Evaluation of the Effects of Pine Cone (*Pinus spp.*) Biochar and Vermicompost on Drought Stress Tolerance in Iranian Borage (*Echium amoenum Fisch. & C.A.Mey*)

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Abstract

Low-input agriculture, through reducing the use of chemical fertilizers and incorporating organic soil amendments, represents an effective strategy for enhancing sustainable crop production and improving plant tolerance to environmental stresses. This study was conducted to evaluate the effects of the integrated application of chemical fertilizer, vermicompost, and biochar on drought stress tolerance in Iranian borage (*Echium amoenum*). The experiment was arranged as a factorial design based on a completely randomized design (CRD) with three replications. Experimental treatments consisted of three drought stress levels (90, 75, and 50% of field capacity) and three fertilization regimes: 100% chemical fertilizer (90 kg NPK ha^{-1}), 50% chemical fertilizer (45 kg NPK ha^{-1}) + vermicompost (25 g pot^{-1}; 0.625% w/w), and 50% chemical fertilizer + vermicompost + biochar (80 g pot^{-1}; 2% w/w). Morphological traits, photosynthetic pigments, and selected biochemical parameters were evaluated. The results showed that soil amendments markedly improved the growth and physiological responses of *E. amoenum* under drought stress. Under mild drought stress (75% field capacity), the application of 50% chemical fertilizer + vermicompost increased root length by 3.1-fold compared with the 100% chemical fertilizer treatment, while under moderate drought stress (50% field capacity), root length increased by 1.9-fold. Leaf area decreased by approximately 40% under moderate drought stress compared with non-stress conditions. Under moderate drought stress, the combined application of 50% chemical fertilizer + vermicompost + biochar increased carotenoid content by 1.7-fold and APX activity by 3.48-fold compared with the 100% chemical fertilizer treatment. Moreover, under moderate drought stress, MDA content in the 50% chemical fertilizer + vermicompost treatment was 55.1-fold higher than that in the 100% chemical fertilizer treatment. In addition, soluble carbohydrate content increased by 1.9-fold under mild drought stress following the application of 50% chemical fertilizer + vermicompost. Overall, vermicompost and biochar, particularly when combined with reduced chemical fertilizer, enhanced growth and physiological responses under drought stress, although their effects varied depending on drought intensity and amendment combination.

Keywords: Antioxidant enzymes, Oxidative stress, Photosynthetic pigments, Field capacity.

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Introduction

Medicinal plants are among the most important natural sources of bioactive compounds and play a significant role in meeting the demands of the pharmaceutical, healthcare, food, and cosmetic industries. Owing to their rich diversity of secondary metabolites, including polyphenols, flavonoids, terpenoids, and essential oils, these plants have long been valued in traditional medicine and have recently attracted

considerable attention in pharmaceutical research and the development of natural products. Among medicinal species, Iranian borage (*Echium amoenum Fisch. & C.A. Mey*.) (Boraginaceae) is one of the most valuable native medicinal plants of Iran. This biennial herb is highly valued not only for its economic importance but also for its sedative, anti-inflammatory, antioxidant, cardioprotective, and cold-relieving *properties,* making it an integral component of traditional Iranian medicine. Recent studies have further highlighted its potential in the production of pharmaceutical and cosmetic products as well as the development of natural health-promoting compounds (Sayyah et al., 2009; Asadi-Samani et al., 2014; Moghadam et al., 2021). In recent decades, climate change, declining precipitation, and increasing temperatures have made drought stress one of the most critical constraints limiting crop growth, productivity, and quality, particularly in medicinal plants. This challenge is especially severe in arid and semi-arid regions, where limited water availability disrupts physiological and biochemical processes, including photosynthesis, nutrient uptake, water balance, and cellular metabolism, ultimately affecting plant growth, productivity, and product quality (Smith et al., 2020). In addition to water scarcity, rising temperatures and the increasing frequency and duration of heat stress may further threaten agricultural production. Ramezani Etedali and Koohi (2026) investigated the potential effects of climate change on heat stress in strategic crops in Qazvin Province, highlighting the importance of developing adaptive strategies to maintain agricultural productivity under changing climatic conditions. Although their study focused on crops rather than medicinal plants, it provides relevant context for understanding the broader challenges associated with climate change. Furthermore, drought stress promotes the excessive generation of reactive oxygen species (ROS), leading to oxidative stress and damage to cellular membranes. Therefore, the development of effective and sustainable strategies to enhance plant tolerance to water deficit has become a major objective of agricultural research (Ahluwalia et al., 2021). Water deficit can adversely affect several interconnected physiological and biochemical processes, including plant growth, photosynthetic pigment accumulation, relative water content, gas exchange, and membrane stability. It may also induce changes in proline accumulation, lipid peroxidation, and antioxidant enzyme activity as plants respond to stress. Research on the medicinal plant Catharanthus roseus has examined the effects of water-deficit stress on morphophysiological and biochemical characteristics, emphasizing the importance of evaluating multiple plant responses when investigating drought tolerance (Haq Nari et al., 2026). Although the responses of individual plant species may differ, such research provides a useful basis for investigating the mechanisms through which medicinal plants respond to water limitation. Within the framework of sustainable agriculture, reducing dependence on chemical fertilizers through the use of organic amendments as alternatives or supplements has emerged as an effective approach for improving soil fertility and ensuring sustainable crop production. Among these amendments, vermicompost and biochar have received considerable attention because of their beneficial effects on the physical, chemical, and biological properties of soil. Vermicompost gradually supplies essential nutrients while improving soil structure, increasing cation exchange capacity, stimulating beneficial microbial activity, promoting root development, and enhancing soil water-holding capacity. These improvements facilitate water and nutrient uptake, thereby increasing plant tolerance to drought stress (Mahmud et al., 2019; Liu et al., 2019; Jabeen & Ahmad, 2019; Pierre-Louis et al., 2021). Biochar is another promising soil amendment produced through the pyrolysis of plant biomass, agricultural residues, and animal manures under oxygen-limited conditions. Its porous structure, relatively large specific surface area, and potential to retain water and nutrients can contribute to improvements in soil physical and chemical properties. Biochar application has been associated with changes in soil aeration, nutrient retention, microbial activity, and root development, although its effects depend on the feedstock, production conditions, application rate, and soil characteristics (Lehmann & Joseph, 2015; Keshavarz Afshar et al., 2017; Murtaza et al., 2023). In this context, Hajebi et al. (2026) investigated organic and inorganic amendments for improving the physical and hydraulic properties of saline soils. Their research addresses the potential role of soil amendments in modifying soil properties

