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How do AMF and Biochar Affect Pepper Growth and Nutrient Content under Biotic and Abiotic Stress?

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ABSTRACT

Salt stress is a significant abiotic stress that adversely affects pepper plant growth which can accelerate the development of plant pathogens and increase plant susceptibility to diseases. *Verticillium dahliae*, which causes pepper wilt disease, is an important biotic stress factor. *Funneliformis mosseae* and biochar organic wastes help to take nutrients from the soil by establishing symbiotic connections with plant roots and, are effective in treating plant diseases, plant growth, and stress tolerance. This study aims to determine the effects of *F. mosseae* (Fm) and 2% biochar (Bc) against *V. dahliae* (Vd) on some plant physiological properties, plant nutrient uptake, soil pH, and EC value in pepper plants grown under salt stress (50mM, 100mM, 150mM). As a result of the study, the use of *F. mosseae* alone or in interaction with 2% biochar significantly increased some physiological parameters and some minerals (P, K, Mg, and Mn) contents of the plant. Moreover, pepper plants showed remarkable resistance to salt and stress factors caused by *V. dahliae*. In addition, the interaction between *F. mosseae* and biochar significantly lowered the soil EC value under conditions of severe salt stress. On the other hand, biochar was more effective than *F.mosseae* in terms of soil pH and Ca/Na ratio. The results showed that biochar and *F. mosseae* were beneficial in reducing biotic (*V. dahliae*) and abiotic stress (salt stress) damage while enhancing plant growth and nutrient absorption. Therefore, this study yields excellent and novel results, particularly in the field of employing beneficial microorganisms for sustainable agriculture.

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Keywords
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AMF ve Biyoçar Biyotik ve Abiyotik Stres Altında Biber Gelişimini ve Besin İçeriğini Nasıl Etkiler?

ÖZET

Tuz stresi, biber bitkisinin büyümesini olumsuz etkileyen, bitki patojenlerinin gelişimini hızlandırabilen ve bitkinin hastalıklara karşı duyarlılığını artırabilen önemli bir abiyotik strestir. Biber solgunluğu hastalığına neden olan *Verticillium dahliae* önemli bir biyotik stres faktörüdür. *Funneliformis mosseae* ve biyoçar organik atıkları, bitki kökleriyle simbiyotik bağlantılar kurarak topraktan besin maddesi alınmasına yardımcı olur ve bitki hastalıklarının kontrolünde, bitki büyümesinde ve stres toleransında etkilidir. Bu çalışma, tuz stresi (50mM, 100mM, 150mM) altında yetiştirilen biber bitkisinde *V. dahliae* (Vd)'ye karşı *F. mosseae* (Fm) ve %2 biyoçarın (Bc) bazı bitki fizyolojik özellikleri, bitki besin elementi alımı, toprak pH'sı ve EC değeri üzerindeki etkilerini belirlemeyi amaçlamaktadır. Çalışma sonucunda, *F. mosseae*'nin tek başına veya %2 biyoçar ile etkileşimli olarak kullanılması, bitkinin bazı fizyolojik parametrelerini ve bazı mineral (P, K, Mg ve Mn) içeriklerini önemli ölçüde artırmıştır. Ayrıca, biber bitkileri tuza ve *V. dahliae*'nin neden olduğu stres faktörlerine karşı kayda değer bir dayanıklılık göstermiştir. Buna ek olarak, *F. mosseae* ve biyoçar arasındaki etkileşim, şiddetli tuz stresi koşulları altında toprak EC değerini önemli ölçüde düşürmüştür. Öte yandan, biyoçar, toprak pH'sı

Bitki Koruma

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Tuz stresi
Verticillium dahliae

ve Ca/Na oranı açısından *F. mosseae*'den daha etkili olmuştur. Sonuçlar, biyoçar ve *F. mosseae*'nin biyotik (*V. dahliae*) ve abiyotik stres (tuz stresi) hasarını azaltmada faydalı olduğunu ve bitki büyümesini ve besin emilimini artırdığını göstermiştir. Dolayısıyla bu çalışma, özellikle sürdürülebilir tarım için faydalı mikroorganizmaların kullanılması alanında mükemmel ve yeni sonuçlar ortaya koymaktadır.

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Graphical Abstract

INTRODUCTION

Pepper (*Capsicum annuum* L.) is an annual herbaceous plant native to South America and a member of the Solanaceae family (Guevara et al. 2021). According to Coskun et al. (2023), pepper ranks third among fruits in terms of production with a global production of 2.5 million tons, and between 6 to 9% of this is produced in Türkiye (Food and Agriculture Organization of the United Nations 2020). Pepper is a very important source of income for growers. On the other hand, biotic and abiotic stress factors that diminish pepper production are a significant issue (Geleta & Labuschagne, 2006). Plant diseases associated with fungi are responsible for 14% of the yield loss in world vegetable production (Tripathi et al., 2024). In areas where peppers are grown, fungi such as *Alternaria* spp., *Fusarium* spp., *Phytophthora capsici*, *Pythium* spp., *Botrytis cinerea* and *Verticillium dahliae* cause major diseases like fruit and root rot and wilt (Nguyen et al. 2010; Coşkun et al. 2021). Of these diseases, *V. dahliae* is one of the most damaging pathogens that cause vascular wilt (Güneş et al. 2024).

Soil-borne fungi *Verticillium dahliae* infection causes symptoms such as discoloration of root and stem structures (dark brown, black in places), stunting, yellowing, and wilting of leaves in pepper plants (Schnathorst, 1981; Tyvaert et al. 2019). It blocks the vascular tissue of the plant, prevents the transport of water and dissolved minerals from the root, and leads to plant death (Tjamos et al. 2000; Pegg & Brady 2002).

The growth and survival of *V. dahliae* may be adversely affected by the presence of salt in the soil (Geleta & Labuschagne, 2006). According to Kacjan et al. (2021), up to a certain point, pepper is salt-sensitive, and pepper plants have a salinity threshold of 1.5 dS m^{-1}, making them moderately salt-sensitive plants. Even at low salt concentrations, a salt-sensitive pepper plant cannot attain the osmotic pressure value created in the solution by the roots (Kotuby-Amacher et al. 2000). Additionally, salt stress alters the ion and water balance in pepper plant cells, causing dryness and adverse effects on plant growth (Etesami & Beattie, 2018).

Therefore, environmentally friendly strategies that promote plant growth, protect natural resources, and aim to reduce waste and environmental impacts should be used to combat such abiotic and biotic stress factors in pepper (Khrieba et al. 2019). Recently, there has been interest in the effects of arbuscular mycorrhizal fungi (AMF) and biochar, which are incorporated into sustainable agricultural practices, against biotic and abiotic stress factors (Elmer et al. 2011; Gunes et al. 2023).

In nutrient-poor conditions such as dry regions, soils containing heavy metals, and salty soils, AMF functions in symbiosis with plant roots. Furthermore, in the case of soil-borne pathogens such as *V. dahliae*, the host plant can be effectively defended by AMF. It can even increase the tolerance or resistance of the plant root at various levels under different conditions (Akköprü & Demir, 2005; Güneş et al. 2022). The combination of AMF + organic waste supports the use of environmentally friendly, economically viable, and sustainable technologies (Demirel et al. 2024). Recent studies have demonstrated the potential of biochar organic waste to control plant pathogens and abiotic stress conditions by altering signaling mechanisms between plants and symbiotic microorganisms (Kolton et al. 2011; Ogundeji et al. 2021).

Providing a safe habitat for protecting AMF against pests, biochar has a direct effect on nutrient uptake and an indirect effect on plant growth (Zhuo et al. 2020). Biochar, which is pyrolysis in an oxygen-free environment, is rich in carbon and possesses an abundant amount of plant nutrients (Ippolito et al., 2012). In addition to inhibiting the development of many plant pathogens, biochar increases water retention capacity and adsorption ability and provides effectiveness against biotic and abiotic stress factors (Graber et al., 2014; Palansooriya et al., 2020). By activating defense systems in response to biotic and abiotic stress signals, AMF+ biochar develops a local and systemic antimicrobial defense (Lamb & Dixon 1997; Low & Merida, 1996). However, studies on the combined effects of pepper grown under the effects of salt and *V. dahliae* stress factors with AMF and biochar have not been sufficiently comprehensive.

Environmentally friendly practices such as AMF and biochar are important in plant-pathogen-environment interaction. The efficacy of single or combined applications of biochar and arbuscular mycorrhizal fungi (AMF) on various parameters of pepper plants against biotic (*V. dahliae*) and abiotic stress (salt stress) factors were evaluated for the first time in this study. Therefore, in this study, the effects of combined treatments on some plant growth parameters, macro and micronutrient contents, soil pH, and EC of pepper under different salt concentrations and *V. dahliae* stress conditions were determined.

The main hypothesis of this study is that the addition of AMF and biochar will have a synergistic effect on the development of pepper plants, as well as on leaf mineral matter content, soil pH, and EC. In particular, this would increase the tolerance of plants to conditions caused by biotic (*V. dahliae*) and abiotic (salt) stress. A controlled experiment was set up to investigate this theory.

MATERIAL and METHOD

Materials

In this study, the commercial cultivar Sera Demre 8 (Iklim Agricultural Products INC., Mersin, Türkiye) was utilized. Virulence (80%) *V. dahliae* isolates (Coşkun et al., 2023) and the highly active *Funneliformis mosseae* FMYYU1 (Fm) AMF inoculum previously isolated (Gunes et al., 2023) from pepper was taken from the culture stock of Van YYU Faculty of Agriculture, Department of Plant Protection (Van/Türkiye). *Medicago sativa* L., the host of AMF, was used to obtain *F. mosseae* inoculum samples, which were then kept at +4 °C. The biochar organic material (Bc) was obtained from Single Carbon Barbecue Coal Production Inc. in a 100% natural form as oak powder at 450 degrees Celsius (Medium pyrolysis type; medium heating rate.). Based on earlier pepper plants were subjected to salt stress at various NaCl (Merck, Germany) concentrations (0 mM, 50 mM, 100 mM, and 150 mM) research (Akay Rastgeldi, 2010). The place of the collection is Gevaş in Van province (Van/Turkey, 38°302′998 "N 43°113′178″E). The soil physico-chemical properties were as follows: pH 7.19, EC 64.6, lime 2.26, organic matter content 0.87/0.044, sand 67.2, clay 17.6, and silt 15.2. The soil is classified as sandy loam by the USDA. pH 8.10, EC 3.42 (dS m-1), moisture 2.48 (%), organic matter content 26.61 (%), organic carbon 15.43 (%), and carbon/nitrogen 23.74 (%) are some of the parameters of biochar.

