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C– Life Sciences and Biotechnology

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
C – Yaşam Bilimleri ve Biyoteknoloji

Volume / Cilt 14 Number / Sayı 1 January / Ocak - 2025

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology
C -Life Sciences and Biotechnology

Estuscience – Life

Volume: 14 / Number: 1 / January - 2025

Eskişehir Technical University Journal of Science and Technology C – Life Sciences and Biotechnology (formerly Anadolu University Journal of Science and Technology C – Life Sciences and Biotechnology) is an **peer-reviewed** and **refereed international journal** by Eskişehir Technical University. Since 2010, it has been regularly published and distributed biannually and it has been published biannually and **electronically only since 2016**.

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CONTACT INFORMATION
Eskişehir Technical Unıversity Journal of Science and Technology
Eskişehir Technical University, Institute of Graduate Programs, 26555 Eskişehir, TURKEY
Phone: +90 222 213 7485
e-mail : btdc@eskisehir.edu.tr

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ABOUT

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Books

Haupt RL, Haupt SE. Practical Genetic Algorithms. 2nd ed. New York, NY, USA: Wiley, 2004.
Kennedy J, Eberhart R. Swarm Intelligence. San Diego, CA, USA: Academic Press, 2001.

Chapters in books

Poore JH, Lin L, Eschbach R, Bauer T. Automated statistical testing for embedded systems. In: Zander J, Schieferdecker I, Mosterman PJ, editors. Model-Based Testing for Embedded Systems. Boca Raton, FL, USA: CRC Press, 2012. pp. 111-146.

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RESEARCH ARTICLE

A REMOTE SENSING APPROACH OF LAND AND WATER CONTENT CHANGE BETWEEN 2014 AND 2024 TO THE PORSUK DAM AND ITS NEAR SURROUNDINGS

Kübra GÜNBEY [1,*] Harun BÖCÜK [2]

[1] Department of Biology, Faculty of Science, Eskişehir Technical University, Eskişehir, Turkey
gunbeykubra@gmail.com - 0000-0003-1589-9699

[2] Department of Biology, Faculty of Science, Eskişehir Technical University, Eskişehir, Turkey
hbocuk@eskisehir.edu.tr - 0000-0002-4480-5295

Abstract

Observing, monitoring, and characterizing land changes in natural ecosystems, affected by many natural and anthropogenic environmental factors, is critical for making effective and sustainable management decisions and for their protection. Today, remote sensing methods, with their many different approaches and techniques, allow for continuous and controlled monitoring of spatial change, especially over large areas, providing cost- and time-effective solutions. This study aimed to determine the changes in the land and water potential of the Porsuk Dam Lake and its surroundings between Eskişehir and Kütahya provinces using remote sensing methods over 10 years. In this context, Landsat satellite data for the years 2014 and 2024 and the days with the least cloudiness were obtained, and normalized difference vegetation index (NDVI) and normalized difference water index (NDWI) calculations were made on these data using the ArcGIS/ArcMap program. Later on, the results, obtained, were compared and the changes in the land and water potential were determined. According to the results of NDVI analysis, the presence of forests (4.78%) and areas with herbaceous vegetation (5.56%) increased in 10 years, while the soil (-2.70%), tree/shrub areas (-1.26%) and water bodies (-5.87%) decreased. According to the results of NDWI analysis, it was determined that dry (2.02%) and moderately dry (10.81%) areas increased, while water bodies (-8.87%) and humid areas (-11.71%) decreased. The results were also supported by surface temperature analysis. Since the results obtained from the study include data on temporal and spatial changes, it is thought that they will contribute to future planning, management, and decision-making processes and studies to be carried out in this field later.

Keywords

Land change,
Ecology,
NDVI,
NDWI,
Time series

Time Scale of Article

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1. INTRODUCTION

The Earth's surface is changing at an unprecedented rate. Anthropogenic impacts have affected more than half of the Earth's land surface [1], while climate change and other disturbances have also affected all land surfaces. Accordingly, changes in land use and land cover are observed on a global scale due to factors such as decrease in biodiversity, soil degradation, decrease in land productivity, soil and water pollution, climate change, urbanization, destruction and reconstruction of agricultural

*Corresponding Author: hbocuk@eskisehir.edu.tr

areas [2-4]. These changes result in the deterioration, fragmentation, extinction and class change of the landscape character within the ecosystem [5]. To detect these changes, effective surface research techniques have been used depending on the land use and area change, such as geospatial and remote sensing techniques [3, 6-9].

Satellite remote sensing is crucial for investigating global land change, as it allows for synoptic and repetitive measurements at many resolutions (spectral, spatial, and temporal) [10-12]. Remote sensing data has also numerous other applications, such as land cover classification, soil moisture measurement, forest type classification, liquid water content measurement, etc. [8]. The availability of present and historical satellite data, the ability of remote sensing systems to monitor land cover and detect spatial changes along with increasing technology, and the availability of spatial planning, land management, processing of data layers, and ground-based modeling systems make it easier to follow spatial changes [8, 13, 14].

Remotely sensed images do not always directly reflect changes in the land surface. At this point, factors such as image recording, atmospheric conditions, natural soil wetness fluctuations, vegetation phenology, topography illumination, sensor and sun affect the spectral change [4]. To eliminate these changes or keep them at a minimum level, analyses are performed with various band combinations. One of these analyses is the normalized difference vegetation index (NDVI) analysis, developed to simplify multi-spectral imagery, which is presently the most widely used index for used to asses and model vegetation, phenology, and distribution [6, 15-17]. It is also possible to see and classify land cover classification, water bodies, open space, shrub areas, and agricultural and forest areas within the reflectance values of NDVI with vegetation features that cannot be directly detected with remote sensing images. (8, 16). The main purpose of the analysis is to obtain information about vegetation and detect plant health and growth with remotely sensed data [18, 19] and information about temporal and spatial changes depending on various factors such as fire, soil degradation, etc. [20]. There are many studies throughout the World in the literature on vegetation evaluations using NDVI analysis [21- 24].

Another analysis most used in the evaluation of land changes is the normalized difference water index (NDWI) analysis. The temporary absence of vegetation and small amounts of ground cover cause soils to be directly exposed to the effects of precipitation events, and the disappearance of vegetation disrupts the natural water balance of the areas [25]. Thanks to NDWI (normalized difference water index) analysis, which is used to determine the water content in the ecosystem, its spatial changes and boundaries over time, the lands within the research area can be evaluated in various class categories (arid, semi-arid, humid, etc.) [26]. There are many studies in the literature on water content evaluations using NDWI analysis [27-29]. In this respect, the results of the NDVI analysis are supported by the results of the NDWI analysis. Thus, it is possible to examine the spatial change situation in the field classification category by comparing two mutual analysis data.

Porsuk Stream is an important stream that flows through the provinces of Kütahya and Eskişehir and feeds one of the most important rivers in Turkey Sakarya River. Porsuk Dam, located on this river, is a very important dam for the region, especially for the accumulation of drinking and utility water. It also plays an important role in preventing possible floods in the region [30]. In this study, it was aimed to determine how the land structure and water content of Porsuk Dam Lake and its near surroundings changed between 2014 and 2024 (within a 10 years) using remote sensing methods (NDVI and NDWI).

2. MATERIALS AND METHODS

2.1. Study area

The study area covers the Porsuk Dam Lake and its nearby surroundings, one part of which is within the borders of Eskişehir and the other part of Kütahya province (Figure 1). The surveyed area is approximately 93,000 hectares in size.

Figure 1. Satellite image of the research area (https://earthexplorer.usgs.gov/)

2.2. Materials

Within the scope of this study, real Landsat 8 satellite images obtained from the official website of the United States Geological Survey (USGS) were used (https://earthexplorer.usgs.gov/). The relevant satellite bands for the region obtained from these satellites were used in the analyses. The images are from the year 2014, and the year 2024 has been chosen as a representation of 10 years. In addition, to avoid any problems with image analysis, an attempt was made to determine days that were close to each other as the dates when both the images were available and the cloudiness rate was lowest. In this context, the dates of 23 May 2014, when the cloudiness rate was 10.53, and 18 May 2024, when the cloudiness rate was 0.08, were determined and the study was carried out using the data of these days.

2.3. Methods

The work plan followed during the study is shown in Figure 2. Accordingly, satellite data were acquired in the first phase. Satellite data were analyzed using the ArcGIS/ArcMap program after pre-processing. In this context, the NDVI and NDWI analyses were performed together with the Surface Temperature Analysis (STA). Then, the analysis results were classified according to their reflection values and classified images were obtained. The area was then calculated by converting these raster data. Finally, the obtained results were compared.

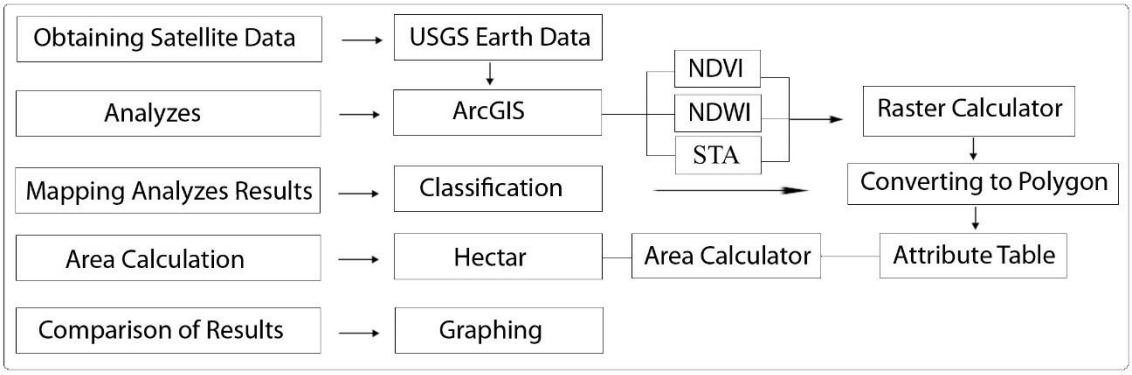

Figure 2. Study work plan

2.3.1 NDVI (Normalized Difference Vegetation Index) Analysis

NDVI analysis was performed on images from 2014 (May 23rd) and 2024 (May 18th) in order to determine and classify the vegetation density in the study area. For this purpose, red and near-infrared bands of the images of the region obtained from the Landsat 8 satellite on the specified dates were used. NDVI analysis was performed based on the formula given below. The calculation data obtained as a result of the analysis were classified into 5 categories as water body, soil area, herbaceous area, tree/shrub and forest areas, based on reflection values, and the obtained results were visualized.

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

2.3.2 NDWI (Normalized Difference Water Index) Analysis

NDWI analysis was performed on images from 2014 (May 23rd) and 2024 (May, 18th) to determine and classify the water situation in the study area. For this purpose, related bands (Band 3 and Band 5) bands of the images of the region obtained from the Landsat 8 satellite on the specified dates were used. NDWI analysis was performed based on the formula given below. The calculated data obtained from the analysis were classified into 4 categories as dry areas, moderately dry areas, humid areas, and water bodies based on reflection values, and the obtained results were visualized.

$$NDWI = \frac{(Band\ 3 - Band\ 5)}{(Band\ 3 + Band\ 5)}$$

2.3.3. ST (Surface Temperature) Analysis

ST analysis was performed on images from 2014 (May, 23rd) and 2024 (May, 18th) to determine and classify the surface temperature in the study area. For this purpose, the related band (Band 10) of the images of the region obtained from the Landsat 8 satellite on the specified dates were used. SA analysis was performed based on the formula given below (Tb: Thermal band, Pv: Proportion of vegetation, ε: Emissivity, Ts: Surface temperature**)**. The calculated data obtained from the analysis were classified into 4 categories, dry areas, moderately dry areas, humid areas, and water bodies based on reflection values, and visualized.

$$Tb = \frac{K2}{\ln(\frac{K1}{L\lambda}+1)} - 273.15$$

$$Pv=(\frac{NDVI-NDVImin}{NDVImax-NDVImin})^2$$

$$\varepsilon TM6 = 0.986 + 0.004\, P\, v$$

$$Ts = \frac{Tb}{1+(\lambda\times\frac{Tb}{\frac{h\times c}{b}})\times ln\varepsilon\lambda}$$

3. RESULTS AND DISCUSSION

3.1. NDVI (Normalized Difference Vegetation Index)

Visual results of NDVI analysis are given in Figure 3 and numerical results are given in Table 1. Accordingly, it is seen that the soil areas in the research area covered 30,966.00 ha in 2014 and 30,130.45 ha in 2024. This means that soil areas have decreased by 2.70% in the last 10-years. It is seen that herbaceous vegetation covered an area of 17,202.20 ha in 2014, and this area reached 18,158.13 ha in 2024. It was determined that the areas with herbaceous vegetation increased by 5.56% in the 10 years. It was seen that areas with tree/shrub vegetation covered 34,285.61 ha in 2014 and 33,854.94 ha in 2024. It means that the areas with tree/shrub vegetation decreased by 1.26% in 10 years. It is seen that forest areas covered an area of 9,008.42 hectares in 2014, and this area reached 9,439.09 hectares in 20024. It was seen that the areas with forest vegetation increased by 4.78% in the 10 years. Finally, when the water body is evaluated, it is seen that it covered an area of 2,050.49 hectares in 2014, and by 2024, this figure decreased to 1,930.11 hectares. This means that over the 10 years, the water bodies have decreased by 5.87%.

Vegetation change is a complex process that mirrors the dynamics of terrestrial ecosystems [24, 25]. The normalized difference vegetation index (NDVI) is an important statistic in satellite remote sensing for monitoring vegetation change. Therefore, it has been widely used to track dynamic vegetation changes [24]. For this reason, many studies have been carried out in Turkey and the world focusing on NDVI to determine the changes in vegetation [15, 19, 22-24, 26, 27, 31].

In a healthy terrestrial ecosystem, vegetation tends to develop towards climax vegetation, while in an unhealthy one, there may be regression and losses in terms of vegetation [32]. When our study is evaluated in this context, the fact that the areas with forest vegetation have increased over 10 years suggests that progressive climax processes are effective in the area and that the vegetation in the research area, which is relatively free from anthropogenic effects, is relatively healthy. In the study, it is seen that while forest areas increase, tree/shrub areas decrease. It is thought that the vegetation in areas with tree or shrub vegetation 10 years ago could not i) reach the reflectance values of the forest, ii) developed in this process and covered more area more densely, and iii) reflectance values of these areas moved to the forest borders.

The water mass in the research area was evaluated in terms of the surface area it covers, since it is difficult to determine the amount of water it has. In this study, it can be seen that the area covered by the lake of the Porsuk Dam has decreased by 5.87% in 10 years. While land areas are expected to increase in areas where water is withdrawn, it is seen that these areas also decrease. It is thought that the soil areas, exposed to the decrease in water, are covered by herbaceous plants, and thus the areas

with herbaceous vegetation increase. In other words, vegetation has responded effectively to change in its environment and exhibits a dynamic movement [5, 24, 26].

3.2. NDWI (Normalized Difference Water Index)

Visual results of NDWI analysis are given in Figure 4 and numerical results are given in Table 2. Accordingly, it is seen that dry areas covered an area of 51,939.28 ha in 2014 and 52,989.54 ha in 2004. It means that dry areas increased by 2.02% in the 10 years. It was determined that moderately dry areas covered an area of 16,423.67 hectares in 2014, and this number reached to 18,198.48 ha in 2024. It was seen that the number of moderately dry areas increased by 10.81% in the 10 years. It was determined that humid areas covered 23,099.28 ha in 2014 and 20,394.59 ha in 2024. It is seen that in the 10 years, humid areas decreased by 11.71%. Finally, when the water body is evaluated, it is seen that it covered an area of 2,050.49 hectares in 2014, and by 2024, this figure decreased to 1,930.11 hectares. This means that the water body decreased by 5.87% over the 10 years.

Today, one of the most important problems in the world is global warming. Global warming brings with it many problems such as area and vegetation change, changes in climate elements, and sustainability. Climate warming accelerates the global hydrological cycle and alters precipitation patterns due to greater evaporation and water vapor from rising temperatures [33, 34]. It is a very difficult process to prevent even with new and harsh measures [4]. According to the results of the NDWI analyses performed in this study, it is seen that the area covered by the water mass decreased by 5.87%. The water mass contributes to the water level of its surroundings, especially by distributing to its near surroundings both as capillaries and as moisture in the atmosphere through evaporation [32]. When water mass decreases, dry or moderately dry areas are expected to increase, as in our study area. In our study, this situation shows that while the dry and moderately dry areas increase, the humid areas decrease.