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relevant to water movement and availability. However, the effects of different amendments may vary according to soil characteristics and amendment composition. Moreover, utilizing forestry residues, such as pine cones, as feedstock for biochar production may provide an opportunity to valorize biomass waste and support more sustainable resource management. The potential benefits of pine-cone-derived biochar for medicinal plants exposed to drought stress nevertheless require direct experimental evaluation. The application of biochar and vermicompost, individually or in combination, may provide complementary benefits for soil quality and plant performance. Biochar may contribute to soil porosity and water and nutrient retention, whereas vermicompost can provide nutrients and biologically active organic matter. Their combined application may therefore influence the soil environment and plant responses differently from either amendment applied alone. Nevertheless, the effectiveness of these amendments depends on their properties, application rates, soil conditions, plant species, and severity of water deficit. Evaluating their effects under controlled drought conditions is therefore necessary to determine whether they can help maintain plant growth and physiological functioning when water availability is limited. Several studies have demonstrated the potential of organic amendments to improve plant growth, physiological performance, and responses to environmental stresses. Khaghani et al. (2020) reported that drought stress affected growth indices of *Thymus daenensis*, whereas Chavoushi et al. (2020) investigated the responses of safflower to chemical treatments under drought stress. Likewise, Munir et al. (2020) reported improvements in chlorophyll content and growth of cucumber following the application of date-palm-derived biochar. Other studies have indicated that biochar and vermicompost, applied individually or alongside other fertilizers, may improve plant growth, physiological characteristics, and productivity under different environmental conditions (Saha et al., 2019; Baiamonte et al., 2020; Gao et al., 2020; Jabborova et al., 2021; Ranjbar et al., 2022; Abdelghany et al., 2023; Mumivand et al., 2023). Research addressing drought stress in medicinal plants further highlights the importance of identifying suitable soil amendments and management practices to sustain plant performance under water-limited conditions. Nevertheless, the effects of organic amendments vary across species and growing conditions, underscoring the need to evaluate specific amendment types and application rates for the target crop. In particular, further research is needed to clarify the individual and combined effects of pine-cone-derived biochar and vermicompost on the drought tolerance of Iranian borage. Considering the importance of developing sustainable strategies to improve drought tolerance in medicinal plants, the present study was conducted to evaluate the effects of pine-cone-derived biochar and vermicompost on the growth, physiological, and biochemical responses of Iranian borage (Echium amoenum Fisch. & C.A. Mey.) under drought stress. The study aimed to determine whether the individual and combined application of these organic amendments could alleviate the adverse effects of water deficit and improve plant performance. The findings are expected to contribute to a better understanding of the potential use of pine-cone-derived biochar and vermicompost in sustainable medicinal-plant production under water-limited conditions.

Materials and Methods

Experimental Site, Experimental Design, and Treatments

This experiment was conducted in the greenhouse of the Bardsir Higher Education Center of Agriculture, Shahid Bahonar University of Kerman, using Iranian borage (*Echium amoenum*). The experiment was arranged as a factorial design based on a completely randomized design (CRD), with three replications for each treatment. The experimental factors included: (1) three drought stress levels (90%, 75%, and 50% of field capacity) and (2) three soil amendment treatments: (i) 100% recommended NPK fertilizer rate (90 kg ha^{-1} each of N, P, and K), (ii) 50% of the recommended NPK fertilizer + vermicompost (25 g pot^{-1}; 0.625 w/w of soil), and (iii) 50% of the recommended NPK fertilizer + vermicompost (25 g pot^{-1}; 0.625 w/w of soil) + biochar (2% w/w of soil). To impose drought stress treatments, soil moisture content was monitored regularly using a Time Domain Reflectometry (TDR) device. Following the determination of soil field

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capacity, irrigation treatments were established at three moisture levels corresponding to 90%, 75%, and 50% of field capacity. Throughout the experimental period, volumetric soil moisture was continuously monitored using a TDR probe. The amount of water lost from each pot was calculated, and the required volume of water was added to maintain soil moisture at the designated field capacity levels.

Biochar Preparation

The biochar used in this study was produced from pine cones (*Pinus eldarica*). Pine cones were collected from public parks in Kerman during the winter season. After collection, the cones were cut into small pieces and thoroughly washed several times with deionized water to remove dust and other surface impurities. The cleaned samples were then air-dried under ambient conditions.

The dried cone pieces, measuring less than 2 cm in length, were wrapped in aluminum foil and subjected to pyrolysis at 500°C for 3 h. The programmed heating period was 20 min, and approximately 25 min was required to reach the target temperature of 500°C. The pyrolysis process was carried out in a laboratory electric furnace equipped with a digital temperature controller under oxygen-limited (semi-aerobic) conditions.

Determination of Physical and Chemical Properties of Biochar

After biochar production, its physical and chemical properties were determined according to the standard methods described by Song and Guo (2012). The results of these analyses are presented in Table 3.

Table 1. Soil physical and chemical characteristics of the experimental farm.

K	P	N	Organic matter	Organic carbon	pH	Ec	Filed capacity	Bulk density	Soil texture	Sand	Silt	Clay
---	$mg.kg^{-1}$	---	%	%		$dS.m^{-1}$	%	$g.cm^{-3}$		-----	%	-----
146	8.27	0.08	1.70	0.99	7.90	1.07	40	1.30	Sandy clay loam	65	12	23

Table 2. Physical and chemical properties of pine cone biochar.

Organic carbon	EC of saturation extract	pH	Apparent density	ash	performance
%	$dS.m^{-1}$		$g.cm^{3}$	%	%
70	2.16	6	71	6.90	76

Soil Preparation, Fertilizer Application, and Plant Cultivation

The soil used in this study was collected from the 0–30 cm surface layer and classified as Typic Petrogypsids. Before planting, the physical and chemical properties of the soil were determined using standard laboratory procedures. The results of the soil analysis are presented in Table 1.

The chemical fertilizers used in this experiment included urea, single superphosphate, and potassium sulfate. The fertilizer application rates were determined based on the soil test results and the nutritional requirements of Iranian borage (*Echium amoenum*), following previously reported fertilization practices for this crop (Amiri et al., 2022). In the 100% fertilizer treatment, NPK fertilizers were applied at a rate equivalent to 90 kg N ha^{-1}, whereas in the 50% fertilizer treatment, the application rate was 45 kg ha^{-1}. Vermicompost and biochar were incorporated into the soil according to the experimental treatments before filling the pots. Biochar was applied at a rate of 80 g pot^{-1}, while vermicompost was applied at a rate of 25 g pot^{-1}. The C/N ratio of the vermicompost was 15.

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Iranian borage seeds were sown at a depth of 2–3 cm, with 10 seeds per pot. The experiment was conducted under greenhouse conditions, where the temperature was maintained at 25 ± 5 °C, relative humidity at 60 ± 5%, and the photoperiod at approximately 12–13 h of light per day. To ensure uniform growth conditions and minimize environmental variation within the greenhouse, the positions of the pots were randomly rearranged twice a week. Nitrogen fertilizer (urea) was applied in two split applications, the first at sowing and the second after seedling thinning, both in conjunction with irrigation (Najafpoor Navaee, 2002).

Measurement of Morphological Traits

Morphological traits were evaluated at the end of the experimental period (150 days after sowing, at the 10-leaf stage). The measured parameters included root length, shoot and root fresh weight, shoot and root dry weight, and leaf area. Fresh weights were determined using a digital analytical balance with a precision of 0.0001 g, while root length was measured using a ruler. After carefully washing the roots to remove adhering soil particles, the plant samples were oven-dried at 70°C for 24 h, and their dry weights were subsequently recorded (Franks et al., 1997). Leaf area (LA) was determined using a leaf area meter. Healthy leaves from each pot were measured individually, and the total leaf area per pot was expressed in cm².