Design of experiment

Pepper seeds were first sown for seedling growth in plastic vials (4.7 x 6.0 cm) containing a 2:1 ratio of peat and perlite. Four weeks later, the pepper seedlings were moved into plastic pots weighing three kilograms. After one hour of autoclaving at 121 °C, the soil was rendered sterile. Considering the results of previous studies (Gunes et al., 2023), the prepared soil was mixed with the best biochar rate of 2% (24 g biochar was mixed for 3 kg pots) before placing the peppers in biochar pots. In the treatments without biochar, only soil was used. For the AMF application, the application method of Schüßler and Walker, (2010) was followed. For *F. mosseae* (150 spores per

1 g soil) treatments, 10 g was deposited in each seedbed, and seedlings were transplanted. The sand was used for all treatments except AMF.

Furthermore, to test for pathogenic applications, the *V. dahliae* isolate was cultured for seven days at 25°C on Potato Dextrose Agar (PDA) (Merck, Darmstadt, Germany). Roots of pepper seedlings were immersed in 1×10^6conidia ml-1 *V. dahliae* spore suspension for 5 minutes, while control plants were only immersed in water (Coşkun et al., 2023). Pepper seedlings were exposed to salt stress at the three-five-leaf stage and five days after planting. Salt treatments were done at doses of 0, 50, 100, and 150. To maintain salt stress levels, a salt solution of 25 mM was prepared for each pot (73.05 g of salt was mixed into 1250 liters of water, and the solution was ready for application). Thus, to avoid osmotic shock in the root zone, 25 mM NaCl (25 ml water) was added to the growth medium every two days until the targeted concentrations were reached (2 times 25 mM for 50 mM, four times for 100 mM, and six times for 150 mM) and the highest salt concentration application was completed on day 12. The control group received the same volume of unsalted water. The research was carried out in a climate chamber at Van Yüzüncü Yıl University Faculty of Agriculture. According to a completely randomized experimental design with six replications, 32 application groups of plants were cultivated in a growth chamber under controlled conditions at 60-70% RH, 22 ± 2 °C, and 16 light/8 dark photoperiods. The implementation design of the study is given in Table 1 below.

Table 1. The study's implementation design
Çizelge 1. Çalışmanın uygulama tasarımı

Treatments
Control
Fm
Bc
50 mM
100 mM
150 mM
Vd
Fm+ Bc
Fm +50 mM
Fm + 100 mM
Fm +150 mM
Bc + 50 mM
Bc + 100 mM
Bc + 150 mM
Fm + Vd
Bc+ Vd
Vd+50 mM
Vd+100 mM
Vd+150 mM
Fm +Bc+50 mM
Fm +Bc+100 mM
Fm +Bc+150 mM
Fm +Bc+Vd
Fm +50 mM+Vd
Fm +100 mM+Vd
Fm +150 mM+Vd
Bc + 50 mM+ Vd
Bc + 100 mM+ Vd
Bc + 150 mM+ Vd
Fm +Bc+50 mM+ Vd
Fm +Bc+100 mM+ Vd
Fm +Bc+150 mM+ Vd

Bc: Biochar Fm: *F. mosseae* Vd: *V. dahliae*

A total of 576 plants were used for the study, with six replications in each treatment group and three plants in each replicate. The experiment was terminated at week 8 because the first symptoms appeared 7 weeks after sowing, and pathogen symptoms started to appear 1 week after the end of salt stress.

Assessment of the Parameters

Before harvesting, leaf number (young and middle), shoot diameter (at the root collar of the plant) parameters (digital caliper Insize, China), leaf area (LICOR, Model: LI-3100, Lincoln, NE, USA), and chlorophyll (taken systematically from the 4th true leaf of each plant) were determined (SPAD 502 Plus), followed by soil pH, soil EC, macro and micronutrients. Tap water was used as irrigation water.

For K, Ca, Mg, Fe, Cu, Zn, Mn, and Na content measurement, dry samples (0.5 g) collected from plant leaves (middle) were burned (Kacar 1984). Using the vanadomolybo-phosphoric yellow technique, the total phosphorus content was determined. To do this, 0.5 g of dried leaf and 1 mL of ethanol were combined (Merck 818,760, Germany). Following the addition of 4 mL of hydrochloric acid, the samples were incubated at 90 °C for 15 minutes (Merck 160 1.05590.2500, Germany). The extracts were filtered before being measured at 430 nm using a spectrophotometer (Jenway 6505 161 UV/vis, UK).

The pH of the soil samples was measured using a 1:2.5 soil-water mixture (Jackson 1958). Soil salinity (EC) was measured using conductivity equipment (U.S. Salinity Laboratory Staff 1954).

Data Analysis

The differences between AMF, biochar, salt concentrations, and *V. dahliae* were assessed using Duncan's multiple comparison test in SAS 9.4 (SAS Institute Inc., Cary, NC, USA 2012). Correlations between the studied traits were determined via Pearson's pairwise correlations using the PAST3 program. The Principal Component Analysis (PCA) was used in conjunction with the XLSTAT statistical program to highlight any similarities or differences that emerged from the applications and to determine how much of these differences could be accounted for by the features taken into consideration in the study. In addition, heatmap clustering (ClustVis) was used to cluster the dependent variable parameters that correspond to the treatments (independent variables).

RESULTS and DISCUSSION

RESULTS

Effects of *F. mosseae* and biochar on plant growth parameters under salt and *V. dahliae* stresses

The effects of *F. mosseae* (Fm) and biochar (Bc) treatments on leaf number (number), shoot diameter (caliper), leaf area (tool), and chlorophyll (Spad) parameters in plants infected with *V. dahliae* (Vd) and grown under salt stress are shown in Table 2. It was determined that there was a statistically significant difference ($p<0.05$) between the treatments ($p<0.05$). The highest values of leaf number, shoot diameter, leaf area, and chlorophyll parameters were found in Fm+Bc and Fm treatments (36.83 plants^{-1}, 4.20 mm, and 413.65 cm^2, respectively), i.e. in treatments without salt and disease stress (Table 2). Vd and conditions with 150 mM salt content were observed as the lowest values. However, despite both stress conditions, it was determined that the value of biochar was higher than AMF, especially in leaf number and shoot diameter, and the opposite situation was observed in leaf area and chlorophyll values. As the salt concentration increased in all Bc, Fm, and Vd applications, a decrease was observed in terms of the number of leaves, shoot diameter, leaf area, and chlorophyll values (Table 2).

Effects of *F. mosseae* and biochar on macro and micronutrient content under salt and *V. dahliae* stresses

The effects of AMF and biochar applications on K, P, Ca, and Mg content in pepper shoots grown under the presence of salt and *V. dahliae* are given in Table 3. As the salt concentration increases, P, Ca, and Mg values change according to the applications, while potassium values decrease. The highest values were found for P in Fm (3264.38 ppm), K in Fm + Bc (768.36 ppm), Ca in Fm + 100 mM (436.14 ppm), and Mg in Fm + 50 mM + Vd (211.07 ppm), respectively.

Therefore, the P, Ca, and Mg values of Fm and Bc alone increased in comparison to the control, and the difference between them was statistically significant ($p<0.05$), especially in P and K mineral substances. When Fm and Bc were compared only in terms of salt concentrations, Fm + 100 mM had the highest value of all mineral substances, while in Bc, the content of all nutrients except P decreased as the salt dose increased (Table 3). In Fm + Bc + Vd treatment, only P, K, and Mg values were found to be statistically insignificant ($p>0.05$), while the Ca value was significant ($p<0.05$). Under high-stress conditions (with salt and Vd interaction), the different treatments of Fm and Bc, Fm + Bc +100 mM + Vd, were highest for Phosphorus (P), while the combination Fm + Bc +150 mM + Vd was highest for other nutrients (K, Ca, and Mg) (Table 3).

Table 2. The effects of AMF, biochar, and *V. dahliae* on the number of leaves (pieces/plant), shoot diameter (mm), leaf area (cm^2) and chlorophyll in pepper grown under salt stress (mean±standard deviation)

Çizelge 2. AMF, biyokömür ve V. dahliae'nin Tuz stresi altında yetiştirilen biberde AMF, biyokömür ve V. dahliae'nin yaprak sayısı (adet/bitki), sürgün çapı (mm), yaprak alanı (cm^2) ve klorofil üzerine etkileri (ortalama±standart sapma)