However, one of the important factors caused by global warming is the issue of climate change. Climate shifts can occur in many different ways at the local level [35, 36]. In this study, although the relatively healthy vegetation developing despite the decrease in the water mass and the water potential in the surrounding area seems like a contradiction, it is actually thought that this situation is due to climate change. Spring rains normally occur in May in the research area [34]. However, in 2024, it was observed that these precipitations occurred at the end of May and early June, rather than in the middle of May as expected (data not shown). Therefore, it is seen that the water mass and moist areas are less in 2024 compared to 2014 due to the delay in the spring rains expected in 2024. For this reason, in this study, which aims to determine what changes have occurred in the area over 10 years, very close days of the year were selected. However, taking into account climate change, conducting an analysis covering June 2024 will take this study further and make the results more clearly understandable.

3.3. ST (Surface Temperature)

Visual results of NDWI analysis are given in Figure 5 and numerical results are given in Table 3. Accordingly, on May 23, 2014, the temperature in the 446.31 ha part of the research area was between -3 and 14.18 ^{0}C, in the 12,649.30 ha area the temperature was between 14.18 and 25.29 ^{0}C, in the 17,888.16 ha area the temperature was between 25.29 and 25.29 ^{0}C. It was determined that the temperature was between 31.58 and 36.82 ^{0}C in the 31456.79 ha area and between 36.82 and 50.45 ^{0}C in the 31,072.16 ha area. On May 18, 2024, the temperature was between 14.01 and 25.12 ^{0}C in the 8,753.12 ha part of the research area, between 25.12 and 29.13 ^{0}C in the 13700.86 ha part, and 29.13 ^{0}C in the 21,557.71 ha part. It was determined that the temperature was between 32.32 and 35.39 ^{0}C in the 28,851.77 ha part and between 35.39 and 44.13 ^{0}C in the 20,649.26 ha part. According to the weighted average calculation based on the midpoints of the temperature spectra and the area where

they are seen, while the average temperature of the area was 34.14 ^{0}C in 2014, this figure was determined to be 32.11 ^{0}C in 2024.

The presence of parts with different temperature values in the area can be explained by different ecological elements in the very wide area. These involve many factors including the difference in the amount of heat retention of water, soil or vegetation, elevation, and bedrock elements [32]. Of course, only one day surface temperature analysis for the years 2014 and 2024 may prevent us from reaching a clear conclusion about the temperature of the region. However, it is clear that it gives an idea about the temperature values of the area. In our study, in general, it can be seen that 2014 was 2 ^{0}C

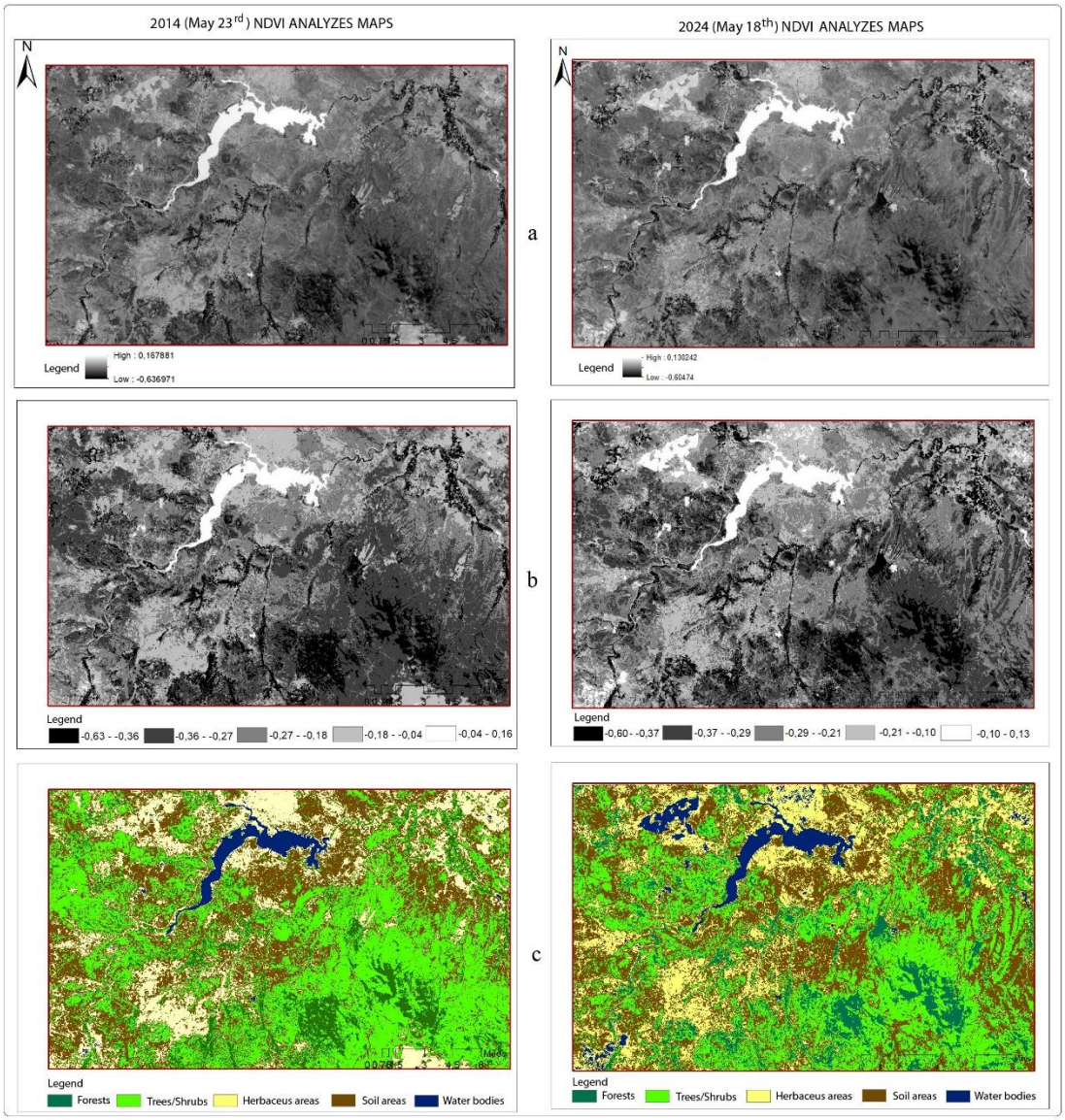

Figure 3. Results of normalized difference vegetation index (NDVI) analyses (2014 and 2024)(a. Maximum/minimum levels of reflection values, b. Classification of reflection values, c. Classification of plant cover and water bodies)

Table 1. Numeric values of plant cover and water bodies belonging to the research area

Categories	2014 (May, 23rd)	2024 (May, 18th)	Change (%)
Soil areas	30,966.00	30,130.45	-2.70
Herbaceous areas	17,202.20	18,158.13	5.56
Trees/Shrubs	34,285.61	33,854.94	-1.26
Forests	9,008.42	9,439.09	4.78
Water bodies			-5.87

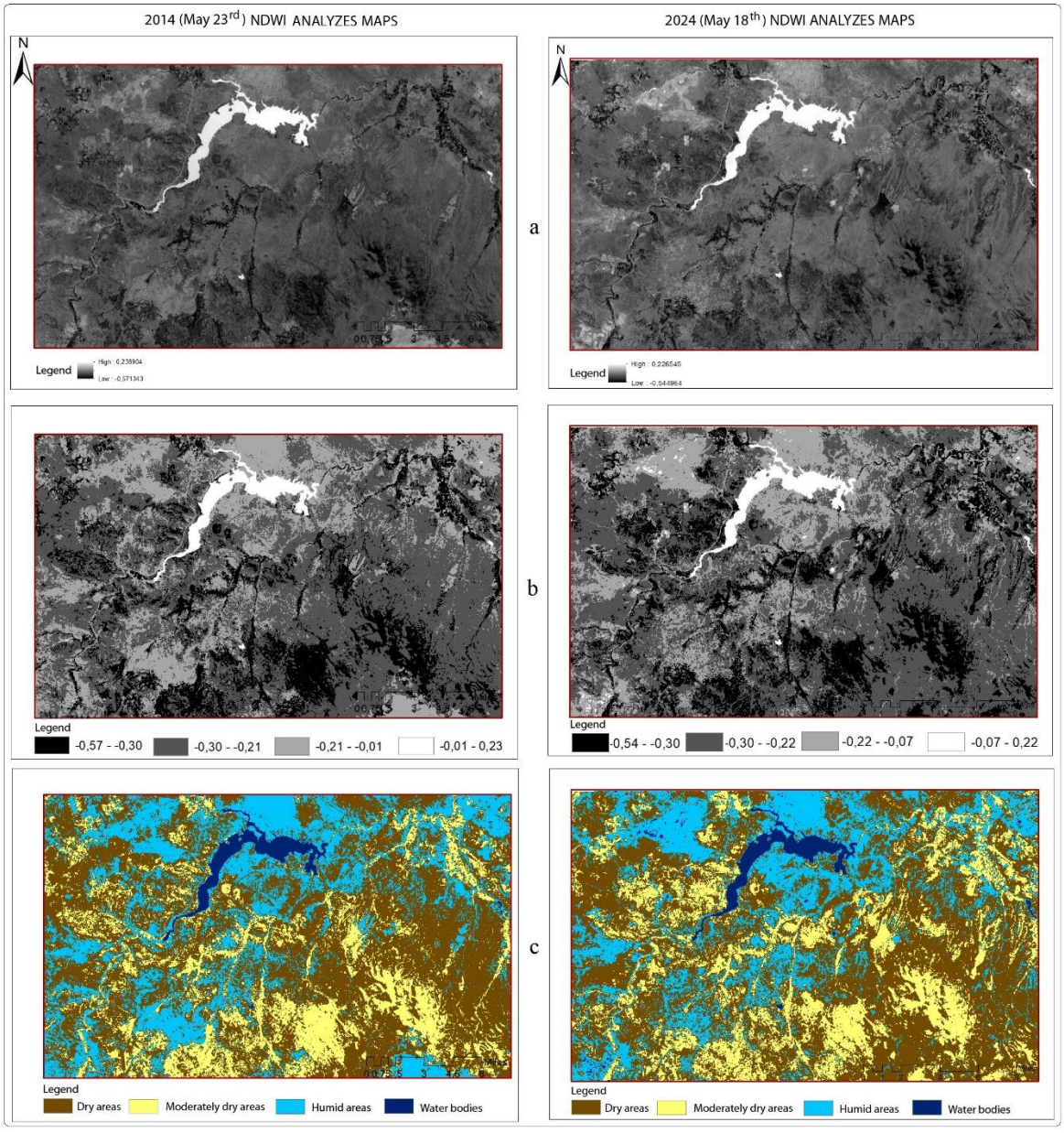

Figure 4. Results of normalized difference water index (NDWI) analyses (2014 and 2024) (a. Maximum/minimum levels of reflection values, b. Classification of reflection values, c. Classification of the areas in terms of water content)

Table 2. Numeric values of plant cover and water bodies belonging to the research area

Categories	2014 (May 23rd)	2024 (May 18th)	Change (%)
Dry areas	51,939.28	52,989.54	2.02
Moderately dry areas	16,423.67	18,198.48	10.81
Humid areas	23,099.28	20,394.59	-11.71
Water bodies	2,050.49	1,930.11	-5.87

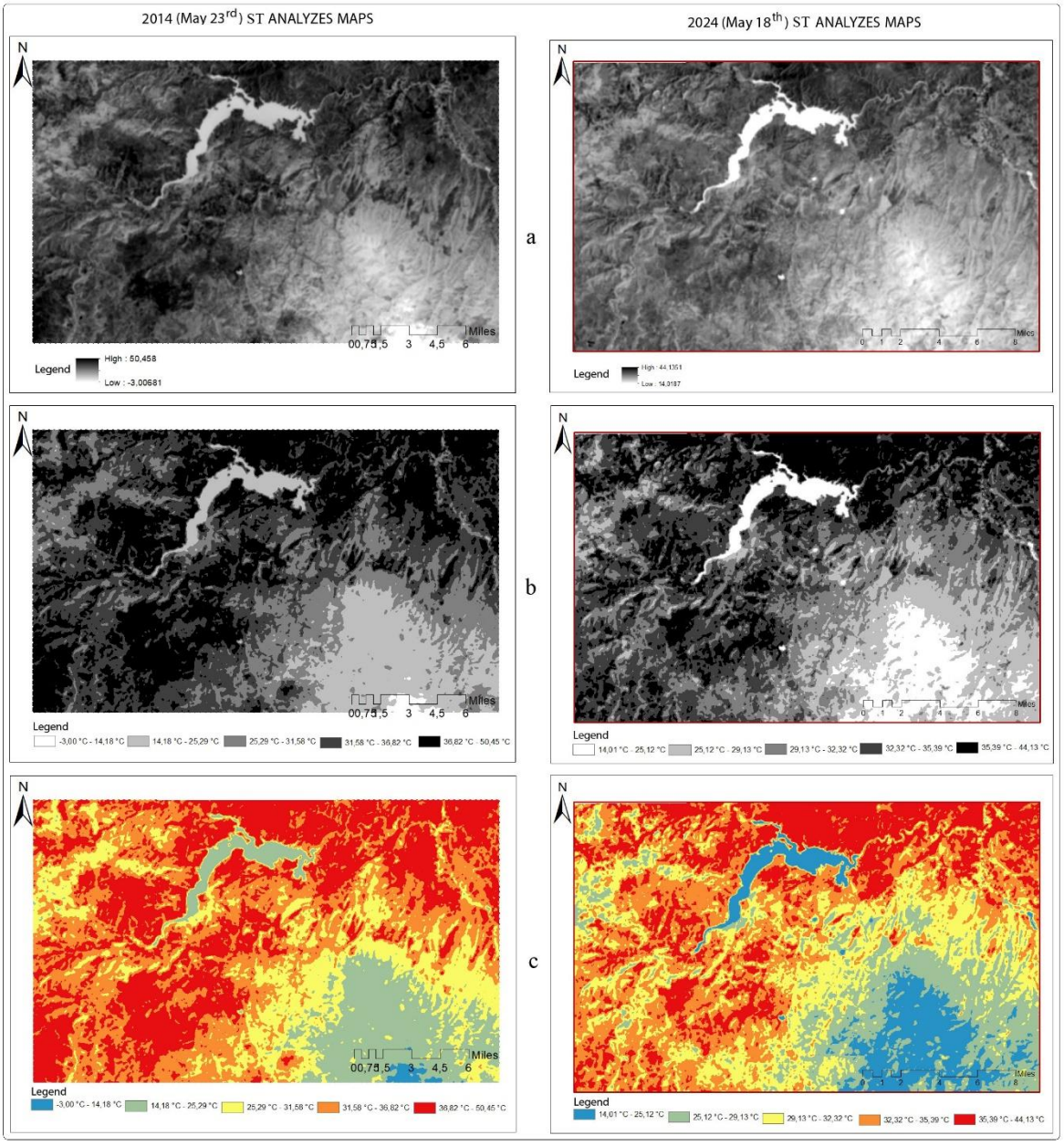

Figure 5. Results of surface temperature (ST) analyses (2014 and 2024) (a. Maximum/minimum levels of reflection values, b. Classification of reflection values, c. Classification of the areas in terms of surface temperature)

Table 3. Temperature spectrum and areas (ha**)**

2014 (May 23rd)		2024 (May 18th)	
Temperature Spectrum (^{0}C)	**Area (ha)**	**Temperature Spectrum (^{0}C)**	**Area (ha)**
-3.00 --- 14.18	446.31	14.01 --- 25.12	8,753.12
14.18 --- 25.29	12,649.16	25.12 --- 29.13	13,700.86
25.29 ---31.58	17,888.16	29.13 --- 32.32	21,557.71
31.58 --- 36.82	31,456.79	32.32 --- 35.39	28,851.77
36.82 --- 50.45	31,072.16	35.39 --- 44.13	20,649.26

warmer for the same period. It is thought that this situation occurred as a result of climate change [35, 36], as explained above.