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Determination of Physiological and Biochemical Traits

Chlorophyll fluorescence parameters, including initial fluorescence (F_0), maximum fluorescence (Fm), and variable fluorescence (Fv), were measured using a PAM-2500 Chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany), and the performance index (PI) was measured at the end of the drought stress period using fully expanded leaves. After dark adaptation of the leaves, the Fv/Fm ratio, representing the maximum quantum yield of photosystem II (PSII), was calculated according to the method described by Baker and Rosenqvist (2004). The performance index (PI), an integrative chlorophyll fluorescence parameter reflecting the functional performance of PSII, was obtained directly from the instrument/software output.

Proline content was determined according to the method of Bates et al. (1973) based on the reaction with ninhydrin, and absorbance was measured at 520 nm. Malondialdehyde (MDA) content, as an indicator of lipid peroxidation, was determined through the thiobarbituric acid (TBA) reaction, and absorbance was recorded at 532 and 600 nm according to the method of Horst and Cakmak (1991).

For soluble sugar determination, alcoholic extracts were prepared from leaf samples, and sugar content was measured using the anthrone reagent at 625 nm according to the method of Paquin and Lechasseur (1979). Furthermore, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were determined after extraction with 80% acetone and measurement of absorbance at 470, 645, and 663 nm, based on the method of Lichtenthaler and Wellburn (1987).

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Determination of Antioxidant Enzyme Activities

For enzyme activity assays, protein extracts were initially prepared from leaf tissues using potassium phosphate buffer. Catalase activity (CAT; EC 1.11.1.6) was determined by monitoring the reduction in hydrogen peroxide (H_2O_2) absorbance at 240 nm according to the method of Velikova et al. (2000).

Guaiacol peroxidase activity (GPX; EC 1.11.1.7) was measured by monitoring the formation of tetraguaiacol at 470 nm following the method of Plewa et al. (1991). Ascorbate peroxidase activity (APX; EC 1.11.1.11) was determined based on the decrease in ascorbate absorbance at 290 nm according to the method of Nakano and Asada (1981). Total protein content was measured using the Bradford (1976) method.

Statistical Analysis

The experimental data were tested for normality and homogeneity of variances before statistical analysis. Data were analyzed using SPSS software version 16. Analysis of variance (ANOVA) was performed based on a factorial arrangement in a completely randomized design (CRD). Mean comparisons were conducted using the Least Significant Difference (LSD) test at a 5% probability level.

Results and Discussion

Morphological and Physiological Measurements

Root Length

Analysis of variance revealed that drought stress and its interaction with soil amendments had statistically significant effects on root length ($P \leq 0.01$ and $P \leq 0.05$, respectively; Table 3).

Table 3. Analysis of variance (mean square) for morphological and physiological traits of *Echium amoenum*.

Source of Variation	Mean Square			
	df	Root length	Dry weight leaf	Leaf area index
Drought	2	53.593**	2.901*	8731/.767**
Soil amendments	2	4.037^{ns}	0.197^{ns}	1025/.783^{ns}
Drought* Soil amendments	4	12.037*	1.403^{ns}	524.777^{ns}
Error	18	3.370	0.716	327.558
CV%		16.23	23.2	19.8

** Significant at the 5% probability level ($P \leq 0.05$); ** significant at the 1% probability level ($P \leq 0.01$); ns, non-significant.*

Mean comparison results showed that the highest root length was recorded under mild drought stress conditions (75% field capacity). In contrast, the lowest value was observed under moderate drought stress (50% field capacity), although the differences among some treatments were not statistically significant (Table 4).

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Table 4. Comparison of mean morphological and physiological traits of Iranian borage

Treatment	Drought			Soil amendments		
	90%	75%	50%	NPK100	N+V	N+V+B
Root length	9.11[a]	9.33[a]	5[b]	8.44[a]	7.11[a]	7.88[a]
Leaf Dry weight	1.51[a]	0.67[b]	0.43[b]	1.01[a]	0.71[a]	0.89[a]
Leaf area	73.72[a]	76.39[a]	21.16[b]	45.11[a]	65.60[a]	60.57[ab]

Columns with the same letters do not show a significant difference.

The evaluation of soil amendment effects showed that under mild drought stress conditions (75% field capacity), the application of 50% chemical fertilizer + vermicompost + biochar (N+V+B) resulted in the highest root length (11), representing a 37.5% increase compared with the100% chemical fertilizer treatment Under non-stress conditions (90% field capacity), the maximum root length was observed in the 100% chemical fertilizer treatment (12), while the N+V and N+V+B treatments resulted in 33.3% and 30.8% lower root lengths, respectively, compared with the control. Under moderate drought stress conditions (50% field capacity), the N+V treatment showed the same root length as the control treatment (5.3), whereas the N+V+B treatment resulted in an 18.9% reduction in root length compared with the100% chemical fertilizer treatment (Fig 1).

The reduction in root growth under drought stress conditions may be attributed to impaired cell division and expansion, decreased turgor pressure, and limited metabolic activity, which ultimately restrict plant growth. The enhanced root development observed in vermicompost-containing treatments can be associated with improvements in soil physical and chemical properties, increased water-holding capacity, enhanced nutrient availability, and the presence of humic compounds. These compounds stimulate cell division and root development, improve water and nutrient uptake, and consequently enhance plant tolerance to drought stress (Atiyeh et al., 2000; Pant et al., 2009).

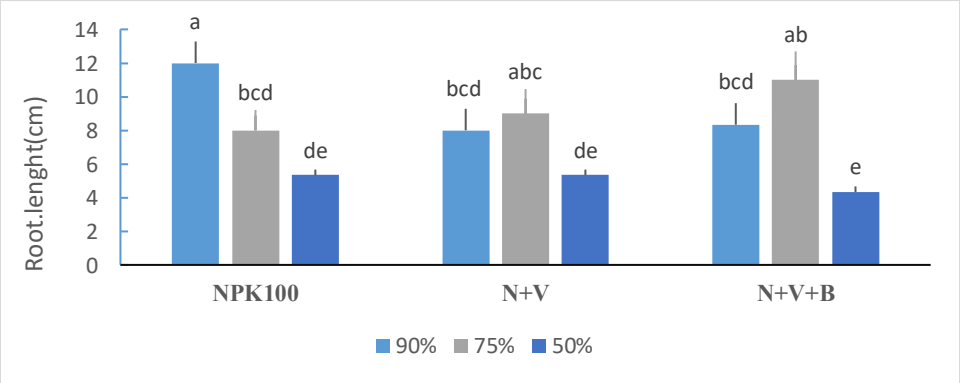


Fig 1. Effects of soil amendment treatments on root length under different drought stress conditions. NPK100, 100% chemical fertilizer (control); N+V, 50% chemical fertilizer + vermicompost; N+V+B, 50% chemical fertilizer + vermicompost + biochar. Drought stress levels were 90%, 75%, and 50% field capacity (FC). Means followed by different letters are significantly different according to the Duncan test at the 5% probability level ($P \leq 0.05$).

Leaf Dry Weight

Analysis of variance indicated that drought stress had a significant effect on leaf dry weight at the 5% probability level ($P \leq 0.05$; Table 3). Mean comparison showed that the highest leaf dry weight was recorded under non-stress conditions. In contrast, the lowest value was observed under moderate drought stress (50% field capacity), and the differences between these stress levels were statistically significant (Table 4).