Treatments	Number of Leaves (number plant^{-1})	Shoot Diameter (mm)	Leaf Area (cm^2)	Chlorophyll (SPAD)
Control	22.33±0.42 e-h	2.87±0.10 b-d	221.63±7.20 d	32.16±1.56 d-j
Fm	33.83±1.07 ab	3.98±0.07 a	367.31±6.47 b	38.54±1.52 a-e
Bc	30.83±0.79 bc	3.12±0.11 bc	287.16±9.83 c	37.28±0.54 a-g
50 mM	26.33±1.99 de	2.52±0.19 d-g	71.11±8.55 ı-k	43.73±1.75 a
100 mM	20.66±2.10 g-k	2.10±0.25 g-ı	67.12±9.51 ı-k	27.85±3.96 h-j
150 mM	15.16±1.66 mn	1.60±0.25 j-l	43.20±7.97 k-m	30.03±2.82 g-j
Vd	20.33±0.84 g-l	2.44±0.08 d-h	104.04±6.87 gh	41.89±1.70 ab
Fm+ Bc	36.83±0.60 a	4.20±0.21 a	413.65±14.84 a	36.13±0.83 a-g
Fm +50 mM	22.83±1.81 e-g	2.44±0.15 d-h	122.44±10.82 fg	34.17±3.33 b-ı
Fm + 100 mM	19.00±1.71 g-m	2.09±0.18 g-ı	69.63±7.92 ı-k	25.63±2.74 j
Fm +150 mM	16.66±1.20 j-n	2.12±0.20 g-ı	68.51±3.08 ı-k	30.23±3.62 f-j
Bc + 50 mM	21.50±1.70 f-i	2.56±0.11 d-g	91.17±6.07 hı	36.35±2.24 a-g
Bc + 100 mM	16.00±1.41 l-n	2.12±0.08 g-ı	58.90±6.63 j-l	28.15±2.39 h-j
Bc + 150 mM	17.33±2.76 i-m	2.35±0.11 e-h	56.86±7.18 j-l	26.59±3.04 ıj
Fm + Vd	27.66±0.71 cd	3.25±0.15 b	221.47±16.64 d	39.77±0.79 a-d
Bc+ Vd	20.00±0.57 g-l	2.46±0.09 d-g	125.05±20.65 fg	40.11±2.72 a-d
Vd+50 mM	21.66±0.71 g-ı	2.21±0.07 f-ı	75.90±3.23 h-j	39.39±1.69 a-e
Vd+100 mM	17.50±1.05 ı-m	2.08±0.09 gh	57.62±2.48 j-l	32.65±0.91 d-j
Vd+150 mM	8.33±1.85 o	1.10±0.14 m	27.32±7.30 m	14.30±2.44 k
Fm +Bc+50 mM	25.83±0.60 d-f	2.07±0.10 g-ı	132.88±9.16 f	41.02±1.61 a-c
Fm +Bc+100 mM	20.00±1.94 g-l	1.33±0.09 k-m	75.79±7.17 h-j	38.33±2.88 a-e
Fm +Bc+150 mM	18.00±0.73 h-m	2.14±0.08 g-ı	70.88±1.44 ı-k	35.24±1.33 b-h
Fm +Bc+Vd	26.33±0.80 de	2.79±0.15 c-e	170.30±8.90 e	35.97±0.76 a-g
Fm +50 mM+Vd	21.83±0.54 f-ı	2.32±0.17 e-h	92.38±2.55 hı	40.20±1.97 a-d
Fm +100 mM+Vd	21.16±0.79 g-j	2.34±0.04 e-h	87.76±3.99 hı	38.53±1.94 a-e
Fm +150 mM+Vd	20.83±0.47 g-k	2.42±0.05 d-h	84.01±6.75 h-j	37.16±1.79 a-g
Bc + 50 mM+ Vd	16.50±0.67 k-n	1.79±0.13 ı-k	67.75±5.93 ı-k	38.07±3.36 a-f
Bc + 100 mM+ Vd	12.50±1.83 n	1.52±0.24 j-m	56.98±7.58 j-l	33.37±2.91 c-j
Bc + 150 mM+ Vd	7.66±2.09 o	1.16±0.30 lm	37.81±7.84 lm	16.94±4.51 k
Fm +Bc+50 mM+ Vd	21.16±0.47 g-j	1.93±0.12 h-j	100.89±6.27 gh	39.65±1.60 a-e
Fm +Bc+100 mM+ Vd	21.66±1.22 f-ı	2.68±0.08 c-f	85.16±7.80 h-j	36.53±1.62 a-g
Fm +Bc+150 mM+ Vd	21.66±1.22 f-ı	2.39±0.09 d-h	82.93±4.29 h-j	31.56±1.73 e-j
$p^{treatment}$	p=0.0001	p=0.0001	p=0.0001	p=0.0001

Bc: Biochar application Fm: *F.mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level
All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to $p<0.05$.

In this study, it was determined that the phosphorus content of the Fm (3264.38 ppm) was higher than in most applications and that Bc had a positive effect with Fm in phosphorus intake in particular being under 50 mM and 100 mM salt conditions (Table 3). In terms of the P nutrient element, Fm + Vd (2552.75 ppm) showed a 63% increase compared to Vd (1566.90 ppm), and Bc + Vd (1357.98 ppm) showed a 13% decrease compared to Vd (1566.90 ppm). There was a 126% increase in K between the lowest value (Bc + 100 mM + Vd) and the highest value (Fm + Bc). Single binary combinations [Fm + 100 mM (0.86 ppm) and Bc + 100 mM (1.76 ppm)] slightly increased the potassium (K) content, while the triple interactions of Fm and Bc [Fm + Bc + 100 mM (2.32 ppm)] led to the significant increase in potassium content (Table 3). In the Ca parameter, a 40% increase was observed between Vd (212.76 ppm) and Fm + Vd (297.52 ppm), and a 7% increase was seen between Bc + Vd (226.83 ppm) (Table 3). This shows that the effect of Fm on the Ca value under the Vd stress was more than that of Bc. There was a 74% increase in Mg between the lowest (Fm+Bc+Vd) and the highest (Fm+150 mM+Vd) treatments (Table 3).

Table 3. The effects of AMF, biochar and *V. dahliae* on content of macro nutrient in pepper grown under salt stress (mean±standard deviation)

Çizelge 3. Tuz stresi altında Tuz stresi altında yetiştirilen biberde AMF, biyokömür ve V. dahliae'nin makro besin içeriği üzerine etkileri (ortalama±standart sapma)

Treatments	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
Control	2722.49±322.73 a-c	593.02±20.48 b-e	194.26±7.57 h	123.35±6.2 d
Fm	3264.38±577.98 a	626.05±33.04 a-d	236.65±2.79 e-h	152.86±12.73 a-d
Bc	2343.83±91.32 b-f	527.82±17.02 b-g	220.09±17.74 f-h	129.24±6.63 cd
50 mM	2050.03±309.08 c-ı	485.34±26.82 d-j	255.63±25.33 b-h	136.98±14.57 b-d
100 mM	1802.59±522.85 d-k	413.96±49.88 f-j	364.66±47.68 a-d	155.67±16.83 a-d
150 mM	2226.31±54.75 b-h	408.39±33.01 f-j	378.64±53.86 a-b	156.87±15.11 a-d
Vd	1566.90±122.95 e-k	444.14±41.76 e-j	212.76±13.37 g-h	128.65±11.31 cd
Fm + Bc	2951.00±757.11 ab	768.36±23.92 a	284.55±14.20 b-h	171.63±13.69 a-d
Fm +50 mM	1416.74±241.79 g-k	406.06±30.74 f-j	325.53±12.46 a-g	162.07±5.03 a-d
Fm + 100 mM	1815.00±45.85 e-k	513.40±36.17 b-ı	436.14±51.79 a	197.03±16.96 a-b
Fm +150 mM	1397.15±100.86 h-k	457.58±24.46 e-j	319.67±44.61 a-h	177.84±9.89 a-d
Bc + 50 mM	2095.51±102.35 c-ı	500.00±12.41 c-j	234.65±35.64 e-h	151.78±18.13 a-d
Bc + 100 mM	1495.09±133.32 f-k	434.72±49.84 e-j	319.47±22.37 a-h	141.14±26.40 b-d
Bc + 150 mM	1906.40±142.24 c-k	427.09±47.35 f-j	307.34±28.97 b-h	131.66±15.45 cd
Fm + Vd	2552.75±329.87 a-d	565.64±70.26 b-f	297.52±40.22 b-h	155.10±9.78a-d
Bc + Vd	1357.98±77.61 h-k	540.98±84.52 b-g	226.83±20.93 b-h	136.21±12.08 b-d
Vd +50 mM	1436.33±216.40 g-k	391.14±73.45 g-j	259.72±8.26 b-h	142.02±15.83 b-d
Vd +100 mM	1371.04±412.98 h-k	438.71±87.03 e-j	336.05±70.59 a-g	187.29±41.63 a-c
Vd +150 mM	1044.60±108.72 k	359.48±43.83 h-j	358.93±64.45 a-e	169.55±10.99 a-d
Fm + Bc +50 mM	1978.21±200.13 c-j	526.63±47.67 b-g	259.38±11.98 b-h	125.74±8.69 cd
Fm + Bc +100 mM	1638.72±107.34 e-k	509.12±33.05 b-ı	300.83±29.20 b-h	152.49±4.14 a-d
Fm + Bc +150 mM	1299.22±116.97 ı-k	375.67±26.58 g-j	303.91±34.43 b-h	136.30±5.81 b-d
Fm + Bc +Vd	2304.65±256.17 b-g	662.13±18.22 ab	246.61±24.59 c-e	121.62±12.14 d
Fm +50 mM+Vd	1958.63±218.23 c-g	653.46±79.25 a-c	293.06±37.97 b-h	150.03±14.88 a-d
Fm +100 mM+Vd	1403.68±106.81 h-k	532.27±8.13 b-g	276.13±29.82 b-h	179.91±21.08 a-d
Fm +150 mM+Vd	1808.47±142.78 d-k	524.02±79.12 b-h	369.09±75.69 a-c	211.07±53.17 a
Bc + 50 mM+ Vd	1083.77±25.00 jk	422.75±46.71 f-j	305.13±28.52 b-h	158.78±15.47 a-d
Bc + 100 mM+ Vd	1606.08±192.05 e-k	340.60±58.62 j	256.03±58.36 b-h	119.79±17.28 d
Bc + 150 mM+ Vd	2441.76±52.77 b-e	353.59±40.38 ı-j	286.00±26.63 b-h	140.18±8.63 b-d
Fm + Bc+50 mM+ Vd	1207.82±98.50 ı-k	380.67±28.66 g-j	242.37±8.50 d-h	147.97±7.82 b-d
Fm + Bc +100 mM+ Vd	1749.71±91.71 d-k	417.89±48.86 f-j	232.98±9.49 e-h	127.74±14.37 cd
Fm + Bc +150 mM+ Vd	1318.81±185.12 ı-k	419.01±45.37 f-j	340.08±24.37 a-f	156.00±17.19 a-d
$p^{treatment}$	p=0.0001	p=0.0005	p=0.0014	p=0.0459

Bc: Biochar application Fm: *F. mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level
All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to p<0.05.

The different combinations of AMF, biochar, salt and *Verticillium* wilt significantly affected (p≤0.05) the Zn, Mn and Fe contents of pepper shoots (Table 4). The lowest Zn value was in the Fm + Bc+ 100 mM (0.16 ppm) application, while the highest value was observed in the Fm + Bc+ Vd (0.58 ppm) application with an increase of 263% (Table 4). A 110% difference was found between the highest Mn value Bc + 150 mM + Vd (4.40 ppm) and the lowest Fm + Vd (2.09 ppm) applications (Table 4). Applications of Bc and Fm showed an increase in Mn compared to the control group (Table 4). While Fm (15.02 ppm) and Bc (16.42 ppm) values in the Fe parameter were higher than in the control (7.43 ppm), there was no statistically significant difference between their interactions [Fm + Bc (7.66 ppm)] (p> 0.05). Bilateral interactions of Fm and Bc in both biotic and abiotic conditions (such as Fm + salt, Fm + Vd, Bc + salt, Bc + Vd) increased the Fe value (Table 4).