In a study conducted in Tokat province, it was noted that the current state of the vegetation can be tracked using remote sensing methods, and the change in vegetation may be observed through future studies [21]. In this study, in addition to the vegetation of the Porsuk dam and its immediate surroundings, water content and surface temperature analysis were also carried out, taking advantage of the opportunities provided by technology, both today and 10 years ago, and the results were compared. In other words, temporal and spatial analysis was applied within the framework of the variables specified in the study area. We believe that this study will provide data for other studies to be carried out in the area in the coming years in terms of determining vegetation, water content and surface temperature, future planning, management and decision-making processes.

4. CONCLUSION

In this study, changes in the vegetation and water content of Porsuk and its immediate surroundings over 10 years were studied by performing NDVI and NDWI analyses. Additionally, the study was supported by surface temperature analysis. It was concluded that the presence of forests and areas with herbaceous vegetation increased over the 10 years, whereas soil, tree/shrub areas and the water body decreased. It was also concluded that dry and moderately dry areas increased, while water bodies and wet areas decreased. The results obtained have been associated with climate change and climate drift.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

CRediT AUTHOR STATEMENT

Kübra Günbey: Formal analysis, Investigation, Visualization, Resources, **Harun Böcük: C**onceptualization, Supervision, Investigation, Validation, Writing – Original Draft.

REFERENCES

[1] Ellis EC, Klein Goldewijk K, Siebert S, Lightman D, Ramankutty N. Anthropogenic transformation of the biomes, 1700 to 2000. Global Ecol Biogeogr 2010; 19: 589–606.

[2] Fischer G, Sun L. Model based analysis of future land-use development in China, Agric Ecosyst Environ 2001; 85 (1-3): 163-176.

[3] Zhang J, Zhang Y. Remote sensing research issues of the National Land Use Change Program of China. ISPRS J Photogramm 2007; 62: 461–472.

[4] Zhu Z, Qiu S, Ye S. Remote sensing of land change: A multifaceted perspective. Remote Sens Environ 2022; 282: 113266.

[5] Krsnik G, Reynolds KM, Murphy P, Paplanus S, Garcia-Gonzalo J, Olabarria JRG. Forest use suitability: Towards decision-making-oriented sustainable management of forest ecosystem services. Geogr Sustain 2023; 4: 414–427.

[6] Beck PS, Atzberger C, Høgda KA, Johansen B, Skidmore AK. Improved monitoring of vegetation dynamics at very high latitudes: A new method using MODIS NDVI. Remote Sens Environ 2006; 100 (3): 321-334.

[7] Ahmadi H, Nusrath A. Vegetation change Detection of Neka river in Iran by using remote sensing and GIS. Journal of Geography and Geology 2012; 2 (1): 58-67.

[8] Meera Gandhi G, Parthiban S, Thummalu N, Christy A. Ndvi: Vegetation change detection using remote sensing and gis – A case study of Vellore District. Procedia Comput Sci 2015; 57: 1199-1210.

[9] Da Ponte E, Roch M, Leinenkugel P, Dech S, Kuenzer C. Emanuel Da vd, (2017), Paraguay's Atlantic forest cover loss e satellite-based change detection and fragmentation analysis between 2003 and 2013. Appl Geogr 2017; 79: 37-49.

[10] Roy DP, Wulder MA, Loveland TR, Ce W, Allen RG, Anderson MC, Helder D, Irons JR, Johnson DM, Kennedy R. et al. Landsat-8: Science and product vision for terrestrial global change research. Remote Sens Environ 2014; 145: 154–172.

[11] Belward AS, Skøien JO. Who launched what, when and why; trends in global land-cover observation capacity from civilian earth observation satellites. . ISPRS J Photogramm 2015; 103: 115–128.

[12] Ustin SL, Middleton EM. Current and near-term advances in Earth observation for ecological applications. Ecol Process 2021; 10: 1–57.

[13] Lu D, Mausel P, Brondizio E, Moran E. 2004. Change detection techniques. Int J Remote Sens 2004; 25: 2365–2401.

[14] Rogan J, Chen D. Remote sensing technology for mapping and monitoring land-cover and land-use change. Prog Plann 2004; 61: 301–325.

[15] Bellón B, Bégué A, Seen DL, de Almeida CA, Simões M. A remote sensing approach for regional-scale mapping of agricultural land-use systems based on NDVI time series. Remote Sens 2017; 9: 600.

[16] Huang S, Tang L, Hupy JP, Wang Y, Shao G. A commentary review on the use of normalized difference vegetation index (NDVI) in the era of popular remote sensing. J For Res 2021; 32(1):1–6.

[17] Lemenkova P, Debeir O. R Libraries for Remote Sensing Data Classification by K-Means Clustering and NDVI Computation in Congo River Basin, DRC. Appl Sci 2022; 12 (24): 12554.

[18] Ichii K, Kawabata A, Yamaguchi Y. Global correlation analysis for NDVI and climatic variables and NDVI trends: 1982–1990. Int J Remote Sens 2002; 23 (18): 3873–3878.

[19] Kasimati A, Psiroukis V, Darra N, Kalogrias A, Kalivas D, Taylor JA, Fountas S. Investigation of the similarities between NDVI maps from different proximal and remote sensing platforms in explaining vineyard variability. Precis Agric 2023; 24:1220–1240.

[20] Saylan İH, Çömert R. Investigation of the success of Sentinel-2A products in mapping of burned forest areas. TUZAL 2019; 1(1), 8-15.

[21] Doğan HM, Kılıç OM, Yılmaz DS. Researching plant density classes of Tokat province by Landsat-7 ETM+ satellite images and geographical information systems. Journal of Agricultural Faculty of Gaziosmanpaşa University 2014; 31(1): 47-53.

[22] Özyavuz M, Bilgili BC, Salıcı A. Determination of vegetation changes with NDVI method. J Environ Prot Ecol 2015; 16(1): 264-273.

[23] Hartoyo APP, Sunkar A, Ramadani R, Faluthi S, Hidayati S. Normalized Difference Vegetation Index (NDVI) analysis for vegetation cover in Leuser Ecosystem area, Sumatra, Indonesia. Biodiversitas 2021; 22(3): 1160-1171.

[24] Yang S, Zhao Y, Yang D, Lan A. Analysis of vegetation NDVI changes and driving factors in the Karst concentration distribution area of Asia. Forests 2024; 15: 398.

[25] Hunault-Fontbonne J, Eyvindson K. (2023), Bridging the gap between forest planning and ecology in biodiversity forecasts: A review. Ecol Ind 2023; 154: 110620.

[26] Özvan H, Arık B, Yeler O, Şatır O, Bostan P. Determining land change using remote sensing and geographical information systems techniques: the case of lake Karataş and its surroundings, Peyzaj 2023; 5(1): 30-39.

[27] Szabó S, Gácsi Z, Balázs B. Spesific features of NDVI, NDWI as reflected in land cover categories. Landscape & Environment 2016; 10(3-4): 194+202.

[28] Özelkan E. Water Body Detection Analysis Using NDWI Indices Derived from Landsat-8 OLI. Pol J Environ Stud 2020; 29 (2): 1759-1769.

[29] Lamani S, Harijan C. Remote sensing and gis applications in ndwi analysis for monitoring water resources in Gadag Taluk. Int J Progressive Res Eng 2023; 3(8): 31-35.

[30] Bakış R, Altan M, Gümüşlüoğlu E, Tucan A, Ayday C, Önsoy H, Olgun K. Investigation of the water potential of Porsuk basin with respect to hydroelectric energy production. Eskişehir Osmangazi Üniversitesi Müh. Mim. Fak. Dergisi 2008; 11 (2): 125-162.

[31] Yıldız H, Mermer A, Ünal E, Akbaş F. Spatial and Temporal Analysis of Turkey Vegetation with NDVI Images. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi 2012; 21 (2): 50-56.

[32] Akman Y, Ketenoğlu O, Güney K, Kurt L, Tuğ GM. Bitki Ekolojisi, Palme Yayıncılık, 2004.

[33] Yang Z, Liu Q. Response of streamflow to climate changes in the Yellow River Basin, China. J Hydrometeorol 2011; 12(5): 1113–1126.

[34] Yetik AK, Arslan B, Şen B. Trends and variability in precipitation across Turkey: a multimethod statistical analysis. Theor Appl Climatol 2024; 155: 473–488.

[35] Lenoir J, Svenning JC. Climate-related range shifts – a global multidimensional synthesis and new research directions. Ecography 2014; 37: 001–014.

[36] Baines PG, Folland CK. Evidence for a Rapid Global Climate Shift across the Late 1960s. J Clim. DOI: 10.1175/JCLI4177.1.

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
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RESEARCH ARTICLE

pH INFLUENCE ON SHELF LIFE OF LIQUID PGPR FORMULATIONS WITH *Bacillus subtilis* STRAINS

Sevgi İŞLEK [1,*], Kemal KARACA [2], Rengin ELTEM [3]

[1] Graduate School of Natural and Applied Sciences, Department of Bioengineering, Ege University, 35040 Bornova, Izmir, Turkey
sevgi.islek95@gmail.com - 0000-0001-7743-7014

[2] Graduate School of Natural and Applied Sciences, Department of Bioengineering, Ege University, 35040 Bornova, Izmir, Turkey
kemalkaraca1@gmail.com - 0000-0003-2193-2854

[3] Faculty of Engineering, Department of Bioengineering, Ege University, 35040 Bornova, Izmir, Turkey
rengin.eltem@ege.edu.tr - 0000-0002-0642-7676

Abstract

Plant Growth Promoting Rhizobacteria (PGPRs) are bacteria that promote plant growth through both direct and indirect mechanisms. The formulation of PGPR inoculants is crucial for the efficacy and commercial success of microbial fertilizers. Formulation aims to optimize the survival of microbial strains under specific environmental conditions and enhance their capacity to promote plant growth. This process ensures protection of bacterial cells against harsh conditions such as high temperatures, desiccation, and storage, thereby extending product shelf life. Proper formulation of PGPR inoculants is a critical component for sustainable agricultural practices, playing a significant role in improving both plant health and productivity.

Among PGPR strains, *Bacillus* species are particularly produced and utilized as microbial fertilizers commercially due to their high efficacy potential and long shelf life. However, for large-scale production, strain-specific PGPR formulations need to be developed and optimized to produce PGPR inoculants with high efficacy potential and extended shelf life.

In this study, acidic liquid formulations were prepared using acetic acid for *B. subtilis* EGE-B-36.5 strain, and alkaline liquid formulations were prepared using calcium acetate-calcium hydroxide for *B. subtilis* EGE-B-1.19 strain. The viable cell count in the liquid formulations was statistically compared with the control. In the acidic liquid formulation, statistically significant changes in viable cell count were observed for *B. subtilis* EGE-B-36.5 strain at pH 4.0 after 12 months and for *B. subtilis* EGE-B-1.19 strain at pH 4.0 after 12 months ($p<0.05$). In the alkaline liquid formulation at pH 9.5 there had been a statistically significant ($p<0.05$) difference between control group of the *B. subtilis* EGE-B-1.19.

Keywords

Bacillus subtilis,
PGPRs,
Microbial fertilizer,
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*Corresponding Author: sevgi.islek95@gmail.com

1. INTRODUCTION

The significant increase in the global population is driving up the demand for food. Therefore, farmers use large amounts of chemical fertilizers and their derivatives to obtain maximum crop yield due to limited land resources. However, the continuous and excessive use of these chemical fertilizers and derivatives negatively affects the natural microflora, such as bacteria, fungi, cyanobacteria, and protozoa, present in the rhizosphere or applied area, causing an imbalance in the natural ecosystem [1]. This damage, which initially provides short-term benefits, ultimately leads to inefficiency in production and poor-quality products [2].

Globally, to mitigate the larger problems that the current negative agricultural practices may cause in the future and to ensure the continuity of the most basic human need nutrition the most innovative solution in agricultural production is microbial fertilizers. These fertilizers are environmentally friendly, harmless to human health, and provide essential elements required by plants, competing with chemical fertilizers. Bacteria that establish a positive relationship with plant roots and positively affect plant development and growth are defined as Plant Growth Promoting Rhizobacteria (PGPRs). PGPRs play an active role in reducing the damage caused by plant pathogenic microorganisms, directly or indirectly facilitating plant growth, promoting plant growth by activating insoluble nutrients in the soil, and minimizing abiotic stress [3].

PGPRs have been documented in the literature as beneficial rhizobacteria for the soil ecosystem due to their high adaptation capabilities to various environments, rapid growth rates, and ability to metabolize a wide range of natural and xenobiotic compounds [4]. Although PGPRs encompass many different types of bacteria, many PGPRs developed for commercial applications are predominantly *Bacillus* species. These products are used in the form of endospores, which provide population stability throughout formulation and shelf life [5].

Among *Bacillus* species, *B. subtilis* strains are the most commonly used PGPRs due to their capacity to produce antibiotics and numerous other beneficial properties, which reduce disease incidence in plants [6]. When aerobic, endospore-forming *Bacillus* species are used in agricultural fields, they contribute to crop productivity directly or indirectly. *Bacillus* species possess many physiological characteristics, such as having a Gram-positive cell wall, forming stress-resistant endospores, secreting peptide antibiotics, and producing peptide signal molecules and extracellular enzymes. Particularly, *Bacillus* species can survive for extended periods under adverse environmental conditions due to their endospore formation mechanism. It is known that most *Bacillus* species promote plant growth. The primary mechanisms of growth promotion include the production of growth-stimulating phytohormones, phosphate solubilization, siderophore production, antibiotic production, inhibition of ethylene synthesis in plants, and induction of systemic resistance against pathogens. Numerous studies have shown that *Bacillus* and *Paenibacillus* species exhibit antagonistic activities that suppress pathogens under both in vitro and in vivo conditions [7].

PGPR inoculants are defined as formulations containing one or more beneficial bacterial strains prepared with an easy-to-use and economical carrier material. The key points in PGPR inoculant technology are the selection of an appropriate carrier for the inoculants and the preparation of a suitable formulation [8]. Biomass production, formulation, and determining shelf life are important steps to consider during the development of PGPR inoculants. It must be ensured that a properly produced, formulated, and applied bioinoculant product will deliver all the benefits it is intended to provide. Generally, many private companies globally offer a variety of efficient and effective bioinoculants for diverse soils in response to increasing demand in the international market. However, these inoculants often tend to be of low quality. In some products used in developing or developed countries, rhizospheric microorganisms may be absent or contaminated with other strains. This inconsistency in the beneficial

effect of bioinoculants in the field creates a negative impression in the market [9]. The heterogeneity of soils poses a significant challenge for bioinoculants. Bacteria inoculated into the soil must compete with the better-adapted native microbiota and survive in the soil microbiome. Therefore, a more suitable microenvironment should be provided for bioinoculants with physicochemical protection. This approach will help prevent rapid declines in the number of live bacterial cells [9].

As a result, the goal of inoculant formulations should be to allow PGPRs to survive better in suitable and available forms during storage and application [9]. An ideal bioinoculant formulation should not have phytotoxic effects on the plants where it is applied, demonstrate high tolerance to adverse environmental conditions, have a cost lower than other products in the market, and be reliable in controlling plant diseases [10].

In this study, indigenous *Bacillus subtilis* EGE-B-1.19 and *Bacillus subtilis* EGE-B-36.5 strains with PGPR properties were used. Following production, the pH values of the culture medium were adjusted to acidic and alkaline levels. The shelf life of liquid formulations at different pH values was monitored over 12 months to determine the optimal pH for these strains and to aim for the production of a viable product with extended shelf life.

2. MATERIALS AND METHODS

2.1. Microorganisms, Culture Conditions and Preformulation

Bacillus subtilis EGE-B-1.19 and *Bacillus subtilis* EGE-B-36.5, which have PGPR properties, were used from microbial culture collection, Department of Bioengineering, Ege University, Izmir, Turkey [11, 12]. Initially, growth of two strains on nutrient agar plates at pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5 was determined. Plates were inoculated and incubated at 30 °C. After incubation, the growth of *Bacillus* strains on petri plates with different pH values was visually graded.

