Produced by Basidiomycetes such as *Fomes fomentarius*. Biosci Biotechnol Biochem. 57(2):278-82. doi: 10.1271/bbb.57.278.

Bojin, L. A., Serb, A. F., Pascariu, M. C., Moaca, A., Kostici, R., Purcărea, V. L., Penescu, M., Ivan, M.V., Georgescu, M., Sisu, E. (2020). Assessment of antioxidant properties of different *Fomes fomentarius* extracts. Farmacia, 68(2), 322-328. 10.31925/farmacia.2020.2.18

Bozdogan, A., Ulukanlı, Z., Bozok, F., Eker, T., Doğan, H.H., Büyükalaca, S., (2018). Antioxidant Potential of Lactarius Deliciosus and Pleurotus ostreatus from Amanos Mountains. Adv. Life Sci. 5(3): 113-120.

Chandler, S. F., Dodds, J. H. (1983). The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasodine in callus cultures of Solanum laciniatum. Plant Cell Reports, 2(4), 205-208. DOI: 10.1007/BF00270105

Chang, S.T., Miles, P.G. (2008). Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact, 2nd ed.; CRC Press: Boca Raton, FL, USA.

Chen, W., Zhao, Z., Chen, S.F., Li, Y.Q. (2008). Optimization for the production of exopolysaccharide from Fomes fomentarius in submerged culture and its antitumor effect in vitro. Bioresour Technol. 99(8):3187-94. doi: 10.1016/j.biortech.2007.05.049.

Elkhateeb, W.A., Elnahas, M.O., Thomas, P.W., Daba, G.M. (2020). Fomes fomentarius and Polyporus squamosus Models of MarvelMedicinal Mushrooms. Biomed Res Rev. 3(1):119. doi: 10.31021/brr.20203119

Erbiai, E. H., da Silva, L. P., Saidi, R., Lamrani, Z., Esteves da Silva, J. C.G., Maouni, A. (2021). Chemical composition, bioactive compounds, and antioxidant activity of two wild edible mushrooms Armillaria mellea and Macrolepiota procera from two countries (Morocco and Portugal). Biomolecules, 11(4):575. doi: 10.3390/biom11040575.

Frankel, E. N., Meyer, A. S. (2000). The problems of using one dimensional methods to evaluate multifunctional food and biological antioxidants. Journal of the Science of Food and Agriculture, 80(13), 1925-1941. DOI: 10.1002/1097-0010(200010)80:13

Fulgoni, V.L. 3rd, Agarwal, S. (2021). Nutritional impact of adding a serving of mushrooms on usual intakes and nutrient adequacy using National Health and Nutrition Examination Survey 2011-2016 data. Food Sci Nutr. 9(3):1504-1511. doi: 10.1002/fsn3.2120.

González-Palma, I., Escalona-Buendía, H.B., Ponce-Alquicira, E., Téllez-Téllez, M., Gupta, V.K., Díaz-Godínez, G., Soriano-Santos, J. (2016). Evaluation of the Antioxidant Activity of Aqueous and Methanol Extracts of Pleurotus ostreatus in Different Growth Stages. Front Microbiol. 7:1099. doi: 10.3389/fmicb.2016.01099.

Grienke, U., Zöll, M., Peintner, U., Rollinger, J.M. (2014) European medicinal polypores - A modern view on traditional uses. J. Ethnopharmacol. 154(3): 564-583. doi: 10.1016/j.jep.2014.04.030.

Gülçin, İ., Büyükokuroğlu, M. E., Oktay, M., Küfrevioğlu, Ö. İ. (2003). Antioxidant and analgesic activities of turpentine of Pinus nigra Arn. subsp. pallsiana (Lamb.) Holmboe. Journal of Ethnopharmacology, 86(1), 51-58. DOI: 10.1016/S0378-8741(03)00036-9

Kalaras, M.D., Richie, J.P., Calcagnotto, A., Beelman, R.B. (2017). Mushrooms: A rich source of the antioxidants ergothioneine and glutathione. Food Chem. 233:429-433. doi: 10.1016/j.foodchem.2017.04.109.

Kolundzic, M., Grozdanic, N.D., Dodevskac, M., Milenkovic, M., Sistoe, F., Mianie, A., Farronato, G., Kundakovic, T. (2016). Antibacterial and cytotoxic activities of wild mushroom Fomes fomentarius (L.) Fr., Polyporaceae. Ind Crops Prod., 79: 110-115. DOI: 10.1016/j.indcrop.2015.10.030

Kolniak-Ostek, J., Oszmiański, J., Szyjka, A., Moreira, H., Barg, E. (2022). Anticancer and Antioxidant Activities in Ganoderma lucidum Wild Mushrooms in Poland, as Well as Their Phenolic and Triterpenoid Compounds. Int J Mol Sci. 23(16):9359. doi: 10.3390/ijms23169359.

Kozarski, M., Klaus, A., Jakovljevic, D., Todorovic, N., Vunduk, J., Petrović, P., Niksic, M., Vrvic, M.M., van Griensven, L. (2015). Antioxidants of Edible Mushrooms. Molecules. 20(10):19489-525. doi: 10.3390/molecules201019489.

Lee, D. H., Kim, J. H., Park, J. S., Choi, Y. J., Lee, J. S. (2004). Isolation and characterization of a novel angiotensin I-converting enzyme inhibitory peptide derived from the edible mushroom Tricholoma giganteum. Peptides, 25(4), 621-627. DOI: 10.1016/j.peptides.2004.01.015

Li, Q.Z., Zheng, Y.Z., Zhou, X.W. (2019). Fungal immunomodulatory proteins: characteristic, potential antitumor activities and their molecular mechanisms. Drug Discov. Today ; 24(1), 307-314).

Mohammadifar, S., Fallahi Gharaghoz, S., Asef Shayan, M.R., Vaziri, A. (2020). Comparison between antioxidant activity and bioactive compounds of Ganoderma applanatum (Pers.) Pat. and Ganoderma lucidum (Curt.) P. Karst from Iran. Iranian Journal of Plant Physiology, 11(1), 3417-3424.

Oviasogie, F. E., Akpaja, E. O., Gbona, K. C., Akonoafua, E. A. (2015). Antimicrobial properties of Ganoderma applanatum (Pers.) Pat. from Benin city, Nigeria. Nigerian Journal of Agriculture, Food and Environment, 11(3), 65-69.

Rahman, M.A., Abdullah, N., Aminudin, N. (2015). Antioxidative effects and inhibition of human low density lipoprotein oxidation in vitro of polyphenolic compounds in flammulina velutipes (golden needle mushroom). Oxid Med Cell Longev. 2015;2015: 403023. doi: 10.1155/2015/403023.

Slinkard, K., Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. American Journal of Enology and Viticulture, 28(1), 49-55.

Suárez-Arroyo, I.J., Loperena-Alvarez, Y., Rosario-Acevedo, R., Martínez-Montemayor, M.M. (2017). Ganoderma spp.: A Promising Adjuvant Treatment for Breast Cancer. Medicines (Basel). 4(1):15. doi: 10.3390/medicines4010015.

Umeno, A., Biju, V., Yoshida, Y. (2017). In vivo ROS production and use of oxidative stressderived biomarkers to detect the onset of diseases such as Alzheimer's disease, Parkinson's disease, and diabetes. Free Radic. Res. 51 (4) 413–427. doi: 10.1080/10715762.2017.1315114.

Wasser, S. P. (2011). Current findings, future trends and unsolved problems in studies of medicinal mushrooms. Applied Microbiology and Biotechnology, 89(5):1323-1332. doi: 10.1007/s00253-010-3067-4.

Zhong-Hua, L., Xiao-Ge, H., Jin-Hui, Z., Le, HE. (2015). Liquid Fermentation of Ganoderma applanatum and Antioxidant Activity of Exopolysaccharides. Open Biomed Eng J. ;9:224-227. doi: 10.2174/1874120701509010224.

Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2024, Volume: 8, Issue: 1, 19-26
Received: 08.09.2023; Accepted: 21.11.2023
DOI: 10.30516/bilgesci.1357510

The Experimental Analysis of The Effect of Geotextile Reinforcement of Cohesive Soil on The Settlement and Bearing Capacity

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Abstract: The engineering properties of soils must be identified to design buildings and foundations in areas where soft clay or loose sand ground conditions predominate. These properties vary widely depending on the type of soil and terrain conditions, such as compaction, water content, consolidation pressure, loading, and drainage conditions. Soils may not always retain the desired properties. Structures built on soils with inadequate bearing capacity can experience excessive settlement or collapse. To reinforce weak soils, either deep foundations or ground improvement methods can be used. This study examined the effect of geotextile reinforcement on clay ground. Experiments were conducted at the Süleyman Demirel University Soil Mechanics Laboratory on clay samples from the provinces of İzmir and İstanbul to determine the index properties, settlement, and sliding resistance parameters of soils. After identifying the settlement and sliding values, geotextile was added, and its effect on settlement and bearing strength values was analyzed. Experiments were conducted by placing single-layered and double-layered geotextile in samples with optimum water content, water content 10% higher than optimum, and water content 10% lower than optimum. The effects on the settlement and bearing capacity of geotextiles mentioned in the article were examined. The results achieved after laboratory experiments are displayed with graphics and compared with each other. As a result of this study, it was observed that geotextile reinforcement increased the bearing capacity of the soil and controlled the settlement behavior.

Keywords: Clay, Geotextile, Bearing Capacity, Settlement, Unconfined compressive strength

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Citation: Fındık, M., Keskin, S.N. (2024). The Experimental Analysis of The Effect of Geotextile Reinforcement of Cohesive Soil on The Settlement and Bearing Capacity. Bilge International Journal of Science and Technology Research, 8(1): 19-26.

1. INTRODUCTION

In geotechnical engineering, soft clay grounds are regarded as problematic grounds in terms of both high compressibility and low load-bearing capacity. Various methods are employed to develop the bearing capacity and settlement behavior of such grounds. There are various ground improvement methods such as mini piles, ground anchoring, stone column, deep mixing, groundwater disposal, ground changing, additive material, filling. One of the methods of soil amendment, which has grown increasingly significant in current years, is the geosynthetic-reinforced grounds. Geotextiles can be applied in various ways to develop the engineering parameters of inconvenient and problematic soils. They, which are classified as woven or non-woven, are often applied in bevels, retaining constructions, highways, railways, fillings that settle on soft floors, and sub-base foundations for improvement (Koerner,1989). Quick and durable solutions can be created with geosynthetics. Experimental and theoretical researches connecting geosynthetics with clay grounds have been carried out.

Mandal and Sah (1992); examined the geogrid reinforcements placed horizontally on the clay ground layers, and the bearing strength of square foundations through model experiments. Improvement was observed at all settlement rates, and notable improvements were observed in the improvement factor in the range of u / B (reinforcement depth/foundation width) =0-0.25.

Ramaswamy and Purushothaman 1992); studied the bearing capacity of model foundations lying on clay ground reinforced with geogrid reinforcement by experimental studies. In clay ground and clay ground with geotextile, the

bearing capacity reduced as the water content increased. While the bearing capacity ratio (BCR) of the clay reinforced with double-layered geogrid reinforcement in the optimum water content was 1.47 compared to the clay ground, this ratio was 1.11 in the wetter condition than the optimum, and it was 1.26 in the drier condition than optimum.

Shin et al. (1993) analyzed the bearing capacities of the strip foundation on a water-saturated clay ground reinforced with geogrid reinforcements by laboratory experiments. Model experiments were conducted on one type of clay, and the alteration of the average water content created changes in the undrained sliding strength. The undrained sliding strength *cu* was identified through the vane test. In laboratory experiments, the critical geogrid layer depth, layer width, primary reinforcement layer depth, and the highest Bearing Capacitiy Ratio BCR value and u/B (reinforce depth/foundation width) was assessed as 0.4.

Das et al. (1994) examined the bearing capacity of strip foundation on the sand and water-saturated clay ground reinforced by geogrid. Grounded on model experiments, optimum primary reinforcement depth, optimum total reinforcement depth, and width were determined for both types of soils. Das et al. reached a ratio between the reinforcement depth and the width of the foundation, B. The total reinforcement depth operating efficiently in the sand was determined to be 2B and 1.75B in clays. The primary reinforcement depth that provided the maximum bearing capacity was 0.30 B in the sands and 0.40B in the clays.

Adams and Collin (1997) performed 34 loading experiments utilizing two different geosynthetics (geogrid and geocell) in order to determine the bearing strength and settlement properties of the surface foundations on geosynthetic reinforced grounds. The number of reinforcement layers, the distance between layers, first reinforcement depth, reinforcement type, and ground density are regarded as variable parameters. From the results achieved from the experiments, the addition of geosynthetic reinforcement has been given to improve the bearing capacity of the sand grounds by about 2.5 times. In the situation where the first reinforcement depth is 0.25B, the highest bearing capacity value is achieved.

Alawaji (2001) examined the strengthening of collapsible sand grounds with geogrid reinforcements, which are exposed to the collapse settlement because of water content. Consequently, Alawaji discovered that the most effective reinforcement design on the collapsible ground is for the situation where the geogrid width is higher four times than the diameter of the loaded area, and the depth of the foundation diameter is 10 %.

Bergado et al. (2001) compared the rise in bearing capacity of soft clay grounds reinforced with geotextile with both experimental and numerical analysis. In the experimental study, they applied the modified California Bearing Ratio (CBR) experimental setup. In their reinforcement studies, they used three different reinforcements of different rigidness. In the modeling, it was estimated that the elasticity modules of clay grounds were higher 315 times than that of the undrained sliding strength. With the parameters applied in these models, values very close to experimental results were achieved.

Dash et al.(2003) studied the effects of geocell on the soft clay ground on the granular filling layer on the small-scale model experiments. Subsequently, it has been discovered that with the convenient settlement of the geocell, the bearing capacity of the circular foundation can be increased seven times.

Noorzad and Mirmoradi (2010) carried out extensive research on clays, including geotextile reinforcement. They examined the effect of geotextiles with various permeability properties, water content, number of geotextile reinforcement, and plasticity on strength parameters of reinforced clay with the triaxial and unconfined compression test. Noorzad and Mirmoradi observed that geotextile reinforcement developed the strength parameters of the clay.

Karakan et al. (2015) studied the behavior of clay grounds reinforced with geotextile reinforcement. With the aim of defining the stress-strain behavior of clay ground samples, unconfined compression experiments have been carried out with and without reinforcement. In the study, in order to show the optimum water content and the change of water content that may happen in the field using clay with low plasticity, it was examined on the dry and wet sides of the compaction curve (± 2, +3, and ± 4). With these parameters, a series of experiment set, including at least three samples were developed. It has been observed that the use of reinforcement develops the mechanical properties of the ground, and the use of geotextiles improves the peak strength.

Çakar (2016) directed a series of unconfined compression tests to define the effects and potential advantages of the use of geotextiles on the mechanical behavior of clay grounds. The effect of the parameters influencing the mechanical properties of the geotextile reinforced material such as geotextile type (woven and non-woven), loading speed, the number of geotextile layers (non-layered, single-layered, two-layered, three-layered and four-layered) was examined.

Aslan (2021) in a study conducted on reinforced earthen walls; hard to get instead of granular filler, marginal filler, which is easier to obtain, was used by changing the filler type. By changing the reinforcement type of this filler (using more resistant reinforcement) and using geosynthetic with higher bending rigidity, an advantage was achieved in terms of construction time and cost of the structure, and it performed as well as granular fillers.

Noori ve Dehganyan (2021) in their study, geogrid and geotextile were placed in clay and sand soils in different numbers, at different depths and in different layers. When the situations of placing one row, two rows and three rows were compared, one row placement had no effect on reducing seating, but they saw a positive contribution in the others.

Demir et al. (2022) in their study, as a result of improving the soil with geotextile produced from hemp, free pressure and triaxial pressure test data showed that the use of

reinforcement had a significant positive effect on soil strength.

In this study, the effect of geotextile reinforcement, which is one of the improvement methods for clay soils, was investigated experimentally. To determine the soil parameters, classification experiments, uniaxial compression tests and consolidation tests were carried out on two different clay soils. Afterwards, clay soils were prepared at three water contents, one of which was optimum water content, and the changes in settlement and shear parameters were examined in case of single and double row geotextile placement.

2. MATERIAL AND METHOD

In the design of the foundations, it is essential to comprehend the engineering and index properties of the ground along with the structure load, load distribution, and structure features. To determine the engineering parameters of soils Sieve analysis, consistency limits and pycnometer tests were carried out. In this context, the index and compaction properties of clays obtained from Istanbul and Izmir were initially defined. At the end of the experiments, the sample from Istanbul province presented Low Plasticity Clay ground (CL) value and the sample from Izmir province High Plasticity Clay ground gave (CH) value. The test results are displayed collectively in Table 1.

Table 1. Index and Compaction Properties of Samples(Dikmen, 2013)

Sample	PL(%)	LL(%)	IP(%)	γ_s (gr/cm^3)	γ_k (gr/cm^3)	w_{opt}
	Plastic limit	Liquid limit	Plasticity Index	Natural unit volume weight	Dry unit volume weight	opt. Water content
İstanbul (CL)	66.5	45	21.5	2.80	1.4	0.295
İzmir (CH)	19	78	59	2.75	1.4	0.3

An odometer test is carried out to measure the amount and speed of consolidation under vertical and axial pressure by providing drainage from the upper and lower surfaces of a water-saturated, disc-shaped and undisturbed soil sample, whose lateral deformation is prevented.In order to define the consolidation parameters of the samples provided by compressing at the maximum dry unit weight, and optimum water content collected from compaction tests, consolidation experiments (oedometer) were carried out. Gradual loading and unloading stages up to 16 kg / cm^2 have been performed on low plasticity and high plasticity clay grounds.

Technical specifications of the woven geotextile used in the experiments are presented in Table 2.

Table 2. Technical properties of geotextiles

Parameters	Value
Thickness	0,7 mm
Wweight	192 g/m^2
Tensile Strength	40 kN/m
Elongation	20 %
Static Puncture Resistance	4,8 kN
Dynamic Performance Resistance	11 mm
Water Permeability	16*10^{-3} m/sn

Later, after settling geotextile at h / 2 height in both ground samples, odometer experiments were repeated.Experimental images are presented in Figure 1.

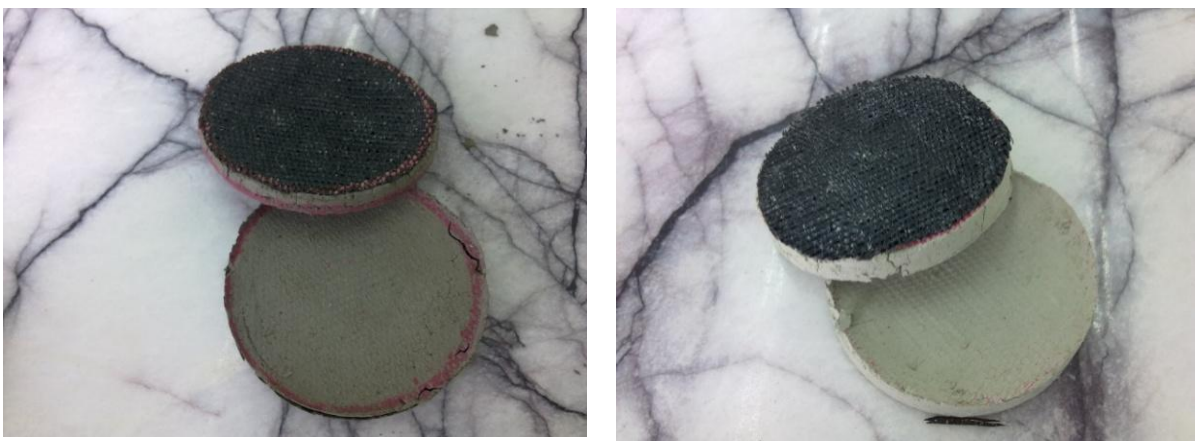
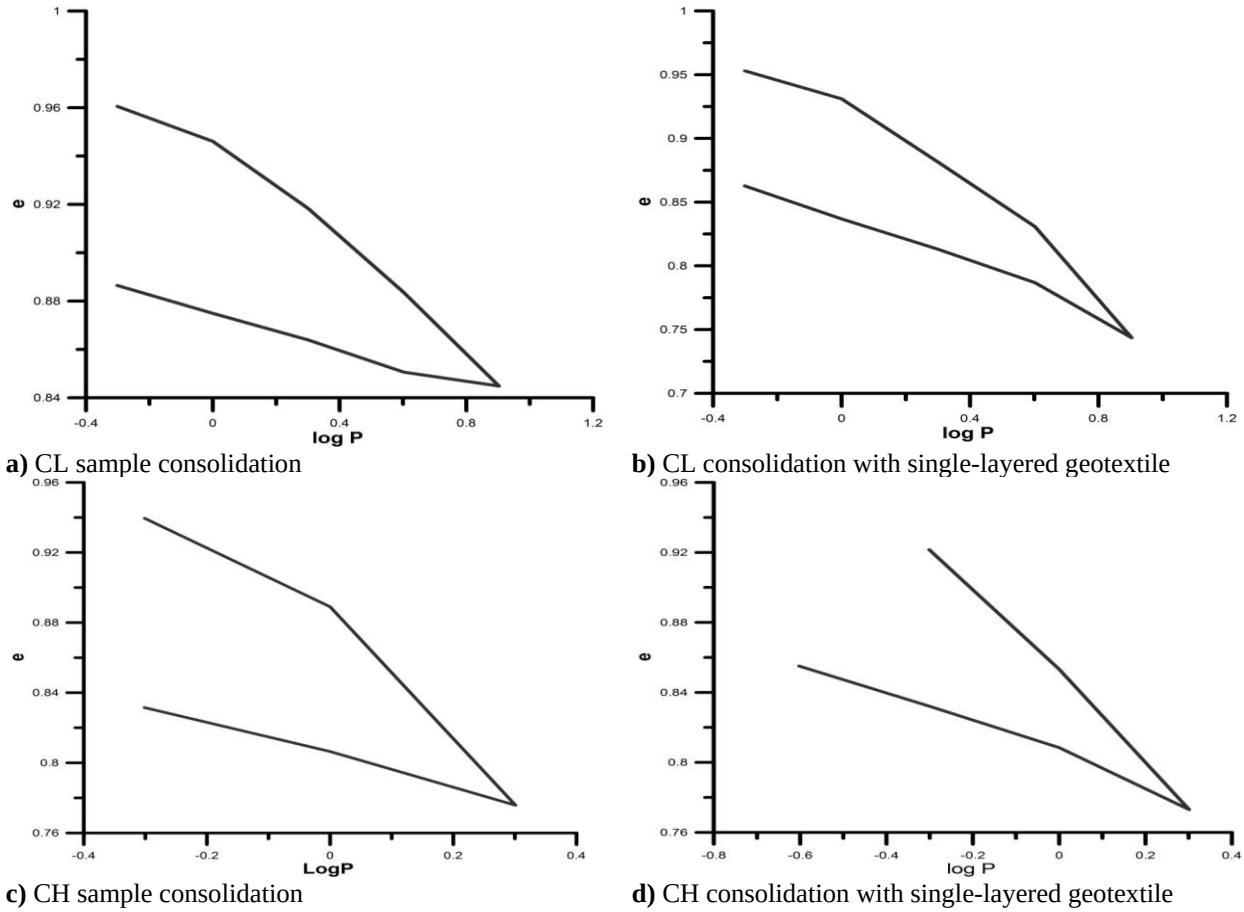


Figure 1. Consolidation test samples placed with geotextile (Dikmen, 2013)

The test results completed on low-plasticity and high-plasticity clay samples and on the series placed with getorextile are presented graphically. The results achieved are displayed in the graphics in Figure 2.



a) CL sample consolidation **b)** CL consolidation with single-layered geotextile

c) CH sample consolidation **d)** CH consolidation with single-layered geotextile

Figure 2. Odometer test result (Dikmen, 2013)

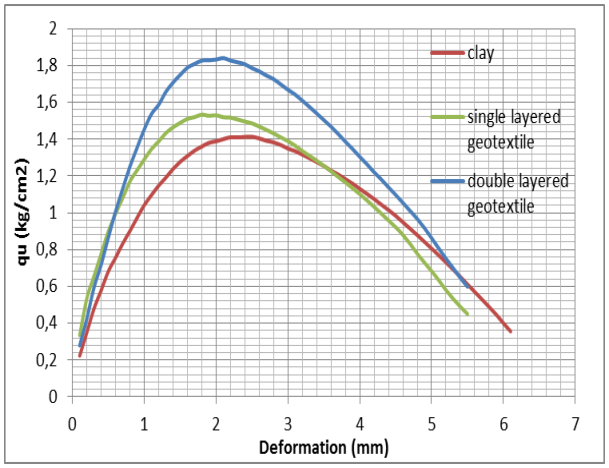
Unconfined compression tests have been conducted to define the undrained sliding strength of grounds. In both samples, experiments were conducted by putting clay on only single-layered geotextile at h / 2 height in clay sample and by placing double-layered geotextiles at h / 3-2h / 3 heights in clay sample. Free pressure test sample images are shown in figure 3.

a) Low plasticity clay sample

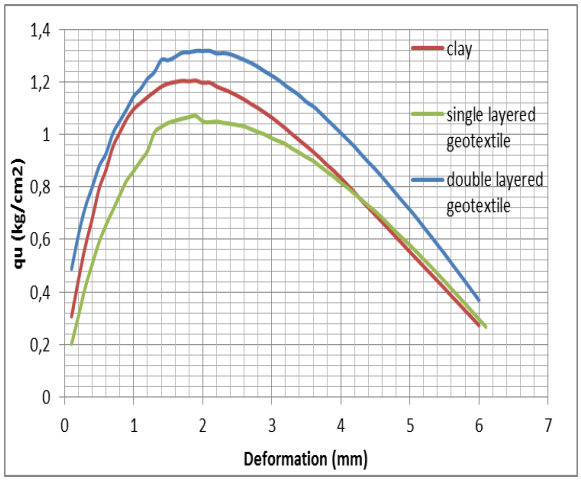
b) High plasticity clay sample

Figure 3. Free pressure test samples (Dikmen, 2013)

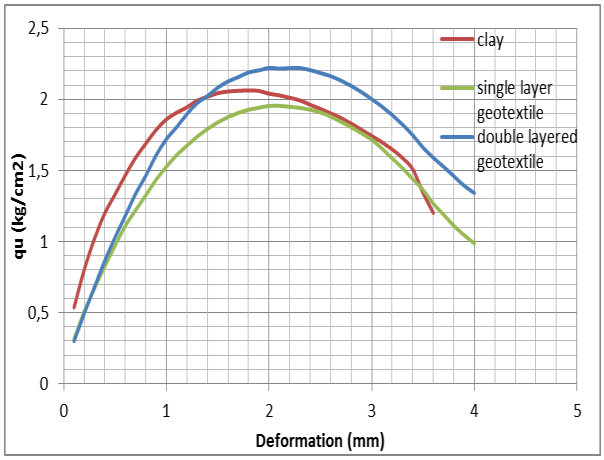
Samples were provided in 3 different water contents such as optimum water content, wopt + 10% more than water content, wopt-10% less than water content. Experiments were repeated for three separate water contents and single-layered and double-layered combinations. The results obtained are classified according to low and high plasticity and water content and are shown in the graphs in Figure 4.



