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Original Article

# Evaluation of biochar and mycorrhiza effects on yield, quality and photosynthetic pigments of coneflower (*Echinacea purpurea* L.) under drought stress condition

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ABSTRACT

Cultivation of coneflower medicinal plant has long been of interest to farmers and researchers. Drought stress is one of the most important environmental stresses in plant cultivation, and providing solutions to combat it is very important. A study was conducted to investigate the effects of biochar and mycorrhiza on yield and some physiological traits of coneflower (*Echinacea purpurea* L.) under drought stress conditions. A split-plot factorial experiment was conducted over two-years (2023-2024) using randomized complete block design with three replications. Drought stress at three levels [D1: normal irrigation (control), D2: 50% F.C. and, D3: 70% F.C.] applied in the main plots and biochar at three levels: [B1: non-consumption (control), B2: 10 t.ha-1, and B3: 20 t.ha-1], and mycorrhiza symbiosis at two levels: [C1: non-consumption (control), C2: 10 g .seedling-1] were placed in subplots. The results showed that organic inputs increased the yields and physiological traits of coneflower and decreased drought stress effects. Interaction of normal irrigation (control) ×biochar (20 t.ha-1) × mycorrhiza improved dry matter yield (60.36%), flower dry weight (98.45%), essential oil yield (156.66%), compared to the drought stress 70% F.C. without biochar and mycorrhiza (lowest one). Moderate (50% F.C.) drought stress increased phenols and flavonoids compared to the other treatments. In general, severe drought stress (70% F.C.) significantly reduces productivity and is not recommended. However, in arid and semi-arid regions, the use of organic fertilizer like mycorrhiza and biochar as an environmental friendly inputs are recommended to reduce drought stress damage.



## 1. Introduction

Nowadays, considering the problems caused by the excessive use of chemical fertilizers in fields and soils, the use of biological sources of fertilizer in agriculture is more and more important than ever. One way to achieve sustainable agricultural goals is to use microorganisms that play an important role in providing the nutritional needs of plants. Although the use of biological fertilizers in agriculture has a long history, the scientific exploitation of such resources is not very recent. Biological fertilizers refer to products resulting from the activity of microorganisms that work in connection with nitrogen fixation or the availability of phosphorus and other nutrients in the soil (Khan et al. 2021). Mycorrhizal fungi are effective in absorbing nutrients such as phosphorus and nitrogen, as well as water absorption under stress conditions, producing plant hormones, modulating the effects of environmental stresses, increasing resistance to plant pathogens, intensifying biological nitrogen fixation activity, and improving quantitative properties (Ahammed et al. 2020). Researchers confirmed that biochar application can promote the quality of Panax quinquefolius by increasing the mycorrhiza symbiosis. This finding provides a theoretical basis for the observed effect of the co-application of biochar and AMF on the growth and active ingredient accumulation of Panax quinquefolius (Chen et al. 2025).

Drought stress and inefficient irrigation methods are the main factors limiting the development of the agricultural sector, especially in arid and semi-arid regions. Drought is one of the important stresses controlling crop yield and has been shown to affect many plant physiological processes. In situations of water scarcity, appropriate nutrient management can reduce the impact of drought on plant performance (Shanthi et al. 2023; Yang et al. 2021). One way to increase water efficiency is to maintain crop production at current levels while reducing water consumption. Adding biochar to soil can improve physical soil properties such as increasing specific surface area, water holding capacity, aeration and reducing soil erosion, chemical properties such as increasing cation exchange capacity, pH, organic carbon, element availability and reducing the risk of soil contaminants, and biological properties such as increasing colonization and protecting microorganisms from predators and increasing soil fertility and crop yield, carbon sequestration and preventing climate change and producing biofuels (Merlin et al. 2020; Khan et al. 2021). The use of biochar improves soil fertility (Melo et al. 2022; Jabborova et al. 2021; Wang et al. 2022). Other studies confirmed that wood biochar application increased green mint (Mentha viridis L.) yield and physico-chemical properties of a soil cultivated (Ouertatani, 2021). Also, wood biochar treatment promoted yield and quality of holy basil (Ocimum sanctum L.) (Singh et al. 2024).