Then, *B. subtilis* EGE-B-1.19 and *B. subtilis* EGE-B-36.5 were grown on *Bacillus* endospore production medium (EPM). This medium contained glucose, 5.0 g; dry yeast, 3.31 g; soy flour, 23.56 g; $(NH_4)_2SO_4$, 0.60 g; glycerol 2.0%; stock salt solution 10 ml; antifoam, 0.1% (w/v); distilled water, 1000 ml; pH 7.0. The stock salt solution was composed of 20.30 g, $MgCl_2.6H_2O$; 10.20 g, $CaCl_2.2H_2O$; 1.00 g, $MnCl_2.4H_2O$; 1000 ml distilled water. Sterilized *Bacillus* EPM was inoculated with each *Bacillus* strain and incubated at 30±2°C. The viable cell count of the culture broth was determined in colony-forming units per milliliter (cfu/ml) using the pour plate method [13]. After production, consecutive serial dilutions ranging from 10^{-1} to 10^{-8} were prepared from the culture liquid, and the viable cell count was determined in colony forming units per milliliter (CFU/ml) using the pour plate method. For this purpose, 1 mL from each dilution ranging from 10^{-4} to 10^{-8} was transferred to sterile glass petri dishes under aseptic conditions. Each dilution was performed in duplicate. Subsequently, the dishes were cooled to 45°-50°C, and approximately 20 mL of nutrient agar medium was poured onto them. After gently swirling the plates by hand until the agar solidified, they were incubated at 28°C for 24-48 hours [14].

Acidic liquid preformulation experiments were carried out with *B. subtilis* EGE-B-36.5 using acetic acid [15], lactic acid [16], propionic acid [16], citric acid [16], and boric acid [17]. *B. subtilis* EGE-B-36.5 culture broth's ($1x10^8$ cfu/ml, pH 8.0) in sterile amber bottles was adjusted to pH 3.0 and 4.0 with lactic acid, citric acid, and propionic acid, pH 3.0, 4.0, and 5.0 with acetic acid, and pH 5.0 with boric acid. Acidic liquid preformulations and culture broth at pH 8.0 as control were stored at room temperature for three months. The viable cell count (cfu/ml) in acidic liquid preformulations and control was analyzed monthly using the pour plate method for three months. In addition, the pH measurements of the preformulations were monitored monthly to determine the relationship between the viable cell count

and the pH change. According to the preformulation results, it was decided which acid should be used to prepare the acidic liquid formulations.

2.2 Experimental Setup for the PGPR Liquid Formulations

The pH of the culture broths ($1x10^8$ cfu/ml, pH 8.0) in sterile amber bottles with *B. subtilis* EGE-B-1.19 and *B. subtilis* EGE-B-36.5 were adjusted to pH 2.0, 3.0, 4.0, and 5.0 with acetic acid. In addition, alkaline liquid formulations were prepared from the culture broth ($1x10^8$ cfu/ml) of only *B. subtilis* EGE-B-1.19 at pH 9.0, 9.5, and 10.0 using calcium acetate-calcium hydroxide. The acidic and alkaline liquid formulations were stored at a room temperature of 25°C. The viable cell count (cfu/ml) of the liquid formulations was monitored monthly using the pour plate method for 12 months. Additionally, the pH values of the liquid formulations were monitored monthly for 12 months using a Milwaukee Mi 150 pH meter.

2.3 Statistical analysis

The experiments were performed in triplicate. The means were statistically analyzed using Tukey's ANOVA ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Investigation of the Growth of *Bacillus* strains at Different pH Levels on Petri Plates

In this study, it was observed that *B. subtilis* EGE-B-1.19 and *B. subtilis* EGE-B-36.5 strains grew well between pH 5.5 – pH 7.5 on NA plates after 48 h of incubation. Although *B. subtilis* EGE-B-1.19 grew well at pH 8.0 and 8.5, it showed weak growth after two weeks of incubation at pH 4.5. *B. subtilis* EGE-B-36.5 showed weak growth at pH 8.0 and 8.5, but it did not grow at pH 4.5, as shown in Table 1. Therefore, acidic and alkaline liquid formulations were designed to be below pH 5.5 and above pH 8.5, where *B. subtilis* strains could not grow well. The purpose of designing acidic and alkaline liquid formulations below pH 5.5 and above pH 8.5 is to allow *Bacillus* strains, which produce endospores, to remain in the endospore form for an extended period without transitioning to the vegetative form.

Table 1. Determination of pH tolerance of *Bacillus subtilis* strains on Nutrient Agar plates

Bacillus subtilis strains	pH								
	pH 4.5	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0	pH 8.5
B. subtilis EGE-B-1.19	+++	+++	+++++	+++++	+++++	+++++	+++++	+++++	+++++
B. subtilis EGE-B-36.5	-	+++	++++	+++++	+++++	+++++	+++++	+++	+++

The pH tolerance of different strains of *B. subtilis* is different, as shown in Table 1. *B. subtilis* can grow at low as pH 4.0 and high as pH 9.0, but 7.8 is where it grows best. Alkaliphile and alkali-tolerant microorganisms grow well in alkali environments above pH 9; however, alkaliphile microorganisms do not show optimal growth below pH 9. It has been shown in studies that *B. subtilis* maintains a cytoplasm pH between 7.3 and 7.6 with its cytoplasmic pH homeostasis ability [18, 19]. Gauvry et al. reported that *the B. subtilis* BSB1 strain grew between pH 4.8 and 9.1 [20]. In our studies, *B. subtilis* EGE-B-1.19 grew at pH 4.5-8.5 and *B. subtilis* EGE-B-36.5 grew at 5.0-8.5. These strains were found to have alkali and acid tolerance but showed different growth rates at different pH values. Accordingly, strain-specific studies should be conducted from formulation development studies even if they belong to the same species.

3.2 Preformulation of *Bacillus subtilis* EGE-B-36.5

The incubation of *Bacillus subtilis* EGE-B-1.19 and *Bacillus subtilis* EGE-B-36.5 in EPM resulted in obtaining spores at concentrations of 5.6 x 10^9 CFU/ml and 1.2 x 10^9 CFU/ml, respectively. Both bacteria were diluted to a concentration of 10^8 CFU/ml through the necessary calculations.

The pH of the culture broth was adjusted to pH 3.0, pH 4.0, and pH 5.0 with lactic acid, propionic acid, citric acid, and acetic acid to prepare the preformulation with *B. subtilis* EGE-B-36.5. In addition, the pH of the culture broth containing *B. subtilis* EGE-B-36.5 was adjusted to pH 5.0 using only boric acid. It has been determined that boric acid must be used in large quantities to adjust the pH of the culture broth to acidic levels such as pH 3.0 and pH 4.0 because it is a weak acid. For this reason, it was decided that using boric acid excessively is not cost-effective (Table 2) As stated in Table 3, preformulations created using five different acids were subjected to statistical analysis using the variance analysis (ANOVA) function in the SPSS package program in the third month to assess the total viable cell count. A significant variation in the viable cell count was observed in the formulation adjusted to pH 3.0 using citric acid ($p < 0.05$). According to the outcomes generated by this software, a second significant change in viable cell count ($p < 0.05$) was identified in the culture medium adjusted to pH 5.0 using acetic acid. In formulations to be conducted on a commercial scale, the unit cost of citric acid is considerably higher than that of acetic acid. Hence, because of the cost-effectiveness of acetic acid, the study proceeded with acetic acid to establish acidic liquid formulations.

Table 2. Monthly viable cell counts (cfu/ml) and pH values of *B. subtilis* EGE-B-36.5 in preformulations

		Preformulation of *B. subtilis* EGE-B-36.5							
Acids	**Storage (Month)**	**0**		**1st month**		**2nd month**		**3rd month**	
		x10⁸ CFU/ml	**pH**	**x10⁸ CFU/ml**	**pH**	**x10⁸ CFU/ml**	**pH**	**x10⁸ CFU/ml**	**pH**
Lactic Acid	pH 3.0	3.3 ± 0.03	3.0 ±0.05	2.3 ±0.9	2.9 ±0.03	2.1 ±0.5	3.6 ±0.04	3.2 ±2.0	3.1 ±0.01
	pH 4.0	3.8 ±0.3	4.1 ±0.05	1.7 ±0.8	4.1 ±0.1	2.2 ±0.05	4.6 ±0.1	2.5 ±0.3	6.4 ±0.14
	pH 5.0	-	-	-	-	-	-	-	-
Propionic Acid	pH 3.0	2.8 ±0.4	3.0 ±0.05	1.2 ±0.2	3.0 ±0.05	1.6 ±0.60	3.0 ±0.05	1.4 ±0.14	3.0 ±0.03
	pH 4.0	2.3 ±0.4	4.0 ±0.04	1.9 ±0.9	3.9 ±0.02	3.0 ±0.47	4.0 ±0.04	1.8 ±0.5	4.0 ±0.02
	pH 5.0	-	-	-	-	-	-	-	-
Citric Acid	pH 3.0	3.9 ±0.089	3.0 ±0.07	1.3 ±0.13	2.9 ±0.05	2.2 ±0.6	3.0 ±0.07	4.1 ±1.25	3.1 ±0.01
	pH 4.0	3.1 ±0.40	4.0 ±0.06	1.5 ±0.3	4.1 ±0.16	1.6 ±0.05	4.0 ±0.06	1.6 ±1.1	6.5 ±0.3
	pH 5.0	-	-	-	-	-	-	-	-
Acetic Acid	pH 3.0	3.2 ±1.1	3.1 ±0.05	1.74 ±0.17	2.9 ±0.04	2.0 ±0.53	3.1 ±0.05	1.9 ±0.5	3.0 ±0.04
Boric Acid	pH 3.0	-	-	-	-	-	-	-	-
	pH 4.0	-	-	-	-	-	-	-	-
	pH 5.0	1.5 ±0.2	4.7 ±0.3	1.4 ±0.25	4.7 ±0.3	1.8 ±0.7	4.7 ±0.25	1.6 ±0.4	4.7 ± 0.3
Control		2.5 ±0.07	8.6 ±0.05	1.7 ±0.2	6.9 ±0.03	3.4 ±1.2	7.5 ±0.06	2.0 ±0.8	7.5 ±0.18

-: Acidic liquid formulation was not prepared.

Table 3. Comparison of viable cell counts (CFU/ml) of *B. subtilis* EGE-B-36.5 in the third month of acidic liquid formulations prepared with different acids

	Population density (x10^8 cfu/ml)		
Acids	pH 3.0	pH 4.0	pH 5.0
Citric Acid	4.1 ± 1.25 *^{a}	1.6 ± 1.1 ^{d}	-
Propionic Acid	1.4 ± 0.14 ^{cd}	1.8 ± 0.5 ^{bcd}	-
Boric Acid	-	-	1.6 ± 0.4 ^{bcd}
Lactic Acid	3.2 ± 2.0 ^{abc}	2.5 ± 0.3 ^{abcd}	-
Acetic Acid	1.9 ± 0.5 ^{bcd}	3.2 ± 0.15 ^{ab}	3.4± 0.9 ^{ab}
Control	2.0 ± 0.8 ^{abcd}		

*In a column, means that are followed by the same letter are statistically similar. NA: not applicable

3.3. Shelf Life of the Acidic Liquid Formulations

Acetic acid formulations of the *B. subtilis* strains were prepared at pH 2.0, 3.0, 4.0, and 5.0, as shown in Tables 4a and 4b. The viable cell count in the formulation at pH 2.0 for both *Bacillus* strains declined in the second month, and no viable cells were observed in the third month. *Bacillus* strains typically thrive in environments with high pH values. Low pH values, such as pH 2.0, represent highly acidic conditions that typically have a negative impact on the growth of most *Bacillus* strains. However, some *Bacillus* species are acidophiles, which means that they can tolerate lower pH values. Extremophilic *Bacillus* species can survive in low pH environments because they have adapted to extreme conditions [21]. As a result, neither of the strains can survive at pH 2.

When viable cell counts were compared with the control group by performing the ANOVA test in the SPSS package program, it was determined that there had been a statistically significant ($p<0.05$) difference between the control and the acetic acid formulation of the *B. subtilis* EGE-B-36.5 strain at pH 4.0 and the acetic acid formulation of the *B. subtilis* EGE-B-1.19 at pH 4.0 (Table 4a and 4b).

Table 4a. Monthly viable cell counts (cfu/ml) and pH values of *B. subtilis* EGE-B-36.5 in acidic liquid formulations

	Acidic liquid formulation of *B. subtilis* EGE-B-36.5									
Storage (Month)	**pH 2.0**		**pH 3.0**		**pH 4.0**		**pH 5.0**		**Control**	
	Population density (x10^8 CFU/ml)	**pH**	**Population density (x10^8 CFU/ml)**	**pH**	**Population density (x10^8 CFU/ml)**	**pH**	**Population density (x10^8 CFU/ml)**	**pH**	**Population density (x10^8 CFU/ml)**	**pH**
0	1.8 ± 1.4	2.0 ± 0	3.2 ± 1.1	3.1 ± 0.05	3.4 ± 0.1	4.0 ± 0.01	4.1 ± 0.05	5.1 ± 0.1	2.5 ± 0.07	8.6 ± 0.05
1	0.8 ± 0.5	2.28 ± 0.01	1.74 ± 0.17	2.9 ± 0.04	2.5 ± 1.42	3.9 ± 0.02	3.1 ± 0.4	8.2 ± 0.2	1.7 ± 0.2	6.9 ± 0,03
2	0.04 ± 0.2	2.03 ± 0.7	2.0 ± 0.53	3.1 ± 0.05	2.2 ± 0.05	4.0 ± 0.01	2.0 ± 1.2	5.1 ± 0.1	3.4 ± 1.2	7.5 ± 0.06
3	-	-	1.9 ± 0.5	3.0 ± 0.04	3.2 ± 0.15	4.0 ± 0.02	3.4 ± 0.9	7.5 ± 0.3	2.0 ± 0.8	7.5 ± 0.18
4	-	-	1.6 ± 0.3	3.0 ± 0.02	2.5 ± 0.5	4.1 ± 0.03	1.9 ± 0.25	7.6 ± 0.3	2.0 ± 0.7	7.5 ± 0.07
5	-	-	1.8 ± 0.1	3.0 ± 0.01	2.2 ± 0.2	4.0 ± 0.01	1.9 ± 0.12	7.6 ± 0.2	1.9 ± 0.15	7.5 ± 0.06
6	-	-	1.5 ± 0.06	3.0 ± 0.02	2.1 ± 0.16	4.0 ± 0.01	1.8 ± 0.2	7.6 ± 0.25	1.8 ± 0.45	7.5 ± 0.03
7	-	-	1.5 ± 0.2	3.0 ± 0.015	2.1 ± 0.1	4.0 ± 0.01	1.5 ± 0.27	7.7 ± 0.16	1.9 ± 0.1	7.6 ± 0.03
8	-	-	1.0 ± 0.1	3.1 ± 0.015	1.8 ± 0.2	4.0 ± 0.015	1.1 ± 0.1	7.8 ± 0.18	1.6 ± 0.2	7.6 ± 0.04
9	-	-	0.9 ± 0.2	3.1 ± 0.01	1.7 ± 0.2	4.0 ± 0.02	0.8 ± 0.1	7.8 ± 0.15	1.4 ± 0.17	7.7 ± 0.08
10	-	-	0,8 ± 0,2	3,1 ± 0,03	1.5 ± 0.14	4.0 ± 0.02	0.5 ± 0.17	7.8 ± 0.4	0.9 ± 0.2	7.8 ± 0.15
11	-	-	0.8 ± 0.25	3.2 ± 0.03	1.4 ± 0.1	4.0 ± 0.02	0.3 ± 0.25	7.8 ± 0.4	0.5 ± 0.14	7.9 ± 0.13
12	-	-	0.6 ± 0.1	3.2 ± 0.04	1.4 ± 0.1	4.0 ± 0.03	0.2 ± 0.1	7.9 ± 0.4	0.5 ± 0.15	7.9 ± 0.29

-: No growth

Table 4b. Monthly viable cell counts (cfu/ml) and pH values of *B. subtilis* EGE-B-1.19 in acidic liquid formulations