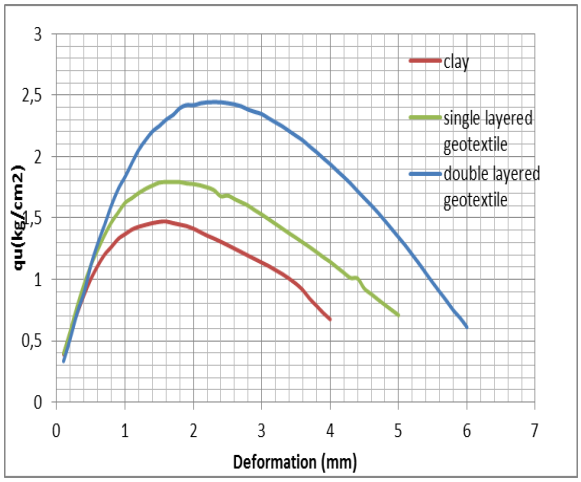
a) CL opt water content



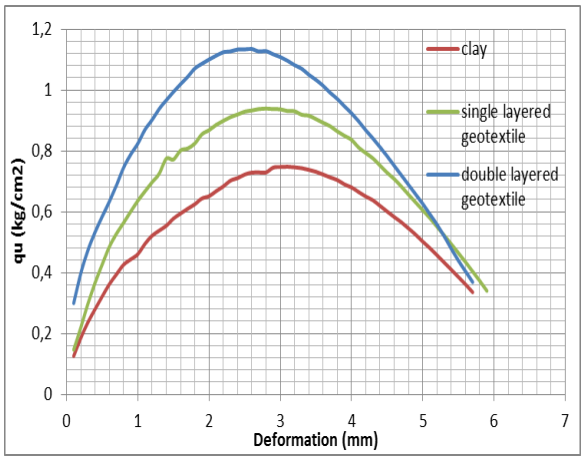
b) CH opt water content



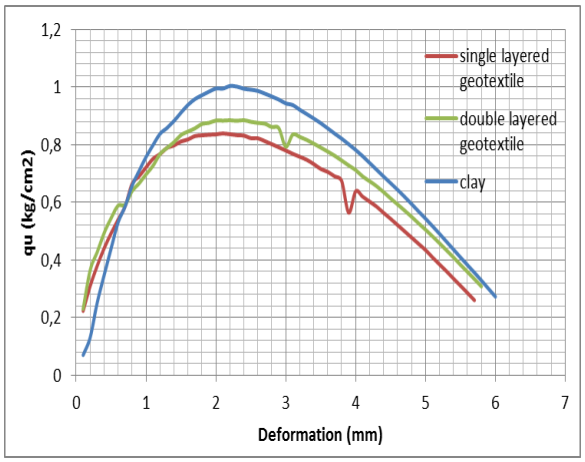
c) CL 10 % less than opt water content



d) CH 10 % less than opt water content



e) CL 10 % more than opt water content



f) CH 10 % more than opt water content

Figure 4. Free pressure test results (Dikmen, 2013)

In the low plasticity clay sample, the unconfined compressive strength obtained at optimum water content was 1.4 kg/cm^2 for clay alone, 1.5 kg/cm^2 for single-layer geotextile reinforcement and 1.8 kg/cm^2 for double-layer geotextile reinforcement. In the low plasticity clay sample, the unconfined compressive strength obtained at a water content of 10% less than the optimum water content was 1.8 kg/cm^2 for clay alone, 2 kg/cm^2 for single-layer geotextile reinforcement, and 2.2 kg/cm^2 for double-layer geotextile reinforcement. In a low plasticity clay sample, the unconfined compressive strength obtained at 10% more water content than the optimum water content is 0.76 kg/cm^2 for clay alone, 0.94 kg/cm^2 for single-layer geotextile reinforcement, 1.15 kg/cm^2 for double-layer geotextile reinforcement. was obtained.

In the high plasticity clay sample, the unconfined compressive strength obtained at optimum water content was 1.1 kg/cm^2 for clay alone, 1.2 kg/cm^2 for single layer geotextile reinforcement, and 1.32 kg/cm^2 for double layer geotextile reinforcement. In the high plasticity clay sample, the unconfined compressive strength obtained at a water content of 10% less than the optimum water content was 1.5 kg/cm^2 for clay alone, 1.8 kg/cm^2 for single-layer geotextile reinforcement, and 2.45 kg/cm^2 for double-layer geotextile reinforcement. In the low plasticity clay sample, the unconfined compressive strength obtained at 10% more water content than the optimum water content was 0.84 kg/cm^2 for clay alone, 0.88 kg/cm^2 for single-layer geotextile reinforcement, and 1.0 kg/cm^2 for double-layer geotextile reinforcement.

3. DISCUSSION AND CONCLUSIONS

In this study, first a literature review was conducted about clay soils and improvement methods. Then, index tests, uniaxial compression tests and consolidation tests were performed on two different clay soils in the laboratory to determine the parameters of the soil. Geotextile offered significant technical and financial advantages when applied correctly. The test results are shown below in detail and comparatively. In general evaluation, geotextile reinforcement reduced the settlement amount in clay soils. Geotextile reinforcement increased the unconfined compressive strength values of the soil. As the number of geotextile layers increases, the strength of the soil also increases, and this increase clearly indicates the bearing capacity. Additionally, as the number of reinforcement increases, the soil becomes more ductile in tension.

The results achieved can be summed up as follows:

- The geotextile in the low plasticity clay ground placed at 0.5h height of the odometer ring decreased the settlement values of the ground by 50% at the same load level.
- The geotextile in the high plasticity clay ground placed at 0.5h height of the odometer ring decreased the settlement values of the ground by 15% at the same load level.
- The unconsolidated-undrained unconfined compressive strength performed in the optimum water content of the low plasticity clay ground rose by 1% in the case of placing a single-layered geotextile, while it rose by 20% in the case of placing a double-layered geotextile.
- In the optimum water content of clay ground with the low plasticity, the unconsolidated-undrained unconfined compressive strength in the water content 10 % less than optimum water content increased by 10% while the double-layered geotextile increased by 30%.
- The unconsolidated-undrained unconfined compressive strength in the optimum water content of the clay with high plasticity rose by 1% in the case of putting a single- layered geotextile, while it increased by 20% in the case of putting a double-layered geotextile.
- In the optimum water content of clay ground with the high plasticity, the unconsolidated-undrained unconfined compressive strength in the water content 10 % less than optimum water content increased by 30% while in the case of placing a single-layered geotextile, it rose by 70%.
- In the optimum water content of the high and low plasticity clay ground, the water content 10 % more than optimum water of unconsolidated-undrained unconfined compressive strength did not noticeably change if single and double-layered geotextile was placed on the ground. Since there was excess water than needed in the ground, the strength values of the clay ground were negatively influenced.

Acknowledgements
I would like to thank Süleyman Demirel University Scientific Research Projects Management Unit for their financial support for this study, which was prepared within the scope of the Scientific Research Project numbered 2999-YL-11.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: Investigation: Material and Methodology, Supervision: Visualization: Writing-Original Draft: Writing-review & Editing: M.F. Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Adams, M.T. ve Collin, J. G., (1997). Large Model Spread Footing Load Tests on Geosynthetic Reinforced Soil Foundation, ASCE Journal of Geotechnical and Geoenvironmental Engineering, 66-72.

Alawaji, H. A., (2001). Settlement and Bearing Capacity of Geogrid – water content 10% higher than optimum Reinforced Sand over Collapsible Soil. Geotextiles and Geomembranes, 19, 75-88.

Aslan E., (2021). Performance of Marginal Fillers Reinforced with Geosythetics Under Static Loads. Bursa Uludağ University Graduate School of Natural and Applied Sciences Department of Civil Engineering. Master's thesis, 123 p.

Bergado D. T., Youwai S., Haic C. N., Voottipruex P., (2001). Interaction of Nonwoven Needle-Punched Geotextiles Under Axisymmetric Loading

Conditions. Geotextiles and Geomembranes, 19, 299-328.

Çakar, G., (2016). Investigation of the behavior of geotextile reinforced clays in the laboratory, Master's Thesis, Balıkesir University Graduate School of Natural and Applied Sciences, Balıkesir.

Das, B.M., Shin, E.C., Omar, M.T., (1994). The Bearing Capacity Of Surface Strip Foundations On Geogrid-Reinforced Sand And Clay – A Comparative Study. Geotechnical And Geological Engineering, 12, 1-14.

Dash S. K., Sireesh S. and Sitharam T. G., (2003). Model Studies on Circular Footing Supported on Geocell Reinforced Sand Underlain by Soft Clay.

Demir, E., Kolay, E. & Karaduman, Y. (2022). Kenevirden İmal Edilen Geotekstilin Sıkıştırılmış Bazı Zeminlerin Dayanımı Üzerindeki Etkisi. Mühendislik Bilimleri ve Araştırmaları Dergisi, 4 (2) , 266-277 . DOI: 10.46387/bjesr.1167983

Dikmen M., (2013). Experimental investigation of the effect of geotextile reinforcement on the settlement and bearing capacity behavior of foundations resting on clay soil using the Plaxis 3D program, Süleyman Demirel University. Institute of Science and Technology. Department of Civil Engineering. Master's thesis, 106p.

Karakan E., Okucu A., Yağcı B., (2015) Stress-strain and strength parameters of geotextile reinforced clays. 6th Geotechnical Symposium, November 2015, Adana

Koerner, R.M., (1989). "Designing with Geosynthetics", Prentice Hall, Englewood Cliffs, New Jersey.

Mandal, J. N., Sah, H., (1992). Bearing Capacity Tests on Geogrid-Reinforced Clay, Geotextiles and Geomembranes. 327-333.

Noorı M.S. and K. Dehghanıan K., (2021). Settlement Analysis of Geosynthetics Reinforced Embankments, Dicle University Journal of Engineering) 12:4, Page 699-709, doi:10.24012/dumf.1002256

Noorzad, R., Mirmoradi S.H., (2010), Laboratory evaluation of the behavior of a geotextile reinforced clay, Geotextiles and Geomembranes, 28,386-392.

Ramaswamy S.D., and Purushothaman P., (1992). Model footings of geogrid reinforced clay. Proceedings of the Indian Geotechnical Conference on Geotechnique Today, 183-186.

Shin E., Das B., Puri S., Yen S., Cook E., (1993). Bearing Capacity of Strip Foundation on Geogrid-Reinforced Clay. Tech. Note, American Society for Testing and Materials, 534-541.

Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2024, Volume: 8, Issue: 1, 27-37
Received: 17.01.2024; Accepted: 13.03.2024
DOI: 10.30516/bilgesci.1421304

Humic Acid Mitigates Drought Stress in Tomato

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Abstract: Drought stress, one of the most important abiotic stresses, severely limits global crop production. To increase tolerance for this stress, environmentally friendly practices are emphasized. Humic acid, one of the most important natural biostimulants, has positive effects on plant growth and yield. Recently, it has also been reported to play an important role in resistance to various abiotic stresses. However, many physiological and molecular mechanisms by which humic acid confers drought resistance have not been fully elucidated. Therefore, the effects of humic acid application (3 ml L^{-1}) on different morphological and physiological stress indicators and some antioxidative enzyme gene expressions of tomato seedlings under drought stress conditions were investigated in this study. It was found that drought stress decreased the shoot fresh/dry weight, root fresh/dry weight, shoot and root length, chlorophyll content and relative water content of plants by 67%, 56%, 31%, 38%, 22%, 20%, 15% and 25%, respectively. Humic acid application significantly increased these parameters, while reducing ion leakage, MDA, and proline levels. The antioxidant enzyme gene expression of tomato seedlings under drought conditions showed no significant difference in *SOD* and *APX* gene expression, whereas *CAT* gene expression increased and *GR* gene expression decreased with humic acid application. Our results showed that humic acid application interacted with stress-related antioxidant enzyme gene expression and may be effective in reducing drought stress.

Keywords: Drought stress, Humic acid, Tomato, Gene expression

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Citation: Aytaç, E., Ünlü, H. Ö., Turan, T., Erkan, İ. E., Akçay, U. Ç., (2024). Humic Acid Mitigates Drought Stress in Tomato. Bilge International Journal of Science and Technology Research, 8(1): 27-37.

1. INTRODUCTION

The agricultural sector worldwide is facing a variety of stresses that are causing major losses in crop productivity and compromising the sustainability of agriculture. Abiotic stresses are known as the most important environmental factors influencing agricultural productivity worldwide, and alone or in combination, they produce excessive amounts of reactive oxygen species (ROS), leading to disruption of redox homeostasis and oxidative stress, which affect plant physiology (Gupta et al., 2022).

Abiotic stresses include pH, high soil salinity, extreme temperature, and drought stress. Drought stress is the most catastrophic stress factor, with severe effects on the yield stability of crops (Manna et al., 2021). Therefore, there is a requirement for a more complete understanding of the responses of plants to abiotic stress and to develop stress tolerant plants and/or practices to improve plant stress tolerance (Alcázar et al., 2020; Manna et al., 2021).

The development of drought-tolerant crop varieties has been possible through genetic engineering, conventional and molecular breeding techniques. However, these processes are laborious and time-consuming, and regulatory concerns about genetically modified crops have prevented their widespread acceptance (Joshi et al., 2020). As an alternative to these methods, the use of biostimulants is one of the most promising strategies for alleviating drought stress (Calvo et al., 2014).

Humic acid is one of the most valuable biostimulants that can be applied externally to increase plant resistance to stress (Arslan et al., 2021). Various field experiments and experimental findings have shown that humic matter can alleviate the effects of abiotic stress (Canellas et al., 2020). Humic acid, which is derived from plant or animal waste, functions as a hormone-like compound, actively promotes the growth and development of plants, and provides protection against abiotic stresses (Arslan et al., 2021).

Humic acid supports plant growth under drought stress by enhancing osmotic adjustment, antioxidant capacity, and photosynthesis (Shen et al., 2020a). It has been suggested that humic acid can improve the hydro-physical properties of soils and increase the drought tolerance of plants, but the underlying molecular process is not yet known (Chen et al., 2022).

Tomatoes are one of the most economically important and widely grown crops in the world (Ansari et al., 2023). With a wide range of health benefits, antioxidant and anti-cancer properties, they are also important products for human well-being (Yadav et al., 2023). As the world's second-most important horticultural crop, both in terms of yield and consumption, tomatoes are also challenged by drought. Tomato plants are affected negatively by drought stress in several biochemical, morphological, physiological, and genetic ways. This not only reduces fruit quality and seed production but also causes significant yield losses (Islam et al., 2023). Therefore, this study was carried out to investigate the effects of humic acid on various morphological and physiological stress indicators and some antioxidative enzyme gene expressions of tomato seedlings under drought stress conditions.

2. MATERIAL AND METHOD

2.1. Plant material and treatments

This research was planned according to a randomised experimental design with 3 replicates and 10 plants in each replicate. Kayra F_1 tomato cultivar (Anamas Seeds, Antalya) seeds were sown in 400 ml polypropylene containers containing sterile perlite. Every two days, irrigation was carried out using Hoagland's solution (Hoagland and Arnon, 1950). Plants were grown for 15 days in a growth chamber with 50% humidity at 24˚C in a 16h light/8h dark cycle. The control treatment had no application other than irrigation. Humic acid (TKİ HÜMAS) was sprayed on the leaves at the rate of 3 ml/L in humic acid treatment. The drought stress started at the end of the 15th day and the water potential of 0 was reached on the 3rd day. 7 days after drought stress samples for analysis have been collected.

2.2. Growth parameters

In order to determine dry weight, the tissues of the shoots and roots were separated, weighed, and dried at 60˚C for 48 hours, then weiged again.

2.3. Relative water content (RWC)

RWC was determined in accordance with the formula given in Smart and Bingham (1974). Accordingly, RWC (%) = (Fresh weight-Dry weight)/ (Turgid weight-Dry weight) X 100. The turgid weight was measured by soaking the leaves in distilled water for 24 hours at room temperature.

2.4. Determination of proline content

The proline content was determined by applying the Bates et al. (1973) method. For this, 0.3g of sample was ground in liquid nitrogen and dissolved by adding 1ml 3% sulphosalicylic acid. 0.1 ml of this mixture was taken and centrifuged. Ninhydrin (0.2 ml), 96% acetic acid (2 ml) and 3% sulphosalicylic acid (0.1 ml) were added. The mixture was kept at 96˚C for 1 hour. After the addition of 1 ml of toluene, centrifugation was repeated and the absorbance of the supernatant obtained was measured at 520 nm with a spectrophotometer.

2.5. Determination of membrane damage

To assess the membrane damage caused by drought stress, malondialdehyde (MDA) levels and membrane electrolyte leakage were determined.

Membrane damage resulting from lipid peroxidation was determined by using the Ohkawa et al. (1979) method to estimate MDA levels. Liquid nitrogen was used to homogenize 0.2 g of the sample, and 1 ml of 5% trichloroacetic acid (TCA) was added. After centrifugation, 0.5% thiobarbituric acid (TBA) was added to the same volume of 20% TCA and kept at 96˚C for 25 minutes. After cooling the samples on ice, the absorbance values were determined at 532 nm. Non-specific absorbance values were read at 600 nm and subtracted from the initial absorbance values.

Electrolyte leakage was determined using the method described in Nanjo et al. (1999). According to this method, 6 leaves were kept in test tubes containing 0.4 M mannitol for 3 hours with shaking, and the electrical conductivity was determined as C1. After 15 minutes in boiling water, the samples were cooled to room temperature and C2 was read. This C2 value has been calculated using the leakage dependent conductivity formula [(C1/C2) X 100]. For electrical conductivity, a Thermo Scientific Orion 013016MD MD 2 condactivimeter probe was used, which can measure in the range 0.01-300 mSs/cm.

2.6. Chlorophyll content determination

The Spad-502 Plus chlorophyll meter was used to measure the amount of chlorophyll content in tomato leaves. Measurements were taken at different points on the leaves of each plant and the results were expressed in SPAD.

2.7. Gene expression analysis

In the study, total RNA was first isolated (Qiagen Rneasy Plant Mini Kit, Qiagen USA) to be used in the semi-quantitative RT-PCR method. The cDNA was then

synthesized (VitaScript cDNA synthesis kit, Procomcure Biotech Austria). PCR amplification was performed with primers specific for four different antioxidant system enzymes, *FeSOD*, *CAT2*, *GR1* and *APX1* genes, prepared using the Primer Premier program (PREMIER Biosoft International, USA). The NCBI Gene Bank reference sequence codes of the genes analyzed in this study are NM_001313769.1, NM_001247257.2, NM_001321393.1, and NM_001247853.2 respectively. The *EF-1* (elongation factor 1 alpha) gene with reference sequence code X14449.1 was used as an internal control in the study.

2.8. RNA isolation

RNA was isolated from tomato leaves using Qiagen RNeasy Plant Mini Kits based on guanidine isothiocyanate lysis and silica membrane purification. Total RNA amounts were determined spectrophotometrically using Nanodrop 2000. The quality of total RNA was determined by separating and visualizing it using 2% agarose gel electrophoresis.

2.9. RT-PCR (Semi-quantitative Reverse Transcription-PCR)

From the RNA molecules obtained, cDNA was synthesized using the VitaScript cDNA synthesis kit with oligodT primers. Primers specific for the genes studied were designed using PrimerPremier 5.0, CA, USA, and PCR amplification was performed using the primers that gave the most appropriate amplification conditions. The bands obtained were separated on a 0.8% agarose gel and visualized using the Biolab UV Tech gel imaging system. The imaged bands were analyzed using ImageJ software developed by the National Institute of Health (NIH) to reveal differences in gene expression levels. The cycle steps and times for PCR analyses are shown in Table 1.

Table 1. PCR step times and cycles

Cycle Step	Temperature	Time	Number of cycles
Initial denaturation	95 ^{0}C	5 min	
Denaturation	95 ^{0}C	1 min	
Annealing	-	45 s	28
Extension	72 ^{0}C	45 s	
Final extension	72 ^{0}C	10 min	

2.10. Data statistical analysis

The Minitab (17) Inc. was used to perform an analysis of variance on the study's data. Using the Tukey test, the differences between the significant means were indicated by distinct letters.

3. RESULTS AND DISCUSSIONS

3.1. Effects of humic acid on the growth parameters

To determine the effects of humic acid on the growth parameters of the tomato plant, measurements of root fresh/dry weight, shoot fresh/dry weight, shoot and root length were carried out and the morphological effects are given in the table below. The effect of the treatments on all these parameters was significant (P<0.05).

Table 2. Effects of treatments on growth parameters

Treatments	Root Fresh Weight (g)	Root Dry Weight (g)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Shoot Length (cm)	Root Length (cm)
Control	0.52 ±0.01^b	0.034 ±0.00^b	2.20 ±0.02^b	0.16 ±0.00^b	17.72 ±0.36^b	18.28 ±0.26^b
Humic Acid	0.64 ±0.00^a	0.046 ±0.00^a	2.44 ±0.04^a	0.17 ±0.00^a	19.06 ±0.16^a	19.04 ±0.50^a
Drought Stress	0.36 ±0.01^d	0.021 ±0.00^d	0.72 ±0.01^d	0.07 ±0.00^d	13.88 ±0.18^d	14.61 ±0.14^d
Drought Stress + Humic Acid	0.45 ±0.01^c	0.030 ±0.00^c	1.08 ±0.00^c	0.11 ±0.00^c	15.09 ±0.04^c	16.80 ±0.22^c

Note: Differences between values shown with different letters are significant at P<0.05 level.

The results of the study showed that shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of tomato plants decreased by 67%, 56.2%, 30.7% and 38.2% respectively when comparing the control and drought-stressed groups of plants (Table 2). Altunlu (2011) in tomato, Kılıçaslan Caşka (2019) in bean, Avşaroğlu (2015) in watermelon, Faaek (2018) in strawberry and Sadak (2018) in pepper reported that drought stress reduced the fresh-dry weights of plant roots and shoots. Shoot fresh-dry weights were found to decrease under drought stress in tomato (Kıran et al., 2014; Zhou et al., 2017; Alp, 2017) and eggplant (Kıran et al., 2016). All these reports support the findings that shoot fresh-dry weight, root fresh-dry weight values decreased with drought stress compared to the control in our study. The stage of development of the plant during the periods of water limitation also varies according to the growth development of the plant and the effect of physiological characteristics (Farooq et al., 2009). Plant growth under water stress is very variable, depending on the duration of the drought. When drought stress begins, the plant increases root growth to access water. In addition, with prolonged drought stress, shoot and root development

stops and leaf area and number of leaves decrease (Anjum et al., 2011).

In the study, drought-stressed tomato plants showed a 21.6% reduction in shoot length and a 20% reduction in root length compared to control plants. Güzel (2006) and Altunlu (2011) reported that shoot and root length decreased in tomato, Ecem (2010) in maize and Avşaroğlu (2015) in watermelon because of drought stress application. Kuşvuran et al. (2020) and Parveen et al. (2019) reported that shoot length decreased with drought stress in pepper and tomato plants, respectively. These results are parallel to our findings.

Humic substances are heterogeneous, large organic complexes that are composed of the components of humus. They play an important role in soil aeration, long-term water retention in the soil, anion and cation exchange, and the chelation of mineral elements (Pettit, 2004). At the same time, it is stated that the effect of humic acids on plant germination and growth, expansion and elongation of root cells, oxygen uptake, respiration, photosynthesis is positive, and they show hormone-like growth (Vaughan, 1985; Garcia et al., 1992; Dell'Amico et al., 1994).

In the study, it was determined that humic acid application increased shoot fresh weight by 10%, shoot dry weight by 6.2%, root fresh weight by 23%, root dry weight by 35.2%, shoot length by 7% and root length by 4.1% in tomato plants (Table 2). Humic acid application has been reported to increase shoot/root fresh and dry weights in tomato (Aksoy, 2019; Khan et al., 2020; Ural, 2020) and strawberry plants (Doğan, 2018). Ashraf and Raddy (2014) found that humic acid application resulted in an increase in root fresh weight in eggplant and tomato plants. In addition, Yaman (2016) in strawberry plants and Qin and Leskovar (2020) in pepper, tomato, watermelon and lettuce plants found that root fresh and dry weights increased as a result of humic acid application. Similarly, Maibodi et al. (2015) reported that humic acid application in grass (*Lolium perenne* L.) resulted in an increase in shoot fresh/dry weight and length, and root fresh weight and length. Humic acid was found to increase root and shoot length in pepper plants by Aslanpay (2011) and in maize plants by Güngör (2018). In addition, Kocamanoğlu (2018) found that the shoot length of purslane and Kalyoncu (2013) found that the root length of mung bean increased with humic acid application. These reports support our findings.

In our study, humic acid treatment against drought stress increased shoot fresh weight by 50%, shoot dry weight by 57%, root fresh weight by 25%, root dry weight by 43%, shoot length by 9% and root length by 15% in tomato plants compared to plants under drought stress. Similar results have been reported for root and shoot lengths in basil and cumin plants (Haghighi et al., 2012). Similar reports have been shown for shoot fresh and dry weights in maize plants (Kaya et al., 2020) and root fresh and dry weights in melon plants (Kıran et al., 2019).

3.2. Effects of humic acid on physiological stress indicators

Physiological stress indicators (MDA, ion leakage, chlorophyll, proline, relative water content) were studied to determine the effects of humic acid on tomato plants under drought stress. As shown in Table 3, the effect of treatments on all these parameters was found to be significant ($P<0.05$).

Table 3. Effects of treatments on physiological stress indicators

TREATMENTS	MDA (nmol/g)	Ion leakage (%)	Chlorophyll (SPAD)	Proline (µmol/g)	RWC (%)
Control	7.27 ±0.11^{d}	8.89 ±0.13^{b}	43.88 ±0.09^{b}	24.78 ±0.46^{c}	74.55 ±0.97^{b}
Humic Acid	7.58 ±0.09^{c}	8.16 ±0.14^{c}	44.75 ±0.14^{a}	22.48 ±0.66^{d}	81.28 ±0.65^{a}
Drought Stress	21.93 ±0.10^{a}	11.78 ±0.15^{a}	37.19 ±0.10^{d}	156.35 ±0.40^{a}	56.20 ±0.35^{d}
Drought Stress + Humic Acid	14.06 ±0.08^{b}	9.19 ±0.17^{b}	41.38 ±0.19^{c}	60.06 ±0.32^{b}	68.45 ±0.45^{c}

Note: Differences between values shown with different letters are significant at $P<0.05$ level.

MDA is a commonly used indicator of oxidative lipid damage and its concentration varies depending on stress (Davey et al., 2005). The osmotic potential of plants is reduced to keep the water content of plants stable during drought stress, and the change that occurs in the plant during drought stress also affects MDA levels. As a result, it has been reported that drought stress influences MDA levels and that the amount of MDA increases with the increase in cell damage (Kayabaşı, 2011). When comparing plants under drought stress with plants in the control group, a 201.6% increase in MDA was observed (Table 3). Similar results have been determined by different researchers in tomato (Alp and Kabay, 2017), grapevine (Koç, 2020), bean (Kabay and Şensoy, 2016), and pepper (Sadak, 2018; Kuşvuran et al., 2020). Compared to drought-stressed plants, plant MDA was reduced by 35.8% when humic acid was applied during drought stress. This positive effect of humic acid was also found in wheat (Arslan, 2018) and in melon (Kıran et al., 2019).