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The reduction in leaf dry weight under water deficit conditions can be attributed to decreased tissue water content, stomatal closure, restricted carbon dioxide assimilation, and consequently reduced photosynthetic activity. In addition, reduced cell division and expansion, impaired nutrient uptake, and accelerated leaf senescence are among the factors contributing to decreased dry matter accumulation.

The results of the present study indicated that the effects of vermicompost-based amendments on root growth were dependent on drought stress intensity. Under mild drought stress (75% field capacity), the application of 50% chemical fertilizer + vermicompost + biochar resulted in the highest root length (11), representing a 37.5% increase compared with the 100% chemical fertilizer control (8). Similarly, the treatment containing 50% chemical fertilizer + vermicompost increased root length by 12.5% compared with the control. These findings suggest that the incorporation of organic amendments may improve root growth under moderate water limitation, possibly through improvements in soil water retention, soil physical conditions, and nutrient availability. This interpretation is consistent with previous reports indicating that vermicompost can partially alleviate drought-induced growth limitations by improving soil structure, increasing water-holding capacity, and enhancing nutrient availability (Kiran, 2019; Durukan et al., 2024). However, the response was not consistent under all drought conditions. At 50% field capacity, the vermicompost treatment produced root length similar to the control (0% change), whereas the vermicompost + biochar treatment resulted in an 18.9% reduction compared with the control. Under non-stress conditions (90% field capacity), both vermicompost-based treatments produced lower root length than the chemical fertilizer control, with reductions of 33.3% and 30.8% for the vermicompost and vermicompost + biochar treatments, respectively. Therefore, the present findings indicate that the beneficial effects of organic amendments were most evident under mild drought stress rather than being consistent across all water regimes. The differences among drought levels may reflect the interaction between amendment type and the severity of water limitation.

Leaf Area

According to the analysis of variance, drought stress had a significant effect on leaf area at the 1% probability level ($P \leq 0.01$; Table 3). Mean comparison revealed that leaf area significantly decreased with increasing drought intensity, with the greatest reduction observed under moderate drought stress (50% field capacity), showing approximately a 40% decrease compared with non-stress conditions (Table 4).

Drought stress reduced leaf area, whereas vermicompost application mitigated the adverse effects of water deficit by improving soil properties, increasing water retention capacity, and enhancing nutrient uptake.

Reduction in leaf area is considered one of the major adaptive mechanisms of plants under drought stress, as it decreases transpiration losses and contributes to water conservation within plant tissues. Under water deficit conditions, reduced turgor pressure, limited cell division and expansion, and decreased photosynthetic capacity restrict leaf development, ultimately resulting in reduced leaf area (Ahmad et al., 2024). The results of the present study are consistent with the findings of Talaat and Abdelsalam (2024), who reported significant reductions in leaf area and wheat biomass under drought stress conditions. They also demonstrated that vermicompost application partially compensated for the negative effects of drought stress by improving soil physical and chemical properties, increasing water-holding capacity, and enhancing nutrient uptake.

Photosynthetic Parameters

Chlorophyll a, Chlorophyll b, and Total Chlorophyll

Analysis of variance revealed that drought stress, soil amendments, and their interaction had significant effects on the contents of chlorophyll a, chlorophyll b, and total chlorophyll at the 1% probability level ($P \leq 0.01$; Table 5).

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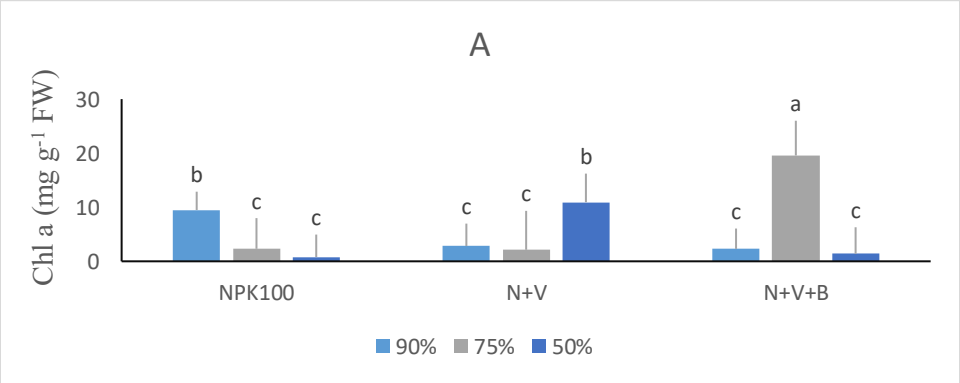
Table 5. Analysis of variance (mean square) for photosynthetic traits of *Echium amoenum*.

Source	Mean Square						
	df	Chl a	Chl b	Chltotal	Carotenoid	FV/FM	PI
Drought	2	34.962^{ns}	106.065**	261.020**	3.051*	0.030**	0.094**
Soil amendments	2	29.685^{ns}	108.818**	248.732**	0.295^{ns}	0.067**	0.007**
Drought* Soil amendments	4	208.082**	171.040**	738.521**	8.961**	0.028**	0.014**
Error	18	11.628	1.726	22.269	0.743	0.002	0.001
CV%		5.61	8.90	10.3	3.65	2.10	2.45

** Significant at the 5% probability level ($P \leq 0.05$); ** significant at the 1% probability level ($P \leq 0.01$); ns, non-significant.*

As shown in Figure 2 under non-stress conditions (90% field capacity), the highest chlorophyll a and total chlorophyll contents were obtained in the 100% chemical fertilizer treatment. However, with increasing drought intensity, the application of vermicompost and biochar significantly enhanced the photosynthetic pigment contents. Under mild drought stress (75% field capacity), the 50% chemical fertilizer + vermicompost + biochar treatment produced the highest chlorophyll a, chlorophyll b, and total chlorophyll contents (Fig 2). Furthermore, under moderate drought stress (50% field capacity), the 50% chemical fertilizer + vermicompost treatment resulted in the highest chlorophyll a content, whereas the combined application of vermicompost and biochar produced the highest chlorophyll b and total chlorophyll contents (fig 2).

The reduction in chlorophyll content under drought stress is primarily attributed to the excessive production of reactive oxygen species (ROS), chloroplast degradation, stomatal closure, and reduced nutrient uptake (Yu-Zheng et al., 2021). In the present study, the application of vermicompost and biochar improved the soil's nutritional and moisture status, thereby preserving photosynthetic pigments under drought conditions. thereby preserving photosynthetic pigments under drought conditions.