	Acidic liquid formulation of *B. subtilis* EGE-B-1.19									
Storage (Month)	**pH 2.0**		**pH 3.0**		**pH 4.0**		**pH 5.0**		**Control**	
	Population density (x10^8 CFU/ml)	**pH**	**Population density (x10^8 CFU/ml)**	**pH**	**Population density (x10^8 CFU/ml)**	**pH**	**Population density (x10^8 CFU/ml)**	**pH**	**Population density (x10^8 CFU/ml)**	**pH**
0	2.9 ± 0.8	2.0 ± 0	3.0 ± 1.3	3.0 ± 0	3.2 ± 0.7	4.0 ± 0	3.5 ± 0.5	5.0 ± 0	3.5 ± 1.14	8.0 ± 0
1	1.3 ± 0.1	2.3 ± 0.01	1.2 ± 0.70	2.9 ± 0.01	2.2 ± 0.5	5.1 ± 0.02	4.1 ± 0.05	7.2 ± 0.3	3.3 ± 0.7	7.9 ± 0.2
2	0.03 ± 0.2	2.2 ± 0.05	0.5 ± 0.2	2.8 ± 0.03	4.0 ± 1.45	5.1 ± 0.07	4.8 ± 0.9	7.0 ± 0.2	3.6 ± 0.15	7.9 ± 0.4
3	-	-	1.1 ± 0.5	2.8 ± 0.01	4.8 ± 1	5.2 ± 0.1	6.5 ± 0.5	7.5 ± 0.6	2.8 ± 0.06	7.8 ± 0.8
4	-	-	0.4 ± 0.24	2.8 ± 0.02	4.6 ± 2.6	5.9 ± 0.7	4.7 ± 1.1	8.3 ± 1.05	4.2 ± 0.4	8.4 ± 0.9
5	-	-	0.1 ± 0.02	3.0 ± 0.02	4.5 ± 0.7	6.2 ± 1.3	3.5 ± 1	8.1 ± 1.03	7.8 ± 0.4	8.7 ± 0.28
6	-	-	0.1 ± 0.04	3.0 ± 0.01	2.8 ± 0.45	6.6 ± 1.7	2.35 ± 0.55	7.8 ± 0.9	1.9 ± 0.75	8.2 ± 0.64
7	-	-	0.1 ± 0.01	3.0 ± 0.01	3.1 ± 1	6.7 ± 1.7	4 ± 1.6	8.1 ± 1.2	3.9 ± 0.2	8.3 ± 0.77
8	-	-	0.4 ± 0.12	2.9 ± 0.03	4.25 ± 0.25	6.8 ± 1.4	2.8 ± 0.95	7.8 ± 1.04	1.75 ± 0.15	8.1 ± 0.53
9	-	-	0.1 ± 0.01	2.9 ± 0.03	3.7 ± 1.6	6.8 ± 1.4	3.7 ± 1.35	7.8 ± 1.1	2.45 ± 0.45	8.2 ± 0.2
10	-	-	0.1 ± 0.08	2.9 ± 0.05	2.9 ± 0.7	6.9 ± 1.3	2.6 ± 0.6	7.7 ± 1.05	1.5 ± 0.3	8.2 ± 0.27
11	-	-	0.15 ± 0.01	2.9 ± 0.01	2.85 ± 0.95	7.0 ± 1.2	1.3 ± 0.6	7.7 ± 1.1	3.2 ± 0.95	8.1 ± 0.4
12	-	-	0.1 ± 0.01	2.8 ± 0.03	3.35 ± 0.55	7.1 ± 1.2	2.4 ± 0.5	7.8 ± 1.1	1.5 ± 0.95	8.2 ± 0.5

-:No growth

In the study conducted by Muis, it was determined that *B. subtilis* grows easily between pH 5.0 and pH 8.0, and the optimum pH is pH 6.0 [22]. Because formulations formed in these ranges will support the growth of bacteria, lower acidic values were used for acetic formulations in our study. In addition, it has been noted that the growth of contamination is inhibited when the culture media is provided at a low pH level [15]. Issahary et al. investigated the activation of *B. cereus* endospores under low pH at different temperatures (50 ℃, 60 ℃ and 70 ℃)[23]. Because of the treatment of endospores at pH 1.0 and all temperatures, they showed that endospores were activated much faster than the control group (water only), but endospores entered the death phase very early. As the temperature increased, the endospores were activated more quickly and entered the death phase more quickly. In addition, in our study, it was determined that the acidic liquid formulation at pH 2.0 did not have viable cells at the end of the 3rd month. In the study, it was observed that the endospores of both *Bacillus* strains were lysed at pH 2.0. Wilks et al. determined the resistance of *B. subtilis* AG174 to extreme acidic and alkaline culture broth *B. subtilis* AG174 was cultured at pH 6.0 and pH 7.0, and the culture broth was adjusted to pH 4.5 [18]. After 2 h, the viability of the strain was determined as 60-100% and 5%–15%, respectively. In addition, the *B. subtilis* strain was cultured at pH 7.0 and pH 9.0, and the culture broth was adjusted to pH 10.0. After 2 h, *B. subtilis* strain viability was determined as 1-5% and 40%–100%, respectively. In this study, they showed the importance of the pH of the growth medium for viability. In our study, *Bacillus* strains in the endospore form were produced under optimum production conditions (pH 7.0, 30 ^{o}C). Therefore, the survival time of the bacteria increased and reached 12 months under similar pH conditions.

Vehapi and Özçimen adjusted the pH of the Luria –Bertani broth (LB) medium to pH 3.0, pH 5.0, and pH 7.0 in their study to investigate the growth of *the B. subtilis* strain [24]. They found that the specific growth rate of the *B. subtilis* strain was seven times higher in the culture medium adjusted to pH 7.0 than in the culture medium adjusted to pH 3 and almost equal to that in the culture medium adjusted to pH 5. The formulations are designed to maintain a steady viable cell count in the strain that will be used. If the pH range of the formulation is suitable for the growth of the strain, the strain may die after a while because of factors such as insufficient nutrients in the environment. Therefore, studies should be conducted on formulations that maintain a steady viable cell count in the strain over time. Furthermore, based on the results obtained in the study, it was concluded that different acidic formulations at various pH values should be developed for both *B. subtilis* strains.

3.4 Shelf Life of the Alkaline Liquid *Bacillus Subtilis* Ege-B-1.19 Formulations

Calcium acetate and calcium hydroxide, which are suitable for use as food additives, are pH regulating substances that enhance bioavailability [25]. In our study for 12 months, it was determined that the alkaline formulations used for *the B. subtilis* EGE-B-1.19 strain provided suitable conditions (Table 5). When the viable cell count was compared with the control group using the ANOVA test in the SPSS package program, it was determined that there had been a statistically significant ($p<0.05$) difference between the control and the alkaline formulation of the *B. subtilis* EGE-B-1.19 strain at pH 9.5 (Table 6)

Table 5. Monthly total number of viable cells (cfu/ml) and pH values of *B. subtilis* EGE-B-1.19 in alkaline liquid formulations

	Alkaline liquid formulation of *B. subtilis* EGE-B-1.19							
Storage (Month)	**pH 9.0**		**pH 9.5**		**pH 10.0**		**Control**	
	Population density ($x10^8$ CFU/ml)	**pH**	**Population density ($x10^8$ CFU/ml)**	**pH**	**Population density ($x10^8$ CFU/ml)**	**pH**	**Population density ($x10^8$ CFU/ml)**	**pH**
0	3.5 ± 0.45	9.0 ± 0	3.7 ± 0.45	9.5 ± 0	3.8 ± 1.45	10.0 ± 0	3.5 ± 1.14	8.0 ± 0
1	3.6 ± 1.05	7.6 ± 0.1	3.8 ± 1,1	9.1 ± 0.15	3.8 ± 0.8	9.7 ± 0.02	3.3 ± 0.7	7.9 ± 0.2
2	4.3 ± 0.7	7.1 ± 0.16	2.7 ± 0.7	8.6 ± 0.04	3.8 ± 0.5	9.4 ± 0.16	3.6 ± 0.15	7.9 ± 0.4
3	4.3 ± 1.1	7.3 ± 0.5	4.8 ± 2.15	7.8 ± 0.65	2.8 ± 0.6	9.2 ± 0.4	2.8 ± 0.06	7.8 ± 0.8
4	5.8 ± 1.55	8.1 ± 0.9	2.0 ± 0.1	7.9 ± 0.4	3.55 ± 0.05	8.9 ± 0.8	4.2 ± 0.4	8.4 ± 0.9
5	3.2 ± 0.1	8.0 ± 0.45	4.7 ± 1.2	7.2 ± 0.1	2.05 ± 0.45	8.1 ± 0.9	7.8 ± 0.4	8.7 ± 0.28
6	3.0 ± 1	7.9 ± 0.7	2.6 ± 0.2	7.3 ± 0.6	2.1 ± 0.8	7.7 ± 0.3	1.9 ± 0.75	8.2 ± 0.64
7	3.5 ± 0.6	7.9 ± 0.8	3.6 ± 0.9	7.2 ± 0.3	3.2 ± 1.45	7.8 ± 0.2	3.9 ± 0.2	8.3 ± 0.77
8	1.85 ± 0.75	7.9 ± 0.9	2.15 ± 0.25	7.1 ± 0.25	0.9 ± 0.2	7.9 ± 0.4	1.75 ± 0.15	8.1 ± 0.53
9	1.9 ± 0	8.1 ± 0.3	1.4 ± 0.2	7.1 ± 0.1	1.55 ± 0.55	8.0 ± 0.15	2.45 ± 0.45	8.2 ± 0.2
10	4.7 ± 0.2	7.9 ± 0.5	3.8 ± 0.1	7.3 ± 0.3	1.3 ± 0.5	8.2 ± 0.1	1.5 ± 0.3	8.2 ± 0.27
11	3.4 ± 1.2	7.8 ± 0.7	1.25 ± 0.25	7.2 ± 0.6	1.1 ± 0.2	8.2 ± 0.1	3.2 ± 0.95	8.1 ± 0.4
12	2.0 ± 0.25	7.9 ± 0.7	3.7 ± 0.3	7.2 ± 0.5	1.7 ± 0.6	8.3 ± 0.2	1.5 ± 0.95	8.2 ± 0.5

Table 6. Statistical values of liquid formulation of *B. subtilis* EGE-B-1.19 and *B. subtilis* EGE-B-36.5

***Bacillus subtilis* EGE-B.1.19**				***Bacillus subtilis* EGE-B.36.5**	
Acidic pH	**Population density ($x10^8$ cfu/ml)**	**Alkaline pH**	**Population density ($x10^8$ cfu/ml)**	**Acidic pH**	**Population density ($x10^8$ cfu/ml)**
Control	1.5 ± 0.95 *[b]	**Control**	1.5 ± 0.95 [b]	**Control**	0.5 ± 0.15 [b]
pH 3.0	0.1 ± 0.01 [c]	**pH 9.0**	2.0 ± 0.25 [ab]	**pH 3.0**	0.6 ± 0.1 [ab]
pH 4.0	3.35 ± 0.55 [a]	**pH 9.5**	3.7 ± 0.3 [a]	**pH 4.0**	1.4 ± 0.1 [a]
pH 5.0	2.4 ± 0.5 [ab]	**pH 10.0**	1.7 ± 0.6 [b]	**pH 5.0**	0.2 ± 0.1 [c]

*In a column, means that are followed by the same letter are statistically similar.

When the literature is examined, it is difficult to suppress growth in neutral and weakly alkaline pH conditions. Chung et al. showed that the strong alkaline structure of the zeolite NaA they used prevented the transformation of endospores into a vegetative form [26].The liquid formulations produced from *Bacillus* strains were adjusted to pH 5.0, pH 9.0, and pH 11.0 and the viable cells count. In the formulations created with pH 5.0 and pH 9.0, there was a decrease in the viable cell count on the 20th day, and no viable cells were found in the formulations after the 60th day. In the formulation created at pH 11.0, they reported that endospores were preserved without growth for up to 60 days.

4. CONCLUSION

These results showed that acidic liquid formulations using acetic acid stabilized the viable cell count of both *B. subtilis*EGE-B-36.5 and *B. subtilis* EGE-B-1.19 strains. The optimum pH value for the shelf life of *B. subtilis* EGE-B-36.5 and *B. subtilis* EGE-B-1.19 was found to be pH 4.0. In addition, the optimum pH for the alkaline formulation of *B. subtilis* EGE-B-1.19 was determined to be pH 9.5. According to this study, the optimum pH value should be specifically determined for each strain when preparing acidic and alkaline liquid formulations.

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CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

CRediT AUTHOR STATEMENT

Sevgi İşlek: Conceptualization, Methodology, Validation, Investigation, Writing – Original Draft, Writing – Review & Editing. **Kemal Karaca:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing. **Rengin Eltem:.** Conceptualization, Methodology, Investigation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition

REFERENCES

[1] Singh M, Singh D, Gupta A, Pandey KD, Singh PK, Kumar A. Plant Growth Promoting Rhizobacteria. PGPR Amelioration in Sustainable Agriculture 2019; 41–66.

[2] Chandini, Kumar R, Kumar R, Prakash O. The impact of chemical fertilizers on our environment and ecosystem. Research trends in environmental sciences 2019; 69-86.

[3] Tabassum B, Khan A, Tariq M, Ramzan M, Iqbal Khan MS, Shahid N, Aaliya K. Bottlenecks in commercialisation and future prospects of PGPR. Applied Soil Ecology 2017; 121: 102–117.

[4] Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. In World Journal of Microbiology and Biotechnology 2012; 28: 1327–1350.

[5] Beneduzi A, Passaglia LM. Genetic and phenotypic diversity of plant growth promoting bacilli. In Bacteria in agrobiology: plant growth responses, Springer, Berlin, Heidelberg 2011; 1-20.

[6] Akinrinlola RJ, Yuen GY, Drijber RA, Adesemoye AO. Evaluation of *Bacillus* Strains for Plant Growth Promotion and Predictability of Efficacy by In Vitro Physiological Traits. International Journal of Microbiology 2018; 1: 5686874.

[7] Kumar A, Prakash A, Johri BN. *Bacillus* as PGPR in Crop Ecosystem, In Bacteria in Agrobiology: Crop Ecosystems, Springer, Berlin, Heidelberg 2011; 37–59.

[8] Malusá E, Sas-Paszt L, Ciesielska J. Technologies for beneficial microorganisms inocula used as biofertilizers, In The Scientific World Journal 2012; 1: 491206

[9] Lobo CB, Juárez Tomás MS, Viruel E, Ferrero MA, Lucca ME. Development of low-cost formulations of plant growth-promoting bacteria to be used as inoculants in beneficial agricultural Technologies. In Microbiological Research 2019: 219: 12–25.

[10] Nakkeeran S, Fernando WD, Siddiqui ZA. Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. PGPR: Biocontrol and biofertilization 2005; 257-296.

[11] Bahadir PS, Liaqat F, Eltem R. Plant growth promoting properties of phosphate solubilizing *Bacillus* species isolated from the Aegean Region of Turkey. Turkish Journal of Botany 2018; 42(2): 183-196.

[12] Oztopuz O, Sarigul N, Liaqat F, Park RD, Eltem R. Chitinolytic *Bacillus subtilis* Ege-B-1.19 as a biocontrol agent against mycotoxigenic and phytopathogenic fungi. Turkish Journal of Biochemistry 2019; 44(3): 323-331.

[13] Liaqat F, Bahadır PS, Elibol M, Eltem R. Optimization of chitosanase production by *Bacillus mojavensis* EGE-B-5.2 i. Journal of basic microbiology 2018; 58(10): 836-847.

[14] Kumar AP, Janardhan A, Radha S, Viswanath B, Narasimha G. Statistical Approach to Optimize Production of Biosurfactant by *Pseudomonas aeruginosa* 2297. 3 Biotech 2015; 5: 71-79.

[15] Reuter CJ. Compositions for stabilizing *Bacillus* spores and methods of use thereof. 2011; U.S. Patent No. 20110200572

[16] Daniels RS. Corn Steep Liquor as a biostimulant composition 2012; U.S. Patent No. 2012015454

[17] Turan M. Mısır maserasyon sıvısı, çinko oksit, borik asit ile bakteri karışımından meydana gelen bir organik gübre. 2017; TR Patent No. 201613931

[18] Wilks JC, Kitko RD, Cleeton SH, Lee GE, Ugwu CS, Jones BD, BonDurant SS, Slonczewski JL. Acid and base stress and transcriptomic responses in *Bacillus subtilis*. Applied and environmental microbiology 2009; 75(4): 981-99.

[19] Bayram S, Aydogan MN. Searching for Versatile Polysaccharide-Degrading Alkali-tolerant or Alkaliphilic *Bacillus* Strains. Journal of the Institute of Science and Technology 2022; 12(1): 133-141.

[20] Gauvry E, Mathot AG, Couvert O, Leguérinel I, Coroller L. Effects of temperature, pH and water activity on the growth and the sporulation abilities of *Bacillus subtilis* BSB1. International Journal of Food Microbiology 2021; 337:108915.

[21] To HTA, Chhetri V, Settachaimongkon S, Prakitchaiwattana C. Stress tolerance-*Bacillus* with a wide spectrum bacteriocin as an alternative approach for food bio-protective culture production. Food Control 2022; 133: 108598.

[22] Muis A. Biomass production and formulation of *Bacillus subtilis* for biological control. Indonesian journal of agricultural science 2006; 7(2): 51-56.