Oxidative stress on the membrane causes an increase in ion leakage during drought stress (Assaha et al., 2016). In this respect, it is believed that one of the best physiological markers of drought stress tolerance is ion leakage, which is an indicator of the stability and integrity of the cell membrane (Kocheva et al., 2004; Bat et al., 2020). Determination of ion leakage is a method used to determine the relationship between environmental stress and growth,

development and genotypic changes in membrane integrity (Bat et al., 2020). Our study showed that drought stress caused a 32.5% increase in ion leakage in tomato plants. Similar situations were reported by Çetin (2018) in wheat, by Can (2017) in cotton and by Ecem (2010) in maize. Also, ion leakage was reduced by 21.9% compared to drought-stressed plants when humic acid was applied under drought-stressed conditions. Like our findings, Abdelaal et al. (2018) reported the positive effect of humic acid onion leakage in barley plants.

Chlorophyll content in leaves is one of the most important factors influencing the efficiency of photosynthesis in plants. It has been reported that significant differences in chlorophyll pigment content occur in plants exposed to drought stress. It has been reported that significant differences in chlorophyll pigment content occur in plants exposed to drought stress (Güzel, 2006). The decrease in chlorophyll content in plants under drought stress may be due to the degradation of chlorophyll (Christ et al., 2014) or changes in enzymatic activities involved in chlorophyll synthesis that slow or inhibit chlorophyll synthesis (Cotrina Cabello et al., 2023). In our study, drought stress caused a 15.2% decrease in leaf chlorophyll content compared to the control. Drought stress was also found to have a negative effect on chlorophyll content in grape (Geçene, 2020) and strawberry plants (Faaek, 2018). In addition, humic acid application to drought-stressed plants was found to increase leaf chlorophyll levels by 11.2% compared to plants under drought stress conditions. Similar findings to our results were reported by Haider et al. (2014) in maize and Korkmaz (2018) in strawberry.

Proline is an essential amino acid that supports plant development and metabolism under abiotic stress conditions. It acts as an antioxidant defence molecule, a molecular chaperone, a signalling molecule that scavenges ROS and activates specific gene functions essential for the plant to recover from stress due to its metal chelating properties. In order to reduce oxidative damage and repair cell structures, plant cells produce a high level of proline, which helps to maintain cellular homeostasis, osmotic adjustment, water uptake, and redox balance (Ghosh et al., 2022). Proline can act as a stress tolerance enhancer, antioxidant, osmolyte and signalling molecule in plants (Kılıç, 2020). Drought stress increased the proline content of the plants by 530.9% compared to the control plants. Different researchers have reported similar results to our findings in Pistacia genotypes (Aljemaa, 2020), soybean (Kayabaşı, 2011) and tomato plants (Sanchez-Radriguez et al., 2010; Khan et al., 2015; Parveen et al., 2019). Humic acid application under drought stress conditions reduced proline levels by 61.5% compared to plants under drought stress conditions. Khorasoninejad et al. (2018) reported similar results in echinacea (*Echinecea purpurea*).

Relative water content (RWC), which is the water retention capacity of tissues, is also an indicator of cell membrane stability and tissue structural integrity (Celikkol Akcay and Okudan, 2023). It is a mechanism that helps regulate the water balance in tissues to protect the plant from stress factors (Bat et al., 2020). Drought stress reduced the relative water content of tomato plants compared to control plants. Similar results have been determined by different researchers in pepper (Cengiz, 2017; Yaban, 2018) and tomato (Altunlu, 2011; Zhou et al., 2017). The positive effect of humic acid on relative water content was observed when plants under drought stress were compared with plants treated with humic acid. Indeed, this positive effect of humic acid was demonstrated in a study on okra plants by Barzegar et al. (2016).

3.3. Effects of humic acid on *EF-1*, *FeSOD*, *APX1*, *CAT2*, and *GR1* antioxidant enzyme genes expression

The gene expressions of FeSOD, APX1, CAT2 and GR1 antioxidant defense enzymes were studied to reveal the effects of drought stress, humic acid and humic acid + drought stress treatments in tomato plants, and *EF-1* was selected as a homebox gene to elucidate these gene expressions. Between treatments, the expression of the *EF-1* gene remained largely the same. This is an indication that EF-1 is a suitable internal control and that the vital activities of the plants continue under the treatments (Figure 1).

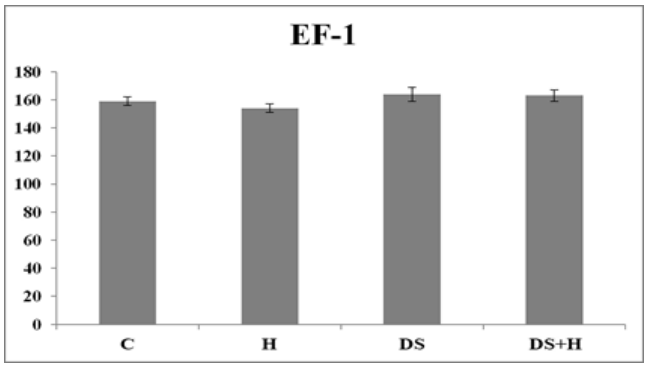

Figure 1. Semi-quantitative *EF-1* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

In the study, when *FeSOD* gene expression levels in tomato plants were analyzed according to treatment, it was found that they varied between 0-91 (Figure 2). However, the highest values were observed in the control, drought stress +humic acid and drought stress treatments, while the lowest value was observed in the humic acid treatment. Drought stress causes the ROS formation such as hydrogen peroxide (H_2O_2) and superoxide (O_2). To scavenge ROS, stressed plants activate both enzymatic and non-enzymatic antioxidants and restore cellular redox homeostasis to reduce oxidative stress. Superoxide dismutases (SODs), which are antioxidant enzymes, are very important attendants of antioxidant biocatalysts through the dismutation of O2- to H2O2. In this way, they increase the tolerance of the plants to stress (Saibi and Brini, 2018). *FeSOD* gene expression levels of tomato plants under drought stress showed a 26.3% decrease compared to the control treatment. Kireçci (2012) in wheat and Çalık (2016) in chickpea reported that drought stress caused a decrease in SOD enzyme activity. It was found that applying humic acid reduced *FeSOD* gene expression levels in tomato plants by 100% compared to the control. Haghighi and Teixeira Da Silva (2013) reported that humic acid application decreased SOD enzyme activity in tomato

compared to control plants. The application of humic acid to tomato plants under drought stress increased the expression of the *FeSOD* gene. Kıran et al. (2019) and Kaya et al. (2020) reported similar results in melon and maize plants, respectively.

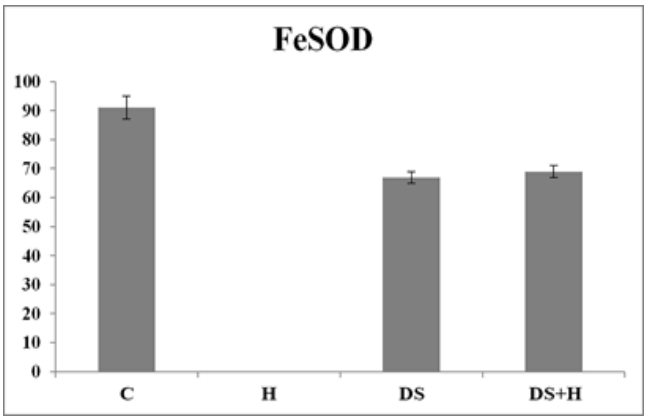

Figure 2. Semi-quantitative *FeSOD* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

When *APX1* gene expression levels were analyzed, the lowest level (0) was observed in the humic acid treatment and the highest level (166) in the drought stress treatment (Figure 3). Ascorbate peroxidase (APX), an enzymatic antioxidant, metabolises stress-induced ROS such as H_2O_2 and controls their potential effects on cellular metabolism and function. APX has a high affinity for H_2O_2 and appears to be an important parameter in the complete destruction of H_2O_2 using ascorbate (AsA) as a specific electron donor in organelles including mitochondria, chloroplasts, peroxisomes, and cytosol (Anjum et al., 2016). *APX1* gene expression in the control plants showed close levels compared to the drought-stressed plants. Similar results to our findings were found in tomato (Aydın, 2015; Alp, 2017; Raja et al., 2020), pepper (Yaban and Kabay, 2019; Kuşvuran et al., 2020) and grapevine (Koç, 2020). Humic acid application reduced *APX1* gene expression by 100% compared to the control plants. Dinler et al. (2016) reported that applying fulvic acid reduced APX1 enzyme activity in soybean plants compared to control group. This statement is in parallel with our findings. Our results showed that there was a 0.6% decrease in *APX1* gene expression because of humic acid application to plants under drought stress. Tartoura (2010) in wheat plants and Aguiar et al. (2016) in sugarcane reported that humic acid application to plants under drought stress reduced APX1 enzyme activity.

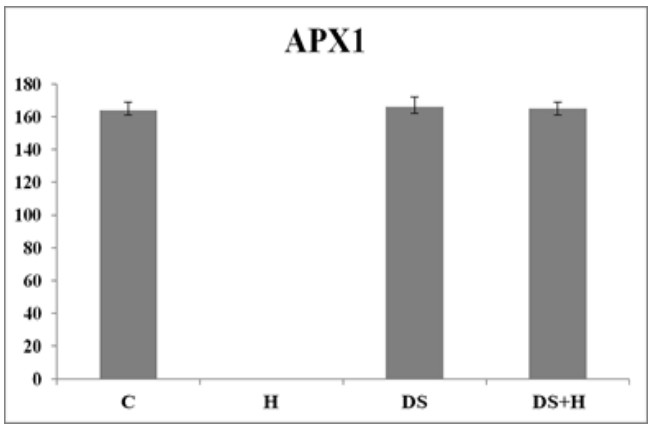

Figure 3. Semi-quantitative *APX1* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

CAT2 gene expression levels were found to vary between 0 and 138 (Figure 4). While the highest value was obtained from drought stress + humic acid, the lowest value was obtained from humic acid treatment. Superoxide dismutase in plant cells forms the first line of defence against ROS (Ighodaro and Akinloye, 2018), catalysing the superoxide radical to molecular oxygen and hydrogen peroxide. This hydrogen peroxide is then removed by catalase (CAT) (Young and Woodside, 2001). In our study, *CAT2* gene expression was found to decrease by 46.1% in drought-stressed plants compared to control. Some researchers reported that drought stress caused a decrease in CAT enzyme activity in tomato (Gökçe Gündüzer, 2015) and wheat (Yediyıldız, 2008; Baltacıer, 2019) plants. There was a 100% decrease in *CAT* gene expression in tomato plants treated with humic acid compared to control plants. Shen et al. (2020b), in their study on millet plants, found that applying humic acid reduced CAT enzyme activity compared to control plants. In addition, Bijanzadeh et al. (2021) found that applications of humic acid and jasmonic acid to wheat plants reduced CAT enzyme activity compared to control plants. These reports support our findings. Also, compared to plants treated with drought stress + humic acid, it was observed that *CAT* gene expression increased by 97.1% in plants under drought stress conditions. Similar results to our findings were found in melon plants by Kıran et al. (2019).

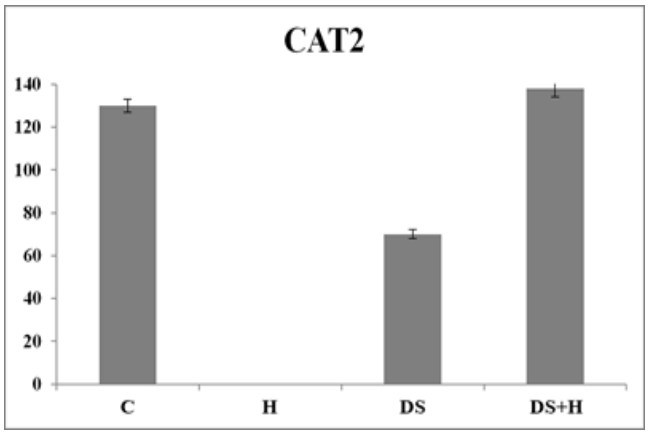

Figure 4. Semi-quantitative *CAT2* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

GR1 gene expression values were lowest in the humic acid treatment (13) and highest in the drought stress (143) (Figure 5). Glutathione reductase (GR) is one of the important antioxidant enzymes that help protect cells against ROS and their reaction products. GR is a NAD(P)H-dependent antioxidant and plays a role in maintaining the reduced glutathione (GSH) and thiol pools in cells. The differential regulation of GR in plants suggests that it is an important component of the plant defence system (Gill et al., 2013). Compared to the control treatment, drought stress increased *GR1* gene expression levels by 10.8%. Taşğın et al. (2017), Çancıoğlu (2014), Çetinkaya (2013) and Özkur (2010) reported an increase in GR enzyme activity with drought stress. Also, our study shows that humic acid application reduced *GR1* gene expression by 89.9% in comparison to drought-stressed tomato plants. Oktay Yiğit (2018) in wheat and Ural (2020) in tomato, found that humic acid applications reduced GR1 enzyme activity compared to control groups. In addition, the expression of the *GR1* gene was decreased by 11.8% when humic acid was applied to plants under drought stress. Tartoura (2010) found a similar situation in wheat plants and reported that this situation may vary depending on applications and doses.

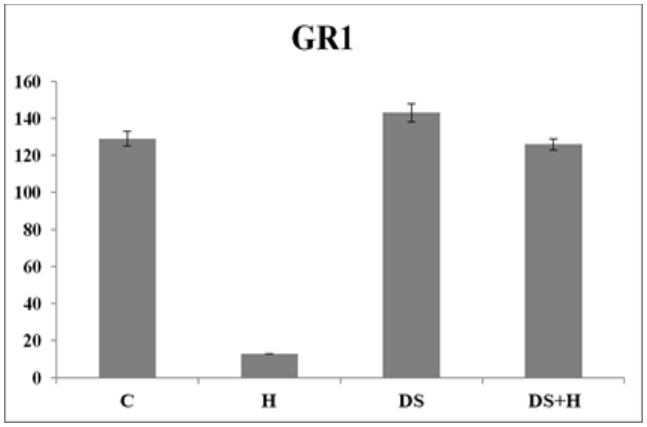

Figure 5. Semi-quantitative *GR1* gene expression of tomato plants under treatments (C: Control, H: Humic acid, DS: Drought stress, DS+H: Drought stress+Humic acid)

4. CONCLUSIONS

Application of humic acid to plants under drought stress resulted in an increase in shoot fresh-dry, root fresh-dry, shoot and root length, chlorophyll and relative water content values. It was also found that MDA, ion leakage and proline levels decreased when humic acid was applied to plants under drought stress. In addition, humic acid application to plants under drought stress did not cause a significant change in *SOD* and *APX* gene expression levels, while it caused an increase in *CAT* gene expression and a decrease in *GR* gene expression. These results indicate that humic acid applications may be effective in reducing the negative effects of drought stress, particularly by increasing *CAT* gene expression. Also, the fact that all the antioxidant enzyme gene expressions disappeared only under the humic acid treatment or were at the lowest level compared to all the treatments, suggests that humic acid positively affects the general physiological and metabolic responses of tomato plants.

Acknowledgements
This study is derived from Esra Aytaç's master's thesis.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: E.A., H.Ö.Ü., U.Ç.A; Investigation: E.A., H.Ö.Ü., U.Ç.A; Material and Methodology: E.A., H.Ö.Ü., U.Ç.A, İ.E.E; Supervision: H.Ö.Ü., U.Ç.A; Visualization: H.Ö.Ü., U.Ç.A; Writing-Original Draft: E.A., H.Ö.Ü; Writing-review & Editing: E.A., H.Ö.Ü; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Abdelaal, K.A.A., Hafez, Y.M., El-Afry, M.M., Tantawy, D.S., Alshaal, T. (2018). Effect of some osmoregulators on photosynthesis, lipid peroxidation, antioxidative capacity, and productivity of barley (Hordeum vulgare L.) under water deficit stress. Environmental Science and Pollution Research, 25, 30199-30211. https://doi.org/10.1007/S11356-018-3023-X

Aguiar, N.O., Medici, L.O., Olivares, F.L., Dobbss, L.B., Torres-Netto, A., Silva, S.F., Novotny, E.H., Canellas, L.P. (2016). Metabolic profile and antioxidant responses during drought stress recovery in sugarcane treated with humic acids and endophytic diazotrophic bacteria. Annals of Applied Biology, 168, 203-213.

Aksoy, E. (2019). The Effects of Humic Acid on Plant Development, Yield, Some Quality and Biochemical Properties on Tomato Grown in Salty Conditions. (Master's Thesis, Harran University Graduate School of Natural and Applied Sciences)

Alcázar, R., Bueno, M., Tiburcio, A.F. (2020). Polyamines: Small amines with large effects on plant abiotic stress tolerance. Cells, 9(11), 2373. https://doi.org/10.3390/cells9112373

Aljemaa, B.A. (2020). Determination of Morphological, Physiological and Biochemical Reactions of Some Pistacia Genotypes in Drought Stress. (Ph.D. Thesis, Selçuk University Graduate School of Natural and Applied Science)

Alp, Y., Kabay, T. (2017). Kuraklık stresinin yerli ve ticari domates çeşitlerinde bazı fizyolojik parametreler üzerine etkileri. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 22(2), 86-96.

Alp, Y. (2017). The Effect of Drought Stress on Early Plant Development in Some Native and Commercial Tomato Genotypes. (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Altunlu, H. (2011). The Effects of Grafting Against Drought Stresse in Tomatoes. (Ph.D. Thesis, Ege University Graduate School of Applied and Natural Sciences)

Anjum, S.A., Xie, X., Wang, L., Saleem, M.F., Man, C., Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stres. African Journal of Agricultural Research, 6, 2026-2032.

Anjum, N.A., Sharma, P., Gill, S.S., Hasanuzzaman, M., Khan, E.A., Kachhap, K., Tuteja, N. (2016). Catalase and ascorbate peroxidase—representative H2O2-detoxifying heme enzymes in plants. Environmental science and pollution research, 23, 19002-19029. https://doi.org/10.1007/s11356-016-7309-6

Ansari, M., Ahmed, S., Abbasi, A., Hamad, N.A., Ali, H.M., Khan, M.T., Haq, I.U., Zaman, Q. (2023). Green synthesized silver nanoparticles: a novel approach for the enhanced growth and yield of tomato against early blight disease. Microorganisms, 11(4), 886. https://doi.org/10.3390/microorganisms11040886

Arslan, E. (2018). The Effect of Drought Stress and Humic Acid on Mrna Expression Levels of Some Genes and DNA Conformation Changes in Bread Wheat (Triticum aestivum L.). (Ph.D. Thesis, Atatürk University Graduate School of Applied and Natural Sciences)

Arslan, E., Agar, G., Aydin, M. (2021). Humic acid as a biostimulant in improving drought tolerance in wheat: The expression patterns of drought-related genes. Plant Molecular Biology Reporter, 39(3), 508-519. https://doi.org/10.1155/2016/3747501

Ashraf, O., Raddy, M.M. (2014). Effect of humic acid as an additive to growing media to enhance the production of eggplant and tomato transplants. Journal of Horticultural Science & Biotechnology, 89(3), 237-244.

Aslanpay, B. (2011). The Effects of Arbuskular Mycorrhizal Fungus (AMF) and Humic Acid on The Growth of Pepper (Capsicum annum L.) Plant And Root Rot Disease Caused By Phytophthora Capsici Leonian (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Assaha, D.V.M., Liu, L., Ueda, A., Nagaoka, T., Saneoka, H. (2016). Triveni Enterprises. Journal of Environmental Biology, 37, 107-114.

Avşaroğlu, Z.Z. (2015). The Effects of Nitric Oxide Applications on the Growth of Local and Wild Types of Watermelon Genotypes Under Drought Conditions. (Master's Thesis, Harran University Institute of Natural and Applied Sciences)

Aydın, M. (2015). Investigation of the Effects of Toxic Boron with Drought Stress on Tomato. (Master's Thesis, Fatih University the Graduate School of Sciences and Engineering)

Baltacıer, G. (2019). Effects of Rosa Canina L. Extract on Antioxidant Activity in Two Wheat Varieties Under Drought Stress. (Master's Thesis, Çanakkale Onsekiz Mart University Graduate School of Natural and Applied Sciences)

Barzegar, T., Moradi, P., Nikbakht, J., Ghahremani, Z. (2016). Physiological response of okra cv. Kano to foliar application of putrescine and humic acid under water deficit stress. International Journal of Horticultural Science and Technology, 3(2), 187-197. https://doi.org/10.22059/İjhst.2017.213448.147

Bat, M., Tunçtürk, R., Tunçtürk, M. (2020). Ekinezya (Echinacea purpurea L.) bitkisinde kuraklık stresi ve deniz yosunu uygulamalarının bazı fizyolojik parametreler üzerine etkisi. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 23(1), 99-107. https://doi.org/10.18016/Ksutarimdoga.Vi.535210

Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39, 205-207.

Bijanzadeh, E., Emam, Y., Pessarakli, M. (2021). Biochemical responses of water-stressed triticale (X triticosecale wittmack) to humic acid and jasmonic acid. Journal of Plant Nutrition, 44(2), 252-269. https://doi.org/10.1080/01904167.2020.1806312

Calvo, P., Nelson, L., Kloepper, J.W. (2014). Agricultural uses of plant biostimulants. Plant Soil, 383, 3-41. https://doi.org/10.1007/s11104-014-2131-8

Can, D. (2017). Determination of The Effects of Deficit Irrigation on Quality, Yield and Yield Components of Some Cotton Variety in Amik Plain Conditions. (Yüksek Lisans Tezi, Harran University Graduate School of Natural and Applied Sciences)

Canellas, L.P., Canellas, N.O., da S. Irineu, L.E.S., Olivares, F.L., Piccolo, A. (2020). Plant chemical priming by humic acids. Chemical and Biological Technologies in Agriculture, 7, 1-17. https://doi.org/10.1186/s40538-020-00178-4

Celikkol Akcay, U., Okudan, N. (2023). Exogenous serotonin improves drought and salt tolerance in tomato seedlings. Plant Growth Regulation, 101, 239-249. https://doi.org/10.1007/s10725-023-01016-x

Cengiz, A. (2017). Determination of the Relationship Between Drought Stress and Nitric Oxide in Pepper Plant. (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Chen, Q., Qu, Z., Ma, G., Wang, W., Dai, J., Zhang, M., Wei, Z., Liu, Z. (2022). Humic acid modulates growth, photosynthesis, hormone and osmolytes system of maize under drought conditions. Agricultural Water Management, 263, 107447. https://doi.org/10.1016/j.agwat.2021.107447

Christ, B., Egert, A., Suessenbacher, I., Kraeutler, B., Bartels, D., Peters, S., Hoertensteiner, S. (2014). Water deficit induces chlorophyll degradation via the 'PAO/phyllobilin'pathway in leaves of homoio-(Craterostigma pumilum) and poikilochlorophyllous (X erophyta viscosa) resurrection plants. Plant, Cell & Environment, 37(11), 2521-2531. https://doi.org/10.1111/pce.12308

Cotrina Cabello, G. G., Ruiz Rodriguez, A., Husnain Gondal, A., Areche, F. O., Flores, D. D. C., Astete, J. A. Q., & Cruz Nieto, D. D. (2023). Plant adaptability to climate change and drought stress for crop growth and production. CABI Reviews, (2023), 1-9. https://doi.org/10.1079/cabireviews.2023.0004

Çalık, B. (2016). The Antioxidant Responces Against to Drought Stress and Orobanche Crenata İnfection on Chickpea (Cicer Arietinum L.) Varieties Which Have Known Diferent Drought Tolerances. (Master's Thesis, Çanakkale Onsekiz Mart University Graduate School of Natural and Applied Sciences)

Çancıoğlu, E. (2014). Investigation of The Effects Carnitine on Reactive Oxygen Species Signalling and Antioxidant System Under Drought Stress. (Master's Thesis, Ege University Graduate School of Natural and Applied Sciences)

Çetin, F. (2018). Investigation of Physiological and Biochemical Parameters of Turkish Wheat Cultivars with Different Ploidy Level Under Drought Stress Conditions. (Master's Thesis, Kastamonu University Graduate School of Natural and Applied Sciences)

Çetinkaya, C. (2013). Physiological Effects of Drought and Rtreatments in Some Strawberry Cultivars. (Master's Thesis, Uludağ University Graduate School of Natural and Applied Sciences)

Davey, M.W., Stals, E., Panis, B., Keulemans, J., Swennen, R.L. (2005). High-throughput determination of malondialdehyde in plant tissues. Analytical biochemistry, 347(2), 201-207. https://doi.org/10.1016/j.ab.2005.09.041

Dell'Amico, C., Masciandaro, G., Ganni, A., Ceccanti, B., Garcia, C., Hernandez, T., Costa, F. (1994). Effects of specific humic fractions on plant growth in humic substances. In: The global environment and implications on human health, ed. Senesi, N. And Miano, T.M., 563-566, Elsevier Science, Amsterdam, The Netherlands.

Dinler, B.S., Gündüzer, E., Tekinay, T. (2016). Pre-treatment of fulvic acid plays a stimülant role in protection of soybean (Glycine max L.) leaves against heat and salt stress. Acta Biologica Cracoviensia Series Botanica, 58(1), 29-41. https://doi.org/10.1515/abcsb-2016-0002

Doğan, M. (2018). Investigation on the Effects of Humic Acid and Silicon Under Cadmium Stress in Strawberry. (Master's Thesis, Harran University Graduate School of Natural and Applied Sciences)

Ecem, N. (2010). Investigation of the Effect of Drought Stress in Different Maize (Zea Mays L.) Cultivars and Lines. (Master's Thesis, Sakarya University Graduate School of Applied and Natural Sciences)

Faaek, M.F.F. (2018). Effects of Drought Stress in Some Strawberry Cultivars. (Master's Thesis, Selçuk University Graduate School of Applied and Natural Sciences)

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development, 29, 185-212.

Garcia, C., Hernandez, T., Costa, F., Ceccanti, B., Dell'Amico, C. (1992). Characterization of the organic fractions of an uncomposted and composted sewage sludge by isoelectric focusing and gel-filtration. Biology and Fertility of Soils, 13, 112-118.