Table 5 displays the effects of applying AMF, biochar, and *V. dahliae* on sodium, potassium/sodium ratio, and calcium/sodium ratio in pepper plants grown under salt stress. Table 5's treatment group discrepancies were statistically different (p< 0.05). The highest value in Na was in Fm + 100 mM (588.31 ppm), and the lowest value was in the control (31.95 ppm) group. The highest value in the K/Na ratio content was in the control (18.64), and the lowest value was in the Fm+100 mM (0.86 ppm) application, with a 95% decrease (Table 5). The highest Ca/Na ratio was in the biochar treatment group (6.61 ppm), and the lowest value was in the Fm +100 mM (0.73 ppm) applications, and the 89% decrease between them was determined to be statistically significant (p<0.05) (Table 5).

Table 4. The effects of AMF, biochar and *V. dahliae* on content of micronutrient in pepper grown under salt stress (mean±standard deviation)

Çizelge 4. Tuz stresi altında AMF, biyokömür ve V. dahliae'nin tuz stresi altında yetiştirilen biberde mikro besin elementi içeriği üzerine etkileri (ortalama±standart sapma)

Treatments	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)
Control	0.34±0.05 b-d	0.08±0.00 a	2.54±0.04 d-f	7.43±0.28 d
Fm	0.17±0.02 cd	0.12±0.01 a	3.77±0.22 a-e	15.02±3.54 b-d
Bc	0.21±0.05 b-d	0.08±0.00 a	3.10±0.12 a-f	16.42±3.02 b-d
50 mM	0.25±0.01 b-d	0.12±0.03 a	4.26±0.59 ab	14.99±4.87 b-d
100 mM	0.23±0.01 b-d	0.13±0.03 a	4.10±0.31 a-c	11.11±1.03 b-d
150 mM	0.25±0.01 b-d	0.13±0.05 a	3.14±0.08 a-f	9.83±0.51 b-d
Vd	0.32±0.05 b-d	0.11±0.02 a	4.02±0.08 a-d	27.41±7.87 a
Fm + Bc	0.27±0.07 b-d	0.11±0.01 a	3.82±0.63 a-e	7.66±0.11 cd
Fm +50 mM	0.19±0.00 cd	0.10±0.00 a	3.50±0.13 a-f	8.37±1.10 cd
Fm + 100 mM	0.20±0.02 b-d	0.09±0.02 a	3.14±0.10 a-f	8.82±1.08 cd
Fm +150 mM	0.21±0.03 b-d	0.14±0.04 a	2.52±0.18 d-f	8.19±0.96 cd
Bc + 50 mM	0.23±0.02 b-d	0.07±0.00 a	3.10±0.12 a-f	18.43±3.88 a-d
Bc + 100 mM	0.23±0.02 b-d	0.08±0.00 a	2.72±0.80 c-f	9.35±1.22 b-d
Bc + 150 mM	0.22±0.01 b-d	0.10±0.01 a	2.76±0.15 c-f	8.54±1.82 cd
Fm + Vd	0.26±0.05 b-d	0.10±0.02 a	2.09±0.48 f	17.47±5.07 a-d
Bc + Vd	0.22±0.03 b-d	0.10±0.02 a	2.88±0.18 b-f	14.75±2.96 b-d
Vd +50 mM	0.22±0.45 b-d	0.09±0.01 a	4.01±0.80 a-d	17.20±4.29 a-d
Vd +100 mM	0.21±0.06 b-d	0.08±0.01 a	3.91±0.44 a-e	14.71±2.13 b-d
Vd +150 mM	0.27±0.07 b-d	0.07±0.00 a	4.37±0.41ab	19.22±4.43 a-c
Fm + Bc +50 mM	0.38±0.07 bc	0.09±0.01 a	2.96±0.28 a-f	12.06±3.72 b-d
Fm + Bc +100 mM	0.16±0.01 d	0.10±0.01 a	2.74±0.06 c-f	7.37±1.14 d
Fm + Bc +150 Mm	0.22±0.01 b-d	0.08±0.00 a	3.58±0.37 a-f	12.52±1.98 b-d
Fm + Bc +Vd	0.58±0.18 a	0.07±0.00 a	2.48±0.19 e-f	7.65±0.67 cd
Fm +50 mM+Vd	0.37±0.08 bc	0.11±0.03 a	2.94±0.15 a-f	6.97±0.24 d
Fm +100 mM+Vd	0.18±0.00 cd	0.10±0.02 a	2.89±0.18 b-f	7.79±1.39 cd
Fm +150 mM+Vd	0.24±0.04 b-d	0.13±0.04 a	2.61±0.39 c-f	8.05±1.78 cd
Bc + 50 mM+ Vd	0.25±0.03 b-d	0.07±0.00 a	2.92±0.51 a-f	8.69±1.32 cd
Bc + 100 mM+ Vd	0.27±0.07 b-d	0.07±0.02 a	2.93±0.40 a-f	8.9±1.48 cd
Bc + 150 mM+ Vd	0.29±0.06 b-d	0.11±0.02 a	4.40±0.81 a	20.64±7.32 ab
Fm + Bc+50 mM+ Vd	0.21±0.01 b-d	0.10±0.03 a	4.38±0.37 ab	17.92±7.30 ad
Fm + Bc +100 mM+ Vd	0.40±0.10 b	0.10±0.01 a	3.23±0.66 a-f	9.59±2.07 bd
Fm + Bc +150 mM+ Vd	0.22±0.03 b-d	0.14±0.02 a	2.93±0.27 a-f	7.97±0.71cd
$p^{treatment}$	p=0.007	p=0.863	p=0.0010	p=0.0042

Bc: Biochar application Fm: *F.mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level
All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to p<0.05.

In the current study, as a result of Bc and Fm interactions, the Fm + Bc + 100 mM (237.34 ppm) application decreased salt stress compared to the Fm + 100 mM (588.31 ppm) application. It was determined that multiple interactions (Fm + Bc + 100 mM + Vd (150.06 ppm)) had much greater effects on Na stress. In general, the Na value of Fm applications was higher than that of Bc (Table 5). In addition, there is a 67% difference between the Fm + Bc (139.31 ppm) application and the Fm + Bc + Vd (45.66 ppm) interaction (Table 5). As the salt concentration increased, the K/Na ratio between applications changed (Table 5). The K/Na ratio of salt treatments was lower than those treated with Vd, and the difference was statistically significant (p<0.05) (Table 5). The effectiveness of Fm and Bc on the K/Na parameter differed according to both salt and Vd stress factors. It was determined that the combined use of Fm and Bc was more effective in the K/Na ratio when the ratios in salt treatments were considered in isolation. Fm alone was ineffective. The K/Na ratio of Fm+Vd (10.68) and Bc+Vd (10.86) increased by 23% and 25%, respectively, compared to Vd (8.68). Especially between Fm + Bc + 50 mM + Vd (4.66 ppm) and Fm + 50 mM + Vd (4.82 ppm), a 3% decrease was observed, while Bc + 50 mM + Vd (1.98 ppm) increased by 135%. While the Ca / Na value in Fm + 100 mM (0.73) and Bc + 100 mM (1.53) increased by 110%, it was determined that Bc was more effective than Fm under salt-stress conditions. However, the Ca/Na value of Fm was higher than Bc in the treatments which involved interaction with Vd.

Table 5. The effects of AMF, biochar and *V. dahliae* on Na (ppm), K / Na (ppm), and Ca / Na (ppm) in pepper grown under salt stress (mean±standard deviation)

Çizelge 5. AMF, biyokömür ve V. dahliae'nin Tuz stresi altında yetiştirilen biberde AMF, biyokömür ve V. dahliae'nin Na (ppm), K/Na (ppm) ve Ca/Na (ppm) üzerine etkileri (ortalama±standart sapma)

Treatments	Na(ppm)	K / Na(ppm)	Ca / Na(ppm)
Control	31.95±1.50 ı	18.64±0.81 a	6.09±0.20 ab
Fm	39.21±3.23 ı	16.19±1.18 b	6.13±0.42 ab
Bc	33.62±2.79 ı	15.92±0.95 b	6.61±0.49 a
50 mM	232.58±53.68 d-g	2.35±0.43 h-j	1.22±0.21 e-ı
100 mM	380.13±4.04 bc	1.20±0.16 ıj	1.04±0.11 g-ı
150 mM	316.87±12.44 b-e	1.29±0.09 ıj	1.18±0.14 e-ı
Vd	53.81±5.07 ı	8.68±1.57 d	3.99±0.20 c
Fm + Bc	139.31±10.84 gı	5.62±0.50 e	2.06±0.21 e-g
Fm +50 mM	168.87±34.19 f-ı	2.76±0.62 f-j	2.22±0.50 de
Fm + 100 mM	588.31±16.27 a	0.86±0.04 j	0.73±0.07 ı
Fm +150 mM	434.32±25.13 b	1.05±0.06 j	0.75±0.13 ı
Bc + 50 mM	232.81±29.80 d-g	2.25±0.29 h-j	1.10±0.27 f-ı
Bc + 100 mM	338.58±115.56 b-d	1.76±0.47 h-j	1.53±0.59 e-ı
Bc + 150 mM	379.28±43.79 bc	1.12±0.01 j	0.82±0.05 ı
Fm + Vd	53.35±1.39 ı	10.68±1.53 c	5.59±0.75 b
Bc + Vd	51.41±6.27 ı	10.86±1.91 c	4.46±0.17 c
Vd +50 mM	231.65±43.60 d-g	1.88±0.50 h-j	1.23±0.21 e-ı
Vd +100 mM	189.92±54.84 e-h	2.54±0.45 g-j	1.90±0.26 e-h
Vd +150 mM	340.75±18.51 b-d	1.05±0.11 j	1.05±0.16 g-ı
Fm + Bc +50 mM	150.98±12.45 f-ı	3.55±0.42 e-ı	1.75±0.07 e-ı
Fm + Bc +100 mM	237.34±37.96 d-g	2.32±0.42 h-j	1.38±0.29 e-ı
Fm + Bc +150 mM	273.31±25.85 c-g	1.40±0.14 ıj	1.14±0.16 f-ı
Fm + Bc +Vd	45.66±1.71 ı	14.54±0.55 b	5.38±0.42 b
Fm +50 mM+Vd	138.72±18.03 gı	4.82±0.48 e-f	2.16±0.22 d-f
Fm +100 mM+Vd	150.94±37.11 f-ı	4.00±0.65 e-h	1.98±0.21 e-g
Fm +150 mM+Vd	279.45±50.93 c-f	1.94±0.20 h-j	1.34±0.16 e-ı
Bc + 50 mM+ Vd	239.49±57.62 d-g	1.98±0.35 h-j	1.44±0.25 e-ı
Bc + 100 mM+ Vd	149.80±23.89 f-ı	2.28±0.14 h-j	1.68±0.15 e-ı
Bc + 150 mM+ Vd	283.07±50.89 c-f	1.34±0.21 ıj	1.16±0.30 e-ı
Fm + Bc+50 mM+ Vd	85.48±10.93 h-ı	4.66±0.60 e-g	3.05±0.57 d
Fm + Bc +100 mM+ Vd	150.06±21.39 f-ı	2.90±0.35 f-j	1.69±0.33 e-ı
Fm + Bc +150 mM+ Vd	393.94±25.62 bc	1.06±0.09 j	0.86±0.05 h-ı
$p^{treatment}$	p=0.0001	p=0.0001	p=0.0001

Bc: Biochar application Fm: *F.mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level
All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to p<0.05.