[23] Issahary G, Evenchik Z, Keynan A. Low-p H Activation of *Bacillus cereus* Spores. Journal of Bacteriology 1970; 101(2): 418-422.

[24] Vehapi M, Özçimen D. Investigation of *B. subtilis* viability at different pH ranges for use in microbial cleaner formulation. Bulletin of Biotechnology 2020; 1(1): 1-7.

[25] Jha S, Singh R, Pandey A, Bhardwaj M, Tripathi SK, Mishra RK, Dikshit A. Bacterial toxicological assay of calcium oxide nanoparticles against some plant growth-promoting rhizobacteria. Int. J. Res. Appl. Sci. Eng. Technol 2018; 6(11): 460-466.

[26] Chung S, Lim JH, Kim SD. Powder formulation using heat resistant endospores of two multi-functional plant growth promoting rhizobacteria *Bacillus* strains having phytophtora blight suppression and growth promoting functions. Journal of the Korean Society for Applied Biological Chemistry 2010; 53(4): 485-492.

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

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RESEARCH ARTICLE

DETERMINATION OF THE ANTIPROLIFERATIVE EFFECT OF *STERNBERGIA LUTEA* (L.) KER GAWL. EX SPRENG. EXTRACTS ON A375 MALIGNANT MELONOMA CELL LINE

Zemheri ŞAMAN [1,*] Sevil YENİOCAK [2] , İrem DEMİR [3] , Ergun KAYA [4] , Nurdan SARAÇ [5]

[1] Muğla Sıtkı Koçman University, Faculty of Science, Molecular Biology and Genetics Departement, 48000, Menteşe, Muğla, Türkiye
zemherisaman@outlook.com - 0000-0003-0165-7824

[2] Muğla Sıtkı Koçman University, Faculty of Science, Molecular Biology and Genetics Departement, 48000, Menteşe, Muğla, Türkiye
sevilyeniocak@outlook.com - 0000-0002-8308-7468

[3] Muğla Sıtkı Koçman University, Faculty of Science, Biology Department, Menteşe, Muğla, Türkiye
iremdemir@posta.mu.edu.tr - 0000-0001-5699-0582

[4] Muğla Sıtkı Koçman University, Faculty of Science, Molecular Biology and Genetics Departement, 48000, Menteşe, Muğla, Türkiye
ergunkaya@mu.edu.tr - 0000-0003-4255-3802

[5] Muğla Sıtkı Koçman University, Faculty of Science, Biology Department, Menteşe, Muğla, Türkiye
nsarac@mu.edu.tr - 0000-0001-7676-542X

Abstract

Epidemiological evidence confirms that plants are primary sources of drugs used to reduce the incidence of cancer and prevent cancer-related deaths. *Sternbergia* species are used for therapeutic purposes due to the amaryllidaceae alkaloids, lectins, phenolic acids, pigments, and volatile components they contain. In this study, the anticancer properties of *S. lutea* extracts were tested on the A375 malignant melonoma cell line. In addition, in the study, the transcriptional expression of *BCL-XL* and *Cas9* genes, which function in cell proliferation and apoptotic pathways, in cells treated with plant extracts were determined by qRT-PCR. According to the cytotoxicity results made by the MTT test, the highest inhibition percentage was determined at the plant's concentration of 500 µg/*m*L. At this concentration, A375 cells were inhibited by 83.63%, and the IC_{50} value of the extract was calculated as 194.64 µg/*m*L. In addition, in qRT-PCR analyses, a statistically significant increase was observed in the mRNA expression levels of Cas9 genes, which are positively correlated with the apoptotic pathway, in the extract and cisplatin-applied groups compared to the control group.

Keywords

Anticancer,
Antiproliferation,
BCL-XL,
Cas9,
Cisplatin

Time Scale of Article

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1 . INTRODUCTION

Researching the effects of medicinal plants on health is important for the discovery or design of new drugs. Plants will continue to be the best source for the production of medicines used to treat different diseases in the past, today and in the future [1-2]. Although there are many synthetic drugs designed from raw materials obtained from plant isolates, the diversity of diseases that people are exposed to and

the fact that people respond differently to diseases increase the importance of drug studies. It is estimated that acceptable therapy is available for only one-third of known human diseases. Therefore, revealing the biological characteristics of medically important species is important for future studies [3-5].

Plants' active ingredients offer antioxidant qualities that help the body combat dangerous free radicals, which are the root cause of many diseases [6-9]. The genus *Sternbergia* is significant among geophytes because it produces a class of naturally occurring antioxidants. The *Sternbergia* is a genus of bulbous monocotyledonous plants in the family Amaryllidaceae. The alkaloids that plants in the Amaryllidace family generate are highly valued in addition to their aesthetic qualities. The genus *Sternbergia* is one of the sources of many alkaloids. Several of these alkaloids have intriguing biological and/or pharmacological characteristics [10-11].

In phytochemical studies on *Sternbergia* species, Amarylidaceae alkaloids, lectins and phenolic acids were obtained. In addition, pigments and volatile components have also been investigated. Among the alkaloids isolated from Sternbergia species, one of the most important ones in terms of treatment is the alkaloid named galantamine. This compound is a competitive cholinesterase inhibitor with long-lasting central action and is used in the treatment of cholinergic-related neurodegenerative diseases such as Alzheimer's disease. Another interesting Amaryllidaceae alkaloid isolated from Sternbergia species is lycorine. Lycorin is antiviral against some RNA and DNA viruses. There are also studies on the interaction of lycorin with DNA and/or RNA and its antitumour activity by using different analysis methods [12-15].

In this context, in the present study, the antiproliferative properties of the plant extracts were investigated in A375 Malignant Melonoma cell lines using *S. lutea* (L.) Ker Gawl. ex Spreng. (Figure 1) extracts. In addition, the expression of *BCL-XL* and *Cas9* genes, which are thought to be involved in cell proliferation and apoptotic pathways, at the transcription level were also examined in the extracted cell lines. This study was produced within the scope of Zemheri Şaman's master's thesis.

Figure 1. Natural population of *S. lutea* (L.) Ker Gawl. ex Spreng (Muğla, Türkiye).

2. MATERIAL and METHODS

2.1. Plant Material

The *S. lutea* natural samples [16-17] were collected in the Menteşe district of Muğla (Türkiye). The bulbs of the collected plants were dried in a dark room under airflow conditions at room temperature (Figure 2).

2.2. Plant Extraction

Dried *S. lutea* samples were physically ground using liquid nitrogen. 10 g of powdered plant material was transferred into a flask and 40 ml of ethanol (96%) was added. It was then extracted in an ultrasonic water bath at 25°C for 30 min at 100% vibration. After extraction, the mixture was centrifuged at 4000 rpm for 5 min and the supernatant was removed. The same procedure was repeated by adding 40 ml ethanol to the pellet. The supernatants obtained from both processes were filtered using Whatman filter paper and the particles were removed. After transferring the supernatant to the beaker, ethanol was removed and the extract obtained was stored at +4°C [18-20].

2.3. Determination of Cytotoxic Activity

2.3.1. Passaged cell cultures

A375 Malignant Melonoma cell line was cultured in 25-well flasks containing DMEM medium containing 10% fetal bovine serum (FBS) and 1% antibiotic. Cells that reached sufficient growth were removed with Trypsin-EDTA, counted with trypan blue and then transferred to 96-well microplates and incubated [3].

Figure 2. *S. lutea* bulb samples used in the study.

2.3.2 Determination of cytotoxic dose

The cytotoxicity of *S. lutea* extract on A375 cell lines was determined by MTT method [21]. Cells were transferred to 96-well plates containing 10000 cells per well and incubated at 37°C for 24 h with 5% CO_2. Cells treated with serial dilutions of the extract were incubated in 5% CO_2 at 37°C for 24 hours.

After 24 hours, 20 µl MTT was applied to the wells and incubated for 3 hours. After incubation, 100 µl dimethyl sulfoxide (DMSO) was applied to the wells and measured at 540 nm in a spectrophotometer kept at room temperature in a shaking incubator for 20 minutes. Test samples were used in the concentration range of 15.625-500 µg/ml. IC_{50} value was calculated statistically [21]. The cisplatin was used as a control to compare the antiproliferative activity of the plant extract.
(%) Vitality = [100 × ($Sample_{abs}$) / ($Control_{abs}$)]
$Sample_{abs}$: Absorbance in wells treated with test material
$Control_{abs}$: Absorbance of the control well
(% inhibition) = 100 - (% viability)
IC_{50} value of *S. lutea* extract was calculated statistically [21].

2.4 Molecular Analyses - Determination of Gene Expression at Transcriptomic Level

RNA was extracted from A375 cell line using Thermo Scientific™ Gene JET RNA Purification Kit (Cat.No. K0732). For cDNA synthesis, total RNAs were reverse transcribed using OneScript® Plus cDNA Synthesis Kit (Cat.No. G236) and oligo-dT primers included in this kit. Amplification of the reverse transcribed RNAs was determined by Real-Time PCR using Ampliqon RealQ Plus 2 × Master Mix Green Kit (Cat. No. A323402) and in the presence of primers for the two genes of interest. The respective genes and the sequences of their forward and reverse primers are given in Table 1. The thermal cycle used in the reaction was denaturation at 95 °C for 30 seconds followed by binding at 55-58 °C for 30 seconds and elongation at 72 °C for 30 seconds. Real-Time PCR was repeated six times and analysed. All groups were analysed for the expression of *β-actin*, a housekeeping gene, and interpreted using the $2^{-\Delta\Delta Ct}$ [2^(-delta delta Ct)] method [22]. The mean of the obtained values (with standard deviation and standard errors) was calculated and a graph was created. The significance of the expression levels of the two target genes obtained after qRT-PCR compared to the control group was statistically analysed by T-Test method. T-Test data with $p \leq 0.05$ were considered statistically significant [23-24].

Table 1. Primers and sequences designed for use in Real-Time PCR [25].

Gene	Primer sequence
β-actin	F: 5’ TCCTCCTGAGCGCAAGTACTC 3’ R: 5’ CTGCTTGCTGATCCACATCTG 3’
BCL-XL	F: 5’ GCTAGCAGACTTTGGACTAGCCAG 3’ R: 5’ AGCTCGGTACCACAGGGTCA 3’
Cas9	F: 5’ GGCTGTCTACGGCACAGATGGA 3’ R: 5’ CTGGCTCGGGGTTACTGCCAG 3’

3. RESULTS

3.1. Anticancer Properties of *S.lutea* Plant Extract

In this study, the cytotoxic activity of the rhizome extract of *S. lutea* on A375 melanoma cells *in vitro* was determined by MTT assay. The highest inhibition among the test concentrations was determined at a concentration of 500 µg/*m*l. At this concentration, A375 cells were inhibited by 83.63%. At the lowest concentration, 20.85% inhibition was observed (Figure 3). IC_{50} value of the extract was calculated as 194.64 µg/*m*l. In order to compare the anti-proliferative activity of the plant extract, A375 cell line was treated with cisplatin as control. Cisplatin was applied at concentrations between 1.6 and 50 µg/ml. The highest inhibition was 85.82% at 50 µg/ml and the lowest inhibition was 10.72% at 1.6 µg/ml (Figure 4).

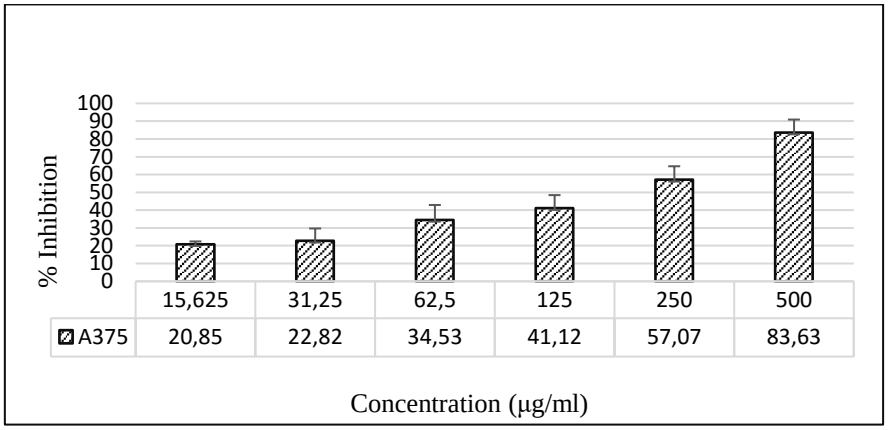

Figure 3. % inhibition values determined by MTT test in A375 cell line treated with *S. lutea* extract.

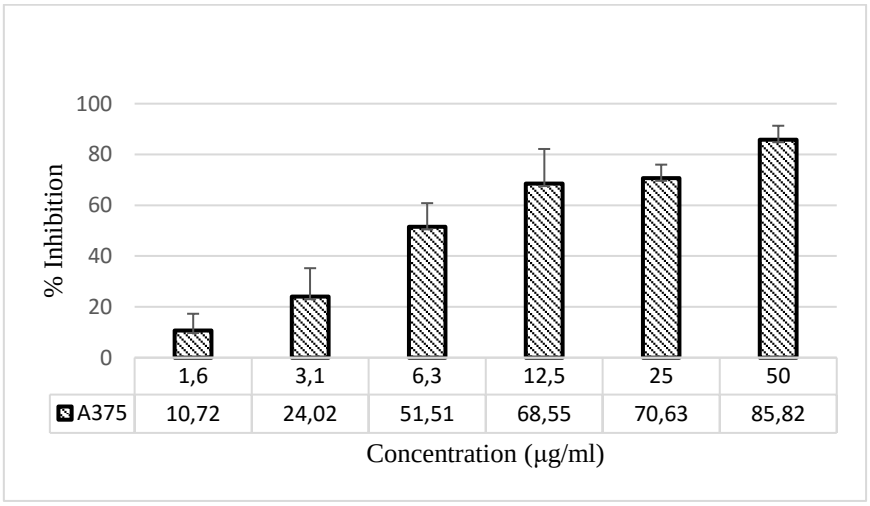

Figure 4. % inhibition values determined by MTT assay in cisplatin-treated A375 cell line.

3.2. Transcriptomic Analysis in A375 Cell Line

The mean of the Ct values (with standard error and standard deviations) obtained after Real-Time PCR to determine the expression levels of *BCL-XL* and *Cas9* genes at mRNA level in A375 cell line were calculated and graphs were created. When the data of A375 cell line were analysed, a decrease was observed in the mRNA expression level of *BCL-XL* gene, which is negatively correlated with the apoptotic pathway, in the extract and cisplatin groups, respectively, compared to the control group. However, this increase was not statistically remarkable. A statistically significant increase was observed in the mRNA expression levels of *Cas9* genes, which are positively correlated with apoptotic pathway, in the extract and cisplatin treated groups compared to the control group. It is a remarkable result in this study that treatment with S. lutea plant extract for 6 hours induced the expression of *Cas9* gene more than cisplatin treatment used as an anti-cancer drug and caused serious side effects (Figure 5).

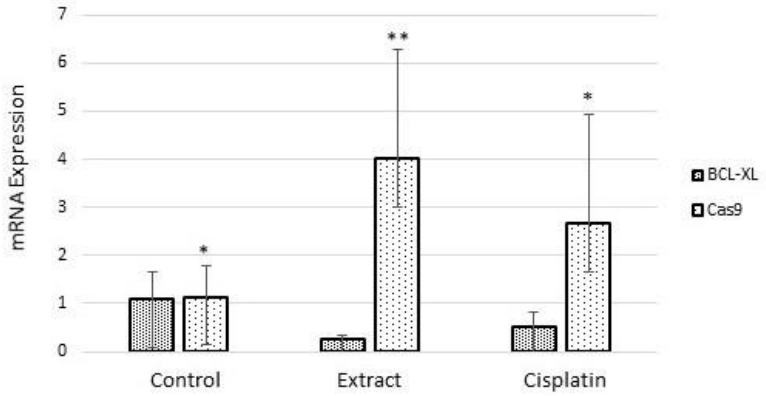

Figure 5. Relative mRNA expression graph of *BCL-XL* and *Cas9* genes in A375 cell line (Control: No substance treatment; Extract: Treated with 192.64 µg/ml *S.lutea* plant extract for 6 h; Cisplatin: Anti-cancer treatment; treated with chemotherapeutic drug at a concentration of 7.87 µg/ml for 6 h).