Geçene, İ. (2020). Determination of Tolerance to Drought Stress Infoxy Grape (Vitis labrusca L.) by Peg Application in Vitro Conditions. (Master's Thesis, Ordu University Institute of Natural and Applied Sciences)

Gill, S.S., Anjum, N.A., Hasanuzzaman, M., Gill, R., Trivedi, D.K., Ahmad, I., Tuteja, N. (2013). Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. Plant Physiology and Biochemistry, 70, 204-212. https://doi.org/10.1016/j.plaphy.2013.05.032

Ghosh, U.K., Islam, M.N., Siddiqui, M.N., Cao, X., Khan, M.A.R. (2022). Proline, a multifaceted signalling molecule in plant responses to abiotic stress: understanding the physiological mechanisms. Plant Biology, 24(2), 227-239. https://doi.org/10.1111/plb.13363

Gökçe Gündüzer, E. (2015). Determination of Catalase Gene mRNA Expression Level with Real Time PCR in Tomato (Solanum lycopersicum L.) Plant Grown Under Salt and Drought Stress. (Ph.D. Thesis, Ankara University Graduate School of Natural and Applied Sciences)

Gupta, A., Bano, A., Rai, S., Mishra, R., Singh, M., Sharma, S., Pathak, N. (2022). Mechanistic insights of plant-microbe interaction towards drought and salinity stress in plants for enhancing the agriculture productivity. Plant Stress, 4, 100073. https://doi.org/10.1016/j.stress.2022.100073

Güngör, K. (2018). Effect of Humic Acid Application on Root Growth and Nutrient Elements Uptake of Maize (Zea mays L.) plant. (Master's Thesis, Ankara University Graduate School of Natural and Applied Sciences)

Güzel, A. (2006). Investigation of Effects of Abscisic Acid (ABA) and Calcium (Ca2+) on the Several Physiological and Growth Parameters in Tomato Plants Exposed to Drouhgt Stress. (Master's Thesis, Mersin University Graduate School of Applied and Natural Sciences)

Haghighi, M., Teixeira Da Silva, J.A. (2013). Amendment of hydroponic nutrient solution with humic acid and glutamic acid in tomato (Lycopersicon esculentum Mill.) culture, Soil Science and Plant Nutrition, 59(4), 642-648. https://doi.org/10.1080/00380768.2013.809599

Haghighi, M., Teixeira Da Silva, J.A., Mozafariyan, M., Roustaii, F. (2012). Humic acid affects the germination of basil and cumin and alleviates the negative impacts of salinity and drought stress. Global Science Books, Medicinal and Aromatic Plant Science and Biotechnology, 6(1), 63-67.

Haider, G., Koyro, H.W., Azam, F., Steffens, D., Müller, C., Kammann, C. (2014). Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations. Plant and soil, 395, 141-157. https://doi.org/10.1007/S11104-014-2294-3

Hoagland, D.R., Arnon, D.I. (1950). The water-culture method for growing plants without soil circular.

California Agricultural Experiment Station, 347(2), 32.

Ighodaro, O.M., Akinloye, O.A. (2018). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria journal of medicine, 54(4), 287-293. https://doi.org/10.1016/j.ajme.2017.09.001

Islam, M.R., Islam, R., Dutta, T., Jhelom, F.R. (2023). Genetic and microbial insights into drought stress alleviation in tomato (Solanum lycopersicum L.). Biology and Life Sciences Forum, 27(1), 22. https://doi.org/10.3390/IECAG2023-15223

Joshi, R.K., Bharat, S.S., Mishra, R. (2020). Engineering drought tolerance in plants through CRISPR/Cas genome editing. 3 Biotech, 10(9), 400. https://doi.org/10.1007/s13205-020-02390-3

Kabay, T., Şensoy, S. (2016). Kuraklık stresinin bazı fasulye genotiplerinde oluşturduğu enzim, klorofil ve iyon değişimleri. Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 26(3), 380-395.

Kalyoncu, Ö. (2013). The Effect of Humic Acid on Growth and Ion Uptake of Mung Bean (Vigna radiata (L.) R. Wilczek) Grown Under Salt Stress. (Master's Thesis, Marmara University Graduate School of Applied and Natural Sciences)

Kaya, C., Şenbayram, M., Akram, N.A., Ashraf, M., Alyemeni, M.N., Ahmad, P. (2020). Sulfur-enriched leonardite and humic acid soil amendments enhance tolerance to drought and phosphorus deficiency stress in maize (Zea mays L.). Scientific Reports, 10, 6432. https://doi.org/10.1038/S41598-020-62669-6

Kayabaşı, S. (2011). Drought Stress in The Soybean (Glyncine max. L.) With Some Physiogical Parameters Investigation of Accumulations Proline. (Master's Thesis, Harran University Graduate School of Natural and Applied Sciences)

Khan, M.A., Asaf, S., Khan, A.L., Jan, R., Kang, S., Kim, K., Lee, I. (2020). Extending thermotolerance to tomato seedlings by inoculation with sa1 isolate of Bacillus cereus and comparison with exogenous humic acid application. Plos ONE, 15(4), E0232228. https://doi.org/10.1371/Journal. Pone.0232228

Khan, S.H., Khan, A., Litaf, U., Shah, A.S., Khan, M.A., Bilal, M., Ali, M.U. (2015). Effect of drought stress on tomato cv. bombino. Journal of Food Processing and Technology, 6(7), 1000465. https://doi.org/10.4172/2157-7110.1000465

Khorasaninejad, S., Ahmadabadi, A.A., Hemmati, K. (2018). The effect of humic acid on leaf morphophysiological and phytochemical properties of Echinacea purpurea L. under water deficit stress. Scientia Horticulturae, 239, 314-323. https://doi.org/10.1016/J.Scienta.2018.03.015

Kılıç, B. (2020). Investigation of the Effects of Proline Pre-treatment on Germination of Black Pine Seeds Under Drought Stress. (Master's Thesis, Artvin Çoruh University Graduate School of Natural and Applied Sciences)

Kılıçaslan Caşka, S. (2019). The Effect of Drought Stress on Plant Growth, Some Physiological and Biochemical Properties of Bean. (Master's Thesis, Atatürk University Graduate School of Natural and Applied Sciences)

Kıran, S., Kuşvuran, Ş., Özkay, F., Ellialtıoğlu, Ş.Ş. (2016). Tuza tolerant ve hassas patlıcan genotiplerinin kuraklık stresi koşullarında bazı morfolojik özelliklerinde meydana gelen değişimler. Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi, 21(2), 130-138.

Kıran, S., Furtana, G.B., Talhouni, M., Ellialtıoğlu, Ş.Ş. (2019). Drought stress mitigation with humic acid in two Cucumis melo L. Genotypes differ in their drought tolerance. Bragantia, Campinas, 78(4), 490-497. https://doi.org/10.1590/1678-4499.20190057

Kıran, S., Özkay, F., Kuşvuran, Ş., Ellialtıoğlu, Ş.Ş. (2014). Tuz stresine tolerans seviyesi farklı domates genotiplerinin kuraklık stresi koşullarında bazı özelliklerinde meydana gelen değişimler. Journal of Agricultural Faculty of Gaziosmanpaşa University, 31(3), 41-48.

Kireçci, O.A. (2012). The Investigation of Some Signal Transduction Biomolecules in Triticum aestivum L. (Wheat Plant) Exposed to Drougt Stress. (Ph.D. Thesis, Inonu University Graduate School of Natural and Applied Sciences)

Kocamanoğlu, Ç. (2018). The Effect of Growing Media and Humic Acid Treatments on Some Yield Properties of Purslane (Portulaca oleracea L.) (Master's Thesis, Ordu University Institute of Natural and Applied Sciences)

Kocheva, K., Lambrev, P., Georgiev, G., Goltsev, V., Karabaliev, M. (2004). Evaluation of chlorophyll fluorescence and membrane injury in the leaves of barley cultivars under osmotic stress. Bioelectrochemistry, 63(1), 121-124. https://doi.org/10.1016/j.bioelechem.2003.09.020

Koç, M. (2020). Physiological Biochemical and Molecular Responses of Some Grape Varieties Against Drought Stress. (Ph.D. Thesis, Tokat Gaziosmanpasa University Graduate School of Natural and Applied Sciences)

Korkmaz, K. (2018). Investigation on the Effects of Humic Acid and Silicon Under Water Stress in Strawberry. (Master's Thesis, Harran University Graduate School of Natural and Applied Sciences)

Kuşvuran, Ş., Kıran, S.U., Altuntaş, Ö. (2020). The morphological, physiological and biochemical effects of drought in different pepper genotypes. Food Science and Technology, 8(6), 1359-1368. https://doi.org/10.24925/Turjaf.V8i6.1359-1368.3375

Maibodi, N.D.H., Kafi, M., Nikbakht, A., Rejali, F. (2015). Effect of foliar applications of humic acid on growth, visual quality, nutrients content and root parameters of perennial ryegrass (Lolium perenne L.). Journal of Plant Nutrition, 38(2), 224-236. https://doi.org/10.1080/01904167.2014.939759

Manna, M., Thakur, T., Chirom, O., Mandlik, R., Deshmukh, R., Salvi, P. (2021). Transcription factors as key molecular target to strengthen the drought stress tolerance in plants. Physiologia Plantarum, 172(2), 847-868. https://doi.org/10.1111/ppl.13268

Nanjo, T., Kobayashi, M.Y., Yoshiba, Y., Kakubar, K., Yamaguchi, S.Y., Shinozaki, K. (1999). Antisense

suppression of the proline degradation improves 123 tolerance to freezing and salinity in Arabidopsis thaliana. Febs Letters, 461(3), 205-210.

Ohkawa, H., Ohishi, N., Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2), 351-358

Oktay Yiğit, Ö. (2018). Effect of Humic Acid on Some Genes in Wheat Seeds in Salinity Temperature Stresses. (Master's Thesis, Mustafa Kemal University Graduate School of Applied and Natural Sciences)

Özkur, Ö. (2010). Antioxidant Defence System in Capparis ovata Plants Under Drought Stress. (Ph.D. Thesis, Ege University Graduate School of Applied and Natural Sciences)

Parveen, A., Rai, G.K., Mushtaq, M., Singh, M., Rai, P.K., Rai, S.K., Kundoo, A.A. (2019). Deciphering the morphological, physiological and biochemical mechanism associated with drought stress tolerance in tomato genotypes. International Journal of Current Microbiology and Applied Sciences, 8(5), 227-255. https://doi.org/10.20546/İjcmas.2019.805.028

Pettit, R. E. (2004). Organic matter, humus, humate, humic acid, fulvic acid and humin: their importance in soil fertility and plant health. CTI Research, 10, 1-7.

Qin, K., Leskovar, D.I. (2020). Humic substances improve vegetable seedling quality and post-transplant yield performance under stress conditions. Agriculture Journal, 10, 254. https://doi.org/10.3390/Agriculture10070254

Raja, V., Qadir, S.U., Alyemeni, M.N., Ahmad, P. (2020). Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses and gene expression in Solanum lycopersicum. 3 Biotech, 10, 208. https://doi.org/10.1007/S13205-020-02206-4

Sadak, A. (2018). The Effect of PGPR on Pepper Seedlings Under Drought Stress. (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Saibi, W., Brini, F. (2018). Superoxide dismutase (SOD) and abiotic stress tolerance in plants: An overview. In Superoxide Dismutase: Structure, Synthesis and Applications. (pp. 101-142)

Sanchez-Rodriguez, E., Rubio-Wilhelmi, M., Cervilla, L.M., Blasco, B., Rios, J.J., Rosales, M.A., Romero, L., Ruiz, J.M. (2010). Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Science, 178, 30-40.

Shen, J., Guo, M., Wang, Y., Yuan, X., Dong, S., Song, X., Guo, P. (2020a). An investigation into the beneficial effects and molecular mechanisms of humic acid on foxtail millet under drought conditions. Plos ONE, 15(6), E0234029. https://doi.org/10.1371/Journal.Pone.0234029

Shen, J., Guo, M.J., Wang, Y.G., Yuan, X.Y., Wen, Y.Y., Song, X.E., & Guo, P.Y. (2020b). Humic acid improves the physiological and photosynthetic characteristics of millet seedlings under drought stress. Plant Signaling & Behavior, 15(8), 1774212. https://doi.org/10.1080/15592324.2020.1774212

Smart, R.E., Bingham, G.E. (1974). Rapid estimates of relative water content. Plant Physiology, 53, 258-260.

Tartoura, K.A.H. (2010). Alleviation of oxidative stress induced by drought through application of compost in wheat (Triticum aestivum L.) plants. American-Eurasian Journal of Agricultural & Environmental Sciences, 9(2), 208-216.

Taşğın, E., Nadaroğlu, H., Adıgüzel, A., Baltacı, M.Ö., Sönmez, Z. (2017). Soya bitkisindeki glutatyon redüktaz aktivitesi ve mrna seviyesinin kuraklık stresinde salisilik asit ile değişimi. Apjes, 5(2), 48-52.

Ural, C. (2020). The Effect of Humic Acid on Some Physiological Parameters and Gene Expressions in Tomato (Solanum lycopersicum) Under Salt Stress. (Master's Thesis, Isparta University of Applied Sciences the Institute of Graduate Education)

Vaughan, D. (1985). Effect of humic substances on metabolic processes in plants. In Humic Substances Effects on Soil and Plants, 54-77.

Yaban, İ., Kabay, T. (2019). Kuraklık stresinin urfa biberinde iyon klorofil ve enzim içerikleri üzerine etkisi. Toprak Su Dergisi, 8(1), 11-17. https://doi.org/10.21657/Topraksu.544650

Yaban, İ. (2018). The Effect of Drought Stress on Some Landraces and Varieties of Urfa Pepper. (Master's Thesis, Yüzüncü Yıl University Graduate School of Applied and Natural Sciences)

Yadav, P., Ansari, M.W., Kaula, B.C., Rao, Y.R., Al Meselmani, M., Siddiqui, Z.H., Kumar, S.B., Rani, V., Sarkar, A., Rakwal, R., Gill, S.S., Tuteja, N. (2023). Regulation of ethylene metabolism in tomato under salinity stress involving linkages with important physiological signaling pathways. Plant Science, 334, 111736. https://doi.org/10.1016/j.plantsci.2023.111736

Yaman, M. (2016). Effects of Humic Acid, Amino Acid, Hormones and Micro Elements Applications on Quality and Yield at Strawberry Seedling. (Master's Thesis, Erciyes University Graduate School of Natural and Applied Sciences)

Yediyıldız, A.G. (2008). Application of Drought and Salt Stresses on Two Wheat Cultivars (Triticum aestivum L. cv. Bayraktar and Atay 85) for Monitoring the Activity of Antioxidative Enzymes. (Master's Thesis, Erciyes University Graduate School of Natural and Applied Sciences)

Young, I. S., Woodside, J. V. (2001). Antioxidants in health and disease. Journal of clinical pathology, 54(3), 176-186.

Zhou, R., Yu, X., Ottosen, C., Rosenqvist, E., Zhao, L., Wang, Y., Yu, W., Zhao, T., Wu, Z. (2017). Drought stress had a predominant effect overheat stress on three tomato cultivars subjected to combined stress. BMC Plant Biology, 17(24), 1-13. https://doi.org/10.1186/S12870-017-0974-X

Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2024, Volume: 8, Issue: 1, 38-49
Received: 22.08.2023; Accepted: 31.03.2024
DOI: 10.30516/bilgesci.1348209

Detection of Antibiotic Residues in Honeys from Different Regions in Türkiye by Liquid Chromatography-Tandem Mass Spectrometry Method

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Abstract: This study aimed to investigate 29 antibiotics and their metabolites in 27 honey samples obtained from different provinces of Turkey by Liquid Chromatography-Tandem Mass Spectrometry Method (LS-MS/MS). This study showed that the correlation coefficients of the calibration graphs were 0.999, the limit of detection (LOD) was 0.94–3.40 ng/g, and the limit of quantification (LOQ) was 3.11–11.22 ng/g. To express the accuracy of the method, intra- and inter-day recoveries were tested using three different concentrations from 0.25 to 1 µg/kg. Intra-day recoveries for antibiotics and metabolites were found to be 95.56–115.56% with relative standard deviation values between 0.43 and 6.58; inter-day recoveries were found to be 90.00–108.89% with relative standard deviation values between 0.54 and 5.31. The analysis results showed that no antibiotic residues were found in any of the honey samples. The honey did not pose any danger to food safety or public health.

Keywords: Antibiotic residue, honey, LC-MS/MS.

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Citation: Seçilmiş Canbay, H., Taşçı, H. (2024). Detection of Antibiotic Residues in Honeys from Different Regions in Türkiye by Liquid Chromatography-Tandem Mass Spectrometry Method. Bilge International Journal of Science and Technology Research, 8(1): 38-49.

1. INTRODUCTION

Two types of honey are produced worldwide: honey produced by traditional honey bees (*Apis mellifera*) and stingless bee honey (Ranneh et al., 2021). Honey is a plant-based natural food containing about 200 substances, including carbohydrates (especially fructose and glucose), enzymes, minerals, vitamins, phenolic acids, amino acids, volatile compounds, flavonoids, carotenoids, and organic acids (gluconic acid and acetic acid) (Ranneh et al., 2021; Valverde et al., 2022; Brar et al., 2023). Besides its rich nutritional content and organoleptic properties, honey provides numerous health benefits such as antifungal, antibacterial, hepatoprotective, antioxidant, hypoglycaemic, antimutagenic, antihypertensive, and anti-inflammatory effects (Ranneh et al., 2021; Brar et al., 2023).

Turkey has approximately 500 nectar plant species for bees. Due to this rich ecosystem, Turkey enjoys favourable conditions for beekeeping activities and thus for the production of various bee products such as honey, propolis, bee bread, bee pollen, and royal jelly (Bayram, 2023). China is the world's leading producer of natural honey with over 472.7 metric tonnes, followed by Turkey, the second largest producer with 96.34 metric tonnes (Anon, 2023).

Food safety is one of the world's major concerns due to rapid urbanisation and population growth. Overpopulation leads to high demand for food production and commercialisation, which in turn calls for attention to maintain food safety and quality control to meet consumer expectations and mitigate the critical problem of foodborne diseases. The main causes of foodborne diseases are attributed to food hazards such as pathogenic microorganisms, heavy metals, toxic substances, pesticides, and veterinary drugs. Therefore, it is very important to detect and identify hazardous substances contained in foods in inspection procedures and food control systems (Hitabatuma et al., 2022).

Organic pollutants that may be contained in foods of natural or anthropogenic origin are divided into four main categories:

pesticides, persistent environmental chemicals, naturally occurring toxins, and veterinary drugs. Honey has been used in the treatment of diseases in recent years due to its natural raw materials and provides numerous health benefits, but it also poses a danger and raises concern due to various contaminants, including antibiotics (Marazuela and Bogialli, 2009; Shoaei et al., 2023; Zergui et al., 2023). Antibiotics are natural, semi-synthetic, and synthetic drugs used to treat diseases caused by bacteria in human and veterinary medicine (Gürel Yücel et al., 2023). Honey may be contaminated with antibiotics due to intensive agricultural and industrial activities or due to their use in beekeeping for the treatment of bacterial diseases (Bonerba et al., 2021; Er Demirhan and Demirhan, 2022). Bees can fly to regions approximately 3-6 km away, and the honey they produce from waters, nectars, and flowers in these regions can be contaminated with environmental pollutants such as heavy metals, radioactivity, and pesticides (Tutun et al., 2019; Savarino et al., 2020). Honey bees are unable to metabolise most of the antimicrobial substances, and drug residues have been reported to be found even after a considerable period in honey harvested after drug administration (Er Demirhan and Demirhan, 2022). Since honey is a natural product widely consumed by all population groups for both nutritional and medicinal purposes, monitoring antibiotic residues and other contaminants in honey is becoming more important to help protect food safety and human health (Bonerba et al., 2021; Cunningham et al., 2022). As these drug residues or metabolites are harmful to humans, animals, and the environment, the most important task is to identify, monitor, and evaluate trace amounts of these residues (Hitabatuma et al., 2022). Liquid chromatography-mass spectrometry (LC-MS) methods, high-performance liquid chromatography (HPLC) methods, and enzyme-linked immunosorbent assays (ELISA) are mostly employed to identify antibiotic residues in different food samples (Shoaei et al., 2023).

Turkey occupies a strong position in the extraction of bee products, with honey being the most popular. Due to uncontrolled and unconscious antibiotic abuse and indirect contamination from the environment, antibiotic residues in the honey lead to significant problems in food safety, public health, and exports. Therefore, this study aimed to determine the presence and levels of various antibiotics in honey by Liquid Chromatography-Tandem Mass Spectrometry Method (LC-MS/MS), to evaluate the results thereof according to national and international legislation, and to compare the data reported in published studies.

2. MATERIAL AND METHOD

2.1. Sampling

A total of 27 commercial honey samples were collected from Marmaris (n = 12), Datça (n = 4), Adana (n = 4), Çanakkale (n = 2), Erzincan (n = 1), Ankara (n = 2), Istanbul (n = 1), and Izmir (n = 1) provinces in Turkey. Honey samples were collected in their original packaging and stored at room temperature and in the dark until analysed.

2.2. Chemicals and reagents

The standards for tetracycline, 4-epi tetracycline, chlortetracycline, 4-epichlorotetracycline, oxytetracycline, 4-epi oxytetracycline, doxycycline, demeclocycline, sulphonamide, sulfamethoxazole, sulfamethazine, penicillin, ampicillin, amoxicillin, streptomycin, neomycin, kanamycin, gentamicin, chloramphenicol, lincomycin, bacitracin A, enrofloxacin, ciprofloxacin, cephalexin, ceftiofur, novobiocin, potassium clavulanate, tylosin, tilmicosin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol, EDTA, acetonitrile and formic acid were obtained from Merck (Darmstadt, Germany). Ultra-purified water was obtained using an H2OPRO-VF-T/Arrium Ultrapure device (Sartorius, Germany).

2.3. Standard solutions

Standard antibiotic solutions (10 µg/mL) were prepared in acetonitrile and stored in amber glass at 2-4ºC. Different working standard solutions were prepared by diluting the stock solutions in the same solvent.

2.4. Sample extraction

For the extraction of honey samples, the method used by Yang et al. (2022) was modified and followed. Each honey sample was weighed at 2 g in polypropylene tubes, and 10 mL of 70% methanol and 200 µL of 0.1 M EDTA were added. The mixture was homogenised by vortexing for 1 minute and then centrifuged at 9000 rpm for 5 minutes. The supernatant (500 µL) was taken into polypropylene tubes, and 2 mL of distilled water was added and mixed. Then, it was filtered through a 0.45 µm filter, and 1.5 mL was taken into vials and made ready to be injected into the LC-MS/MS system.

2.5. Device used and operating conditions

Antibiotics and their metabolites in honey samples were chromatographically separated according to the method used by Tasci et al. (2021)**.** The extracts were injected 1 µL into the LC-MS/MS system.

2.6. Validation

The correlation equation, correlation coefficient (R^2), limit of detection (LOD), limit of quantification (LOQ), recovery rate, intra-day precision, and inter-day precision were determined as quality parameters (ICH, 2005).

2.7. Statistical analysis

Data were analysed by descriptive statistics using Minitab for Windows Version Release 16.1 (Minitab Inc., 2011).

3. RESULTS

The correlation equation, correlation coefficient values (R^2), the limit of detection (LOD), the limit of quantification (LOQ), recovery rate, and intra-day and inter-day precision of the graphs of antibiotics analysed in 27 honey samples are shown in Table 1 and Table 2 respectively. No antibiotic species were detected in the 27 honey samples analysed. .

Table 1. Validation parameters of antibiotic and metabolites analyses in honey samples.

Antibiotics	Calibration equation	R^2	LOD (ng/g)	LOQ (ng/g)
Tetracycline	$Y=2.12*10^{-6}x+1.29*10^{-4}$	0.999	3.40	11.22
4-epitetracycline	$Y=3.13*10^{-6}x+2.24*10^{-5}$	0.999	1.54	5.10
Chlortetracycline	$Y=7.25*10^{-6}x+6.21*10^{-5}$	0.999	1.03	3.41
4-epichlorotetracycline	$Y=2.89*10^{-6}x+6.08*10^{-5}$	0.999	1.02	3.35
Oxytetracycline	$Y=1.24*10^{-6}x+2.29*10^{-5}$	0.999	0.94	3.11
4-epioxytetracycline	$Y=4.13*10^{-6}x+5.01*10^{-5}$	0.999	1.37	4.50
Doxycyline	$Y=2.00*10^{-6}x+5.31*10^{-5}$	0.999	3.06	10.11
Demecloycline	$Y=2.00*10^{-6}x+5.24*10^{-4}$	0.999	2.73	9.01
Sulfonamide	$Y=2.13*10^{-6}+3,18*10^{-5}$	0.999	3.18	10.51
Sulfamethoxazole	$Y=2.04*10^{-6}x+4.43*10^{-4}$	0.999	0.94	3.11
Sulfamethazine	$Y=1.95*10^{-6}x+2.25*10^{-5}$	0.999	2.06	6.79
Penicilin	$Y=4.04*10^{-6}x+8.89*10^{-4}$	0.999	1.84	6.09
Ampicilin	$Y=1.00*10^{-6}x+5.52*10^{-4}$	0.999	1.76	5.80
Amoxicilin	$Y=4.21*10^{-6}x+1.53*10^{-5}$	0.999	1.42	4.70
Streptomycin	$Y=1.32*10^{-6}x+8.35*10^{-5}$	0.999	1.02	3.35
Neomycin	$Y=2.15*10^{-6}x+3.05*10^{-5}$	0.999	1.11	3.65
Kanamycin	$Y=6.21*10^{-6}x+7.04*10^{-5}$	0.999	1.96	6.47
Gentamicin	$Y=2.03*10^{-6}x+2.46*10^{-5}$	0.999	0.96	3.16
Chloramphenicol	$Y=4.33*10^{-6}x+3.12*10^{-5}$	0.999	2.75	9.09
Lincomycin	$Y=7.23*10^{-6}x+4.08*10^{-5}$	0.999	1.18	3.89
Baciratcin A	$Y=4.79*10^{-6}x+3.07*10^{-4}$	0.999	2.36	7.80
Enrofloxcain	$Y=9.55*10^{-6}x+2.25*10^{-5}$	0.999	1.59	5.23
Ciprofloxacin	$Y=8.12*10^{-6}x+3.12*10^{-4}$	0.999	1.11	3.68
Cephalexin	$Y=1.78*10^{-6}x+2.12*10^{-5}$	0.999	2.23	7.35
Ceftiofur	$Y=5.21*10^{-6}x+3.29*10^{-4}$	0.999	1.01	3.33
Novobiocin	$Y=1.01*10^{-6}x+1.21*10^{-5}$	0.999	1.98	6.52
Potasyum clavulanate	$Y=1.23*10^{-6}x+8.29*10^{-4}$	0.999	2.53	8.34
Tylosin	$Y=1.21*10^{-6}x+8.22*10^{-5}$	0.999	1.31	4.34
Tilmicosin	$Y=4.12*10^{-6}x+1.25*10^{-5}$	0.999	1.40	4.61

Explanations; R^2: Correlation Coefficient; LOD: Limit of Detection; LOQ: Limit of Quantification

Table 2. Precision and repeatability results for antibiotics and their metabolites in honey samples.