Soil pH and EC values

Table 6 displays how AMF, biochar, and *V. dahliae* applications affected the soil's pH (mS/cm) and EC (dSm-1) levels in peppers grown under salt stress. According to Table 6, the variations in soil pH and EC values across treatments are statistically significant (p<0.05). As the salt concentration increased, the pH values of Bc, Fm, and Vd applications decreased, and EC values increased (Table 6). The highest pH and EC values were in Bc+Vd (7.90 mS/cm) and Fm+150 mM applications, respectively. In our study, the pH values of the applications made with Vd and Fm were found to be higher than in the control group, and the difference was found to be statistically significant (p<0.05) (Table 6). In the EC parameter, an increase in EC values was observed as the salt concentration increased. It was determined that the EC values of Fm or Bc in individual salt interaction applications (Fm+salt or Bc+salt) were higher than for the triple combination (Fm+Bc+salt). Therefore, it was determined that the Fm + Bc interaction decreased the EC value despite the increasing salt doses (Table 6).

Correlation and Principal Components Analysis (PCA)

As a size reduction technique, PCA (Principal Components Analysis) is generally interpreted by considering the first two or three basic components. The connection between parameters and the differences between treatments was examined in our study using PCA analysis. Correlation analysis was conducted for the 17 features that were the subject of the study's examination. The correlation values of the relationships between these parameters are given in Figure 1. Correlations enclosed in rectangles are statistically significant. Blue indicates a positive correlation, and red indicates a negative correlation. Strong correlations are boxed and circles which indicate weak correlations are not boxed (Figure 1). The correlation coefficient takes values between -1 and +1. r=-1 denotes a negative linear relationship, r= +1 indicates a positive linear relationship, r=0 indicates no relationship between two variables. A value of 0.00 denotes no relationship, 0.01-0.29 indicates a low level of relationship, 0.30-0.70 indicates a medium level of relationship, 0.71-0.99 indicates a high level of relationship, and 1.00 indicates a relationship.

Table 6. The effects of AMF, biochar and *V. dahliae* on EC (dSm-1) and pH (mS/cm) in the soil in pepper grown under salt stress (mean±standard deviation)

Çizelge 6. AMF, biyokömür ve V. dahliae'nin Tuz stresi altında yetiştirilen biberde AMF, biyokömür ve V. dahliae'nin topraktaki EC (dSm-1) ve pH (mS/cm) üzerine etkileri (ortalama±standart sapma)

Treatments	Soil pH (mS/cm)	EC (dSm^{-1})
Control	7.26±0.08 f-k	661.00±103.76 j
Fm	7.64±0.06 bc	618.00±56.69 j
Bc	7.89±0.05 a	535.75±85.72 j
50 mM	7.18±0.05 h-k	2395.00±82.20 hı
100 mM	7.19±0.05 hk	4992.50±274.23 ef
150 mM	6.90±0.15 l	7305.00±498.77 c
Vd	7.54±0.05 b-d	358.50±22.48 j
Fm + Bc	7.48±0.10 c-f	1598.75±444.06 ıj
Fm +50 mM	7.27±0.11 f-j	3145.75±228.12 gh
Fm + 100 mM	7.05±0.03 kl	4372.50±462.73 fg
Fm +150 mM	7.06±0.02 j-l	12580.00±225.16 a
Bc + 50 mM	7.50±0.06 b-e	2587.50±185.53 hı
Bc + 100 mM	7.51±0.02 b-e	4897.50±506.48 ef
Bc + 150 mM	7.32±0.01 d-ı	7805.00±879.30 c
Fm +Vd	7.70±0.01 ab	440.75±47.45 j
Bc + Vd	7.90±0.12 a	491.00±65.58 j
Vd +50 mM	7.28±0.01 e-j	2617.50±108.50 hı
Vd +100 mM	7.26±0.05 f-k	3492.50±247.63 gh
Vd +150 Mm	7.18±0.01 h-k	5170.00±504.71 ef
Fm + Bc +50 mM	7.36±0.03 d-h	2662.50±220.24 hı
Fm + Bc +100 mM	7.38±0.03 d-h	5810.00±301.52 de
Fm + Bc +150 mM	7.24±0.01 g-k	6877.50±622.62 cd
Fm + Bc +Vd	7.40±0.12 d-h	930.75±115.84 j
Fm +50 mM+Vd	7.12±0.03 ı-k	2812.50±115.92 hı
Fm +100 mM+Vd	7.07±0.04 j-l	5537.50±410.637 d-f
Fm +150 mM+Vd	7.14±0.04 ı-k	9372.50±663.46 b
Bc + 50 mM+ Vd	7.47±0.04 c-f	2705.00±133.69 hı
Bc + 100 mM+ Vd	7.50±0.05 b-e	4342.50±742.61 fg
Bc + 150 mM+ Vd	7.42±0.03 c-g	6730.00±125.63 cd
Fm + Bc+50 mM+ Vd	7.54±0.09 b-d	2401.00±412.83 hı
Fm + Bc +100 mM+ Vd	7.33±0.05 d-ı	5577.50±590.35 d-f
Fm + Bc +150 mM+ Vd	7.41±0.08 d-h	6845.00±1127.16 cd
$p^{treatment}$	p=0.0001	p=0.0001

Bc: Biochar application Fm: *F.mosseae* Vd: *V. dahliae* Pn: P value indicating the importance level
All data were subjected to the Duncan multiple comparison test, and the difference between the treatment groups marked with the same letter was insignificant according to p<0.05.

According to Figure 1, there is a strong correlation relationship between SD - NL, LA- NL, LA- SD, Ca/Na - K/Na. CHL- NL, CHL – SD, P-NL, P-SD, P-LA, Fe-Mn, Na-SEC, K-NL, K-SD, K-LA, K-P, Ca- Na, Ca-Mg, K/Na-NL, K/Na- SD, K/Na- LA, K/Na- SpH, K/Na- P, K/Na- K, Ca/Na-NL, Ca/Na-SD, Ca/Na-LA, Ca/Na-SpH, Ca/Na-P, Ca/Na-K (Figure 1). SpH - SD, SpH-LA, Cu-SEC, Fe-SpH, K-CHL, Mg-SEC, Mg-Na, Ca-SEC, and Ca/Na-CHL show moderate correlation (Figure 1).

SEC-NL, SEC-SD, SEC-CHL, SEC-LA, SEC-SpH, P-SEC, Na-NL, Na-SD, Na-CHL, Na-LA, Na-SpH, Na-P, K-SEC, K-Na, Mg-SpH, Mg- Zn, Ca-NL, Ca-SD, Ca-CHL, Ca-LA, Ca- SpH, K/Na-SEC, K/Na-Na, K/Na-Mg, K/Na-Ca, Ca/Na- SEC, Ca/Na-Na, Ca/Na-Mg, Ca/Na-Ca all have a negative linear relationship (Figure 1). For the data showing a strong correlation relationship, shoot diameter, number of leaves, leaf area, Ca/Na - K/Na are effectively linearly related to each other despite salt stress conditions.

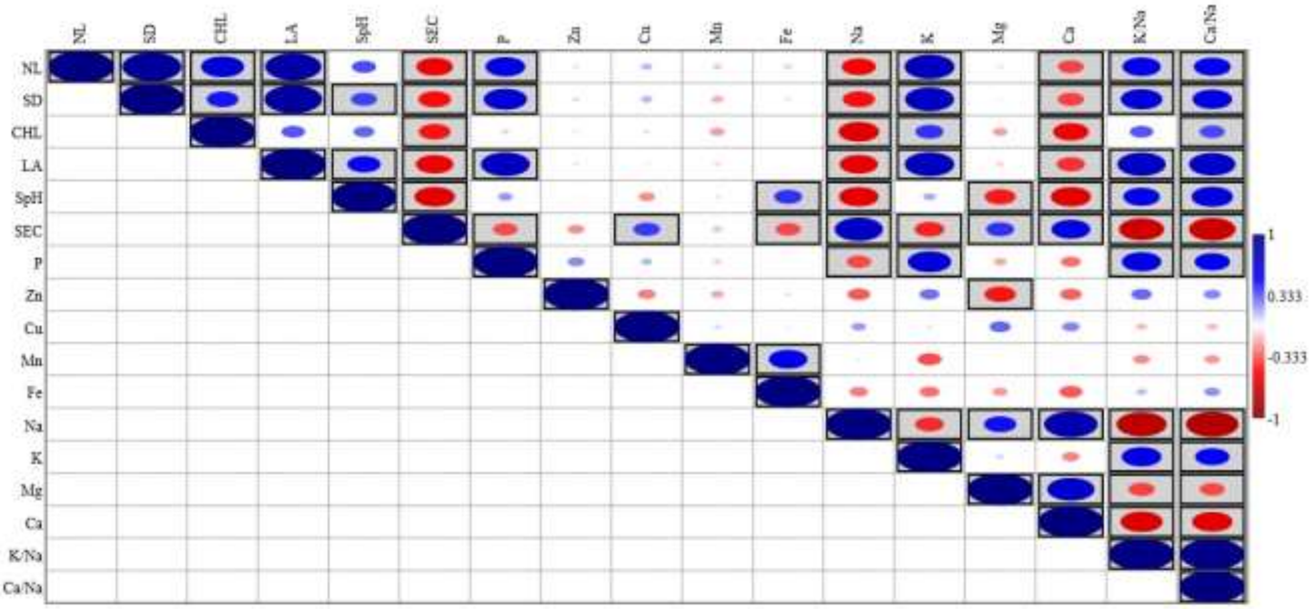

Figure 1. Pearson correlation coefficients between features.
Şekil 1. Özellikler arasındaki Pearson korelasyon katsayıları.
Values in bold frame are different from 0 and the significance level is alpha: 0.05.
NL: Number of Leaves, SD: Shoot Diameter, CHL: Chlorophyll, LA: Leaf Area, SpH: Soil pH, SEC: Soil EC, P: Phosphorus, Zn: Zinc, Cu: Copper, Mn: Manganese, Fe: Iron, Na: Sodium, K: Potassium, Mg: Magnesium, Ca: Calcium, K/Na: Potassium Sodium Ratio, Ca/Na: Calcium Sodium Ratio