The importance of medicinal plants lies in the presence of bioactive compounds contained in them. Use nutrients correctly during the growth stages of these plants not only raises the yield but also promotes the biosynthesis of the active ingredients quantitatively and qualitatively. Thus, proper fertilizer application (like biochar and mycorrhizal) play an important role in the successful cultivation of medicinal plants (Wu et al. 2021). Coneflower (*Echinacea purpurea* L.) is a medicinal plant commonly used for stamina enhancement and various diseases treatments. Coneflower products are widely used herbal remedies purported to alleviate common cold symptoms, bronchitis, and upper respiratory tract infections. Coneflower has also showed anti-inflammatory properties in various experimental models (Arifin et al. 2022; Vieira et al. 2023; Liu et al. 2021).Understanding the mechanisms of plant response to drought stress is essential in drought-prone areas. Since studies conducted to investigate the effects of organic inputs (biochar as a soil amendment and mycorrhiza as a biological fertilizer), and the interaction effects of these treatments under water deficit conditions on the growth and yield of the coneflower. In addition, their use leads to a reduction in the use of chemical fertilizers in agroecosystems. Also, the rate of water absorption and retention in this plant are limited, this experiment was conducted to investigate the physiological traits and quantitative and qualitative performance of the coneflower medicinal plant under non-stress and drought stress conditions.

## 2. Materials and Methods

### *2.1. Experimental design and agronomic practices*

This experiment was performed at the research farm of the Faculty of Agriculture and Natural Resources, Islamic Azad University of Karaj (Karaj, Iran), over two-years (2023-2024) on coneflower (*Echinacea purpurea* L.). The geographic coordinates of the experimental farm are 35˚49' N and 51˚6' E, with an altitude of 1321 m above sea level. The temperature and humidity conditions for the two years of the experiment are illustrated in Fig. 1. The soil was sampled before planting from a depth of 0-30 cm, and the soil characteristics are presented in Table 1.

**Table 1.** Physicochemical properties of the soil in the field before planting (0–30 cm depth)

| Soil Characteristic | 2023 | 2024 |
|---|---|---|
| Soil texture | Sandy clay | Sandy clay |
| pH | 7.5 | 7.7 |
| N ($mg.kg^{-1}$) | 6.71 | 5.28 |
| P ($mg.kg^{-1}$) | 8.42 | 8.56 |
| K ($mg.kg^{-1}$) | 342 | 356 |
| EC ($ds.m^{-1}$) | 1.6 | 1.5 |
| O.M (%) | 0.43 | 0.51 |

O.M: Organic matter, EC: Electrical Conductivity.

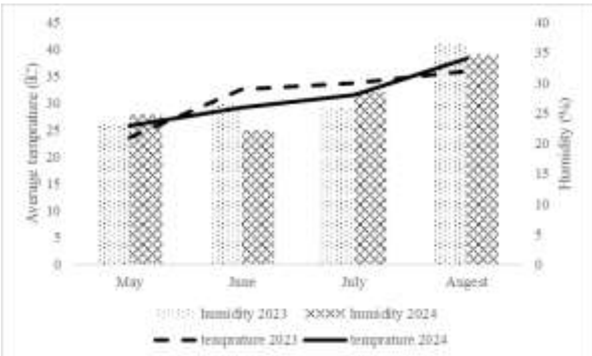


**Fig. 1** Climatology curve for Karaj city from May to August (2023-2024).

The experiment was conducted as a split-plot factorial with a completely randomized design with three replications. The experiment was carried out in two years (2023-2024), using the same location in both years and with the exact same treatment combinations. The treatments were: drought stress at three levels [$D_1$: normal irrigation (control), $D_2$: 50% F.C. and, $D_3$: 70% F.C.] as the main plots and biochar at three levels: [$B_1$: non-consumption (control), $B_2$: 10 $t.ha^{-1}$, and $B_3$: 20 $t.ha^{-1}$], and mycorrhizal symbiosis at two levels: [$C_1$: non-consumption (control), $C_2$: 10 g $seedling^{-1}$] were placed in subplots.