Antibiotics	Spiked level (µg/kg)	Intra-day Recovery (%,n:3)	Intra-day RSD (%)	Inter-day Recovery (%,n:3)	Inter-day RSD (%)
Tetracycline	0.45	102.22	1.26	97.78	2.27
		100.00	1.28	95.56	2.32
		102.22	1.24	100.00	2.13
	0.75	100.00	0.78	98.67	0.78
		98.67	0.70	98.67	0.70
		98.67	0.68	97.33	0.70
	1	99.00	1.54	99.00	0.59
		98.00	1.59	98.00	0.60
		101.00	1.43	98.00	0.54
4-epitetracycline	0.45	100.00	1.26	100.00	1.26
		102.22	1.29	102.22	1.29
		102.22	1.18	102.22	1.28
	0.75	98.67	1.33	98.67	1.33
		100.00	1.20	100.00	1.31
		101.33	1.19	101.33	1.26
	1	101.00	1.98	101.00	1.98
		103.00	2.02	103.00	2.02
		99.00	1.82	99.00	2.04
Chlortetracycline	0.45	100.00	1.26	97.78	2.27
		102.22	1.28	100.00	2.30
		102.22	1.16	95.56	2.21
	0.75	100.00	2.03	98.67	1.57
		102.67	1.88	98.67	1.59
		98.67	1.86	96.00	1.62
	1	110.00	5.55	101.00	1.54
		108.00	5.66	99.00	1.52
		99.00	5.60	98.00	1.51
4-epichlorotetracycline	0.45	97.78	2.59	97.78	2.22
		97.78	2.64	100.00	2.24
		102.22	2.42	102.22	2.22
	0.75	98.67	2.02	97.33	2.83
		101.33	1.82	96.00	2.74
		102.67	1.80	101.33	2.77
	1	106.00	4.90	99.00	2.65
		109.00	4.95	103.00	2.59
		99.00	4.46	98.00	2.62
Oxytetracycline	0.3	96.67	3.89	93.33	2.09
		96.67	3.93	93.33	2.15
		103.33	3.82	90.00	2.05
	0.5	108.89	2.32	108.89	2.39
		108.89	2.09	108.89	2.41
		113.33	2.03	104.44	2.44
	0.75	101.33	1.53	98.67	0.79
		101.33	1.58	97.33	0.80
		98.67	1.42	97.33	0.79
4-epioxytetracycline	0.45	100.00	1.26	100.00	1.26
		102.22	1.28	102.22	1.29
		102.22	1.24	102.22	1.18
	0.75	98.67	1.33	98.67	1.33
		100.00	1.20	100.00	1.20
		101.33	1.17	101.33	1.19
	1	101.00	1.98	101.00	1.98
		103.00	2.04	103.00	2.00
		99.00	1.84	99.00	1.80
Doxycyline	0.5	102.00	1.92	98.00	1.19
		104.00	1.96	96.00	1.21
		106.00	1.91	96.00	1.19
	0.75	98.67	0.79	98.67	0.78
		97.33	0.78	98.67	0.79
		97.33	0.79	97.33	0.80
	1	108.00	5.79	97.00	0.59
		109.00	5.85	98.00	0.61
		98.00	5.57	98.00	0.60
Demecloycline	0.45	100.00	1.30	97.78	2.66
		97.78	1.33	97.78	2.72
		97.78	1.29	93.33	2.61
	0.75	98.67	0.79	98.67	0.79
		97.33	0.78	97.33	0.72
		97.33	0.79	97.33	0.69
	1	105.00	3.13	99.00	1.02
		104.00	3.23	98.00	1.04

		99.00	3.20	97.00	0.94
Sulfonamide	0.3	96.67	1.97	96.67	4.17
		100.00	2.01	90.00	4.22
		96.67	1.88	90.00	3.83
	0.5	108.89	3.01	108.89	2.08
		113.33	2.87	106.67	1.93
		115.56	2.79	104.44	1.91
	0.75	101.33	1.53	98.67	0.78
		101.33	1.55	98.67	0.80
		98.67	1.53	97.33	0.79
Sulfamethoxazole	0.5	98.00	3.10	98.00	2.42
		102.00	3.16	94.00	2.50
		96.00	2.90	94.00	2.38
	0.75	98.67	1.55	97.33	1.57
		98.67	1.39	100.00	1.58
		101.33	1.38	97.33	1.60
	1	108.00	4.41	101.00	2.55
		105.00	4.45	99.00	2.58
		99.00	4.01	96.00	2.55
Sulfamethazine	0.5	98.00	4.19	96.00	2.08
		96.00	4.23	94.00	2.13
		104.00	4.11	98.00	1.95
	0.75	97.33	2.05	98.67	1.33
		98.67	1.85	100.00	1.20
		101.33	1.80	101.33	1.19
	1	101.00	5.01	103.00	3.24
		108.00	5.17	98.00	3.27
		98.00	4.66	97.00	2.94
Penicilin	0.45	100.00	3.42	102.22	1.27
		102.22	3.45	100.00	1.30
		95.56	3.35	100.00	1.24
	0.75	98.67	2.31	98.67	2.05
		98.67	2.08	101.33	2.01
		102.67	2.02	97.33	2.04
	1	109.00	6.38	99.00	1.18
		112.00	6.58	97.00	1.19
		99.00	5.93	97.00	1.14
Ampicilin	0.3	103.33	3.33	96.67	2.04
		100.00	3.37	93.33	2.06
		96.67	3.33	93.33	2.00
	0.5	106.67	1.21	108.89	2.08
		106.67	1.18	106.67	1.88
		104.44	1.13	104.44	1.82
	0.75	98.67	0.78	98.67	0.79
		98.67	0.78	97.33	0.81
		100.00	0.78	97.33	0.73
Amoxicilin	0.3	100.00	1.97	93.33	2.04
		96.67	2.03	93.33	2.06
		96.67	1.97	96.67	2.00
	0.45	100.00	2.22	97.78	1.30
		97.78	2.12	97.78	1.17
		102.22	2.05	100.00	1.14
	0.75	100.00	0.76	98.67	0.78
		101.33	0.77	98.67	0.80
		101.33	0.78	100.00	0.72
Streptomycin	0.5	102.00	2.00	100.00	3.16
		100.00	2.02	96.00	3.19
		98,00	2.00	94.00	3.10
	0.75	98.67	1.55	98.67	0.79
		101.33	1.50	97.33	0.71
		98.67	1.52	97.33	0.69
	1	107.00	3.36	99.00	1.02
		101.00	3.30	98.00	1.05
		101.00	3.33	97.00	0.95
Neomycin	0.3	96.67	3.89	93.33	2.04
		96.67	3.97	96.67	2.06
		103.33	3.64	93.33	1.87
	0.5	113.33	3.10	108.89	2.08
		108.89	2.78	104.44	1.93
		106.67	2.76	106.67	1.91
	0.75	100.00	0.78	97.33	0.78
		98.67	0.79	98.67	0.80
		98.67	0.71	98.67	0.79
Kanamycin	0.5	102.00	2.32	98.00	2.08
		98.00	2.37	96.00	2.10
		98.00	2.18	94.00	2.04

	0.75	101.33	1.55	98.67	0.78
		98.67	1.39	98.67	0.71
		98.67	1.38	97.33	0.69
	1	105.00	4.41	101.00	1.54
		108.00	4.50	99.00	1.59
		99.00	4.05	98.00	1.43
Gentamicin	0.45	97.78	1.30	97.78	1.32
		100.00	1.34	95.56	1.34
		97.78	1.30	97.78	1.30
	0.75	98.67	0.78	97.33	1.35
		100.00	0.74	100.00	1.22
		98.67	0.72	98.67	1.18
	1	105.00	3.70	99.00	3.76
		110.00	3.73	105.00	3.88
		113.00	3.77	98.00	3.49
Chloramphenicol	0.45	100.00	1.27	97.78	1.33
		102.22	1.29	95.56	1.35
		100.00	1.25	95.56	1.33
	0.75	101.33	2.31	98.67	0.79
		101.33	2.08	97.33	0.76
		97.33	2.02	97.33	0.77
	1	99.00	5.86	101.00	2.11
		111.00	6.04	98.00	2.07
		103.00	5.44	97.00	2.09
Lincomycin	0.3	96.67	3.89	96.67	2.01
		103.33	3.97	93.33	2.06
		96.67	3.64	96.67	1.89
	0.45	102.22	1.26	97.78	1.30
		102.22	1.14	97.78	1.17
		100.00	1.13	100.00	1.16
	0.75	98.67	1.55	98.67	0.78
		101.33	1.58	100.00	0.79
		98.67	1.42	98.67	0.71
Baciratcin A	0.5	98.00	1.19	102.00	3.53
		98.00	1.20	96.00	3.57
		96.00	1.09	96.00	3.47
	0.75	98.67	0.78	98.67	0.79
		100.00	0.72	97.33	0.71
		98.67	0.71	97.33	0.69
	1	105.00	2.23	99.00	1.02
		105.00	2.27	98.00	1.05
		101.00	2.25	97.00	0.95
Enrofloxcain	0.3	103.33	1.88	96.67	2.01
		100.00	1.92	96.67	2.06
		103.33	1.85	93.33	1.89
	0.45	97.78	1.30	102.22	3.45
		97.78	1.18	97.78	3.10
		100.00	1.15	95.56	3.07
	0.75	101.33	1.53	98.67	0.78
		101.33	1.56	98.67	0.79
		98.67	1.41	97.33	0.71
Ciprofloxacin	0.3	100.00	1.95	96.67	3.89
		100.00	1.99	96.67	3.97
		96.67	1.82	103.33	3.82
	0.45	100.00	1.29	97.78	1.32
		97.78	1.16	97.78	1.20
		100.00	1.15	95.56	1.17
	0.75	100.00	1.33	98.67	0.78
		101.33	1.35	98.67	0.80
		98.67	1.21	97.33	0.72
Cephalexin	0.3	103.33	1.84	96.67	5.21
		106.67	1.86	103.33	5.31
		103.33	1.81	93.33	4.87
	0.45	111.11	3.08	108.89	2.39
		113.33	2.77	108.89	2.15
		106.67	2.69	104.44	2.13
	0.75	101.33	1.53	97.33	1.35
		101.33	1.58	100.00	1.37
		98.67	1.42	98.67	1.23
Ceftiofur	0.25	100.00	2.28	96.00	2.44
		104.00	2.30	96.00	2.46
		100.00	2.23	92.00	2.26
	0.5	100.00	1.15	100.00	1.17
		102.00	1.03	98.00	1.04
		100.00	1.00	98.00	1.03
	0.75	100.00	0.78	98.67	0,79
		98.67	0.80	97.33	0.80

		98.67	0.72	97.33	0.72
Novobiocin	0.3	103.33	1.88	96.67	4.08
		103.33	1.90	96.67	4.20
		100.00	1.85	90.00	4.12
	0.45	102.22	2.22	97.78	2.27
		97.78	2.01	95.56	2.05
		100.00	1.91	100.00	1.99
	0.75	100.00	0.78	98.67	0.79
		98.67	0.78	97.33	0.81
		98.67	0.71	97.33	0.73
Potasyum clavulanate	0.75	98.67	0.78	98.67	0.79
		100.00	0.78	97.33	0.80
		98.67	0.76	97.33	0.74
	1	105.00	2.50	101.00	1.54
		104.00	2.25	99.00	1.39
		109.00	2.18	98.00	1.37
	1.25	98.40	0.47	99.20	0.81
		99.20	0.48	98.40	0.82
		99.20	0.43	97.60	0.74
Tylosin	0.5	98.00	3.46	98.00	2.39
		98.00	3.57	98.00	2.44
		104.00	3.40	94.00	2.37
	0.75	101.33	1.32	98.67	0.79
		102.67	1.33	97.33	0.80
		100.00	1.34	97.33	0.80
	1	112.00	5.25	103.00	3.50
		105.00	5.31	97.00	3.46
		101.00	5.25	97.00	3.50
Tilmicosin	0.5	102.00	3.01	94.00	1.22
		104.00	3.05	96.00	1.23
		98.00	2.96	94.00	1.20
	0.75	98.67	0.78	98.67	0.78
		98.67	0.70	98.67	0.71
		100.00	0.68	97.33	0.69
	1	103.00	2.50	102.00	2.67
		101.00	2.58	98.00	2.76
		98.00	2.32	97.00	2.48

RSD: Relative Standard Deviation

4. DISCUSSION

The calibration equation was established by providing reference standards at various concentrations (0.10 to 10 µg/mL), and the corresponding peak fields were obtained. The calibration curves showed good linearity, characterised by a high correlation coefficient ($R^2 > 0.999$). The LOD value for antibiotic residues was calculated as 0.94–3.40 ng/g and the LOQ value as 3.11–11.22 ng/g. To express the accuracy of the method, intra- and inter-day recoveries were tested using three different concentrations from 0.25 to 1 µg/kg. Intra-day recoveries for antibiotics and metabolites were found to be 95.56–115.56% with relative standard deviation values between 0.43-6.58; inter-day recoveries were found to be 90.00–108.89% with relative standard deviation values between 0.54–5.31. Recoveries and relative standard deviation values show the accuracy of the method. The validation parameters herein were compared with those in other studies. Günes et al. (2008) found a correlation coefficient of 0.996, a LOD of 6 ng/g, a LOQ of 20 ng/g, and a recovery rate of 85-89% for erythromycin in honey samples. Güneş et al. (2009) found that the LOD for oxytetracycline in honey was 10 ng/g, recovery 89–92%, and the LOD for sulphonamides was 6–12 ng/g, recovery 80–87%. Erdoğdu et al. (2011) determined correlation coefficient values of 0.9999, recovery rates of 50.7–62.2%, LOD values of 1.56-2.2 µg/kg, and LOQ values of 5.20–6.83 µg/kg for sulphonamide-derived antibiotics in honey samples. Ahmed et al. (2023) found that the accuracy rates for sulphonamides and tetracyclines in honey were between 83.07–86.93% and 86.90–91.19%, respectively, and the precision rates were below 9.54% with R^2 values between 0.978 and 1.00. The validation parameters determined herein were found to be compatible with those in other studies.

None of the antibiotics were found in the 27 honey samples analysed. Several studies have been conducted in Turkey and worldwide using different techniques to determine the presence and level of antibiotics in honey, and Table 3 and Table 4 summarise the results of these studies. A meta-analysis study conducted by Shoaei et al. (2023) to investigate antibiotic residues in honey showed that the antibiotic concentrations were fluoroquinolone 8.59 µg/kg, tetracycline 5.68 µg/kg, sulphonamides 5.54 µg/kg, and macrolides 4.19 µg/kg. There are differences between the results obtained herein and of other studies. These differences are correlated with factors such as difference in legislation and methods for determining antibiotic residues in honey, the use of various antibiotics for preventive and therapeutic purposes in beekeeping, indirect contamination from the environment, incorrect beekeeping practises, insufficient training of beekeepers on the dangerous effects of antibiotics, and inadequate monitoring system (Derebaşı et al., 2014; Savarino et al., 2020; Shoaei et al., 2023). Environmental conditions such as potable water around the apiary, surface, depth, and type of soil, as well as the type of plants grown therein, play an active role in antibiotic residues in honey samples (Ahmed et al., 2015). Chiesa et al. (2018) reported that close proximity of hives to agricultural activities due to different farming practices had a significant effect on antibiotic residues and honey quality. The level of antibiotic residues in honey samples herein was determined to be similar to the results of Güneş et al. (2009) and Kutlu et al. (2017) and much lower

than the results of other researchers (Table 3 and Table 4). This may be due to the absence of residues in honey samples, the breakdown of antibiotics in honey over time, or the lower concentration than the detected concentrations of the method employed.

Table 3. The summary results of the studies related to the antibiotic residues in honey analysed by different methods in Türkiye.

Location	Types of antibiotics	Analyses Methods	No. of honey sample	Incidence rate n (%)	Range (mean)	Reference
Erzurum	Tetracyclin, Neomicin	ELISA	79	37 (46.8)	Tetrasiklin: 2.1-47.08 (9.33) ppb Neomisin: 0	Aydemir Atasever and Yüksel, 2022
Sivas	Tetracyclin group Streptomycin group	ELISA	60	Tetracycline: 22 (73.3) in packaged honey, 18 (60) in open sold honey; streptomycin: 30 (100) in packaged honey, 28 (93.3) in open sold honey	Tetracycline: 0.12-371.44 (13.91) ppb in packaged honey; 0.02-13.32 (1.75) ppb in open honeys. Streptomycin: 1.30-250.2 (25.8) ppb in packaged honey; 0.19-22.71 (8.2) ppb in open honeys.	Ağaoğlu et al., 2020
Bingöl	Sulfonamide group	LC-HRMS	13	n.r. (n.r.)	Sulfamethoxazole: 0.96-5.1 (n.r.) µg/kg	Kırkan et al., 2020
Kars	Multiple aminoglycoside and macrolide groups of antibiotics	Biochip array biosensors	45	Neomycin: 11 (24.4) Tylosin B: 2 (4.4) Amikacin: 8 (17.8) Lincosamides: 6 (13.3) Erythromycin: 41 (91.1 Streptomycin: 10 (22.2)	Neomycin: < 1.0-8.4 (0.81) ppb Tylosin B: < 1.0-55.2 (1.45) Amikacin: < 1.0-33.7 (4.11) Lincosamides: < 1.0-10.7 (1.23) Erythromycin: < 1.0-38.1 (6.71) Streptomycin: < 1.0-1000 (59.9)	Aksoy, 2019
Bitlis	Tetracylines, Sulfonamides	LC/MS/MS	20	0 (0)	0 (0)	Kutlu et al., 2017
Muğla	Amphenicols, Sulfonamides, Tetracyclines	UPLC-ESI-MS/MS	50	n.r. (n.r.)	Sulfamethazine: 647 µg/kg tetracycline: 968 µg/kg epitetracycline: 197 µg/kg oxytetracycline: 743 µg/kg epioxytetracycline: 158 µg/kg	Kıvrak et al., 2017
Black Sea Region	Streptomycin, Tetracyclines, Sulphonamides	Charm II tests	209	68 (32.5)	n.r. (n.r.)	Derebaşı et al., 2014
İzmir	Sulfonamide group	LC-MS/MS	536	Sulfanilamid: 2 (0.37) Sulfadiazin: 1 (0.19) Sulfametazine: 108 (20.15) Sulfamethoxazole: 9 (1.68) Sulfadimethoxie: 6 (1.12)	Sulfanilamid: 6.9-198 (102.45) µg/kg Sulfadiazin: 24.86 µg/kg Sulfametazine: 6.2-13356.7 (597.34) µg/kg Sulfamethoxazole: 10.66-70.1 (25.01) µg/kg Sulfadimethoxine: 25.4-542.5 (157.25) µg/kg	Erdoğdu et al., 2011
Southern Marmara region	Oxytetracyclin, Sulphonamides	LC-MS/MS	50	0 (0)	0 (0)	Gunes et al., 2009
Southern Marmara region	Erythromycin	LC-ESI-MS	50	4 (8)	50-1776 (n.r.) ng/g.	Gunes et al., 2008

n.r.: results not reported by author.

Table 4. The summary results of the studies related to the antibiotic residues in honey analysed by different methods in worldwide.

Location	Types of antibiotics	Analyses Methods	No. of honey sample	Incidence rate n (%)	Range (mean)	Reference
East Tennessee (USA)	Tetracycline, Erythromycin	cELISA	Tetracycline:9 Erythromyci:9	Tetracycline:9/9 (n.r.) Erythromycin:9/ 9 (n.r.)	Tetracycline: n.r. (77.86) µg/kg Erythromycin: n.r. (0.68) µg/kg	Sarkar et al., 2023
Egypt, Libya, Saudi Arabia	Sulfonamides, Tetracyclines	HPLC–DAD, and HPLC–MS/ MS	Egyptian 33 Saudi Arabian 18 Libyan 24	Egyptian 19(57.6), Saudi Arabian 14(75), Libyan 18 (77.77)	Egyptian < d.l -275.080 (< d.l -96.825) µg/kg Saudi Arabian < d.l-151.066 ((< d.l-100.313) µg/kg Libyan < d.l -462.476 (< d.l-157.323)µg/kg	Ahmed et al., 2023
Iran	Erythromycin	ELISA	80	8 (10.66)	7.50-120 (20.32) ppb	Mehrabi et al., 2022
China	Quinolones, Sulfonamides, Tetracyclines, Phenicols	TQMS	94	All antibiotics: 79 (84.0)	All antibiotics: 0.04-7.84 (n.r.) ng/g	Wang et al., 2022
Italy	Amphenicols, Lincosamides, Macrolides, Nitroimidazoles, Pleuromutilins, Quinolones, Sulfonamides, Tetracyclines	LC-QTOF	55	3 (n.r.)	Sulfathiazole 0.5 µg/kg, Sulfamethazine 1.3 µg/kg, Tetracycline 0.5 µg/ kg; Oxytetracycline 1.1 µg/kg Tetracycline 0.5 µg/kg.	Paoletti et al., 2022
Indian	Oxytetracycline, Erythromycin, Chloramphenicol	HPLC	100	Oxytetracycline: 24/100 (24) Erythromycin: 2/100 (2) Chloramphenicol: 0/0 (0)	Oxytetracycline: 4.8-204 (69.3) ng/g Erythromycin: 51.0-55.0 (53.0) ng/g Chloramphenicol: 0(0)	Kumar et al., 2020a
Northwestern Himalayan Region of India	Oxytetracycline, Erythromycin, Chloramphenicol	HPLC	150	Oxytetracycline: 23/150 (15.3) Erythromycin: 8/150 (5.3) Chloramphenicol: 0/0 (0)	Oxytetracycline: 9-69 (28.9) ng/g Erythromycin: 50-112.0 (78.8) ng/g Chloramphenicol: 0(0) ng/g	Kumar et al., 2020b

n.r.: results not reported by author; < d.l.: below the detection limit

The European Union (EU) legislation has not set maximum residue limits (MRLs) for antimicrobial substances in honey, and therefore the European community does not allow the use of antibiotics in beekeeping. Therefore, the absence of MRLs means "zero tolerance" for antibiotic residues in honey, which is the same as the analytical method's limit of detection (EU, 2010). The legislation on veterinary drug residues for Turkey, prepared in compliance with EU legislation, has not set MRLs for antimicrobial substances in honey (TFC, 2017). However, some countries allow the use of a limited number of antibiotics in beekeeping and have set MRLs for these antibiotics. Although the Food and Drug Administration (FDA) in the United States authorises lincomycin 750 ppb, oxytetracycline (sum of tetracycline residues) 750 ppb, and tylosis 500 ppb for antibiotic residues in honey, it has not approved the use of chloramphenicol, nitrofurans, and/or fluoroquinolones to treat honey bees (FDA, 2023). Canada allows fumagillin at 0.025 ppm, oxytetracycline at 0.3 ppm, and tylosin at 0.2 ppm (Health Canada, 2022), while Australia and New Zealand allow oxytetracycline in honey at 0.3 ppm (FSANZ, 2016). As this study detected no antibiotic residues in honey samples, it was found to conform to national and international legislation and concluded that honey produced in Türkiye is safe for human consumption globally.

5. CONCLUSIONS

The absence of antibiotic residues in all the honey analysed in this study is satisfactory for food safety and public health, and the honey were found to be safe for human consumption. Implementation of antibiotic residue monitoring programmes to meet food safety requirements and scientific beekeeping practices is vital for the assessment of potential risks to human health. Therefore, appropriate antibiotic use and sale should be controlled, conscious beekeeping should be promoted, certified production should be compulsory, and legal inspections should be is recurrent. Hives should be placed at an appropriate distance from agricultural environment, and antibiotic residues in honey should be checked regularly.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
Conceptualization: H.S., FT; Investigation: H.S., F.T.; Material and Methodology: H.S., F.T.; Original Draft: H.S., F.T.; Writing-review & Editing: H.S., F.T.; Other: All authors have read and agreed to the published version of manuscript.

REFERENCES

Ağaoğlu, S., Alemdar, S., Ercan, N. (2020). Antibiotic residues in filtered honeys. Turkish Journal of Agriculture-Food Science and Technology, 8(11), 2408-2415. https://doi.org/10.24925/turjaf.v8i11.2408-2415.3687

Ahmed, M.B., Rajapaksha, A.U., Lim, J.E., Vu, N.T., Kim, I.S., Kang, H.M., Lee, S.S., Ok, Y.S. (2015). Distribution and accumulative pattern of tetracyclines and sulfonamides in edible vegetables of cucumber, tomato, and lettuce. Journal of Agricultural and Food Chemistry, 63, 398–405. https://doi.org/10.1021/jf5034637. Epub 2015 Jan 9.

Ahmed, M.B.M., Taha, A.A., Mehaya, F.M.S. (2023). Method validation and risk assessment for sulfonamides and tetracyclines in bees' honey from Egypt, Libya and Saudi Arabia. Environ Geochem Health, 45, 997-1011. https://doi.org/10.1007/s10653-022-01258-0

Aksoy, A. (2019). Simultaneous screening of antibiotic residues in honey by biochip multi-array technology. Medycyna Weterynaryjna, 75(9), 567-571. https://doi.org/dx.doi.org/10.21521/mw.6240

Anon (2023). Leading producers of natural honey worldwide in 2021*(in 1,000 metric tons). https://www.statista.com/statistics/report-content/statistic/812172

Aydemir Atasever, M., Yüksel, A.T. (2022). Determination of some antibiotic residues in honey produced in Erzurum region. Veterinary Sciences and Practices, 17(3), 76-80. https://doi.org/10.5152/VetSciPract.2022.220105

Bayram, N.E. (2023). Nectar honey from Turkey: crystallization and physicochemical profile. European Food Research and Technology, 249,1049-1057. https://doi.org/10.1007/s00217-022-04194-6

Bonerba, E., Panseri, S., Arioli, F., Nobile, M., Terio, V., Di Cesare, F., Tantillo, G., Maria Chiesa, L. (2021). Determination of antibiotic residues in honey in relation to different potential sources and relevance for food inspection. Food Chemistry, 334, 127575. https://doi.org/10.1016/j.foodchem.2020.127575

Brar, D.S., Pant, K., Krishnan, R., Kaur, S., Rasane, P., Nanda, V., Saxena, S., Gautam, S. (2023). A comprehensive review on unethical honey: Validation by emerging techniques. Food Control, 145, 109482. https://doi.org/10.1016/j.foodcont.2022.109482

Chiesa, L.M., Panseri, S., Nobile, M., Ceriani, F., Arioli, F. (2018). Distribution of POPs, pesticides and antibiotic residues in organic honeys from different production areas. Food Addit Contam A 35:1340-55.

Cunningham, M.M., Tran, L., McKee, C.G., Polo, R.O., Newman, T., Lansing, L., Griffiths, J.S., Bilodeau, G.J., Rott, M., Guarna, M.M. (2022). Honey bees as biomonitors of environmental contaminants, pathogens, and climate change. Ecological Indicators, 134, 108457. https://doi.org/10.1016/j.ecolind.2021.108457

Derebaşı, E., Bulut, G., Col, M., Güney, F., Yaşar, N., Ertürk, Ö. (2014). Physicochemical and residue analysis of honey from Black Sea region of Turkey. Fresenius Environmental Bulletin, 23(1), 10-17.

Er Demirhan, B., Demirhan, B. (2022). Detection of antibiotic residues in blossom honeys from different regions in Turkey by LC-MS/MS Method. Antibiotics, 11, 357. https://doi.org/10.3390/ antibiotics11030357

Erdoğdu, A.T., Coşkun, Y., Güven, S.İ. (2011). Determination of sulphonamide antibiotics residues in honey presented for consumption. The Journal of Bornova Veterinary Science, 33, 37-44.

EU (2010). Commission regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Off J EU 2009, L15, 72.