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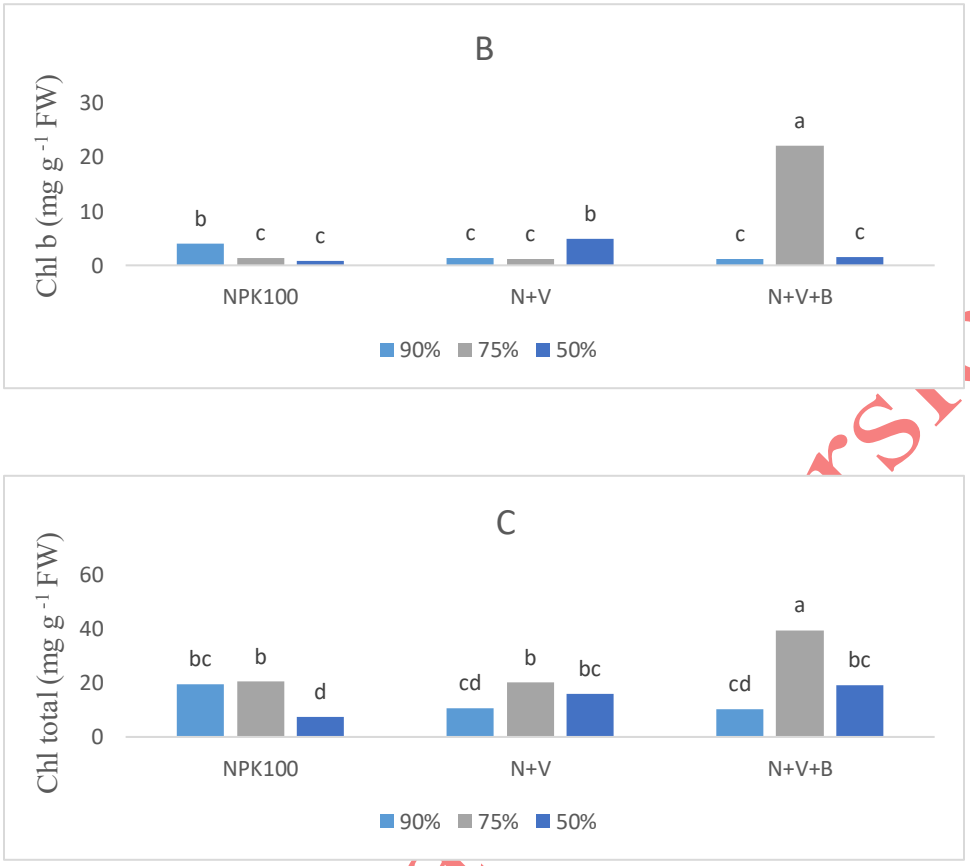


Fig 2. Effects of soil amendment treatments on leaf chlorophyll content under different drought stress conditions. (A) Chlorophyll a, (B) chlorophyll b, and (C) total chlorophyll. NPK100, 100% chemical fertilizer (control); N+V, 50% chemical fertilizer + vermicompost; N+V+B, 50% chemical fertilizer + vermicompost + biochar. Drought stress levels were 90%, 75%, and 50% field capacity (FC). Chlorophyll contents are expressed as mg g^{-1} FW (fresh weight). Means followed by different letters are significantly different according to the Duncan test at the 5% probability level ($P \leq 0.05$).

Carotenoids

Analysis of variance indicated that drought stress and the interaction between drought stress and soil amendments had significant effects on carotenoid content at the 1% probability level ($P \leq 0.01$; Table 5). As illustrated in Figure 3, carotenoid content decreased with increasing drought intensity. The highest carotenoid content was observed under non-stress conditions and mild drought stress (75% field capacity) in the 100% chemical fertilizer treatment. However, under moderate drought stress (50% field capacity), the 50% chemical fertilizer + vermicompost + biochar treatment exhibited the highest carotenoid content, resulting in a 1.7-fold increase compared with the 100% chemical fertilizer treatment.

Carotenoids are among the most important protective pigments in plants, playing a crucial role in scavenging reactive oxygen species (ROS) and preventing lipid peroxidation, thereby protecting chloroplasts and the photosynthetic apparatus from oxidative damage. The decline in carotenoid content under drought stress is primarily attributed to enhanced oxidative stress and chloroplast degradation. In the present study, the application of vermicompost and biochar maintained carotenoid content under drought conditions, which was likely associated with improved soil moisture and nutrient status, leading to reduced oxidative damage (kammann and Koyro, 2011).

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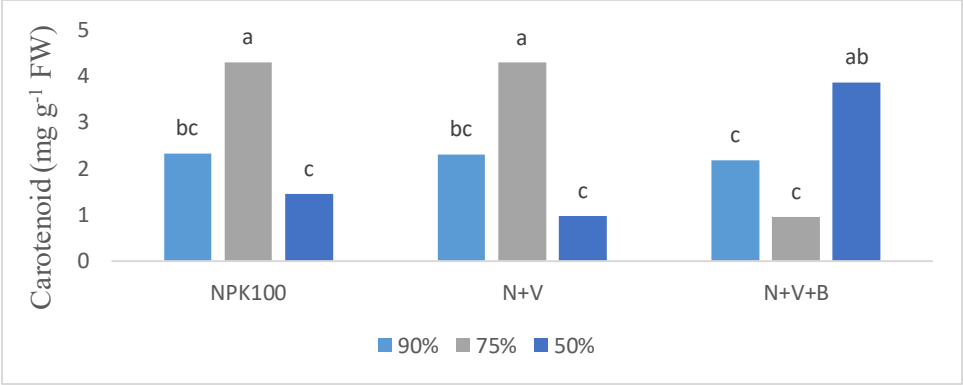


Fig 3. Effects of soil amendment treatments on leaf carotenoid content under different drought stress conditions. NPK100, 100% chemical fertilizer (control); N+V, 50% chemical fertilizer + vermicompost; N+V+B, 50% chemical fertilizer + vermicompost + biochar. Drought stress levels were 90%, 75%, and 50% field capacity (FC). Carotenoid content is expressed as mg g^{-1} FW (fresh weight). Means followed by different letters are significantly different according to the Duncan test at the 5% probability level ($P \leq 0.05$).

Chlorophyll Fluorescence Parameters

Analysis of variance revealed that drought stress, soil amendments, and their interaction had significant effects on chlorophyll fluorescence parameters (Fv/Fm and PI) at the 1% probability level ($P \leq 0.01$; Table 5). The performance index (PI) is an integrative chlorophyll fluorescence parameter reflecting the functional performance of photosystem II (PSII). As shown in Figures 4 and 7, both Fv/Fm and PI values decreased with increasing drought stress intensity. The highest Fv/Fm values under mild (75% field capacity) and moderate (50% field capacity) drought stress were recorded in the 50% chemical fertilizer + vermicompost treatment, whereas under non-stress conditions (90% field capacity), the 50% chemical fertilizer + vermicompost + biochar treatment exhibited the highest Fv/Fm value. Similarly, the highest PI value under non-stress conditions was observed in the 100% chemical fertilizer treatment, while under mild and moderate drought stress, the 50% chemical fertilizer + vermicompost + biochar treatment produced the highest PI values (Fig 4).

Chlorophyll fluorescence parameters are among the most reliable indicators for evaluating the performance of photosystem II (PSII) and the photosynthetic efficiency of plants. Reductions in Fv/Fm and PI indicate damage to PSII, disruption of electron transport, and a decline in photochemical efficiency under drought stress conditions (Baker, 2008). In the present study, the application of vermicompost and biochar improved these fluorescence parameters under drought stress, which is likely attributable to enhanced soil water and nutrient status, reduced oxidative stress, and improved stability of chloroplast membranes (Murchie & Lawson, 2013).