The seeds of the coneflower were sown in straw trays in early February. The seeds germinated in early March and after 60 days (in May) the seedlings were prepared and then transferred to the filed. The applied biochar has been made by the pyrolysis method, from forest trees wood. The applied biochar plus has been made by pyrolysis method, from forest trees wood, made in Mazandaran, Iran. The results of the chemical analysis of biochar are shown in Table 2. The arbuscular mycorrhizal fungi (AMF) spores number (~20 per 10 g soil) which was produced by Pishtaz Varian biotechnology company under the license and direct supervision of the Soil and Water Institute of Iran, in equal proportions of the three genus included *Funeliformis mossea*, *Rhizaphagus intraradices* and *Claroideoglomus etunicatum* (former scientific names, respectively: *Glomus mosseae*, *G. intraradices* and *G. etunicatum*) spores and hyphea, each other mycorrhized root fragments applied an stationary bearer (Attarzadeh et al., 2019).

**Table 2.** Chemical analysis of biochar

| Biochar characteristic | 2023 | 2024 |
|---|---|---|
| N (%) | 0.35 | 0.39 |
| P (%) | 0.19 | 0.18 |
| K (%) | 0.41 | 0.38 |
| Iron (%) | 0.46 | 0.41 |
| Calcium (%) | 4.59 | 4.72 |
| Oxygen (%) | 44.86 | 43.05 |
| Carbon (%) | 48.72 | 46.83 |
| Silicon (%) | 1.52 | 1.41 |
| Aluminium (%) | 0.19 | 0.18 |

Land preparation was done in April and manual planting (transplanting) was done in early May. Each experimental unit had 10 planting lines with a distance of 35 cm and a distance of 30 cm between plants in a row, the distance between plots within a block was one meter and between blocks was two meters. Drought stress was applied in the following way: first, the planting rows were irrigated in both non-stressed and drought-stressed conditions, and the volume of water consumed was measured by placing a graduated beaker under one of the holes in the beaker. Sampling was done from the root development depth (0-30 cm) after 24 hours of irrigation and weighing. The samples were transferred to an oven at 100°C and the soil moisture content was determined at the field capacity. Drought stress was applied at three levels (Rezaie Alulu et al. 2020). Biochar was added to the field soil before planting and mixed with the soil. Mycorrhiza was also given to the plants (10 g per plant) after the plants were established (14 days after planting). Weeds were controlled manually by hand without using any chemical inputs. No pesticides were used during the growth of the plants.

### *2.2. Yield and yield components of coneflower*

After 100 days (after full flowering), the coneflower plants were harvested. At the time of harvest, total dry matter yields (leaf + stem+ flower) were measured. Plant samples were harvested from a 1m$^2$ area of each plot and placed at room temperature (at 25°C) for 14 days. The number of flowers in plants were counted manually. Stem diameter was measured using a digital caliper.

### *2.3. Essential oil percentage and yield*

At harvesting time, the essential oil percentage was measured by extracting it from the dry plant (flowers) using the distillation method with water by the Clevenger device (for 3 hours), and finally, the essential oil content was calculated in milliliters per 100 g of dry matter. Essential oil content was obtained from the product of the flower dry matter yield in the essential oil content.

### *2.4. Photosynthesis pigments*

In 50% flowering stage, 0.5 g of leaf was mixed with 80% acetone and centrifuged, and after preparing the extract, the amount of light absorption was read by a spectrophotometer at wavelengths of 663, 645, and 470 nm for chlorophyll a, b, and carotenoids respectively and calculated by Equation 1, 2 and 3. Flower carotenoids were also measured using the leaf carotenoid method, with the difference that petal samples were used (Arnon, 1967).