Principal component analysis (PCA) was used to identify the variables that make up the variation. According to the analysis, the contribution of the eigenvalue and variance values to the features differentiating the applications was established (Table 7). The first four components with an eigenvalue larger than 1.00 in the PCA analysis of the 17 different features investigated in the study explained 77.15% of the total variation. To ascertain the suitable quantity of principle components in PCA, one may select components with eigenvalues exceeding 1 or those that account for a minimum of 67% of the total variance (Özdamar, 2010). The first two components in the current study accounted for 60.18% of the general variation (Table 7). The first component (PCA1) accounted for 43.66% of the variation, and the features that best explained the variation were the number of leaves, shoot diameter, leaf area, soil pH, soil EC, Na, K/Na, Ca/Na. Although the second component (PCA2) was responsible for 16.52% of the variation, this component's Cu, K, Mg, and Ca made the most significant contribution to justifying the variation. The third component (PCA3) explained 10.03% of the variation and provided the greatest contribution to variation in all parameters except Zn, Mn, and Fe. In the fourth component (PCA4), the chlorophyll, and P were the most explanatory features (Table 7).

By using a loading plot composed of PCA1 and PCA2 components, the interrelationships between the 17 variables evaluated in the study were determined (Figure 2). In this context, according to Figure 2, there is a positive correlation between Fe, Zn, K/Na, and Ca/Na. There is also a positive relationship between plant growth parameters, chlorophyll, P, and K. However, these parameters had a negative correlation with Ca, Mg, Cu, Mn, Na, and soil EC.

The effect of PCA1 and PCA2 components Fm and Bc on soil EC-pH, leaf mineral content, chlorophyll, and Vd, and growth parameters under salt stress were visualized using a score plot (Figure 3). It was observed that control, Bc, Vd, and 50 salt and Fm+ Bc+ 100+ Vd applications were located close to each other. Interactions with Vd and high salt dose were in the PCA1 negative and PCA2 positive regions, respectively. Fm was determined in the PCA1 and PCA2 positive regions of Fm+Bc, Fm+Vd, and Fm+50+Vd treatments. This was found to be correlated with how biotic and abiotic stress variables interacted (Figure 3). As a result, it was discovered that Fm was better than Bc at mitigating the damage caused by salt stress (Figure 3).

Table 7. PCA results regarding characters used in this study
Çizelge 7. Çalışmada kullanılan karakterlere ilişkin PCA sonuçları

	PCA-1	PCA-2	PCA-3	PCA-4
Eigenvalue	7.423	2.808	1.705	1.179
Variability (%)	43.664	16.520	10.032	6.935
Cumulative (%)	43.664	60.184	70.217	77.152
Factor loadings of parameters				
Number of Leaves	**0.290**	-0.266	0.142	0.148
Shoot Diameter	**0.289**	-0.273	0.138	0.005
Chlorophyll	0.212	-0.033	0.008	**0.661**
Leaf Area	**0.310**	-0.212	0.159	-0.145
Soil pH	**0.237**	0.232	0.142	0.139
Soil EC	**-0.290**	-0.188	-0.091	0.010
P	0.237	-0.217	0.041	**-0.465**
Zn	0.114	0.104	**-0.487**	-0.327
Cu	-0.063	**-0.326**	0.295	0.070
Mn	-0.060	0.218	**0.538**	-0.255
Fe	0.056	0.379	**0.458**	-0.147
Na	**-0.311**	-0.178	0.028	-0.143
K	0.256	**-0.336**	-0.114	-0.077
Mg	-0.157	**-0.369**	0.246	0.045
Ca	-0.269	**-0.280**	0.054	-0.152
K/Na	**0.327**	0.022	-0.072	-0.157
Ca/Na	**0.323**	0.062	-0.019	-0.098

* Numbers in bold are the highest values of the attributes.

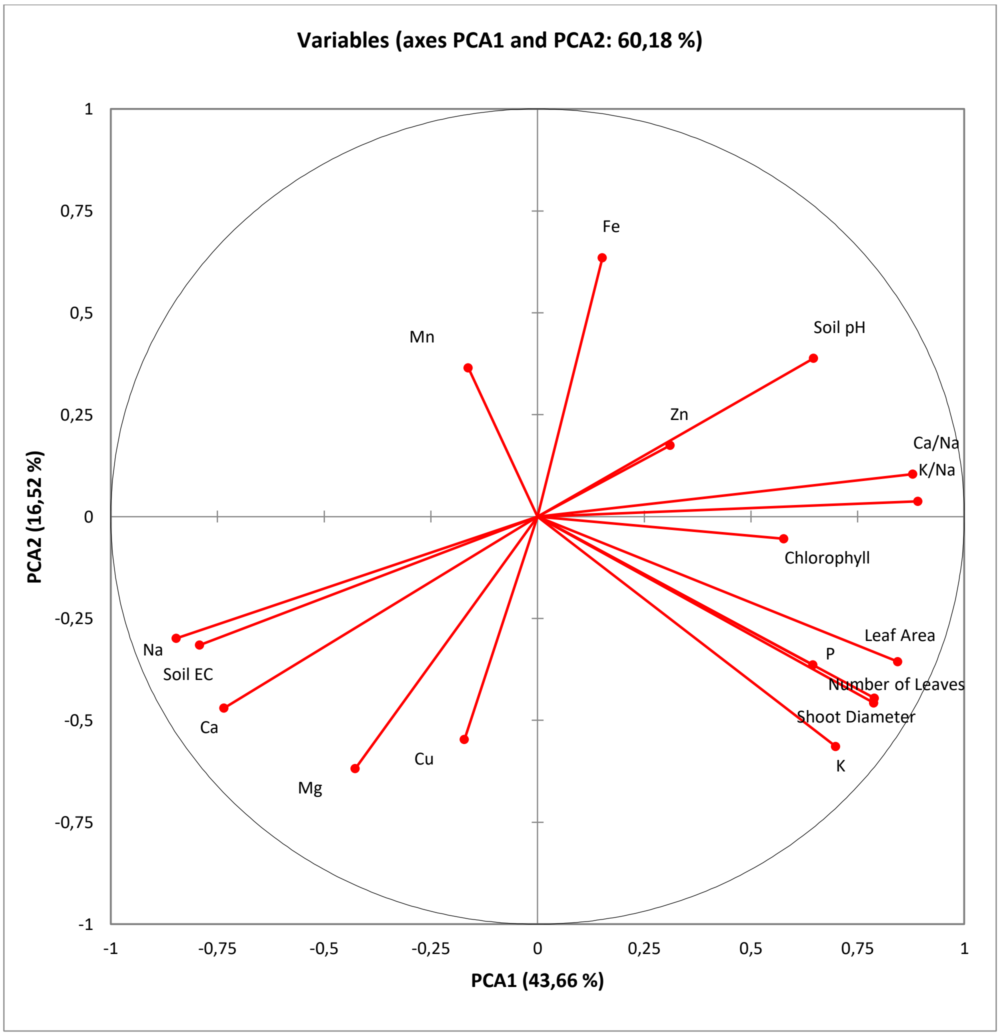

Figure 2. Character-based PCA loading plot for the first two major components
Şekil 2. İlk iki ana bileşen için karakter tabanlı PCA yükleme grafiği