4. DISCUSSION and CONCLUSION

The cytotoxic effect of the extract obtained from the rhizome parts of *S. lutea* was investigated on A375 Human Malignant Melanoma cells. Inhibition was found to increase depending on the dose. This type of cancer, which was rare in the past, has become a more common cancer type day by day [26-27]. The cause of 75% of deaths due to skin cancer is melanoma cancer [28]. The Amaryllidacea family, including S. lutea, is known to contain many alkaloids. One of them is hypamine and it is a type of alkaloid that can be obtained from *S. lutea* [29]. Another type of alkaloid, lycorine, has been found to have anti-proliferative effect as a result of studies [30]. S. lutea contains many other alkaloids [31]. In a study by Masi et al. [32], inhibition values were investigated using MTT method on different alkaloids. In this study, SK-MEL-3 cell line, a different type of skin cancer, was used and the presence of anti-cancer activity was determined as a result of the study. IC_{50} value was given as >50 µM.

In this study, the IC_{50} value of the ethonolic extract of S. lutea on A375 cells was calculated as 194.64 µg/mL. The difference in IC_{50} values between the two studies is thought to be due to the difference in cell lines. The cytotoxicity of the ethanolic extract of *S.luta*, whose IC_{50} value was determined in this study, on cancer cells can be investigated in future studies.

As a result of this study, it was observed that the % inhibition rates of A375 Human Malignant Melanoma cells treated with *S. lutea* extract *in vitro* for 24 hours increased depending on the dose increase. In the light of the data obtained from this study, it will be possible to investigate the effect of *S. lutea* plant, which has cytotoxic effect on melanoma cells, on other cancer cells and to create pioneering data for new drug searches.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

CRediT AUTHOR STATEMENT

Zemheri Şaman: Investigation, Formal analysis, Writing - original draft, Visualization**, Sevil Yeniocak:** Investigation, Formal analysis, Visualization**, İrem Demir:** Investigation, Formal analysis, Visualization**, Ergun Kaya:** Conceptualization, Supervision, Investigation, Formal analysis, Writing - original draft, **Nurdan Saraç:** Conceptualization Investigation, Formal analysis, Writing - original draft.

REFERENCES

[1] Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. Molecules (2016); 21(5): 559.

[2] Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, Dzobo K. Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. International Journal of Molecular Sciences 2018; 19(6): 1578.

[3] Abdul Ghafoor N, Galatali S, Yeniocak S, Kaya E, Sarac N, Ugur A. Investigating anticancer potency of *in vitro* propagated endemic *Thymus cilicicus* Boiss. & Bal. extract on human lung, breast, and prostate cancer cell lines. Biologia 2022; 77: 3229-3239.

[4] Najmi A, Javed SA, Al Bratty M, Alhazmi HA. Modern Approaches in the Discovery and Development of Plant-Based Natural Products and Their Analogues as Potential Therapeutic Agents. Molecules 2022; 27(2): 349.

[5] Chaachouay N, Zidane L. Plant-Derived Natural Products: A Source for Drug Discovery and Development. Drugs and Drug Candidates 2024; 3(1): 184-207.

[6] Kaya E, Galatalı S, Güldağ S, Öztürk B, Ceylan M, Çelik O, Aktay İ. Mass production of medicinal plants for obtaining secondary metabolite using liquid mediums via bioreactor systems: SETISTM and RITA®. Turkish Journal of Scientific Reviews 2018; 11(2): 5-10.

[7] Kivrak S, Göktürk T, Kivrak I, Kaya E, Karababa E. Investigation of phenolic profiles and antioxidant activities of some *Salvia* species commonly grown in Southwest Anatolia using UPLC-ESI-MS/MS. Food Science and Biotechnology 2019; 39: 423-431.

[8] Mucha P, Skoczyńska A, Małecka M, Hikisz P, Budzisz E. Overview of the Antioxidant and Anti-Inflammatory Activities of Selected Plant Compounds and Their Metal Ions Complexes. Molecules 2021; 26(16): 4886.

[9] Yeniocak S, Galatalı S, Demir İ, Uğur A, Saraç N, Kaya E. Investigation of biological activities of *in vitro* grown Sesamum orientale plant extract on the cell cultures: wound healing and antiproliferation. Advances in Traditional Medicine 2024; 1-14.

[10] Youssef S, Mahmood A, Vela E. On the genus Sternbergia (Amaryllidaceae) in Iraq. Anales Del Jardín Botánico De Madrid 2017; 74(1): e053.

[11] Desgagné-Penix I. Biosynthesis of alkaloids in Amaryllidaceae plants: a review. Phytochemistry Reviews 2021; 20: 409–431.

[12] Nikolova M, Gevrenova, R. Determination of phenolic acids in Amaryllidaceae spesies by high performance liquid chromatography. Pharmaceutical Biology 2005; 43(3): 289-291.

[13] Berkov S, Bastida J, Tsvetkova R, Viladomat F, Codina C. Alkaloids from *Sternbergia colchiciflora*. Zeitschrift für Naturforschung 2009; 64c: 311-316.

[14] Kükcüoglu M, Baser KHC. Headspace Volatiles of Three Turkish Plants. Journal of Essential Oil Research 2010; 22: 389-392.

[15] Kaya G. Chemical compounds and biological activities of *Sternbergia* Waldst. & Kit. species. Marmara Pharmaceutical Journal 2014; 15(2): 52-57.

[16] Varol Ö, Dogru A, Kaya, E. Yilanli Dağı (Muğla)'nin florasi. Ekoloji 2004; 13(50): 23-32.

[17] Kaya E, Varol Ö, Aktaş-Aytepe H. Urban Flora of Muğla (Muğla, Turkey). Flora Mediterranea 2008; 18: 127-148.

[18] Saraç N, Şen B. Antioxidant, mutagenic, antimutagenic activities, and phenolic compounds of *Liquidambar orientalis* Mill. var. orientalis. Industrial Crops and Products 2014; 53: 60-64.

[19] Polat MM, Kaya E, Kivrak I. Comparative chromatographic analysis of phenolic compounds of *Liquidambar orientalis* plant cultivated under *in vitro* salt stress. International Journal of Secondary Metabolite 2023; 10(4): 570-582.

[20] Çelik O, Kaya E. mRNA Transcription Analyses of ROS Genes of *Olea europaea* L. *in vitro* Cultures Treated with Different Boron Salts. Journal of Aegean Agricultural Research Institute 2024; 34(1): 24-32.

[21] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods 1983; 65: 55-63.

[22] Livak KJ, Schmittge TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 2001; 25(4): 402-408.

[23] Çiçek S, Ağar H, Galatalı S, Kaya E. Transcriptomic analysis of *AREB1* and *AREB2* genes playing important roles in drought stress tolerance in tomato under *in vitro* drought stress. Environmental Analysis & Ecology Studies 2023; 10: 1203-1209.

[24] Galatali S, Kaya E. Investigation of the Cold Stress Effect on mac_4 (HSP80-Like) Gene at Transcriptional Level for *Mentha* × *piperita* L. Modern Concepts & Developments in Agronomy 2022; 10(5): 1057-1059.

[25] Wei Y, Zhang L, Wang C, Li Z, Luo M, Xie G, Gong JN. Anti-apoptotic protein BCL-XL as a therapeutic vulnerability in gastric cancer. Animal Models and Experimental Medicine 2023; 6(3): 245-254.

[26] Alves da Costa F, Ramos A, Bernardo C, Cardoso Borges F, Costa Miranda A. Epidemiological and clinical characterization of a population-based cohort of cutaneous malignant melanoma patients in the South Region of Portugal. Scientific Reports 2023; 13(1): 5641.

[27] Rastrelli M, Tropea S, Rossi CR, Alaibac M. Melanoma: epidemiology, risk factors, pathogenesis, diagnosis and classification. In vivo 2014; 28(6): 1005-1011.

[28] Grazziotin TC, Lovatto L, Riccardi F, Pizzol A, dos Santos AP. Melanoma. In Dermatology in Public Health Environments: A Comprehensive Textbook 2017; 341-366. Cham: Springer International Publishing.

[29] Ferri D, Ubaldi C, Marcozzi G, Fasciani P, Bacchetta L, Pace L. Chemical characterization of *Narcissus poeticus* from Sirente–Velino (Apennines-Italy): Galantamine accumulation and distribution of allergenic compounds in the flower. Natural Product Communications 2017; 12(1): 15-18.

[30] Lamoral-Theys D, Andolfi A, Van Goietsenoven G, Cimmino A, Le Calvé B, Wauthoz N, Evidente A. Lycorine, the main phenanthridine Amaryllidaceae alkaloid, exhibits significant antitumor activity in cancer cells that display resistance to proapoptotic stimuli: an investigation of structure− activity relationship and mechanistic insight. Journal of medicinal chemistry 2009; 52(20): 6244-6256.

[31] Citoglu GS, Yilmaz BS, Bahadir O. Quantitative analysis of lycorine in Sternbergia species growing in Turkey. Chemistry of Natural Compounds 2008; 44: 826-828.

[32] Masi M, Gunawardana S, van Rensburg MJ, James PC, Mochel JG, Heliso PS, Evidente A. Alkaloids isolated from *Haemanthus humilis* Jacq., an indigenous South African Amaryllidaceae: Anticancer activity of coccinine and montanine. South African Journal of Botany 2019; 126: 277-281.

ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ
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RESEARCH ARTICLE

NEW DATA ON TWO SPIDER SPECIES (ARANEAE) FROM ULUDAĞ MOUNTAIN, BURSA

Rahşen S. KAYA [1,*]

[1] Department of Biology, Faculty of Arts and Science, Bursa Uludağ University, Bursa, Türkiye
rkaya@uludag.edu.tr - 0000-0002-3769-9105

Abstract

In this study, mimetid spider *Ero cambridgei* Kulczyński, 1911 is recorded from Türkiye for the first time. A male *E. cambridgei* was collected in the orb-web of a female *Cyclosa algerica* Simon, 1885. Morphological diagnosis, along with images of both species are provided. Additionally, the copulatory organs of *C. algerica* Simon, 1885 are compared with those of *C. sierrae* Simon, 1870 for diagnostic purposes with their photographs. Furthermore, the record of *C. algerica* in the Uludağ Mountain remarks the northernmost point of its distribution range in Türkiye.

Keywords

Cyclosa algerica,
C. sierrae,
Ero cambridgei,
Fauna,
Marmara Region

Time Scale of Article

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1. INTRODUCTION

Members of Mimetidae comprise 163 species in 8 genera and are distributed worldwide, primarily in the tropics of Central and South America [1]. However, the biodiversity of Mimetidae remains poorly understood, most likely due to their small size and cryptic lifestyle. In Türkiye, only four species of mimetids have been reported: *Ero aphana* (Walckenaer, 1802), *E. flammeola* Simon, 1881, *E. furcata* (Villers, 1789), and *Mimetus laevigatus* (Keyserling, 1863) [2].

The pirate spider *Ero* C. L. Koch, 1836 is a cosmopolitan genus with 43 currently recognized species [1]. The main characters that have traditionally been used to distinguish *Ero* from the closely related genus *Mimetus* Hentz, 1832 are the height of the clypeus (higher in *Ero* than in *Mimetus*) and the length of the forelegs (in *Ero* legs I and II are subequal, while in *Mimetus* legs I are the longest) [3].

Araneidae Clerck, 1757 is the third largest family of spiders, with 3131 species in 191 genera worldwide [1]. In Türkiye, 55 species in 20 genera have been recorded [2]. The Araneid spider *Cyclosa* Menge, 1866 is a rich and globally distributed genus with 176 species. Four species of this genus are known from Türkiye: *C. algerica* Simon, 1885, *C. conica* (Pallas, 1772), *C. oculata* (Walckenaer, 1802), and *C. sierrae* Simon, 1870.

*Corresponding Author: rkaya@uludag.edu.tr

Cyclosa algerica is a Mediterranean species whose records for Europe so far concern only Portugal, Spain, France Algeria, Tunisia, Italy (Sicily), Greece, Azerbaijan, Iran and Bulgaria [1, 4]. The species has been recently recorded from Antalya province by Lecigne [5].

This paper presents the following findings: the first record of *E. cambridgei* in Türkiye; the first documented finding of *E. cambridgei* in the web of the araneid spider *C. algerica*; confirmation of the occurrence of *C. algerica* in Türkiye, along with the reporting of its northernmost distribution point.

2. MATERIALS AND METHODS

The samples examined in this study were collected from Uludağ Mountain (Bursa) in the Marmara region of Türkiye (Figure 1). Spiders were collected by hand collection. They were preserved in 70% ethanol and deposited in the Zoological Museum of the Bursa Uludağ University, Türkiye (ZMUU, R.S. Kaya).

The digital images were taken with a Leica DFC295 digital camera attached to a Leica S8APO stereo microscope and Leica M205 C. Measurements were taken from the dorsal side of the body and all measurements are in millimeters.

The nomenclature follows the World Spider Catalog [1], and the terminology of male palp follows Levi [6], Thaler et al. [7], and Marusik [8].

Figure 1. The locality where the specimens were collected from the Marmara Region of Türkiye.

3. RESULTS

3.1. Family Mimetidae Simon, 1881

Genus *Ero* C. L. Koch, 1836

***Ero cambridgei* Kulczyński, 1911**
Figures 2a–e

Ero cambridgei Roberts [9]: page 170, figure 75a (♂♀).
Ero cambridgei Roberts [10]: page 258 (♂♀).
Ero cambridgei Thaler et al. [7]: page 360, figures 23-24, 28-29, 34-35, 60-61 (♂♀).
For a complete list of synonyms, see the World Spider Catalog [1].

Determination. Thaler et al. [7].

Material examined. Türkiye: • 1♂, Bursa Prov., Uludağ Mountain Range, Seferiışıklar-Göynükbelen area, 40°01'15"N, 29°06'10"E, 587 m, 07.05.2006 (R.S. Kaya).
Diagnosis. *Ero cambridgei* is closely related to *E. furcata* (Villers, 1789). The male of *E. cambridgei* differs from those *E. furcata* by having paracymbium basally without bipartite process (cf. with bipartiate process, figure 22 in Thaler et al. [7]).
Description.
Male. Total length 1.90. Carapace 0.8 long and 0.8 wide. Abdomen 1.2 long 0.9 wide. Carapace yellowish-brown, cephalic region darker. Sternum yellowish-brown, with light median stripe and dark spots. Chelicerae dark brown. Legs brown, and leg joints with dark annulations. Abdomen globular and yellowish-brown, dorsum with darker spots posteriorly.

Palp as in Figures 2a–e; femur long, 1.5 times longer than tibia; tibia approximately 3 times longer than wide; cymbium oval, with finger-like slender proximal cybial extension; paracymbium rectangular with horizontal distal branch, basally not bipartiate; subtegulum oval; tegulum rounded; conductor with prolateral furrow, its retrolateral tip slender and finger-like, ventral tip trapezoid; embolus strongly sclerotized, originating from the position at 6 o'clock and ending at between 11 and 12 o'clock.
Note. The dorsal extension of the cymbium, along with the other characteristics of the palp, fit well with those shown in figures 24 and 35 of Thaler et al. [7].

Distribution. From Canary Islands east to Maritime Prov. of Russia and Japan (Honsu), new to Türkiye [1].

Comments. There are three species of *Cyclosa* are collected in the studied area during the study period. These are *C. algerica, C. sierrae* and *C. conica*. When I was searching the webs of *Cyclosa* species, I noticed a small spider, *E. cambridgei*, in the orb-web of a female *C. algerica*.
The other mimetids collected in the Uludağ Mountain region by the author are *E. aphana* and *M. laevigatus*, but any hunting behavior was not observed on mentioned species during the study period.