Equation 1: Chl.$a$ (mg L$^{-1}$) = (12.25 * $A_{663}$) - (2.79* $A_{647}$)

Equation 2: Chl.$b$ (mg L$^{-1}$) = (21.5* $A_{647}$) - (5.1* $A_{663}$)

Equation 3: Carotenoids = 100 ($A_{470}$) - 3.27 (mg chl. $a$) – 104 (mg chl. $b$)/227

### *2.5. Total flavonoids and phenols compounds*

The aluminum chloride colorimetric method was used to determine the amount of flavonoids. The solutions were left at room temperature for 30 minutes. The absorbance of each reaction compound was measured at 415 nm with a spectrophotometer. A standard curve was prepared with methanolic quercetin solutions and obtained according to a linear relationship in mg dry weight (Chang et al., 2002). The amount of total phenolic compounds was determined using the Folin-Ciocaltio reagent. To measure the amount of total phenolics, about 1 g of powdered dry leaves of the plant was ground in 14 ml of methanol for 2 minutes and the resulting solution was centrifuged after passing through filter paper. Their absorbance was read at 765 nm with a spectrophotometer (Verza et al., 2007).

### *2.6. Statistics*

The data from the experiment was analyzed based on split-plot on randomized complete block design by using SAS statistical software package (ver. 9.4). The comparison of treatment means was made using Duncan test at a 5% probability level. Bartlett test results was shown in table 3.

**Table 3.** Bartlett test results

| Traits | *P* | *chi-square* |
|---|---|---|
| Plant height | 0.371 | 0.243 |
| Flower number in plant | 0.828 | 0.465 |
| Main stem diameter | 0.465 | 0.326 |
| Dry matter yield | 0.135 | 0.104 |
| Flower dry weight | 0.266 | 0.109 |
| Essential oil percentage | 0.298 | 0.165 |
| Essential oil yield | 0.465 | 0.305 |
| Total flavonoids | 0.765 | 0.565 |
| Total phenols | 0.809 | 0.325 |
| Chlorophyll *a* | 0.598 | 0.297 |
| Chlorophyll *b* | 0.627 | 0.408 |
| Total Chlorophyll | 0.329 | 0.198 |
| Carotenoids | 0.765 | 0.538 |
| Flower carotenoids | 0.326 | 0.155 |