FDA-Food and Drugs Administration (2023). Code of Federal Regulations - Title 21 - Food and Drugs, Chapter I--Food and Drug Administration Department of Health and Human Services Subchapter E - Animal Drugs, Feeds, and Related Products Part 556 Tolerances for Residues of New Animal Drugs in Food., https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm, (Accessed May 10, 2023)

FSANZ-Food Standards Australia and New Zealand (2016). Australia New Zealand food standards code - Standard 1.4.2. schedule 20 - Maximum residue limits (2016), https://www.foodstandards.gov.au/code/Pages/default.aspx., (Accessed April 10, 2023)

Gunes, N., Cibik, R., Gunes, M.E., Aydin, L. (2008). Erythromycin residue in honey from the Southern Marmara region of Turkey. Food Additives and Contaminants, 25(11), 1313-1317. https://doi.org/10.1080/02652030802233472

Gunes, M.E., Gunes, E., Cibik, R. (2009). Oxytetracycline and sulphonamide residues analysis of honey samples from Southern Marmara Region in Turkey. Bulgarian Journal of Agricultural Science, 15, 163-167.

Gürel Yücel, M., Seçilmiş, H., Tasci, F. (2023). Investigation of tylosin and tilmicosin residues in meat by high-performance liquid chromatography method. Polish Journal of Veterinary Sciences 26(1), 39-46. https://doi.org/10.24425/pjvs.2023.145005

Health Canada (2022). List of Maximum Residue Limits (MRLs) for Veterinary Drugs in Foods, https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/maximum-residue-limits-mrls/list-maximum-residue-limits-mrls-veterinary-drugs-foods.html, (Accessed April 11, 2023)

Hitabatuma, A., Wang, P., Su, X., Ma, M. (2022). Metal-organic frameworks-based sensors for food safety. Foods, 11, 382. https://doi.org/10.3390/foods11030382

ICH-International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (2005). Validation of analytical procedures: text and methogology Q2 (R1). In: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. IFPMA, Geneva, Switzerland, pp 1-13.

Kırkan, E., Tahir, A.O., Bengu, A.Ş., Aslan, H., Ciftci, M., Aydoğan, C. (2020). Rapid determination of sulfonamide residues in honey samples using non-targeted liquid chromatographyhigh resolution mass spectrometry. Separation Science Plus, 1–9. https://doi.org/10.1002/sscp.202000051

Kıvrak, İ., Kıvrak, Ş., Harmandar, M. (2017). Development of a rapid method for the determination of antibiotic residues in honey using UPLC-ESI-MS/MS. Food Science and Technology (Campinas), 36(1), 90-96. https://doi.org/10.1590/1678-457X.0037

Kumar, A., Gill, J.P.S., Bedi, J.S., Chhuneja, P.K. (2020a). Residues of antibıotics in raw honeys from different apiaries of Northern India and evaluation of human health risks. Acta Alimentaria, 49(3), 314-320.

Kumar, A., Gill, J.P.S., Bedi, J.S., Chhuneja, P.K., Kumar, A. (2020b). Determination of antibiotic residues in Indian honeys and assessment of potential risks to consumers. Journal of Apicultural Research, 59(1), 25-34. https://doi.org/10.1080/00218839.2019.1677000

Kutlu, M.A., Gül, A., Özdemir, F.A., Kılıç, Ö. (2017). Determination of antibiotic residues from honey produced in Hizan District, Bitlis Province. Turkish Journal of Agricultural and Natural Sciences, 4(4), 523-527.

Marazuela, M.D., Bogialli, S. (2009). A review of novel strategies of sample preparation for the determination of antibacterial residues in foodstuffs using liquid chromatography-based analytical methods. Analytica Chimica Acta, 645, 5-17.

Mehrabi, A., Mahmoudi, R., Khoshmaram, N.B., Norian, R., Mosavi, S., Ebrahimi, H., Alizadeh, A., Kazemi, M. (2022). ELISA evaluation of erythromycin residues in honey samples collected from different areas of Qazvin, Iran. Journal of Chemical Health Risks, 12. https://doi.org/10.22034/jchr.2022.1905023.1158

Paoletti, F., Sdogati, S., Barola, C., Giusepponi, D., Moretti, S., Galarini, R. (2022). Two-procedure approach for multiclass determination of 64 antibiotics in honey using liquid chromatography coupled to time-of-flight mass spectrometry. Food Control, 136, 108893. https://doi.org/10.1016/j.foodcont.2022.108893

Ranneh, Y., Akim, A. M., Hamid, H. A., Khazaai, H., Fadel, A., Zakaria, Z. A., Albujja, M., Bakar, M. F. A. (2021). Honey and its nutritional and anti-inflammatory value. BMC Complementary Medicine and Therapies, 21(1), 30. https://doi.org/10.1186/s12906-020-03170-5

Sarkar, S., Souza, M.J., Martin-Jimenez, T., Abouelkhair, M.A., Kania, S.A., Okafor, C.C. (2023). Tetracycline, sulfonamide, and erythromycin residues in beef, eggs, and honey sold as “antibiotic-free” products in East Tennessee (USA) farmers’ markets. Veterinary Sciences,10, 243. https://doi.org/10.3390/vetsci10040243

Savarino, A.E., Terio, V., Barrasso, R., Ceci, E., Panseri, S., Chiesa, L.M., Bonerba, E. (2020). Occurrence of antibiotic residues in apulian honey: potential risk of environmental pollution by antibiotics. Italian Journal of Food Safety, 9(1), 8678. https://doi.org/10.4081/ijfs.2020. 8678

Shoaei, F., Talebi-Ghane, E., Ranjbar, A., Mehri, F. (2023). Evaluation of antibiotic residues in honey: a systematic review and meta-analysis. International Journal of Environmental Health Research, 1-12. https://doi.org/10.1080/09603123.2023.2197285

Tasci, F., Secilmis Canbay, H., Doganturk, M. (2021). Determination of antibiotics and their metabolites in milk by liquid chromatography-tandem mass spectrometry method. Food Control, 127, 108147. https://doi.org/10.1016/j.foodcont.2021.108147

TFC-Turkish Food Codex (2017). Turkish food codex regulation on classification of pharmacological active substances that can be found in animal foods and maximum residue limits. Official gazette, 7 Mar 2017 Tuesday, No: 30000. http://www.resmigazete.gov.tr/default.aspx.

Tutun, H., Kahraman, H.A., Aluc, Y., Avci, T., Ekici, H. (2019). Investigation of some metals in honey samples from West Mediterranean region of Turkey. Veterinary Research Forum, 10(3), 181-186. https://doi.org/10.30466/vrf.2019.96726.2312

Valverde, S., Ares, A.M., Stephen Elmore, J., Bernal, J. (2022). Recent trends in the analysis of honey constituents. Food Chemistry, 387, 132920. https://doi.org/10.1016/j.foodchem.2022.132920

Wang, Y., Dong, X., Han, M., Yang, Z., Wang, Y., Qian, L., Huang, M., Luo, B., Wang H., Chen, Y., Jiang, Q. (2022). Antibiotic residues in honey in the Chinese market and human health risk assessment. Journal of Hazardous Materials, 440, 129815. https://doi.org/10.1016/j.jhazmat.2022.129815

Yang, Y., Lin, G., Liu, L., Lin, T. (2022). Rapid determination of multi-antibiotic residues in honey based on modified QuEChERS method coupled with UPLC–MS/MS. Food Chemistry, 374, 131733. https://doi.org/10.1016/j.foodchem.2021.131733

Zergui, A., Boudalia, S., Joseph, M.L. (2023). Heavy metals in honey and poultry eggs as indicators of environmental pollution and potential risks to human health. Journal of Food Composition and Analysis, 119, 105255. https://doi.org/10.1016/j.jfca.2023.105255

Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2024, Volume: 8, Issue: 1, 50-55
Received: 07.01.2024; Accepted: 31.03.2024
DOI: 10.30516/bilgesci.1415844

A Study of The Anticancer Effect of 1,8 Cineole: Molecular Docking Analysis

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Abstract: Since cancer is a serious disease that affects many people around the world, scientists focus on studies on the diagnosis and treatment of cancer. Plants have been used for therapeutic purposes for many years. Plants that form the basis of traditional medicine contain therapeutic compounds. These compounds have important properties such as anticancer, anti-inflammatory, analgesic, antimicrobial and antioxidant. Essential oils obtained from various plants are known to have therapeutic effects. Terpenes make up the largest part of the composition of plant essential oils. Terpenes have various beneficial effects such as anti-anxiety, anti-depressant, anti-inflammatory, anti-bacterial, anti-cancer, analgesic and mood-boosting. 1,8 cineole is one of the monoterpene compounds found in essential oils. 1,8 cineole is an important compound with various properties such as antioxidant, anti-inflammatory and anticancer. The molecular docking method is one of the computational modeling methods used in drug development programs. In this study, the interactions of 1,8 cineole, which is known to have anticancer properties, with various receptors prominent in anticancer studies (Estrogen receptor beta (ER-β), Epidermal growth factor receptor (EGFR), Receptor tyrosine-protein kinase erbB-2 (HER2) and Tankyrase 1) were examined with the help of the molecular docking method, the interaction profile was determined and presented in comparison with literature studies. As a result of docking studies, it was predicted that the interaction with Tankyrase-1 would be stronger.

Keywords: Essential oil, Molecular docking, Cancer, Plant

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Citation: Bıçak, B. (2024). A Study of The Anticancer Effect of 1,8 Cineole: Molecular Docking Analysis. Bilge International Journal of Science and Technology Research, 8(1): 50-55.

1. INTRODUCTION

Cancer is one of the deadliest diseases in the world, resulting in the death of thousands of people every year. Scientists have been continuing their work on discovering new drugs to fight cancer for a long time. Scientists continue to research agents obtained from nature as well as chemically synthesized pharmaceutical agents in new drug discoveries (Dehelean et al., 2021).

Plants have been used for therapeutic purposes for a long time. Plants, which form the basis of traditional medicine, contain therapeutic compounds. These compounds have various properties such as anticancer, anti-inflammatory, analgesic, antimicrobial and antioxidant (Hoch et al., 2023). Many essential oils obtained from plants are also known to have medicinal benefits. It has been reported in literature studies that 1,8-cineole, which is prominent in the content of essential oils, has pharmacological effects (Murata et al., 2013). 1,8 cineol (eucalyptol), found in the essential oils of various plants such as eucalyptus, rosemary, thyme, and sage, has various properties such as anti-inflammatory, antioxidant, antimicrobial, analgesic and anticancer (Hoch et al., 2023). The anticancer properties of 1,8 cineole have been exhibited by cytotoxicity studies associated with colon (Murata et al., 2013), lung (Rodenak-Kladniew, Castro, Crespo, Galle, & de Bravo, 2020), ovarian (Abdalla et al., 2020), skin (Sampath et al., 2018) and liver (Rodenak-Kladniew, Castro, Stärkel, Galle, & Crespo, 2020) cancers.

The molecular docking method is used as a predictive tool in drug development studies. With this method, the interactions, and binding affinities of drug candidate molecules with target receptors are determined. A prediction profile can be created by examining the interactions of molecules thought to be effective against the disease for which drug development is targeted and disease-related

macromolecules and can be used to support experimental studies.

In cancer studies, receptors are determined according to the cancer type being studied. For example, Estrogen receptor β is an important receptor in the expression of cancer-related genes and ovarian cancer. In a study on ovarian cancer, it was reported that ER-β activated apoptosis and reduced proliferation and migration (Schüler-Toprak, Moehle, Skrzypczak, Ortmann, & Treeck, 2017; Treeck et al., 2007). EGFR activation is associated with tumor growth, invasion and metastasis (Normanno et al., 2006; Sasaki, Hiroki, & Yamashita, 2013). EGFR is known to be overexpressed in non-small cell lung cancer (Lee, 2006). In clinical studies, it has been reported that EGFR is often overexpressed in advanced stages of colon cancer (de Castro-Carpeño et al., 2008). HER-2 activation is known to play a role in tumor development, and its overexpression has been reported in ovarian (Slamon et al., 1989), lung (Riudavets, Sullivan, Abdayem, & Planchard, 2021), liver (Shi et al., 2019) and colorectal cancer (Ivanova et al., 2022). It is known that tankyrase inhibition also plays a role in the antiproliferative effect by affecting some signals in colorectal cancer (Solberg et al., 2018).

In this study, molecular docking studies of 1,8 cineole, which is known to have anti-cancer properties in the literature, were carried out with ER-β, EGFR, HER2 and Tankyrase 1 receptors used as targets in cancer studies, and its interaction profile and binding affinities were determined. Additionally, the interactions of some drugs used in cancer treatment and 1,8 cineole with target receptors were comparatively examined.

2. MATERIAL AND METHOD

Considering that 1,8 cineole has an anticancer effect, molecular docking studies were carried out to examine the interactions of 1,8 cineole with various cancer targets. These targets were selected as ER-β (PDB ID: 1X7J), EGFR (PDB ID: 1M17), HER2 (PDB ID: 3RCD), human tankyrase 1 (PDB ID: 4W6E). In the preparation step of the study, 1,8 cineole (PubChem ID: 2758) was optimized with DFT/B3LYP/6-311++G(d,p) basis set using Gaussian09 (Frisch et al., 2009) and receptors were downloaded from PDB DataBank (https://www.rcsb.org/). 1,8 Cineole and the selected receptors were prepared for docking analysis via AutoDock Tools 1.5.6. All molecular docking studies were realized using AutoDock Vina (Trott & Olson, 2010). The molecular docking studies were completed successfully, and the visualizations of molecular docking results were realized with the help of Pymol (DeLano, 2002) and Discovery Studio Visualizer 2019 (Studio, 2008).

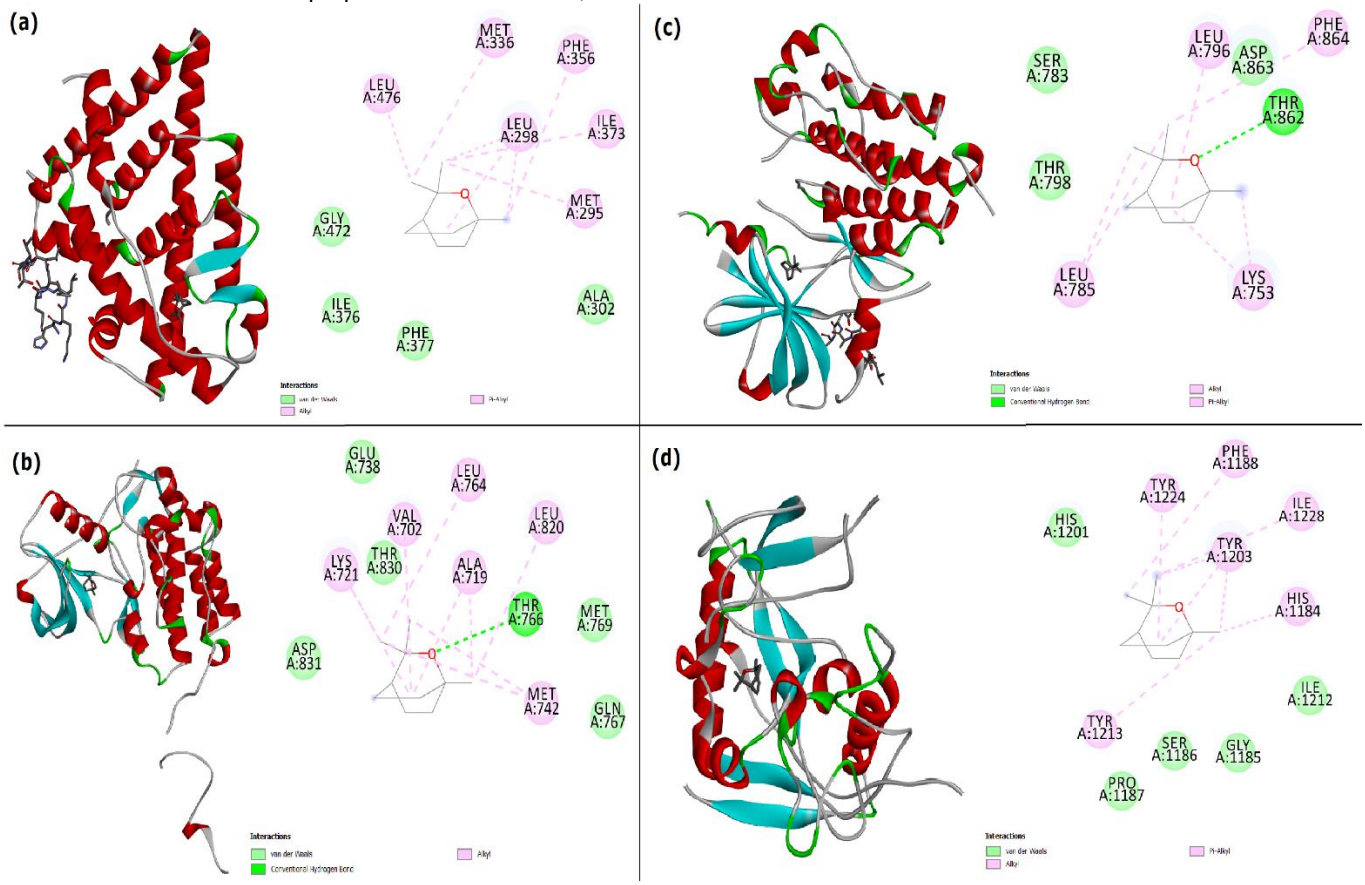

Figure 1. The close interactions of 1,8 Cineole at ERβ (a), EGFR (b), HER2 (c) and TNKS 1 (d) active sites.

Table 1. Results of molecular docking studies of 1,8 Cineole.

Receptor	Interaction residues	Binding Affinity (kcal/mol)
1X7J	**Alkyl:** Leu-476, Met-336, Leu-298, Ile-373, Met-295 **Pi-Alkyl:** Phe-356 **VdW:** Ala-302, Gly-472, Ile-376, Phe-377	-5.6
1M17	**H-Bond:** Thr-766 **Alkyl:** Lys-721, Val-702, Leu-764, Ala-719, Leu-820, Met-742 **VdW:** Glu-738, Thr-830, Asp-831, Met-769, Gln-767	-5.6
3RCD	**H-Bond:** Thr-862 **Alkyl:** Leu-796, Lys-753, Leu-785 **Pi-Alkyl:** Phe-864 **VdW:** Asp-863, Ser-783, Thr-798	-5.4
4W6E	**Alkyl:** Ile-1228 **Pi-Alkyl:** Tyr-1224, Phe-1188, Tyr-1203, His-1184, Tyr-1213 **VdW:** His-1201, Pro-1187, Ser-1186, Gly-1185, Ile-1212	-6.2

3. RESULTS

The first molecular docking study was carried out with ER-β. As a result of the docking study, the binding energy was determined as -5.6 kcal/mol, and the fact that the RMSD values were below 2 Å is an indication that the docking study was successful. In the study, 1,8 Cineole docked to the active site of ER-β made pi-alkyl interaction with the Phe-356 residue and alkyl interaction with the Leu-476, Met-336, Leu-298, Ile-373, Met-295 residues of ER-β (see Figure 1 and Table 1). Additionally, as a result of the docking study, it was determined that 1,8 cineole has van der Waals (VdW) interaction with Ala-302, Gly-472, Ile-376, Phe-377 residues of ER-β. 1,8 Cineole also had pi interactions, alkyl interactions, and VdW interactions with the same residues of ER-β as genistein (reference compound) (Manas, Xu, Unwalla, & Somers, 2004).

In the molecular docking study of 1,8-cineole and EGFR, it was determined that the binding energy was calculated as -5.6 kcal/mol, and RMSD values was below 2 Å. 1,8 Cineole made hydrogen bond, alkyl and VdW interactions in the active site of EGFR. 1,8 Cineole interacted with Thr-766 residue of EGFR and formed hydrogen bond having 3.0 Å. In addition, it was observed that alkyl interactions with Lys-721, Val-702, Leu-764, Ala-719, Leu-820, Met-742 residues and VdW interactions with Glu-738, Thr-830, Asp-831, Met-769, Gln-767 residues (see Figure 1 and Table 1).

In the study conducted with HER2 (PDB ID: 3RCD), the binding affinity was determined as -5.4 kcal/mol, similar to other docking studies. 1,8 Cineole formed hydrogen bond with Thr-862 residue of HER2. Other interactions were determined as alkyl, pi-alkyl and VdW. 1,8 Cineole interacted with Lys-753 (alkyl), Leu-796 (alkyl), Leu-785 (alkyl), Phe-864 (pi-alkyl), Asp-863 (VdW), Ser-783 (VdW), Thr-798 (VdW).

In the docking study performed with Tankyrase-1 (PDB: 4W6E), 1,8 Cineole made alkyl, pi-alkyl and VdW interactions (see Figure 1). Pi-alkyl interactions formed between 1,8 Cineole and Tyr-1224, Phe-1188, His-1184, Tyr-1213 residues of Tankyrase-1. Alkyl interaction occurred between 1,8 Cineole and Ile-1228 residue. In addition, VdW interactions occurred between 1,8 Cineole and His-1201, Pro-1187, Ser-1186, Gly-1185 and Ile-1212 residues. Binding affinity was calculated as -6.2 kcal/mol, and RMSD values gave very good results for docking study.

4. DISCUSSION AND CONCLUSIONS

Molecular docking is a preferred method in drug design. It is a useful and supportive method in new drug discovery studies. In the molecular docking method, the appropriate orientation and binding affinity of the ligand (drug candidate) in the active site of the target receptor is predicted (Korkmaz & Ayaz, 2023). In this study, considering that 1,8 Cineol is in various types of cancer (Abdalla et al., 2020; Murata et al., 2013; Rodenak-Kladniew, Castro, Crespo, et al., 2020), the receptors selected are ERβ, EGFR, HER2 and Tankyrase 1. ER-β has a wide distribution in different body regions (Hsu, Chu, & Kao, 2017; Lazennec, 2006; Siegfried, 2001; Williams, DiLeo, Niv, & Gustafsson, 2016). It has been reported in the literature that estrogen has a special place in cancers such as lung, colon and ovarian (Hsu et al., 2017; Lazennec, 2006; Siegfried, 2001; Williams et al., 2016). EGFR activation is associated with tumor growth, invasion, and metastasis (Normanno et al., 2006; Sasaki et al., 2013). Therefore, it is expressed in many types of cancer (Bethune, Bethune, Ridgway, & Xu, 2010; Glaysher et al., 2013; Rego et al., 2010). Due to these properties, it is among the important targets in anticancer studies. HER2 belongs to the EGFR tyrosine kinase family and is another important receptor chosen as a target in antitumor studies (Iqbal & Iqbal, 2014). HER-2 activation is associated with tumor development. It has been presented in literature studies that HER-2 is overexpressed in various types of cancer (Ahcene Djaballah, Daniel, Milani, Ricagno, & Lonardi, 2022; Riudavets et al., 2021; Slamon et al., 1989). Tankyrases are involved in a number of cellular functions such as telomere homeostasis, Wnt/β-catenin signaling, viral replication. Tankyrases, which play a role in disease-related cellular processes, have become one of the important targets in drug discovery studies (Kamal, Riyaz, Kumar Srivastava, & Rahim, 2014). Tankyrase is one of the prominent targets in different cancer studies. Varying levels of tankyrase expression have been reported in various types of cancer (Mehta & Bhatt, 2021; Verma, Kumar, Chugh, Kumar, & Kumar, 2021).

According to the results of the docking study performed with ERβ, it has been determined that genistein, the reference compound of ER-β (PDB ID: 1X7J), and 1,8 cineole have similar close interactions in the active site of ER-β (Manas et al., 2004). As a result of a successful docking study with EGFR, when the interaction profiles were compared with erlotinib, a cancer drug, it was seen that it has similar interactions (PDB ID: 1M17) (Stamos, Sliwkowski, & Eigenbrot, 2002). In the molecular docking study performed with HER2 (PDB ID: 3RCD), 1,8 cineole has similar interaction profiles as reference compounds (Ishikawa et al., 2011; Prabhavathi et al., 2022). When compared to neratinib, a cancer drug, and TAK-285 (reference compound in the PDB file), it was observed that 1,8 cineole, like TAK-285 (Ishikawa et al., 2011), made hydrogen bonds with Thr-862 and alkyl interactions with Leu-785. Additionally, 1,8 cineole was found to have alkyl interactions with Leu-796 and Lys-753, like both reference compounds. In the molecular docking study performed with Tankyrase-1, 1,8 cineole made similar interactions with similar residues as the reference compound in the PDB (PDB ID: 4W6E) (Johannes et al., 2015). When compared to the docking study performed with caffeic acid, 1,8 cineole was observed to have different types of interactions with similar residues (Neagu, Stefaniu, Albulescu, Pintilie, & Pirvu, 2021).

1,8 cineole is an important compound that has antimicrobial, anti-inflammatory, and anticancer properties and is found in different amounts in various essential oils. In this study, we focused on the anticancer effect of 1,8 cineole, and its interactions with ERβ, EGFR, HER2 and Tankyrase-1, which are prominent targets in cancer research, were examined by molecular docking method. Interaction profiles were compared with reference compounds and similar interaction profiles were obtained.

It has been reported in literature studies that 1,8 cineole has an apoptotic effect on human colon cancer cell lines and is associated with the inactivation of survivin and Akt and the activation of p38 in treatment (Murata et al., 2013). It is also known from literature studies that tankyrase inhibition blocks the Wnt/β-catenin pathway, which is activated in almost all human colorectal cancer, and reverses the resistance to PI3K and Akt inhibitors in colorectal cancer (Arqués et al., 2016). It was determined that the study in which 1,8 cineole had the best binding energy and RMSD value was the molecular docking study performed with Tankyrase 1. Considering the literature studies, this study predicts that 1,8 cineole may have a role in the apoptotic effect on colorectal cancer cells.

With further application of experimental in vitro studies of different cancer types, theoretically targeting different receptors depending on the cancer type and examining the best binding profiles can be guiding in pre-clinical studies.

Acknowledgements
I would like to thank Prof. Dr. Uğur Gündüz and Prof. Dr. Serda Kecel Gündüz for their support.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions
All process steps such as conceptualization, investigation, analysis, visualization, methodology and writing were written by Bilge Bicak. The author has read and agreed to the published version of manuscript.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding
The authors declared that this study has received no financial support.

REFERENCES

Abdalla, A. N., Shaheen, U., Abdallah, Q. M., Flamini, G., Bkhaitan, M. M., Abdelhady, M. I., . . . Bader, A. (2020). Proapoptotic activity of Achillea membranacea essential oil and its major constituent 1, 8-cineole against A2780 ovarian cancer cells. *Molecules, 25*(7), 1582.

Ahcene Djaballah, S., Daniel, F., Milani, A., Ricagno, G., & Lonardi, S. (2022). HER2 in colorectal cancer: the long and winding road from negative predictive factor to positive actionable target. *American Society of Clinical Oncology Educational Book, 42*, 219-232.

Arqués, O., Chicote, I., Puig, I., Tenbaum, S. P., Argilés, G., Dienstmann, R., . . . Silberschmidt, D. (2016). Tankyrase inhibition blocks Wnt/β-catenin pathway and reverts resistance to PI3K and AKT inhibitors in the treatment of colorectal cancer. *Clinical cancer research, 22*(3), 644-656.