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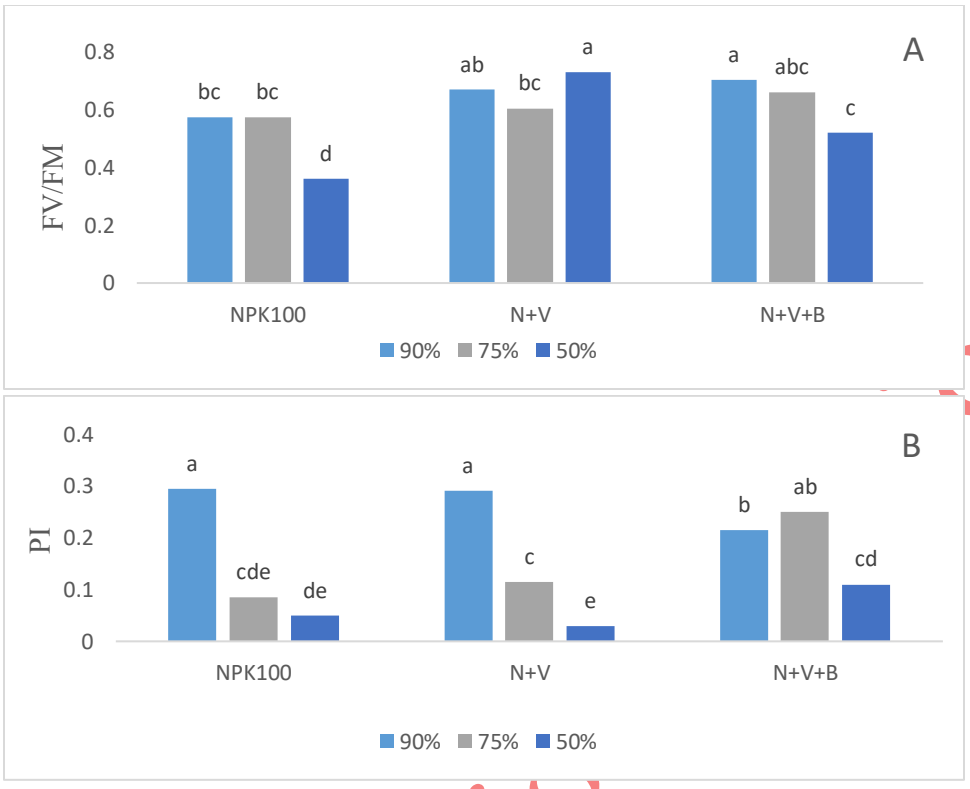


Fig 4. Effects of soil amendment treatments on chlorophyll fluorescence parameters under different drought stress conditions. (A) Maximum quantum yield of photosystem II (Fv/Fm) and (B) performance index on an absorption basis (PI). NPK100, 100% chemical fertilizer (control); N+V, 50% chemical fertilizer + vermicompost; N+V+B, 50% chemical fertilizer + vermicompost + biochar. Drought stress levels were 90%, 75%, and 50% field capacity (FC). Means followed by different letters are significantly different according to the Duncan test at the 5% probability level (P ≤ 0.05).

Biochemical Traits

Proline Content

Analysis of variance revealed that drought stress had a significant effect on proline content at the 1% probability level (P ≤ 0.01; Table 6). Proline is one of the most important osmo-protectants in plants, playing a crucial role in maintaining membrane stability, regulating osmotic balance, and alleviating damage caused by reactive oxygen species (ROS) (Ashraf & Foolad, 2007). In the present study, the reduction in proline content was likely associated with the severity of drought stress and the disruption of plant metabolic processes. It has also been reported that the proline response to drought stress depends on plant species as well as the intensity and duration of stress, and therefore does not always exhibit an increasing trend (Hayat et al., 2012).

Table 6. Analysis of variance (mean of squares) for biochemical traits of *Echium amoenum*.

Source	Mean Square							
	df	Proline	Carbohydrate	Protein	Catalaz	GPX	APX	MDA
Drought	2	0.831**	652.2256**	0.422**	0.000^{ns}	0.000*	0.037**	0.560**
Soil amendments	2	0.211^{ns}	339.794**	0.026^{ns}	1.168^{ns}	1.168^{ns}	0.011**	0.226**

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Drought* Soil amendments	4	0.158^{ns}	820.625**	0.090**	0.000^{ns}	0.000**	0.028**	0.242**
Error	18	0.119	4.088	0.013	2.943	2.943	0.001	0.000
CV%		22.45	12.43	9.27	10.7	18.44	20.23	0.00

** Significant at the 5% probability level (P ≤ 0.05); ** significant at the 1% probability level (P ≤ 0.01); ns, non-significant.*

Soluble Carbohydrate Content

Analysis of variance revealed that drought stress, soil amendments, and their interaction had significant effects on soluble carbohydrate content at the 1% probability level (P ≤ 0.01; Table 6). Soluble carbohydrate content decreased with increasing drought stress intensity. Under mild (75% field capacity) and moderate (50% field capacity) drought stress, the 50% chemical fertilizer + vermicompost treatment increased soluble carbohydrate content by 1.9-fold and 1.11-fold, respectively, compared with the 100% chemical fertilizer treatment. Under non-stress conditions (90% field capacity), the highest soluble carbohydrate content was observed in the 50% chemical fertilizer + vermicompost + biochar treatment (Fig 5).

Soluble carbohydrates are among the most important osmotic regulators in plants, contributing to the maintenance of turgor pressure, stabilization of cellular membranes, and mitigation of oxidative damage (Slama et al., 2007). The increased soluble carbohydrate content observed in treatments amended with vermicompost and biochar may be attributed to improved plant nutritional status, enhanced soil water retention, and the maintenance of photosynthetic activity. In addition to their protective role, soluble carbohydrates are involved in regulating plant physiological responses to drought stress (Devi et al., 2007). However, some studies have reported a reduction in soluble carbohydrate content following biochar application (Hafez et al., 2020).

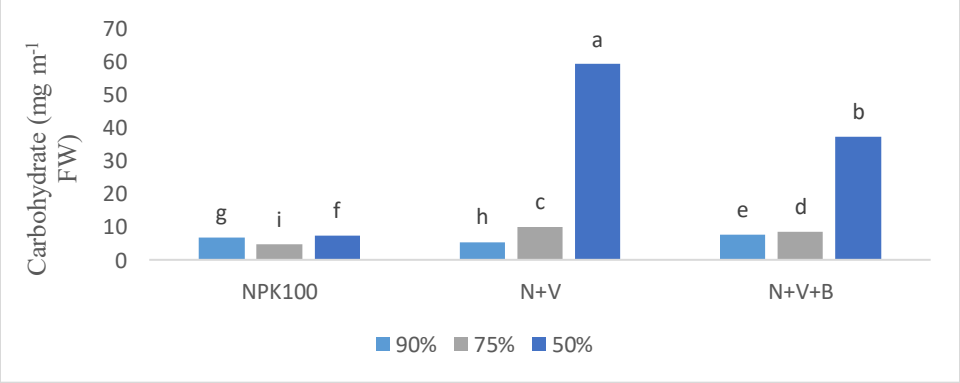


Fig 5. Effects of soil amendment treatments on soluble carbohydrate content under different drought stress conditions. NPK100, 100% chemical fertilizer (control); N+V, 50% chemical fertilizer + vermicompost; N+V+B, 50% chemical fertilizer + vermicompost + biochar. Drought stress levels were 90%, 75%, and 50% field capacity (FC). Soluble carbohydrate content is expressed as mg g^{-1} FW (fresh weight). Means followed by different letters are significantly different according to the Duncan test at the 5% probability level (P ≤ 0.05).