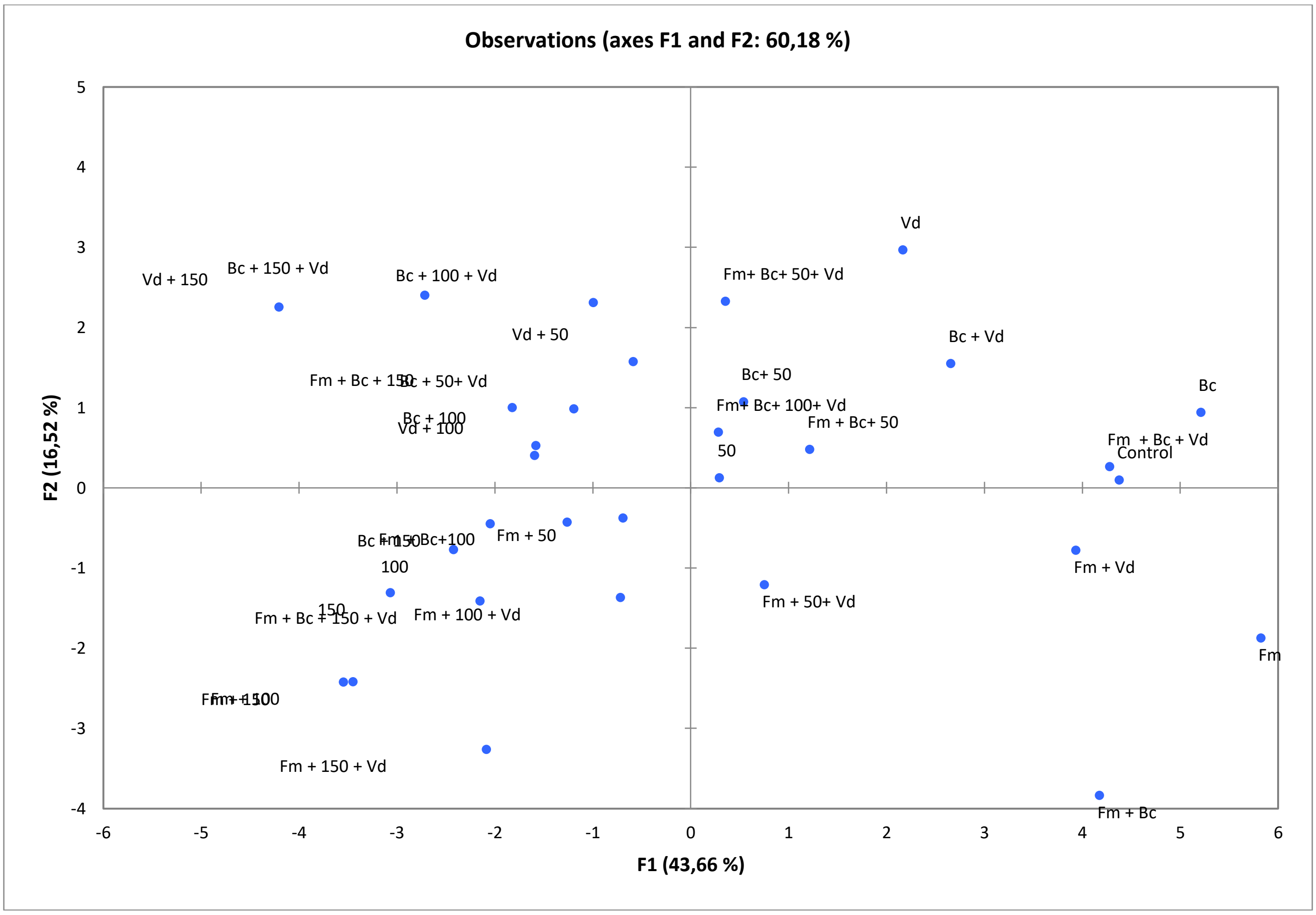

Figure 3. The score plot for the PCA analysis's initial two principal components

Şekil 3. PCA analizinin ilk iki temel bileşeni için skor grafiği

A heatmap graph was created to determine the relationship between 32 applications and 17 parameters (Figure 4). In the graph, the blue color indicates a low value, and the red color indicates a high value. The heatmap for the effects of *F. mosseae* and biochar against salt stress and *V. dahliae* on plant growth and nutrient content in pepper is given in Figure 4. The cluster is divided into two basic groups (top two rows) for parameters and two basic groups (five left rows) for applications. The first is the group from top to bottom between Fm+50 and Bc+50+Vd, and the other is the group between Bc+50 and Bc (Figure 4).

For the parameters in the first group (top right), treatments with 100 mM and 150 mM salt were found to result in colors (red) indicating higher temperatures. Vd also interacts with both Fm and Bc when high salt concentrations (100 and 150 mM) are used (Figure 4). In the first group (red color), there was a high correlation between Mg mineral matter Vd+100 and Fm 150+ Vd as well as between soil EC and Fm+150 (Figure 4). It was also found that the treatments in the first group were positively correlated with all mineral substances (except Zn) and negatively correlated with other parameters (Figure 4).

For the parameters in the second group (bottom right), Control groups (control, Fm, Bc) and low-stress (50 mM) treatments were found to have lower color (blue) temperatures (Figure 4). In the second group (blue color), a high correlation was discovered between Zn and Fm+Bc+100+Vd, Zn and Fm+Bc+50, Zn+Bc+Vd, Soil pH and Bc+Vd, Fe and Vd, K and Fm+50+Vd (bottom row from left) (Figure 4).

In the second group of treatments (bottom row from left), the physiological parameters of pepper (number of leaves, shoot diameter, leaf area, and chlorophyll), soil pH, and Zn values were associated with low-stress conditions or control groups (Figure 4). These parameters were more strongly associated with Vd treatments compared with salt concentrations (Figure 4).

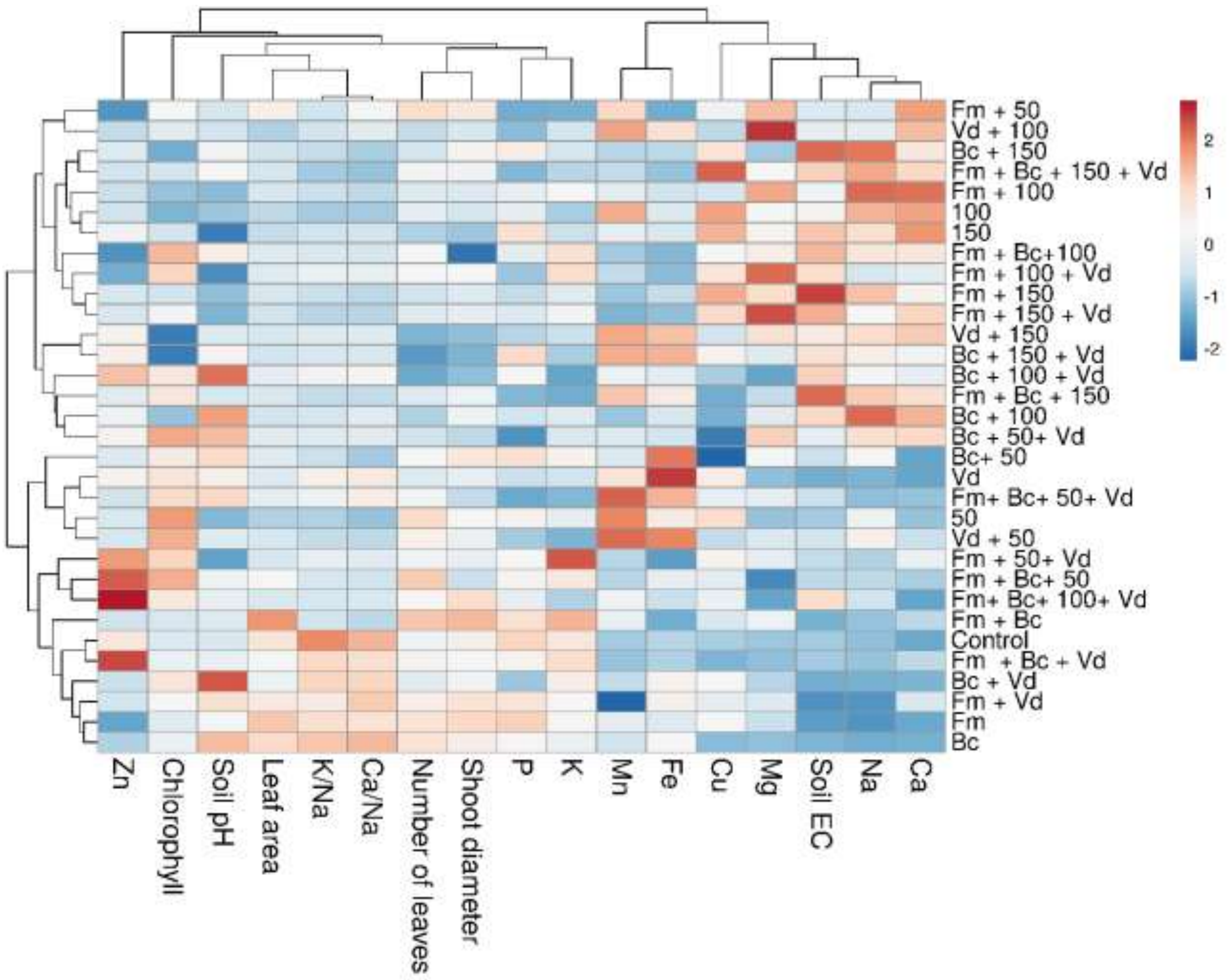

Figure 4. Heatmap of the effects of *V. dahliae* and *F. mosseae* and biochar on plant growth and nutrient content against salt stress in pepper

Şekil 4. Biberde tuz stresine karşı V. dahliae ve F. mosseae ile biyokömürün bitki büyümesi ve besin içeriği üzerindeki etkilerinin ısı haritası

DISCUSSION

This is the first study to investigate the effects of *F. mosseae* (Fm) and biochar (Bc) on plant growth, nutrient content, soil pH, and soil EC in peppers grown under different concentrations of salt stress and *V. dahliae* (Vd). As salt concentration increased, leaf number, leaf area, and chlorophyll values decreased, indicating that high salt stress concentration negatively affected plant growth (Table 2). In this study, the interaction of *Funneliformis mosseae* with 2% biochar increased the physiological parameters of pepper (leaf number 65%, shoot diameter 46%, leaf area 86%, and chlorophyll 36%). This can be explained by the change in plant cell structure and the inhibition of growth caused by excess salt in the soil (Sagar et al., 2021).

Plants that associate with AMF have been found to have increased resistance or tolerance to biotic and abiotic stresses (Jung et al., 2012; Li et al., 2013). Induced systemic resistance is the alteration of plant hormonal balance by beneficial microorganisms that affect pathogen performance in distant tissues (Van der Ent et al., 2009). Tolerance has been measured as an index that scores the physical difference between damaged and undamaged plants as the plant develops vegetatively despite pathogen infection (Gruntman and Novoplansky, 2011). AMF can stimulate systemic and local resistance against many pathogens or under abiotic stress conditions such as salt stress, as well as intervene or inhibit stress development directly (Köhl et al., 2019). Strategies and mechanisms by which AMF can influence disease development include competition with the pathogen, altering microbial density in the rhizosphere, and inducing resistance in plants (Pozo & Azcón-Aguilar, 2007). AMF, therefore, plays a protective role in plant-pathogen-abiotic stress interaction. AMF is a biotechnological strategy for plant protection optimization (Pozo et al., 2010).

Worldwide, the biochar + AMF combination has attracted attention for its wide range of applications, especially for nutrient cycling improvement (Castañeda et al., 2020), tolerance to abiotic stress factors (such as salt, drought, heavy metals) (Hashem et al., 2019) and use against plant pathogens (Gujre et al., 2021). In addition to the positive impact of biochar + AMF applications on soil improvement and plant growth, biochar provides a habitat for AMF. As a result, there are both complementary and opposing effects between them (Jaafar, 2014).