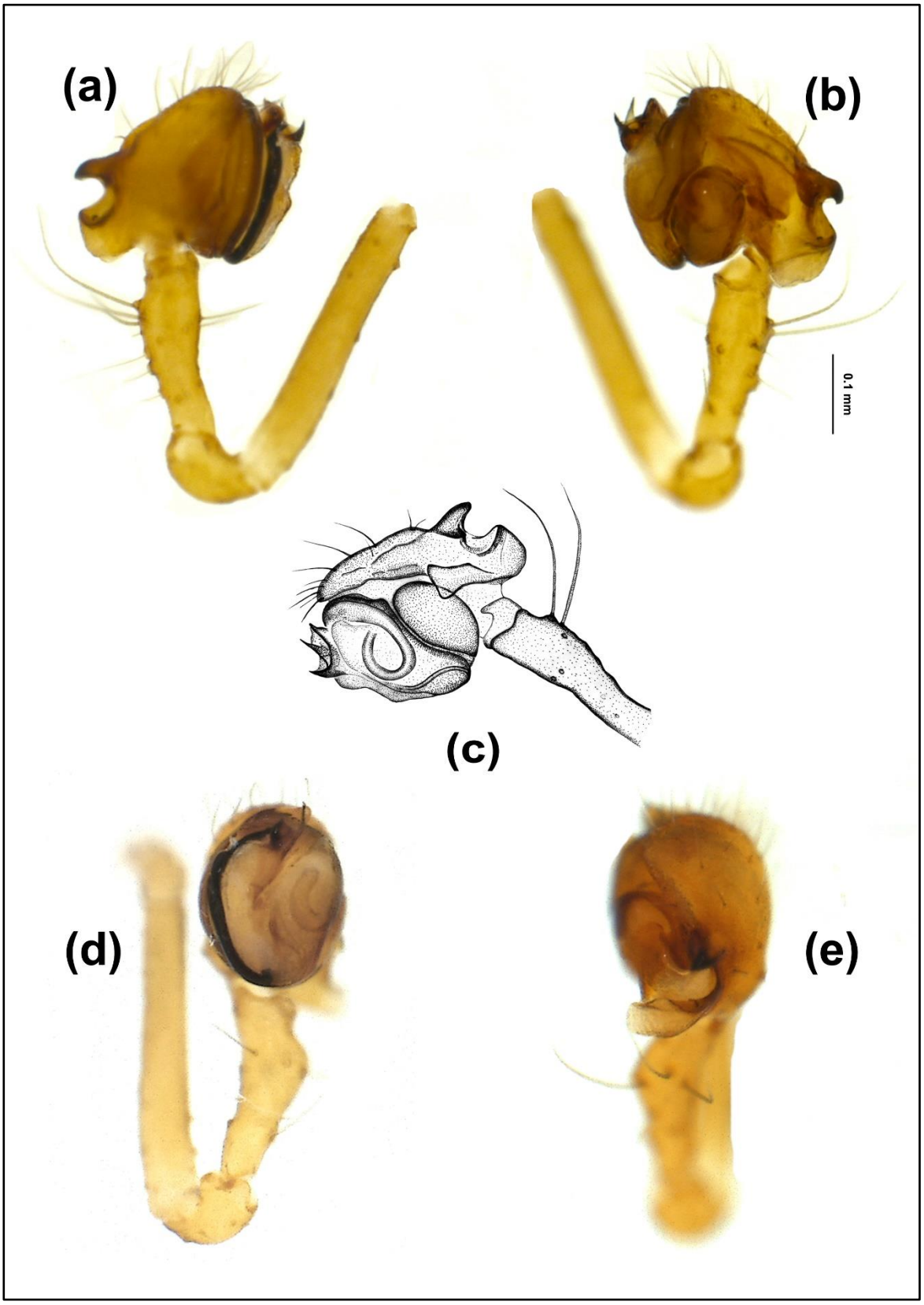

Figure 2. Male palp of *Ero cambridgei*.
(a) Prolateral view; (b, c) Retrolateral view; (d) Ventral view; (e) Dorsal view.

3.2. Family Araneidae Clerck, 1757

Genus *Cyclosa* Menge, 1866

***Cyclosa algerica* Simon, 1885**
Figures 3a–c, 4a–c

Cyclosa algerica Levi [6]: page 79, figures 34-37(♂♀).
For a complete list of synonyms, see the World Spider Catalog [1].

Determination. Levi [6] and Marusik [8].
Material examined. Türkiye: • 2♂3♀, Bursa Prov., Uludağ Mountain Range, Seferiışıklar-Göynükbelen area, 40°01'15"N, 29°06'10"E, 587 m, 07.05.2006 (R.S. Kaya); • 1♂3♀, same locality, 27.05.2007 (R.S. Kaya); • 1♀, same locality, 05.05.2008 (R.S. Kaya); • 1♀, same locality, 10.05.2010 (R.S. Kaya); • 2♂1♀, Güneybudaklar Vill., 14.05.2006 (R.S. Kaya).

Comparative material. *Cyclosa sierrae*, Türkiye: • 2♂2♀, Bursa Prov., Uludağ Mountain Range, Kirazlı Vill., 15.06.2005 (R.S. Kaya).

Diagnosis. This species is most similar to *C. sierrae* by general habitus and shape of copulatory organs. The male differs by having broad (Figure 3a–b) and circular distal tip of median apophysis (vs. slender and conical, figure 3d–e), tooth of median apophysis short and triangular-shaped (vs. tooth long with strongly curved pointed tip, figure 3d–e). The female of *C. algerica* differs by having epigynal plate approximately as long as wide (vs. wider than long, figure 3f), medially with sclerotized lobe on each side (vs. posteriorly with sclerotized lobe on each side, figure 3f), epigynal scape wide (Figure 3c) and apically not tapering towards the end (vs. scape narrower and apically tapering towards the end, figure 3f)

Description.
Male. Total length 4.0. Carapace 2.0 long and 1.6 wide. Abdomen 1.9 long and 1.2 wide. Carapace dark brown to black. Chelicerae dark brown. Sternum dark brown to black with dark margin. Legs brown, with dark annulations. Abdomen with a single dorsal protuberance posteriorly, dorsum yellowish with dark brown median marking, venter dark brown with two light markings (Figure 4a).

Palp as in Figures 3a–b, 4b–c; patella with only one bristle; tibia short; cymbium rather flat, medially broad and apically prolonged; paracymbium short and finger-like; conductor sclerotized, flattened and large, about 1.6 times longer than wide in prolateral view; conductor lobe apically slightly curved; embolus filamentous, long and thin; median apophysis large and long, about 5.7 times longer than wide in retrolateral view, its distal tip broad and circular, tooth of median apophysis short and triangular-shaped.

Female. Total length 6.0. Carapace 1.9 long and 1.4 wide. Abdomen 4.1 long and 2.9 wide. As male, except for the lighter color in general habitus.

Epigyne as in Figure 3c; epigynal plate approximately as long as wide, medially with sclerotized lobe on each side; epigynal scape slightly wrinkled, approximately 5 times longer than wide, with parallel sides, scape not reaching the posterior margin of the median plate; anterior margin of the plate as wide as posterior margin.

Habitat. The specimens were collected from the webs. The locality open and dry area with shrub vegetation.

Distribution. Portugal, Spain, France, Algeria, Tunisia, Italy (Sicily), Greece, Türkiye, Azerbaijan, Iran [3].

Comments. The general appearance and shape of copulatory organs show that, *C. algerica* is very similar to other Mediterranean species *C. sierrae*. These similarities may have confused in the identification of the two species. Additionally, upon checking the record of *C. algerica* from Türkiye by Lecigne [5], it became evident that the figure of male palp presented by Lecigne [5] corresponds to the figure of *C. sierrae* palp presented by Levi [6] and Marusik [8], rather than to those of *C. algerica*.

The specimens reported in this study represent the northernmost record of the known zoogeographical range in Türkiye. This species was previously recorded in Antalya province in Türkiye [5]. It is distributed across the countries of the Mediterranean Basin. Its observation of Uludağ Mountain Range in the Marmara Region can be considered an interesting record in a zoogeographical perspective. However, the collection sites where these specimens were collected exhibit a Mediterranean climate and it is observed that the species is locally well distributed in the region. This new locality for the species suggests a preference for open and dry habitats.

Based on the present study and the studies by Kaya & Uğurtaş [11] and Marusik [8], the other Araneidae spider species that occurred in the Uludağ Mountain region include: *Aculepeira ceropegia* (Walckenaer, 1802), *Agalenatea redii* (Scopoli, 1763), *Araneus diadematus* Clerck, 1757, *Araniella alpica* (L. Koch, 1869), *A. cucurbitina* (Clerck, 1757), *Argiope bruennichi* (Scopoli, 1772), *Cercidia prominens* (Westring, 1851), *C. algerica*, *C. conica*, *C. sierrae*, *Gibbaranea bituberculata* (Walckenaer, 1802), *Glyptogona sextuberculata* (Keyserling, 1863), *Hypsosinga albovittata* (Westring, 1851), *Larinioides cornutus* (Clerck, 1757), *L. suspicax* (O. P.-Cambridge, 1876), *Leviellus stroemi* (Thorell, 1870), *Mangora acalypha* (Walckenaer, 1802), *Neoscona adianta* (Walckenaer, 1802), *Nuctenea umbratica* (Clerck, 1757), *Zilla diodia* (Walckenaer, 1802) and *Zygiella x-notata* (Clerck, 1757).

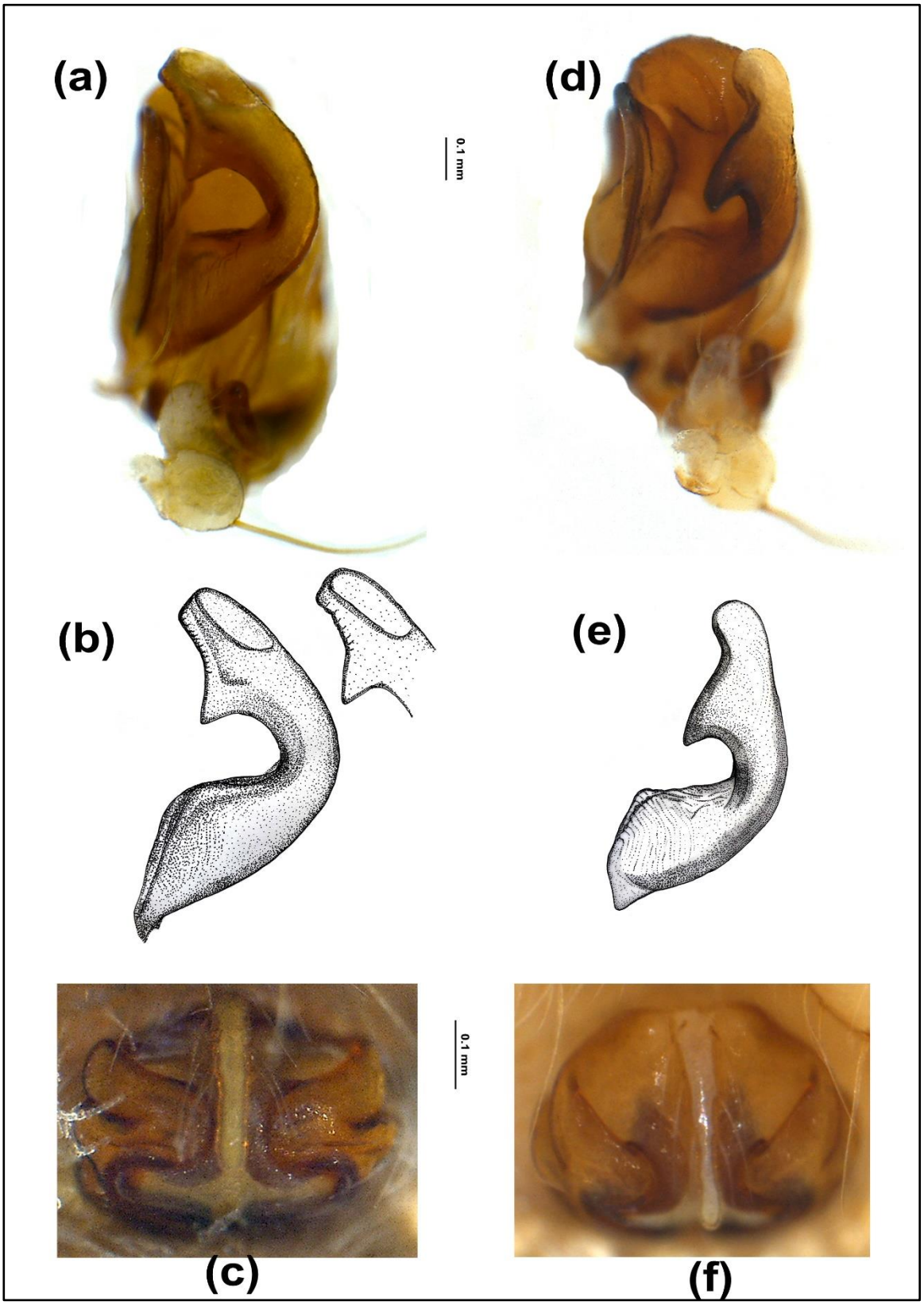

Figure 3. Copulatory organs of *Cyclosa algerica* (a - c) and *C. sierrae* (d - f).
(a, d) Male palp, ventral view; (b, e) median apophysis, ventral view; (c, f) Epigyne, ventral view.

Figure 4. Male of *Cyclosa algerica*.
(a) General habitus, dorsal view; (b) Male palp, ventro-retrolateral view; (c) Male palp, ventro-prolateral view.

4. DISCUSSION

This study reports *E. cambridgei* (Mimetidae) for the first time and confirms the occurrence of *C. algerica* (Araneidae) in Türkiye [1, 2]. The discovery of *E. cambridgei* in Uludağ Mountain range represents a significant addition to the spider fauna of Türkiye, marking the first record of this species in the country.
Additionally, the record of *C. algerica* from Uludağ Mountain range represents the northernmost known distribution point of this species in Türkiye.

Ero cambridgei and *C. algerica* are considered as rarely collected species in the collections [4]. The discovery of both species in the same habitat suggests that the Uludağ Mountain range may be an important area for many spider species. This underscores the need to conserve such habitats, which support biodiversity and provide environments where rarely or poorly known species can thrive. Future studies should focus on the ecological requirements of these spiders to gain a better understanding of their distribution and the factors contributing to their rarity.

The spider genus *Ero* is represented by only three species in Türkiye [2]. The addition of *E. cambridgei* to this list underscores the richness of Türkiye's spider fauna and the potential for further discoveries. The diverse microhabitats of the Uludağ Mountain range, shaped by its unique climatic and geographical features, likely play a crucial role in supporting these spider populations.

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ETHICS COMMITTEE APPROVAL

Ethics committee approval is not required for this study.

CONFLICT OF INTEREST

The author state that there is no conflict of interest regarding the publication of this article.

CRediT AUTHOR STATEMENT

Rahşen S. Kaya: Investigation, Resources, Writing – Original draft, Writing – Review & Editing, Visualization, Conceptualization.

REFERENCES

[1] World Spider Catalog. Version 25.0. Natural History Museum Bern, online at http://wsc.nmbe.ch, accessed on {28.11.2024}.

[2] Danışman T, Kunt KB, Özkütük RS, Coşar İ. The Checklist of the Spiders of Turkey. Version 2024. http://www.spidersofturkey.info [Accessed on 28.11.2024]

[3] Benavides LR, Hormiga G. A morphological and combined phylogenetic analysis of pirate spiders (Araneae, Mimetidae): evolutionary relationships, taxonomy, and character evolution. Invertbr Syst 2020; 34(2): 144-191.

[4] Nentwig W, Blick T, Bosmans R, Gloor D, Hänggi A, Kropf C. Spiders of Europe. Version 8.2024. https://www.araneae.nmbe.ch [accessed on 28.11.2024].

[5] Lecigne S. A new species of *Sintula* (Linyphiidae), redescription of *Brigittea innocens* (Dictynidae) and eight spider species newly recorded for Turkey (Araneae). Arachnol Mitt 2021; 62: 11-34.

[6] Levi HW. The American orb-weaver genera *Cyclosa*, *Metazygia* and *Eustala* north of Mexico (Araneae, Araneidae). Bull Mus Com Zool 1977; 148: 61-127.

[7] Thaler K, Harten A van, Knoflach B. Pirate spiders of the genus *Ero* C.L. Koch from southern Europe, Yemen, and Ivory Coast, with two new species (Arachnida, Araneae, Mimetidae). Denisia 2004; 13: 359-368.

[8] Marusik YM. Spiders (Araneae) new to the fauna of Turkey. 6. New species and genera records of Araneidae. Turk J Arach 2009; 2(4): 12-16.

[9] Roberts MJ. The spiders of Great Britain and Ireland, Volume 1: Atypidae to Theridiosomatidae. Harley Books Colchester, England, 1985, 229 pp.

[10] Roberts MJ. Collins Field Guide: Spiders of Britain & Northern Europe. Harper Collins, London, 1995, 383 pp.

[11] Kaya R, Uğurtaş İH. The orb-weaver spiders (Araneae, Araneidae) of Uludağ mountain, Bursa. Turk J Arach 2008; 1(2): 160-165.