Bethune, G., Bethune, D., Ridgway, N., & Xu, Z. (2010). Epidermal growth factor receptor (EGFR) in lung cancer: an overview and update. *Journal of thoracic disease, 2*(1), 48.

de Castro-Carpeño, J., Belda-Iniesta, C., Sáenz, E. C., Agudo, E. H., Batlle, J. F., & Barón, M. G. (2008). EGFR and colon cancer: a clinical view. *Clinical and Translational Oncology, 10*, 6-13.

Dehelean, C. A., Marcovici, I., Soica, C., Mioc, M., Coricovac, D., Iurciuc, S., . . . Pinzaru, I. (2021). Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. *Molecules, 26*(4), 1109.

DeLano, W. L. (2002). Pymol: An open-source molecular graphics tool. *CCP4 Newsl. Protein Crystallogr, 40*(1), 82-92.

Frisch, M., Trucks, G., Schlegel, H., Scuseria, G., Robb, M., Cheeseman, J., . . . Petersson, G. (2009). Gaussian 09 Revision A. 02, 2009, Gaussian Inc. *Wallingford CT, 66*, 219.

Glaysher, S., Bolton, L., Johnson, P., Atkey, N., Dyson, M., Torrance, C., & Cree, I. (2013). Targeting EGFR and PI3K pathways in ovarian cancer. *British journal of cancer, 109*(7), 1786-1794.

Hoch, C. C., Petry, J., Griesbaum, L., Weiser, T., Werner, K., Ploch, M., . . . Wollenberg, B. (2023). 1, 8-cineole (eucalyptol): A versatile phytochemical with therapeutic applications across multiple diseases. *Biomedicine & Pharmacotherapy, 167*, 115467.

Hsu, L.-H., Chu, N.-M., & Kao, S.-H. (2017). Estrogen, estrogen receptor and lung cancer. *International journal of molecular sciences, 18*(8), 1713.

Iqbal, N., & Iqbal, N. (2014). Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. *Molecular biology international, 2014*.

Ishikawa, T., Seto, M., Banno, H., Kawakita, Y., Oorui, M., Taniguchi, T., . . . Miki, H. (2011). Design and synthesis of novel human epidermal growth factor receptor 2 (HER2)/epidermal growth factor receptor (EGFR) dual inhibitors bearing a pyrrolo [3, 2-d] pyrimidine scaffold. *Journal of medicinal chemistry, 54*(23), 8030-8050.

Ivanova, M., Venetis, K., Guerini-Rocco, E., Bottiglieri, L., Mastropasqua, M. G., Garrone, O., . . . Ghidini, M. (2022). HER2 in metastatic colorectal cancer: pathology, somatic alterations, and perspectives for novel therapeutic schemes. *Life, 12*(9), 1403.

Johannes, J. W., Almeida, L., Barlaam, B., Boriack-Sjodin, P. A., Casella, R., Croft, R. A., . . . Hawkins, J. L. (2015). Pyrimidinone nicotinamide mimetics as selective tankyrase and wnt pathway inhibitors suitable for in vivo pharmacology. *ACS medicinal chemistry letters, 6*(3), 254-259.

Kamal, A., Riyaz, S., Kumar Srivastava, A., & Rahim, A. (2014). Tankyrase inhibitors as therapeutic targets for cancer. *Current Topics in Medicinal Chemistry, 14*(17), 1967-1976.

Korkmaz, T. B., & Ayaz, F. (2023). Molecular docking: A powerful tool for predicting protein-ligand interactions. *Advanced Engineering Days (AED), 7*, 4-6.

Lazennec, G. (2006). Estrogen receptor beta, a possible tumor suppressor involved in ovarian carcinogenesis. *Cancer letters, 231*(2), 151-157.

Lee, S. (2006). Is EGFR expression important in non-small cell lung cancer? In (Vol. 61, pp. 98-99): BMJ Publishing Group Ltd.

Manas, E. S., Xu, Z. B., Unwalla, R. J., & Somers, W. S. (2004). Understanding the selectivity of genistein for human estrogen receptor-β using X-ray crystallography and computational methods. *Structure, 12*(12), 2197-2207.

Mehta, C. C., & Bhatt, H. G. (2021). Tankyrase inhibitors as antitumor agents: a patent update (2013–2020). *Expert Opinion on Therapeutic Patents, 31*(7), 645-661.

Murata, S., Shiragami, R., Kosugi, C., Tezuka, T., Yamazaki, M., Hirano, A., . . . Ohkohchi, N. (2013). Antitumor effect of 1, 8-cineole against colon cancer. *Oncology reports, 30*(6), 2647-2652.

Neagu, G., Stefaniu, A., Albulescu, A., Pintilie, L., & Pirvu, L. C. (2021). Antiproliferative Activity of Stokesia laevis Ethanolic Extract in Combination with Several Food-Related Bioactive Compounds; In Vitro (Caco-2) and In Silico Docking (TNKS1 and TNKS2) Studies. *Applied Sciences, 11*(21), 9944.

Normanno, N., De Luca, A., Bianco, C., Strizzi, L., Mancino, M., Maiello, M. R., . . . Salomon, D. S. (2006). Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene, 366*(1), 2-16.

Prabhavathi, H., Dasegowda, K., Renukananda, K., Karunakar, P., Lingaraju, K., & Raja Naika, H. (2022). Molecular docking and dynamic simulation to identify potential phytocompound inhibitors for EGFR and HER2 as anti-breast cancer agents. *Journal of Biomolecular Structure and Dynamics, 40*(10), 4713-4724.

Rego, R., Foster, N., Smyrk, T., Le, M., O'connell, M., Sargent, D., . . . Sinicrope, F. (2010). Prognostic effect of activated EGFR expression in human colon carcinomas: comparison with EGFR status. *British journal of cancer, 102*(1), 165-172.

Riudavets, M., Sullivan, I., Abdayem, P., & Planchard, D. (2021). Targeting HER2 in non-small-cell lung cancer (NSCLC): a glimpse of hope? An updated review on therapeutic strategies in NSCLC harbouring HER2 alterations. *ESMO open, 6*(5), 100260.

Rodenak-Kladniew, B., Castro, A., Stärkel, P., Galle, M., & Crespo, R. (2020). 1, 8-Cineole promotes G0/G1 cell cycle arrest and oxidative stress-induced senescence in HepG2 cells and sensitizes cells to anti-senescence drugs. *Life sciences, 243*, 117271.

Rodenak-Kladniew, B., Castro, M. A., Crespo, R., Galle, M., & de Bravo, M. G. (2020). Anti-cancer mechanisms of linalool and 1, 8-cineole in non-small cell lung cancer A549 cells. *Heliyon, 6*(12).

Sampath, S., Subramani, S., Janardhanam, S., Subramani, P., Yuvaraj, A., & Chellan, R. (2018). Bioactive compound 1, 8-Cineole selectively induces G2/M arrest in A431 cells through the upregulation of the p53 signaling pathway and molecular docking studies. *Phytomedicine, 46*, 57-68.

Sasaki, T., Hiroki, K., & Yamashita, Y. (2013). The role of epidermal growth factor receptor in cancer metastasis

and microenvironment. *BioMed research international, 2013*.

Schüler-Toprak, S., Moehle, C., Skrzypczak, M., Ortmann, O., & Treeck, O. (2017). Effect of estrogen receptor β agonists on proliferation and gene expression of ovarian cancer cells. *BMC cancer, 17*, 1-9.

Shi, J. H., Guo, W. Z., Jin, Y., Zhang, H.-P., Pang, C., Li, J., . . . Zhang, S. J. (2019). Recognition of HER2 expression in hepatocellular carcinoma and its significance in postoperative tumor recurrence. *Cancer medicine, 8*(3), 1269-1278.

Siegfried, J. M. (2001). Women and lung cancer: does oestrogen play a role? *The lancet oncology, 2*(8), 506-513.

Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., . . . Ullrich, A. (1989). Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science, 244*(4905), 707-712.

Solberg, N. T., Waaler, J., Lund, K., Mygland, L., Olsen, P. A., & Krauss, S. (2018). TANKYRASE inhibition enhances the antiproliferative effect of PI3K and EGFR inhibition, mutually affecting β-CATENIN and AKT signaling in colorectal cancer. *Molecular Cancer Research, 16*(3), 543-553.

Stamos, J., Sliwkowski, M. X., & Eigenbrot, C. (2002). Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. *Journal of biological chemistry, 277*(48), 46265-46272.

Studio, D. (2008). Discovery studio. *Accelrys [2.1]*.

Treeck, O., Pfeiler, G., Mitter, D., Lattrich, C., Piendl, G., & Ortmann, O. (2007). Estrogen receptor β1 exerts antitumoral effects on SK-OV-3 ovarian cancer cells. *Journal of Endocrinology, 193*(3), 421-433.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry, 31*(2), 455-461.

Verma, A., Kumar, A., Chugh, A., Kumar, S., & Kumar, P. (2021). Tankyrase inhibitors: emerging and promising therapeutics for cancer treatment. *Medicinal Chemistry Research, 30*, 50-73.

Williams, C., DiLeo, A., Niv, Y., & Gustafsson, J.-Å. (2016). Estrogen receptor beta as target for colorectal cancer prevention. *Cancer letters, 372*(1), 48-56.

Bilge International Journal of Science and Technology Research
ISSN: 2651-401X
e-ISSN: 2651-4028
Research Article
2024, Volume: 8, Issue: 1, 56-64
Received: 22.01.2024; Accepted: 13.03.2024
DOI: 10.30516/bilgesci.1423992

Determination of Bending Moment Resistance of L-Type Doweled Joints Reinforced With Glass-Fiber-Reinforced Polymer Woven Fabrics (GFRPWF) and Basalt-Fiber-Reinforced Polymer Woven Fabrics (BFRPWF)

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Abstract: In this study investigated the bending moment resistance of L-type doweled joints reinforced with glass-fiber-reinforced polymer woven fabrics (GFRPWF) and basalt-fiber-reinforced polymer woven fabrics (BFRPWF). Dowels produced from Scots pine, oak, beech and chestnut wood were used in the doweled joints. While the GFRPWF and BFRPWF were fixed with epoxy adhesive, the dowels were fixed with polyvinyl acetate (PVAc-D3/D4) glue. Test were carried out to determine the bending moment resistance of doweled joints. Experimental results showed that joints connected with oak dowel has been the highest bending moment resistance, and the joints of Scots pine dowel has been the weakest bending moment resistance. The bending moment resistance of oak dowel was approximately 23%, 33%, and 61% higher than for joints constructed with beech, Chestnut and Scots pine, respectively. The bending moment resistance value reinforced with the BFRPWF (55.62 N.m), and the lowest was in unreinforced joints (32.06 N.m). The mean bending moment resistance of reinforced joints (GFRPWF, BFRPWF) was 31% and 74% higher than unreinforced samples (control), respectively. In general, it has been found that the bending moment resistance of doweled joints is influenced by wooden dowel species and FRP types.

Keywords: BFRPWF, dowel, GFRPWF, polyvinyl acetate, Scots pine, wooden.

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Citation: Karaman, A. (2024). Determination of bending moment resistance of L-type doweled joints reinforced with glass-fiber-reinforced polymer woven fabrics (GFRPWF) and basalt-fiber-reinforced polymer woven fabrics (BFRPWF). Bilge International Journal of Science and Technology Research, 8(1): 56-64.

1. INTRODUCTION

Nowadays, only wood material and no other building material can be used. It is possible to build a house without using it. other structure Materials may not have such wide usage opportunities. Of wood; From facade cladding to interior spaces, from ceilings to floors, kitchen Dozens of usage areas from furniture to verandas and pergolas available (Beram, 2021).

Wooden structures are vulnerable to degradation over time due to prolonged exposure to loading conditions and fluctuations in environmental factors, such as temperature and humidity. These deteriorations can compromise the structural integrity of wood-based materials, giving rise to safety concerns that necessitate their early identification (Beram, 2023).

Wood material, as a natural, renewable and sustainable resource, has been in human life for thousands of years and still is. It is a building and engineering material that maintains its importance. From ancient times to the present, human beings have needed both shelter and comfort. It needed wood material and a wide variety of products produced from this material for life (İçel and Beram, 2016).

Fiber reinforced polymer (FRP) is composite with mature technology and wide application. FRP was widely used in civil industry, such as transportation industry bridges (Yang et al., 2020), roads (Mohamed and Masmoudi, 2011), light industry, chemical industry (Gilby, 1999), medical treatment (Jiang et al., 2021) electricity and other industries. FRP has the light weight, corrosion resistance, fatigue resistance, enabling FRP to be used as a structural material (Passos et al., 2021).

The application of FRPs for the reinforcement of timber structures has proven its effectiveness in increasing the load-bearing capacity and, in some cases, the rigidity of structural members, thus providing cost-effective and competitive alternatives in both new design and retrofitting existing historic buildings (Saad and Lengyel, 2022). Among all FRP products, basalt fiber reinforced polymer (BFRP), carbon fiber-reinforced polymer, unidirectional glass fiber-reinforced polymer (GFRP), and aramid fiber-reinforced polymer, especially E-Glass fibers, seem to be the most effective in reinforcing wooden beams due to their low cost and favorable mechanical properties (Bywalski et al., 2020). BFRP has a higher strength and modulus, a similar cost, and more chemical stability compared to E-glass FRP (GFRP) (Wu et al., 2009). Basalt-based materials are environmentally friendly and non-hazardous. The current production technology for continuous basalt fibers is very similar to the technology used in the production of E-glass. The main difference is that E-glass is made from a complex pile of material, while the basalt filament is made from smelting basalt rock without any other additives, resulting in a cost-effective one. Their specific mechanical properties are comparable to or better than those of E-glass (Fiore et al., 2011). As an innovative material, basalt fiber reinforced polymer has the advantages of extremely good modulus and high strength (Wu et al., 2009; Wei et al., 2011; Fiore et al., 2011; Borhan, 2012; Wang et al., 2013). Based on the ultimate stress obtained from monotonic tensile tests, it is explained that basalt fiber fabrics have higher elongation resistance than both carbon-based and glass-based laminates (Palmieri et al., 2009; Wu et al., 2010; Dorigato and Pegoretti, 2012), basalt fibers have higher tensile strength than E-glass fibers (Wei et al., 2010; Carmisciano et al., 2011; Lopresto, 2011; Dorigato and Pegoretti, 2012) basalt fibers exhibit mechanical properties completely comparable to those of glass, with the elastic modulus of basalt being higher than that of glass fibers (Dorigato and Pegoretti, 2012). Various experimental studies show that reinforcing wooden structures provides an increase in load-bearing capacity, hardness and ductility over a wide range, most likely due to the organic structure of wood. Most studies show an increase in capacity of 20% to 50% (Gentile et al., 2002; Osmannezhad et al., 2015), a negligible increase in hardness (Amy et al., 2004; Fiorelli and Dias, 2003) or sometimes much higher (Gentile et al., 2002; Osmannezhad et al., 2015; Fiorelli et al., 2011). Goa et al. (2023) studied the mechanical properties and applications of glass fiber-reinforced polyurethane composites (GFRP) in communication pole line engineering. The results show that GFRP has high hardness, light weight, high strength and durability.

There are several studies using glass fiber-reinforced polymers and basalt fiber-reinforced polymers as reinforcement reinforcement for wood beams (Yusof and Saleh 2010; Fiorelli et al., 2011; Alhayek et al., 2012; Alshurafa et al., 2012; Morales-Conde et al., 2015; Osmannezhad et al., 2015: O'Ceallaigh et al., 2019; Wang et al., 2019; Bywalski et al., 2020; Balmori et al., 2020; Balmori et al., 2021; Karaman, 2021; Camargo et al., 2023; Ezika et al., 2023; Shekarchi et al., 2023).

When the studies related to the bending moment capacity of L-type doweled joints with reinforced the BFRPWF and the GFRPWF are examined in the literature review, However, it has been seen that studies on the the bending moment capacity of L-type two-pin dowel joints with reinforced investigate that are not applied and it is considered that there is a deficiency in the literature and at the same time the study to be carried out on this subject will be original and contribute to the literature. The aim of study was to investigate that the effects of wooden dowel species and the BFRPWF and GFRPWF on the bending moment resistance of the L-type doweled joints.

2. MATERIAL AND METHOD

For the study, the beech wood (*Fagus orientalis* Lipsky), widely employed in the furniture sector, was chosen as the wooden material (Figure 1e). Wood species used in the production of dowels are Scots pine (*Pinus sylvestris* Lipsky) (Figure 1h), beech (*Fagus orientalis* Lipsky) (Figure 1f), oak (*Quercus petrea* Lieble) (Figure 1g), and chestnut (*Castanea sativa* Mill) (Figure 1ı) . The selection of wood materials were conducted randomly from timber merchants located in Siteler-Ankara Turkey. Some physical and mechanical properties of wood materials are given Table 1.

Table 1. Some physical and mechanical properties of wood species used in the study (Bozkurt and Erdin, 2000).

Some physical and mechanical properties	Wooden dowel species			
	Beech	**Sessile oak**	**Scots pine**	**Chestnut**
Air-dry density (D_{12}) (g/cm^3)	0.66	0.69	0.52	0.56
Tension strength (MPa)	135	90	104	135
Compression strength (MPa)	62	60	55	50
Bending strength (MPa)	105	94	87	77
Modulus of elasticity (MPa)	16000	12300	12000	9000
Shear strength (Mpa)	8	11	10	8

The GFRPW and BFRPWF for 200 gr/m^2 plain materials used in the study was obtained by Dost Chemical Industry Raw Material Industry and Trading Company (Turkey, Istanbul) (Figure 1c and d). Some physical and mechanical properties of the BFRPWF and GFRPWF are shown in Table 2.

Table 2. Some physical and mechanical properties of the BFRPWF and GFRPWF (Valentino et al., 2014; Dong, 2019).

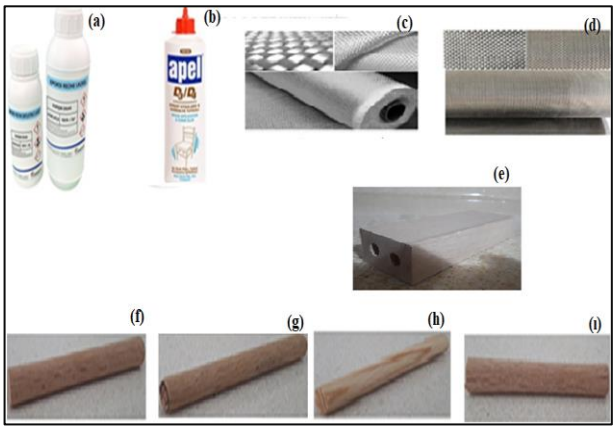
Property	FRPs	
	BFRPWF	GFRPWF
Density (g/cm^3)	2.8	2.58
Tensile strength (GPa)	2.80	3.45
Shear modulus (GPa)	21.7	30
Elastic modulus (GPa)	89	72
Elangation at break (%)	3.15	4.7

The FRPs were fastened with epoxy adhesive and hardener. The type of epoxy resin used in the matrix material was MGS L285 resin and hardener was MGS H285 (Dost Chemical Industry Raw Material Industry and Trading Co., Istanbul Türkiye (Figure 1a). The polyurethane adhesive (PUR) used in this the study was obtained by Beta Chemical Industrial Industry and Trade Company (Turkey, Istanbul) (Figure 1b). The technical parameters of the adhesives are given in Table 3.

Table 3. Technical data and characteristics of the adhesives.

Technical Data	Polyvinyl acetate (PVAc-D3/D4)	Epoxy (L285 Resin+ H285 Hardener)
Viscosity (mPas)	19000±5000	600 - 900
Working time (min)	35-40 at 21 °C	45-240 at 25 °C
Density (g/cm^3)	1.055±3 at 21 °C	1.21 at 25 °C
Solids content (%)	51±2	-
pH	2.5-3.5	-
Main agent/Hardener Ratio (w/w)	100/5	100/50

Figure 1. Materials used in experiments. (a) Epoxy adhesive. (b) Polyvinyl acetate (PVAc-D3/D4) adhesive. (c) GFRPWF. (d) BFRPWF. (e) Beech wood. (f) Beech dowel. (g) Oak dowel. (h) Scots pine dowel, and (ı) Chestnut dowel.



Preparation of test specimens

The wood materials were kept in the air conditioning room at 20±2°C and 65±3% relative humidity until their weight stabilized (TS 2470, 1976). Then, 1000x11x11 mm pieces were cut from beech sapwood, oak sapwood, chestnut sapwood and scotch pine sapwood for the dowel, and dowels with a diameter of 8 mm were produced from these pieces using a dowel machine. A diagram of the tested two-pin dowel joints is shown in Figure 2. Joint specimens were constructed of beech wood. The specimens consisted of two structrual parts, namely, a rail member anda post member. The rail part measured 150 mm long 50 mm width 25 mm thick, whereas the post member measured 125 mm long 50 mm width 25 mm thick. The dowels with a diameter of 8 mm and a length of 50 mm were used as the joining elements. The members were jointed to each other with 2 pieces of 8 mm diameter and 40 mm length dowels with the PVAc-D3/D4 adhesive. The rail and post members were drilled with a drilling machine. Depths of the dowel holes in both the post and the rail was 21 mm. Then the holes in the member were cleaned with compressed air. The adhesive was spread over the dowel surfaces and dowel holes with approximately 200 g/m^2 calculation. In all of the samples, a piece of wax paper was included between the two members to prevent any possibility of the members adhesion. Then, areas where the BFRPWF and GFRPWF were to be placed were bonded with an average of 200 ± 10 gr/m² with a brush with a blend of epoxy adhesive and hardener. Joint instances were left to dry for two days.

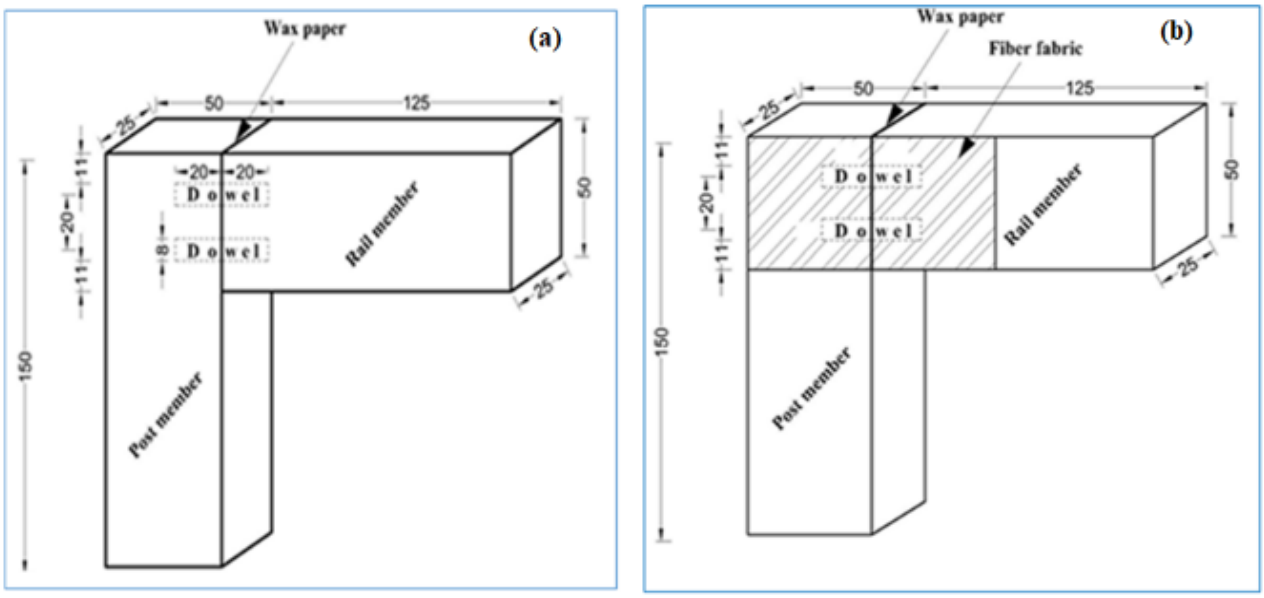


Figure 2. General configuration of L type dowel joints (dimensions in mm). (a) Unreinforced and (b) Reinforced with FRP.

According to this, four wooden dowel species, and two fiber woven fabrics (BFRPWF, GFRPWF, and control), and 5 samples of each material (4 x 3 x 5) were the variables, totally a number of 60 samples were constructed in this research. Prior to testing, all of the specimens obtained were conditioned at 20 °C ± 2 °C and 65 % ± 5 % percent relative humidity so that they could reach the equilibrium moisture content.

Test method

All of the bending tests were carried out on a 50 kN capacity universal testing machine (Shimadzu Autograph AG-IS, Sydney, Australia) in the laboratory of Karabuk University Sefik Dizdar Safranbolu Vocational High School. Figure 3 shows the set up for bending tests. A concentrated load was applied to the rail of each specimen at a point 100 mm from the front edge of the post, i.e., the moment arm was 100 mm. The loading speed was 8 mm/min. Loading was continued until breakage or separation occurred in the specimens. The loads carried by joints were recorded in newtons (N). The loads were then converted to corresponding bending moment values by means of the expression. The resistance of the joints (modulus of rupture) to bending forces was calculated using the following Eq. (1)

$$M = F \times L \quad (1)$$

where M is the bending moment resistance (N·m), F is the ultimate applied force (N) and L is the moment arm (m).

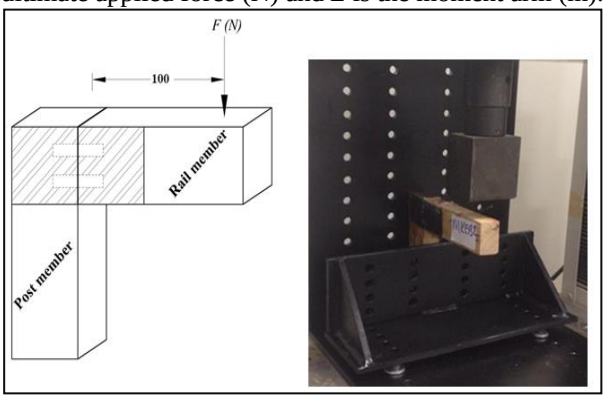


Figure 3. Way of support and loading the specimens of the L type dowel corner joints.

Statistical analyses

A two way analysis of variance (ANOVA) was made in wood L type corner joints in order to determine the effects of wooden dowel species, and FRP types. In case of mutual interactions of sources of variance, significant
at $\alpha = 0.05$, Tukey test was used to identify which factors made the difference.

3. RESULTS AND DISCUSSION

Mean values of the bending moment capacity of the tested L-type dowel joints with their standard deviation and coefficients of variation are shown in the following Table 4.

Table 4. Bending moment resistance (N.mm) of L-type doweled joints (N.m).