The results indicate that the interaction of pepper plants with Vd, whose resistance decreased due to salt stress, negatively affected plant growth criteria. It may be due to the synergistic interaction between biotic and abiotic stress factors. On the other hand, Fm by itself or in combination with Bc increased plant growth in both salt and Vd environments (Table 2). According to Akhter et al. (2015) and Giono et al. (2021), organic wastes not only lessen stress damage to plants but also aid nutrient intake for AMF development and even contribute to the symbiotic relationship. At the same time, it has been determined that the mineral substances absorbed by the biochar are transferred to the host plant via AMF hyphae, and the effect of the combination of AMF and biochar on plant growth is positive (Hammer et al., 2014; Zhuo et al., 2020; Were et al., 2021). Research conducted by Graber et al. (2010) determined that biochar contributes to the development of pepper plants being grown in nutrient-poor soils. Demir et al. (2015) reported that the interaction of AMF with different organic wastes increased plant growth parameters despite *V. dahliae* wilt and that this interaction could be an alternative application to combat biotic stress conditions.

Table 3 shows that phosphorus content, Ca, K, and Mg values varied according to the AMF inoculum and biochar ratio under biotic and abiotic stress conditions. The P value showed that Fm increased the phosphorus value compared to the control groups despite the salt stress, especially in the environment with Vd (Table 3). Beltrano et al. (2013) stated that the effect of AMF on plant growth is largely related to phosphorus uptake, that AMF increases the phosphorus value at all salinity levels, and that the phosphorus value of the plant is low at non-mycorrhizal high salt levels (100 mM and 150 mM). These results, which are compatible with our study, were discovered to be related to one of the mechanisms that increase the plant's tolerance to salinity. Phosphorus averages of three interactive applications (Fm + Bc + 50 mM etc.) were higher than the applications with four interactions (Fm + Bc + 100 mM + Vd etc.), and the difference between these applications was statistically significant ($p<0.05$) (Table 3). It has been reported that the pyrolysis temperature of the biochar affects the P uptake in the plant, the P value of the biochar pyrolyzed at low temperatures is high, and the P value of the biochar pyrolyzed at high temperatures is low (Xu et al., 2016).

Mycorrhizal fungi are at the heart of terrestrial food webs that support life on Earth. Helping to transport nutrients between ecosystems, mycorrhizal fungi are natural as well as ecologically important organisms (Hawkins et al., 2023). As a biotic component, AMFs obtain carbohydrates necessary for their vital activities from plant roots, while their hyphae act as capillary roots and are effective in the uptake of water and nutrients, especially some nutrients such as phosphorus (P), zinc (Zn), iron (Fe) and copper (Cu) (Fiorilli et al., 2015; Celik, 2023). Although the increase in nutrient content has no direct effect on the plant pathogen, these increases prevent the plant pathogen from further damaging the host plant (Bennett et al., 2006). The tendency of AMF to increase plant vigor may provide biological protection against pathogens. Although research findings on the effects of AMF and organic matter on nutrients under various stress situations differ, our investigation revealed that Fm had a greater impact on the Ca value in the medium with Vd than Bc (Table 3). In other studies, it has been determined that mycorrhiza applications increase the values of Mg, Cu, Mn, N, P, K, and Ca elements in some abiotic stress factors (Zhang et al., 2019a; Zhao et al., 2021). Karagiannidis et al. (2002) determined that under biotic stress conditions (*V. dahliae*), Ca, Mg, and K mineral substance values were similar to the control group, and the difference between them was statistically insignificant. Nzanza et al. (2012) reported that applications made including the interaction of *F.mosseae* and compost did not effect on the nutritional values of Ca, B, Cu, Mn, Na, and Zn.

For the K and Mg parameters, on the other hand, as salt doses increase, a decrease in K values and a change in Mg values are observed (Table 3). Biró et al. (2000) stated that Bc altered Ca, Mg, Fe, Zn, Cu, and S values according to mycorrhizal applications. However, in a different study, it was determined that mycorrhizal applications increased the K concentration in saline soils and the K, Ca, and Mg values of organic wastes (Giri et al., 2007). However, it has been reported in some studies that the opposite is true and that the Zn content of the applications containing Vd is lower than the control (Kesimci et al., 2019; Coşkun, 2021). Abd El-Mageed et al. (2020) reported that biochar increased the Zn value of pepper grown under salt stress and facilitated nutrient uptake. Therefore, in our study, the increase in the Zn in Fm + Bc + Vd interaction is associated with Bc despite Vd.

According to Table 4, Cu content was not statistically different between treatments, but the Manganese (Mn) value increased in Bc and Fm treatments compared to that of the control group. Nzanza et al. (2012) determined that *F.mosseae* and organic waste interaction applications did not affect Ca, B, Cu, Mn, Na, and Zn uptake in leaves, which supports the Cu uptake findings obtained in the study. In this study, the interaction of *Funneliformis* *mosseae* with 2% biochar increased the content of some mineral substances of pepper (P 63%, K 126%, Mg 74%, and Mn 110%). Cu values did not differ between treatment groups. Similarly, Abd El-Mageed et al. (2020) reported a decrease in Cu and Mn nutritional values in biochar applications. *V. dahliae* pathogenic treatments applied to different plants showed significant decreases in nutrient mineral values, especially Cu and Mn contents in comparison to the control (Demir et al., 2015).

In both biotic and abiotic conditions, the iron (Fe) element value of binary interactions (such as Fm + salt, Fm + Vd, Bc + salt, Bc + Vd) in the leaf showed an increase (Table 4). In another study by Vahedi et al. (2022), it was stated that the combination of AMF + biochar significantly increased the Fe (2.38) and Zn (1.29) values compared to the control group in terms of mineral substance value, while Biró et al. (2000) reported that the Fe concentration value of the groups that did not receive AMF was higher than the control. In line with these studies, it is believed that the applications made involving Bc increase the value of the Fe element.

In our study, Fm and Bc had a diminishing effect on the uptake mechanism at high salt concentrations. The effect of Bc treatments on the Na value varied according to the treatment groups (Table 5). In different studies, it has been determined that biochar promotes plant growth, increases salt tolerance, improves soil properties, facilitates nutrient uptake, and reduces Na uptake in both salt stress and non-stress conditions (Ali et al., 2017; Farhangi-Abriz and Torabyan, 2018).

In terms of the Na parameter of our study, Na values were lower in the treatments with Fm, Bc, and Vd interactions (Table 5). Giri et al. (2007) determined that the Na concentration value of the plant grown in a salty environment in mycorrhiza applications was lower than in the control group.

In general, the K/Na values in salt concentration applications were lower than those of the control groups. As the salt concentration increased, the K/Na ratio between applications varied (Table 5). Wu et al. (2010) determined that the Na value of the plant grown under salt stress decreased significantly, and the K, Mg, and K/Na ratios increased in applications with *F.mosseae*. Abdel Latef et al. (2014) stated that biochar facilitates plant nutrient uptake (N, P, Ca, and Mg) and increases the K/Na ratio. It was determined that the Ca/Na ratio changed according to the Bc and Fm application groups under stress conditions (Table 5). In similar studies, it has been reported that biochar incorporated into the soil to manage salt stress balances the water content in the soil due to its porous structure, increases the Ca value, and positively changes the Na amount (Zhang et al., 2019a). *F.mosseae* was determined to increase both the Ca/Na ratio and the Mg/Na ratio (Hajiboland et al., 2010; Wu et al., 2010).

As the salt concentration increased, the soil pH values of Bc, Fm, and Vd applications decreased, and soil EC values increased (Table 6). In the study conducted by Zhang et al. (2019b), the pH value did not increase while investigating the effect of biochar against salt stress; It was observed that the difference between other applications was insignificant and that the pH value of pyrolyzed biochar at 300 ^{0}C was higher than 600 ^{0}C. In our study, the soil pH values of the applications made with Vd and Fm were found to be higher than the control group (Table 6). In parallel with this, it is stated in some studies that the pH value in the soil may affect the microorganism density (Rousk et al., 2009; Dilegge et al., 2019). At the same time, it was determined that biochar, which increases the alkalinity level in the soil, inhibits the growth of fungi that prefer acidic environments (Yao et al., 2017; Shi et al., 2018; Ogundeji et al., 2021). In other studies, it is stated that biochar application can create an antagonistic effect against plant pathogens, change the chemical properties of the soil, and provide a better habitat for fungi (Liu et al., 2015; Dilegge et al., 2019; Ogundeji et al., 2021).

Fm or Bc had higher EC values in applications of individual salt interactions (Fm+salt or Bc+salt) than in the triple combination (Fm+Bc+salt). Thus, it was determined that, despite increased salt doses, the Fm + Bc interaction decreased the soil EC value (Table 6). Moreover, the interaction between *Funneliformis mosseae* and biochar reduced the soil EC value by 78% under severe salt stress conditions (150 mM). Regarding soil pH and Ca/Na ratio, biochar was shown to be more effective than *Funneliformis mosseae*. Azeem et al. (2019) found that high pH and EC values are related to the rate of biochar mixed into the soil. In other studies, conducted within this context, it was stated that mycorrhiza applications increase the salinity tolerance and decrease the soil EC value (Oztekin et al., 2013). Additionally, it has been found that biochar, which has a high cation exchange capacity and strong absorbent characteristics, removes toxic ions from the soil and releases beneficial ions (Novak et al., 2012). Therefore, these study findings support those of our study, and it is thought that the high pH value in applications using Bc is correlated with the pyrolysis temperature of the biochar.

CONCLUSION

In conclusion, biochar and *Funneliformis mosseae* application can minimize the severity of *Verticillium* wilt and enhance plant growth and stress tolerance in pepper plants grown under salt stress. Mycorrhizae in the soil and their interaction with biochar are a tremendous advantage for agricultural biodiversity considering climate change.

It works particularly well against plant diseases that are challenging to treat, such as the soil-borne *Verticillium* pathogen. Alternative approaches to sustainable agriculture are suggested for pepper production, such as the combination of AMF + biochar, which supports plant growth and resistance, is beneficial for the soil ecologically and environmentally friendly, and has a significant economic impact. This approach will impact different pathosystems, and the results will be meaningful for sustainable agriculture. Nonetheless, to completely comprehend the mechanisms underlying these benefits and to make the most effective use of these treatments in agricultural systems, further research is required.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors declare that there is no conflict of interest between them.

Research involving human participants and/or animals: Not applicable. The research involved no human participants or animals.

Competing interests There is no conflict of interest, according to the writers.

Data sets generated and analyzed during the current study will be provided by the corresponding author upon request of the editor or reviewers.

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