Total Protein Content

Analysis of variance revealed that drought stress and the interaction between drought stress and soil amendments had significant effects on total protein content at the 1% probability level (P ≤ 0.01; Table 6). Under non-stress conditions and moderate drought stress (50% field capacity), the highest total protein content was observed in the 50% chemical fertilizer + vermicompost + biochar treatment, representing

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approximately 1.0-fold and 1.2-fold increases, respectively, compared with the100% chemical fertilizer treatment. Under mild drought stress (75% field capacity), the highest total protein content was recorded in the 100% chemical fertilizer treatment, and significant differences were observed among the treatments (Fig 6).

The reduction in protein content under drought stress is likely attributable to the inhibition of protein synthesis and the enhanced degradation of proteins induced by oxidative stress. In contrast, the application of vermicompost and biochar helped maintain protein content by improving the nutritional status of the plants, enhancing nutrient availability, and reducing oxidative damage.

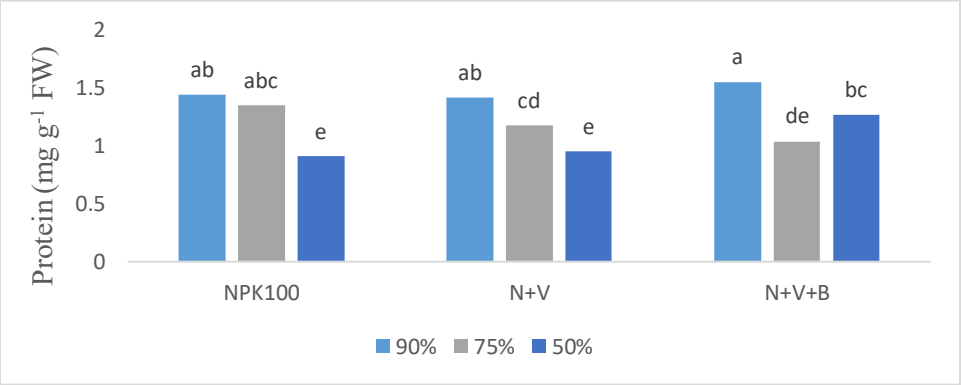


Fig 6. Effects of soil amendment treatments on soluble protein content under different drought stress conditions. NPK100, 100% chemical fertilizer (control); N+V, 50% chemical fertilizer + vermicompost; N+V+B, 50% chemical fertilizer + vermicompost + biochar. Drought stress levels were 90%, 75%, and 50% field capacity (FC). Soluble protein content is expressed as mg g^{-1} FW (fresh weight). Means followed by different letters are significantly different according to the Duncan test at the 5% probability level ($P \leq 0.05$).

Catalase (CAT) Activity

Analysis of variance indicated that drought stress, soil amendments, and their interaction had no significant effects on catalase (CAT) activity ($P > 0.05$; Table 6).

The absence of significant changes in CAT activity suggests that, under the conditions of the present study, the antioxidant defense system of *Echium amoenum* was likely mediated primarily through other antioxidant enzymes or non-enzymatic antioxidant compounds. Furthermore, the intensity and duration of drought stress may have influenced the lack of a significant CAT response (Gill & Tuteja, 2010; Hasanuzzaman et al., 2020).

Guaiacol Peroxidase (GPX) Activity

Analysis of variance revealed that drought stress and the interaction between drought stress and soil amendments had significant effects on guaiacol peroxidase (GPX) activity at the 1% probability level ($P \leq 0.01$; Table 6). Under non-stress conditions and mild drought stress (75% field capacity), the highest GPX activity was observed in the 50% chemical fertilizer + vermicompost treatment, showing a significant increase compared with the 100% chemical fertilizer treatment. Under moderate drought stress (50% field capacity), however, the highest GPX activity was recorded in the 100% chemical fertilizer treatment (Fig 7).

Guaiacol peroxidase is one of the key antioxidant enzymes involved in scavenging reactive oxygen species (ROS), thereby preventing lipid peroxidation and protecting cellular membranes from oxidative damage (Loreto & Schnitzler, 2010). The enhanced GPX activity observed in vermicompost-amended treatments may be attributed to improved plant nutritional status and a reduction in oxidative stress.

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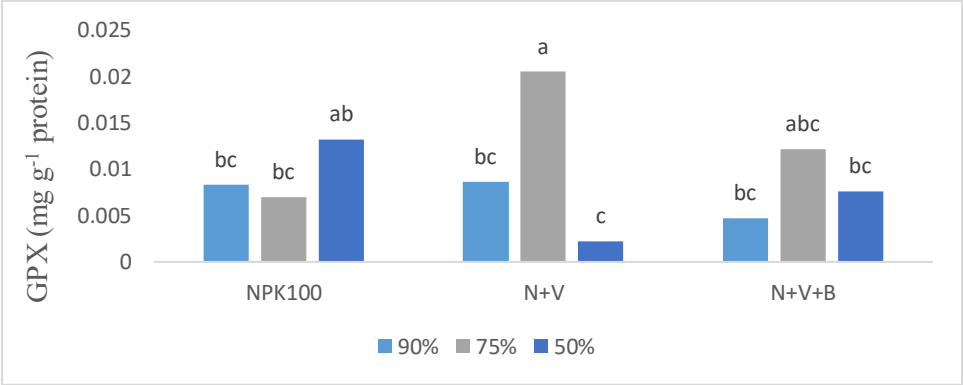


Fig 7. Effects of soil amendment treatments on guaiacol peroxidase (GPX) activity under different drought stress conditions. NPK100, 100% chemical fertilizer (control); N+V, 50% chemical fertilizer + vermicompost; N+V+B, 50% chemical fertilizer + vermicompost + biochar. Drought stress levels were 90%, 75%, and 50% field capacity (FC). Means followed by different letters are significantly different according to the Duncan test at the 5% probability level (P ≤ 0.05).

Ascorbate Peroxidase (APX) Activity

Analysis of variance revealed that drought stress, soil amendments, and their interaction had significant effects on ascorbate peroxidase (APX) activity at the 1% probability level ($P \leq 0.01$; Table 6). Under moderate drought stress (50% field capacity), the 50% chemical fertilizer + vermicompost + biochar treatment increased APX activity by 3.48-fold compared with the. the100% chemical fertilizer treatment Under mild drought stress (75% field capacity), the highest APX activity was observed in the 50% chemical fertilizer + vermicompost treatment, whereas under non-stress conditions (90% field capacity), the 100% chemical fertilizer treatment exhibited the highest APX activity (Fig 8).