Woo*den* Dowel Species	**FRP Types**	**Mean**	**SD**	**COV (%)**
	Control (Unreinforced)	34.37	3.84	11.19
Beech	GFRPWF	43.87	4.91	11.19
	BFRPWF	54.06	6.50	12.01
	Control (Unreinforced)	29.28	2.51	8.58
Chestnut	GFRPWF	41.28	5.73	13.89
	BFRPWF	51.87	8.37	16.13
	Control (Unreinforced)	41.03	5.37	13.08
Oak	GFRPWF	50.69	9.63	19.00
	BFRPWF	71.00	7.33	10.32
	Control (Unreinforced)	23.56	4.18	17.74
Scots pine	GFRPWF	31.94	3.77	11.81
	BFRPWF	45.56	7.49	7.70

SD: Standart deviation COV (%): Coefficient of variation.

According to multiple comparisons on the bending moment capacity, the highest bending moment resistance value was obtained from jointed with an oak dowel and reinforced with the BFRPWF (71 N.m), while the lowest value was acquired in joint with Scots pine dowel and unreinforced (control) (23.56 N.m).

The results of the multi-way ANOVA analysis of the wooden dowel species and reinforced fiber woven fabrics on the bending moment resistance of the L-type, two pin dowel joints under the compression load were given in the Table 5.

Table 5. Summary of the ANOVA results for bending moment resistance.

Source	**df**	**Sum of Squares**	**Mean Square**	**F**	**P**
Wooden dowel species (A)	3	3283.128	1094.376	28.972	.000
FRP types (B)	2	5599.991	2799.996	74.125	.000
A×B	6	220.176	36.696	.971	.455
Error	48	1813.145	37.774		
Total	60	122945.364			
Corrected Total	59	10916.439			

R Squared = .834 (Adjusted R Squared = ,796) df: Degrees of Freedom.

According to the analysis of variance as presented in Table 3, the effects of the main factors including, the wooden dowel species (A) and the FRP (B), were found to be statistically significant at the level of 0.05. Interactions of the wooden dowel species and the FRP (A×B) were not found to be statistically significant at the level of 0.05. Tukey test was carried out in order to determine these differences. The bending moment resistance means according to independent effects of test variables were given in Table 6.

Table 6. The results from the Tukey's test for independent effects of test variables (N.m)

Source		**Bending moment resistance**	**HG**
Wooden dowel species	Oak	54.24	A
	Beech	44.10	B
	Chestnut	40.81	B
	Scots pine	33.69	C
FRP types	BFRPWF	55.62	A
	GFRPWF	41.95	B
	Control	32.06	C

HG: Homogeneity groups,

For the wooden dowel species, the oak dowel showed significantly higher the bending moment resistance value than other dowels (Table 6), The bending moment resistance value of oak was approximately 23%, 33% and 61% higher than for joints constructed with beech, chestnut, and Scots pine, respectively, The situation with the species of lumber used for dowels can explain with the structural properties of the materials, The reasons for these may be based on the density of wooden materials, As a general rule, mechanical properties increase as the density of solid wood material increases, There is an increasing-linear relationship between bending strength, modulus of elasticity and shock resistance and density.

According to FRP types, the highest bending moment resistance value were obtained in the BFRPWF (55.62 N.m), and the lowest was in control samples (32.06 N.m), The mean bending moment resistance of joints with the BFRPWF was 33 % and 73 % higher than joints with GFRP, and not reinforced joints (control), respectively.

The cost of elements used in traditional reinforcement methods is low compared to that of FRP materials, But in the long run, elements such as bolts, nails, etc, may not be effective on timber, Therefore, it is more convenient to use FRP materials instead of traditional reinforcement methods as they require maintenance and repair over time and have low durability.

Failure modes

After testing, all connections were visually inspected in order to identify the failure mode of the dowels, In the bending moment resistance test of L-type doweled joints construction with Beech wood in not reinforced samples deformations as in Figure 4 were observed, while there was no deformation in the wooden members, bending deformation in the dowel used for the joint was observed. For all of the joint types, failures initially occurred as opening at the inner face of joints when those joints were subjected to bending moment. The width of the gap between the rail and the post was measured to obtain the degree of decay of the dowel joints. As result, it was seen that the highest rate of the gap was in Oak and Chestnut dowel test samples (see Figure 4a, 4b),the Scots pine and beech dowel samples followed respectively (see Figure 4d, 4c).

According to the reinforced joints, GFRPWF has prevented cracking. It was observed for all of the joint types, failures not occurred as opening at the inner face of joints when those joints were subjected to bending moment, the gaps were much shorter than the not reinforced samples. For the samples of the Chestnut dowel+GFRPWF, cracks occurred on the inner face of the face members (Figure 4e). The deformations of dowel joints the reinforced with GFRPWF strengthened are than not reinforced samples. It is seen that the deformation of the dowel joints reinforced with GFRPWF is less than the samples in reinforcement.

According to test results, the failures that occurred as a split of particleboard in both the face and butt members, In the joints of the test samples reinforced with the BFRPWF, failures have occured on the outer face of the basalt woven fiber fabric, In all of the samples the beech dowel joints reinforced with the BFRPWF (Figure 4i), the failures are almost identical, it is seen that the failures occurred as a result of cracking at the junction of the middle of the BFRPWF are more in the beech dowel (Figure 4i), and the Scots pine dowel (Figure 4k) samples.

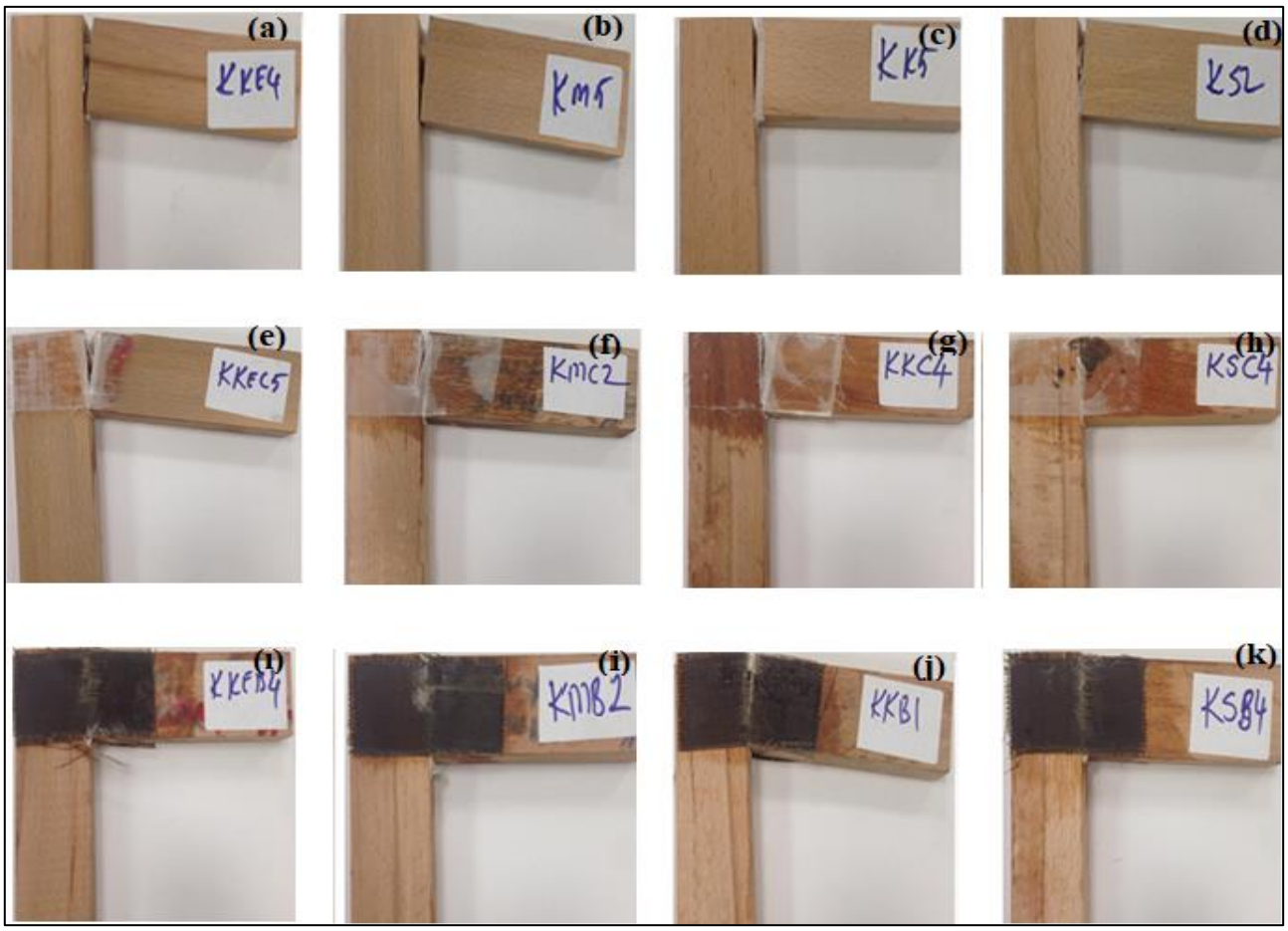


Figure 4. Failure modes of the experimental samples under bending strength test. (a) Chestnut dowel joints. (b) Oak dowel joints. (c) Beech dowel joints. (d) Scots pine dowel joints. (e) Chestnut dowel + GFRPWF joints. (f) Oak dowel + GFRPWF joints. (g) Beech dowel + GFRPWF joints. (h) Scots pine dowel + GFRPWF joints. (ı) Chestnut dowel + BFRPWF joints. (i) Oak dowel + BFRPWF joints. (j) Beech dowel + BFRPWF joints, and (k) Scots pine dowel + BFRPWF joints.

4. DISCUSSION AND CONCLUSIONS

For this aim, it is an ideal reinforcement element for wood materials since it has a high degree of hardness and higher strength compared to its light weight and it is a non-abrasive corrosion resistant flexible material which ensures the reduction of long-term maintenance costs and provides fast installation on site, Also, these materials demonstrate high durability in corrosive environments thanks to their high resistance to fatigue, The production of reinforced wood materials with high economic value and their increasing use can benefit economically,

The bending moment resistance of L-type doweled joints constructed four wooden dowel species and reinforced with the BFRPWF and GFRPWF was investigated. Experimental results indicated that traditional glued Oak dowel joints yielded the highest bending moment resistance among beech dowel, Chestnut dowel and Scots pine dowel joints. Scots pine dowel joints had the lowest bending moment resistance
among the joints evaluated. The mean comparison showed that beech dowel joints could produce a higher bending moment resistance than chestnut dowel joints. The bending moment resistance value of reinforced joints (for the GFRPWF and BFRPWF joints, respectively) were 31 % and 74 % higher than unreinforced joints.

Fiorelli and Alves (2002) explained that the increase in the rigidity of beams reinforced with GFRP is between 15% and 30%. Speranzini et al. (2010) examined solid timber beams externally reinforced with carbon, glass, basalt, hemp and flax FRP under a four-point bend test (the increase in flexural strength was 24.6% and 23.2% for glass and basalt, respectively). Yusof and Saleh (2010) reinforced the beams with GFRP rods placed in the slots on the underside. In this case, the tested bearing resistance increase was between 20% and 30%, and the hardness increase was between 24% and 60%. Borri et al. (2013) investigated low-grade and high-grade timber beams reinforced with linen and basalt FRP. The results showed a 38.6% and 65.8% increase in the bending strength of low-grade timber beams reinforced with FFRP and BFRP, respectively. In addition, strength increases were 29.2% and 25.9%, respectively, and maximum mid-deflection increases were 9.1% and 14.5%, respectively. Monaldoa et al. (2019) explained that beams reinforced with BFRP have a bending ultimate load higher of by about 20 % than the case of GFRP. Shekarchi et al. (2020) performed a series of tests on a wooden beam that was unreinforced and reinforced with GFRP. The test results showed that the bending performance of glulam is effectively enhanced by the binding of GFRP; Here, the bending stiffness, ductility, and energy absorption of reinforced beams increased by up to 59%, 79%, and 209%, respectively, compared to unreinforced beams.

Researchers could be provide a range of optimum values, for the parameters (four different wooden dowel species, FRP types) affecting frame furniture joint bending moment resistance and this colud be helpful for engineering design of furniture structures, Future studies will have to investigate the bending moment resistance of L-shaped two-pin dowel joints reinforced with different FRP materials.

Ethics Committee Approval
N/A

Peer-review
Externally peer-reviewed.

Author Contributions

The manuscript was written by the corresponding author. The author declares that the materials and methods used in this study do not require ethical committee approval or legal-specific permission.

Conflict of Interest
The authors have no conflicts of interest to declare.

Funding

The authors declared that this study has received no financial support.

REFERENCES

Alhayek, H., Svecova, D. (2012). Flexural stiffness and strength of GFRP-reinforced timber beams. Journal of Composites for Construction, 16(3), 245-252. https://doi.org/10.1061/(ASCE)CC.1943-5614.0000261

Andor, K., Bellovics, B. (2020). Analysis of modulus of elasticity of spruce beams under bending with and without fibre reinforcement. Wood Research, 65(1), 101-110. https://doi.org/10.37763/wr.1336-4561/65.1.101110

Amy, K., Svecova, D. (2004). Strengthening of dapped timber beams using glass fibre reinforced polymer bars. Canadian Journal of Civil Engineering, 31(6), 943-955.

Balmori, J. A., Basterra, L. A., Acuña, L. (2020), Internal GFRP reinforcement of low-grade maritime pine duo timber beams. Materials, 13(3), 571. https://doi.org/10.3390/ma13030571

Balmori, J. A., Branco, J. M., Basterra, L. A. (2021). Behaviour of the adhesive bond between low-grade wood and GFRP reinforcements using epoxy resin. Construction and Building Materials, 271, 121516 https://doi.org/10.1016/j.conbuildmat.2020.121516

Basterra, L. A., Balmori, J. A., Morillas, L., Acuña, L., Casado, M. (2017). Internal reinforcement of laminated duo beams of low-grade timberwith GFRP sheets. Construction and Building Materials, 154, 914–920 https://doi.org/10.1016/j.conbuildmat.2017.08.007

Beram, A. (2021). Dünyada ve Türkiye'de Geçmişten Günümüze Ahşap Yapı Sektörü. Ormancılık ve Ziraat Alanında Sürdürülebilirlik Temelli Yaklaşımlar, ed. Beram, A. and Beram-Ceyda, R., 1-14, SRA Academic Publishing, Lithuania.

Beram, A. (2023). Evaluating the strength properties of standing trees through fractometry. Eurasian Journal of Forest Science, 11(3), 137-150.

Borhan, T. M. (2012). Properties of glass concrete reinforced with short basalt fibre. Materials and Design, 42, 265-271. https://doi.org/10.1016/j.matdes.2012.05.062

Bozkurt, A.Y., Erdin, N. (2000). Wood Anatomy. Istanbul University publication number: 4263. Faculty of forestry publication number: 466, Istanbul University Press.

Bywalski, C., Drzazga, M., Kaźmierowski, M., Kamiński, M. (2020). Shear behavior of concrete beams reinforced with a new type of glass fiber reinforced polymer reinforcement: experimental study. Materials, 13(5), 1159. https://doi.org/10.3390/ma13051159

Carmisciano, S., De Rosa, I. M., Sarasini, F., Tamburrano, A., and Valente, M. (2011). Basalt woven fiber reinforced vinylester composites: Flexural and electrical properties. Materials and Design, 32(1), 337-342. https://doi.org/10.1016/j.matdes.2010.06.042

Camargo, M. V. D., Christoforo, A. L., Barcarolo, L. R. D. V., Moura, J. D. D. M. (2023). Experimental analysis of the performance of doweled connections reinforced with glass-fiber-reinforced polymer (GFRP) in wood pinus spp. Forests, 14(5), 931. https://doi.org/10.3390/f14050931

de la Rosa García, P., Escamilla, A. C., García, M. N. G. (2013). Bending reinforcement of timber beams with composite carbon fiber and basalt fiber materials. Composites Part B: Engineering, 55, 528-536. https://doi.org/10.1016/j.compositesb.2013.07.016

Dong, C. (2019). Numerical study of hybrid composites containing basalt fibres under flexural loading. SN Applied Sciences, 1(4), 287.

Dorigato, A., Pegoretti, A. (2012). Fatigue resistance of basalt fibers-reinforced laminates. Journal of Composite Materials, 46(15), 1773-1785. https://doi.org/10.1177/0021998311425620

Ezika, A. C., Sadiku, E. R., Ray, S. S., Oyeoka, H. C., Ibenta, M. E., Okpechi, V. U. (2023). Wood Fiber-Reinforced Polyester Composite. In Polyester-Based Biocomposites (pp. 137-160). CRC Press.

Fernando, D., Frangi, A., Kobel, P. (2016), Behaviour of basalt fibre reinforced polymer strengthened timber laminates under tensile loading. Engineering Structures, 117, 437-456.

Fiorelli, J., Dias, A.A. (2003). Analysis of the strength and stiffness of timber beams reinforced with carbon fiber and glass fiber. Materials Research, 6 (2), 193-202.

Fiore, V. I. N. C. E. N. Z. O., Di Bella, G., Valenza, A. (2011). Glass–basalt/epoxy hybrid composites for marine applications. Materials and Design, 32(4), 2091-2099. https://doi.org/10.1016/j.matdes.2010.11.043

Gao, H., Sun, Y., Jian, J., Dong, Y., Liu, H. (2023). Study on mechanical properties and application in communication pole line engineering of glass fiber reinforced polyurethane composites (GFRP). Case Studies in Construction Materials, 18, e01942. https://doi.org/10.1016/j.cscm.2023.e01942

Gentile, C., Svecova, D., Rizkalla, S. H. (2002). Timber beams strengthened with GFRP bars: development and applications. Journal of Composites for Construction, 6(1), 11-20. https://doi.org/10.1061/(ASCE)1090-0268(2002)6:1(11)

Gilby, J. (1999). Cooling tower shows end users the benefits of FRP. Reinforced Plastics, 9(43), 34-36.

Jiang, H., Zhang, Z., Lin, Z., Gong, X., Guo, H., Wang, H. (2021). Modification of polyurethane sponge filler using medical stones and application in a moving bed biofilm reactor for ex situ remediation of polluted rivers. Journal of Water Process Engineering, 42, 102189. https://doi.org/10.1016/j.jwpe.2021.102189

İçel, B., Beram, A. (2016). Non-destructive evaluation methods that can be used for the determination of some properties of historical wooden structures. Turkish Journal of Forestry, 17(2), 201-207.

Karaman, A. (2021). Bending moment resistance of T-type joints reinforced with basalt and glass woven fabric materials. Maderas. Ciencia y tecnología, 23, 1-12. https://www.scielo.cl/scielo.php?pid=S0718-221X2021000100444andscript=sci_arttextandtlng=en

Lopresto, V., Leone, C., De Iorio, I. (2011). Mechanical characterisation of basalt fibre reinforced plastic. Composites Part B: Engineering, 42(4), 717-723. https://doi.org/10.1016/j.compositesb.2011.01.030

Mohamed, H. M., Masmoudi, R. (2011). Deflection prediction of steel and FRP-reinforced concrete-filled FRP tube beams. Journal of Composites for Construction, 15(3), 462-472. https://doi.org/10.1061/(ASCE)CC.1943-5614.0000172

Monaldo, E., Nerilli, F., Vairo, G. (2019). Basalt-based fiber-reinforced materials and structural applications in civil engineering. Composite Structures, 214, 246-263. https://doi.org/10.1016/j.compstruct.2019.02.002

Morales-Conde, M. J., Rodríguez-Liñán, C., Rubio-de Hita, P. (2015), Bending and shear reinforcements for timber beams using GFRP plates. Construction and

Building Materials, 96, 461-472. https://doi.org/10.1016/j.conbuildmat.2015.07.079

O'Ceallaigh, C., Sikora, K., McPolin, D., Harte, A. M. (2019). The mechano-sorptive creep behaviour of basalt FRP reinforced timber elements in a variable climate. Engineering Structures, 200, 109702. https://doi.org/10.1016/j.engstruct.2019.109702

Osmannezhad, S., Faezipour, M., Ebrahimi, G. (2014). Effects of GFRP on bending strength of glulam made of poplar (Populus deltoids) and beech (Fagus orientalis). Construction and Building Materials, 51, 34-39. https://doi.org/10.1016/j.conbuildmat.2013.10.035

Passos, A. C., Arouche, M. M., Aguiar, R. A. A., Costa, H. R. M., de Barros, S., Sampaio, E. M. (2021). Adhesion of epoxy and polyurethane adhesives in pultruded composite material. Journal of Advanced Joining Processes, 3, 100045. https://doi.org/10.1016/j.jajp.2021.100045

Saad, K., Lengyel, A. (2022). Strengthening timber structural members with CFRP and GFRP: A state-of-the-art review. Polymers, 14(12), 2381. https://doi.org/10.3390/polym14122381

Shekarchi, M., Vatani Oskouei, A., Raftery, G.M. (2020). Flexural behavior of timber beams strengthened with pultruded glass fiber reinforced polymer profiles. Composite Structures, 241,112062. https://doi.org/10.1016/j.compstruct.2020.112062

TS 2470. (1976). For Physical and Mechanical Experiments in Wood Sampling Methods and General Features Test. Institute of Turkish Standards, Ankara, Türkiye.

Valentino, P., Sgambitterra, E., Furgiuele, F., Romano, M., Ehrlich, I., Gebbeken, N. (2014). Mechanical characterization of basalt woven fabric composites: numerical and experimental investigation. Frattura ed Integrità Strutturale, 8(28), 1-11. https://doi.org/10.3221/IGF-ESIS.28.01

Wang, X., Wu, Z., Wu, G., Zhu, H., Zen, F. (2013). Enhancement of basalt FRP by hybridization for long-span cable-stayed bridge. Composites Part B: Engineering, 44(1), 184-192. https://doi.org/10.1016/j.compositesb.2012.06.001

Wang, B., Bachtiar, E. V., Yan, L., Kasal, B., Fiore, V. (2019), Flax, basalt, E-Glass FRP and their hybrid FRP strengthened wood beams: An experimental study. Polymers, 11(8), 1255. https://doi.org/10.3390/polym11081255

Wei, B., Cao, H., Song, S. (2011). Degradation of basalt fibre and glass fibre/epoxy resin composites in seawater. Corrosion Science, 53(1), 426-431. https://doi.org/10.1016/j.corsci.2010.09.053

Wei, B., Cao, H., Song, S. (2010). Tensile behavior contrast of basalt and glass fibers after chemical treatment. Materials and Design, 31(9), 4244-4250. https://doi.org/10.1016/j.matdes.2010.04.009

Wu, Z., Wang, X., Wu, G. (2009, July). Basalt FRP composite as reinforcements in infrastructure. In Proc., 17th International Conference on Composites/Nano Engineering (ICCE-17) (pp. 21-24), New Orleans.

Wu, Z., Wang, X., Iwashita, K., Sasaki, T., Hamaguchi, Y. (2010). Tensile fatigue behaviour of FRP and hybrid FRP sheets. Composites Part B: Engineering, 41(5), 396-402. https://doi.org/10.1016/j.compositesb.2010.02.001

Yang, Y., Fahmy, M. F., Guan, S., Pan, Z., Zhan, Y., Zhao, T. (2020). Properties and applications of FRP cable on long-span cable-supported bridges: A review. Composites Part B: Engineering, 190, 107934. https://doi.org/10.1016/j.compositesb.2020.107934

Yusof, A., Saleh, A. L. (2010). Flexural strengthening of timber beams using glass fibre reinforced polymer. Electronic Journal of Structural Engineering, 10, 45-56. https://doi.org/10.56748/ejse.10124

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Article in periodical journals / Periyodik dergilerde makale

Akyıldırım, O., Gökce, H., Bahçeli, S., Yüksek, H. (2017). Theoretical and Spectroscopic (FT-IR, NMR and UV-Vis.) Characterizations of 3-p-chlorobenzyl-4-(4-carboxybenzylidenamino)-4,5-dihydro-1H-1,2,4-triazol-5-one Molecule. Journal of Molecular Structure, 1127: 114-123.

Tan, S., Williams, C.T. (2013). An In Situ Spectroscopic Study of Prochiral Reactant–Chiral Modifier Interactions on Palladium Catalyst: Case of Alkenoic Acid and Cinchonidine in Various Solvents. J. Phys. Chem. C, 117(35): 18043–18052.

Book / Kitap

Özkan, K. (2016). Biyolojik Çeşitlilik Bileşenleri (α, ß, γ) Nasıl Ölçülür? Süleyman Demirel Üniversitesi, Orman Fakültesi Yayın No: 98, ISBN: 976-9944-452-89-2, Isparta, 142 s.

Whittaker, E. T. (1988). A treatise on the analytical dynamics of particles and rigid bodies. Cambridge University Press.

Reference to a chapter in an edited book / Kitapta bölüm

Westhoff, V., Van Der Maarel, E. (1978). The braun-blanquet approach in classification of plant communities, Reinhold Tüxen (Ed.), Handbook of Vegetation Science, Springer Netherlands, pp. 619-704.

Şencan, A., Sevindir, H.C., Kiliç, M., Karaboyaci, M. (2011). Biosorption of CR+ 6 from Aqueous Solution with Activated Sludge Biosolids (Ref. NO: MT11-OP-475), Gökçekus, H., Türker, U., LaMoreaux, J.W., (Ed, Survival and Sustainability, 973-984.

Thesis and dissertation / Tez

Gülsoy, S. (2011). *Pistacia terebinthus* L. subsp. *palaestina* (Boiss.) Enler (Anacardiaceae)'in Göller Yöresi'ndeki Yetişme Ortamı Özellikleri ve Yetişme Ortamı-Meyve Uçucu Yağ İçeriği Etkileşimleri. SDÜ, Fen Bilimleri Enstitüsü, Orman Mühendisliği Anabilim Dalı, 194 s.

Özdemir, S. (2015). Ovacık Dağı Yöresi'nde Türk Kekiği (*Origanum onites* L.) ve Büyük Çiçekli Adaçayı (*Salvia tomentosa* Miller) Türlerinin Ekolojik Özellikleri. SDÜ, Fen Bilimleri Enstitüsü, Orman Mühendisliği Anabilim Dalı. 74s.

Conference proceedings / Konferans bildirisi

Özkan, K., Kavgacı, A. (2009). Küresel ısınmanın orta dağlık alanlarda tür çeşitliliği üzerine olası etkileri (Acıpayam yöresi örneği). I. Ulusal Kuraklık ve Çölleşme Sempozyumu (Eds: Palta, Ç.,), 16-18 Haziran 2009, Konya, Türkiye, 277-284.

Özkan, K., Negiz, M.G., Şentürk, Ö., Kandemir, H. (2012). Göller Bölgesi'ndeki Bazı Önemli Rekreasyon Alanları ve Onların Ekolojik Özellikleri, I. Rekreasyon Araştırmaları Kongresi 2012, Bildiri Kitabı, 12-15 Nisan, 587-596, Detay Yayıncılık, Kemer-Antalya.

Electronic reference / Elektronik kaynak

FAO, (2016). Sustainable Food and Agriculture. Food and Agriculture Organization of the United Nations, Rome, http://www.fao.org/sustainability/en/, Accessed: 14.06.2016.

Milliparklar, (2017). Doğa Koruma ve Milli Parklar Genel Müdürlüğü. http://www.milliparklar.gov.tr/korunanalanlar/kavramlar.htm, Erişim Tarihi: 18.06.2017

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