Ascorbate peroxidase (APX) is one of the major antioxidant enzymes in plants, playing a crucial role in detoxifying hydrogen peroxide (H_2O_2) and protecting cells against oxidative damage under stress conditions. The enhanced APX activity observed in treatments amended with vermicompost and biochar indicates an improved antioxidant defense system, which contributes to increased drought tolerance by mitigating oxidative stress.

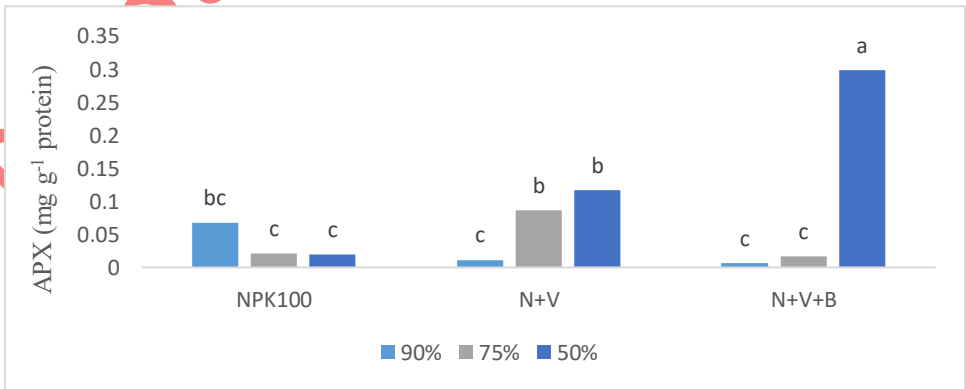


Fig 8. Effects of soil amendment treatments on ascorbate peroxidase (APX) activity under different drought stress conditions. NPK100, 100% chemical fertilizer (control); N+V, 50% chemical fertilizer + vermicompost; N+V+B, 50% chemical fertilizer + vermicompost + biochar. Drought stress levels were 90%, 75%, and 50%

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field capacity (FC). Means followed by different letters are significantly different according to the Duncan test at the 5% probability level (P ≤ 0.05).

Malondialdehyde (MDA) Content

Analysis of variance revealed that drought stress, soil amendments, and their interaction had significant effects on malondialdehyde (MDA) content at the 1% probability level (P ≤ 0.01; Table 6). Mean comparison results showed that MDA content increased with increasing drought stress intensity. The highest MDA content was observed in the 50% chemical fertilizer + vermicompost treatment, whereas the lowest value was recorded in the 100% chemical fertilizer treatment. Furthermore, under moderate drought stress (50% field capacity), the 50% chemical fertilizer + vermicompost treatment exhibited the highest MDA content, showing a significant difference compared with the100% chemical fertilizer treatment. Under non-stress conditions and mild drought stress (75% field capacity), the highest MDA content was observed in the 50% chemical fertilizer + vermicompost + biochar treatment (Fig 9).

Malondialdehyde is considered one of the most important indicators of lipid peroxidation and oxidative damage to cellular membranes in plants exposed to stress conditions. An increase in MDA content reflects enhanced oxidative damage caused by reactive oxygen species (ROS) accumulation (Gapinska et al., 2008). In the present study, increasing drought stress intensity was accompanied by elevated MDA accumulation, indicating an increase in oxidative stress in Iranian borage plants. However, the response of this parameter to vermicompost and biochar application was inconsistent with some previous studies that reported reductions in MDA content following the application of organic amendments (Mamnabi et al., 2020). This discrepancy may be attributed to differences in drought stress severity, biochar characteristics, amendment application rates, experimental conditions, or the specific response mechanisms of Iranian borage plants.

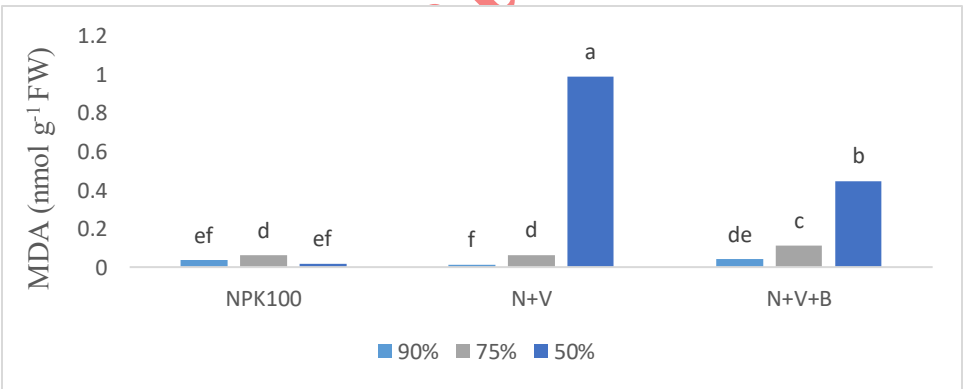


Fig 9. Effects of soil amendment treatments on malondialdehyde (MDA) content under different drought stress conditions. NPK100, 100% chemical fertilizer (control); N+V, 50% chemical fertilizer + vermicompost; N+V+B, 50% chemical fertilizer + vermicompost + biochar. Drought stress levels were 90%, 75%, and 50% field capacity (FC). Means followed by different letters are significantly different according to the Duncan test at the 5% probability level (P ≤ 0.05).

Conclusion

The results of this study demonstrated that drought stress significantly affected most morphological, physiological, and biochemical traits of Iranian borage (*Echium amoenum*). Among the morphological traits, the application of 50% chemical fertilizer + vermicompost improved root length, particularly under mild (75% FC) and moderate (50% FC) drought stress, promoting root-system development and enhancing water-uptake capacity. Furthermore, the combined application of vermicompost and biochar with 50%

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chemical fertilizer improved chlorophyll content, carotenoid concentration, and chlorophyll fluorescence parameters, thereby mitigating some of the adverse effects of drought stress on photosynthetic performance. At the biochemical level, the amended treatments enhanced the activity of specific antioxidant enzymes — notably ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) — and increased soluble-carbohydrate and total-protein contents, indicating a strengthened defense response, while catalase (CAT) activity was not significantly affected. Notably, the response of malondialdehyde (MDA) differed between the two amended treatments. Under severe drought (50% FC), the 50% chemical fertilizer + vermicompost treatment resulted in a pronounced accumulation of MDA, reflecting substantial oxidative damage to membranes. In contrast, when biochar was applied together with vermicompost and 50% chemical fertilizer, MDA accumulation was substantially moderated, indicating that the addition of biochar helped buffer oxidative damage and partly attenuated the stress-induced lipid peroxidation. This pattern suggests that the vermicompost + biochar combination offers more balanced protection of membrane integrity than vermicompost alone, and it underscores the value of including biochar in the amendment strategy. Overall, the integrated application of vermicompost and biochar with 50% of the recommended chemical fertilizer reduced the chemical-fertilizer requirement by half while largely maintaining growth and physiological performance, and it moderated drought-induced oxidative damage more effectively than vermicompost alone. This approach therefore represents a promising strategy for sustainable, low-input production of this valuable medicinal plant in arid and semi-arid regions. Given that the study was conducted under greenhouse conditions in a single season at one location, field-scale, multi-year validation is needed before recommendations can be extended to growers. Future research should also examine soil microbial dynamics, the long-term effects of biochar on soil fertility and water retention, and the economic feasibility of this low-input system.

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