**Table 4.** ANOVA for yield, yield compounds and essential oil of coneflower (*Echinacea purpurea* L.)

| S.O.V. | DF | Plant height | Dry matter yield | Flower dry weight | Flower number in plant | Main stem diameter | Essential oil percentage | Essential oil yield |
|---|---|---|---|---|---|---|---|---|
| Year | 1 | 5.83$^{ns}$ | 10.24$^{ns}$ | 15.97$^{ns}$ | 6.98$^{ns}$ | 1.35$^{ns}$ | 1.04$^{ns}$ | 16.72$^{ns}$ |
| Replication (year) | 4 | 10.35 | 16.83 | 28.12 | 13.84 | 4.06 | 3.16 | 21.48 |
| Drought stress (D) | 2 | 371.52$^{**}$ | 587.29$^{**}$ | 704.39$^{**}$ | 18.72$^{**}$ | 10.24$^{**}$ | 10.38$^{**}$ | 102.56$^{**}$ |
| Year × D | 2 | 12.48$^{ns}$ | 16.82$^{ns}$ | 28.76$^{ns}$ | 10.39$^{ns}$ | 2.55$^{ns}$ | 3.69$^{ns}$ | 10.39$^{ns}$ |
| Replication (year × D) | 8 | 11.83 | 21.90 | 35.43 | 15.43 | 5.61 | 8.45 | 15.77 |
| Biochar (B) | 2 | 405.69$^{**}$ | 605.43$^{**}$ | 713.59$^{**}$ | 97.34$^{**}$ | 11.82$^{**}$ | 11.03$^{**}$ | 205.36$^{**}$ |
| Year × B | 2 | 10.76$^{ns}$ | 9.73$^{ns}$ | 32.68$^{ns}$ | 22.46$^{ns}$ | 2.39$^{ns}$ | 2.53$^{ns}$ | 19.82$^{ns}$ |
| Mycorrhiza (M) | 1 | 249.83$^{**}$ | 556.63$^{**}$ | 468.99$^{**}$ | 87.59$^{**}$ | 10.46$^{**}$ | 9.72$^{**}$ | 176.91$^{**}$ |
| Year × M | 1 | 13.27$^{ns}$ | 11.52$^{ns}$ | 13.73$^{ns}$ | 38.60$^{ns}$ | 0.97$^{ns}$ | 1.56$^{ns}$ | 18.35$^{ns}$ |
| D × B | 4 | 305.89$^{**}$ | 428.61$^{**}$ | 807.54$^{**}$ | 10.35$^{ns}$ | 5.35$^{ns}$ | 3.83$^{ns}$ | 145.27$^{**}$ |
| Year × D × B | 4 | 9.73$^{ns}$ | 13.73$^{ns}$ | 32.91$^{ns}$ | 18.41$^{ns}$ | 10.09$^{ns}$ | 4.55$^{ns}$ | 13.86$^{ns}$ |
| D × M | 2 | 506.45$^{**}$ | 637.44$^{**}$ | 721.89$^{**}$ | 24.39$^{ns}$ | 8.63$^{ns}$ | 0.98$^{ns}$ | 392.54$^{**}$ |
| Year × D × M | 2 | 17.62$^{ns}$ | 25.39$^{ns}$ | 36.12$^{ns}$ | 32.45$^{ns}$ | 11.32$^{ns}$ | 1.28$^{ns}$ | 18.36$^{ns}$ |
| B × M | 2 | 635.31$^{**}$ | 635.72$^{**}$ | 684.19$^{**}$ | 10.77$^{ns}$ | 6.07$^{ns}$ | 5.19$^{ns}$ | 155.73$^{**}$ |
| Year × B× M | 2 | 22.74$^{ns}$ | 22.03$^{ns}$ | 44.25$^{ns}$ | 26.83$^{ns}$ | 8.34$^{ns}$ | 2.45$^{ns}$ | 19.61$^{ns}$ |
| D × B× M | 4 | 580.59$^{**}$ | 563.51$^{**}$ | 371.35$^{**}$ | 11.28$^{ns}$ | 4.85$^{ns}$ | 5.33$^{ns}$ | 238.94$^{**}$ |
| Year × D × B × M | 4 | 13.61$^{ns}$ | 10.86$^{ns}$ | 15.59$^{ns}$ | 16.05$^{ns}$ | 7.13$^{ns}$ | 9.21$^{ns}$ | 21.35$^{ns}$ |
| Error | 60 | 20.75 | 19.76 | 21.64 | 24.16 | 15.61 | 12.79 | 32.49 |
| C.V (%) | | 12.83 | 11.49 | 13.11 | 8.97 | 10.32 | 9.76 | 13.06 |

**Significant at $p < 0.01$. ns, no significance (at $p < 0.05$)

**Table 5.** Interaction of drought stress, biochar and mycorrhiza on plant height, dry matter yield, flower dry weight, essential oil yield and total flavonoids of coneflower (*Echinacea purpurea* L.)

| Treatments | | | Plant height (cm) | Dry matter yield ($g.m^{-2}$) | Flower dry weight ($g.m^{-2}$) | Essential oil yield ($g.m^{-2}$) | Total flavonoids ($mg.g^{-1}$) |
|---|---|---|---|---|---|---|---|
| Drought Stress | Biochar ($ton.ha^{-1}$) | Mycorrhiza(10 g $seedling^{-1}$) | | | | | |
| Normal irrigation (control) | 20 | 10 | 82.23$^{a}$ | 387.33$^{a}$ | 232.67$^{a}$ | 215.93$^{a}$ | 39.78$^{c}$ |
| | | 0 | 79.41$^{b}$ | 353.67$^{b}$ | 214.26$^{b}$ | 201.49$^{b}$ | 48.24$^{b}$ |
| | 10 | 10 | 79.06$^{b}$ | 350.43$^{b}$ | 212.37$^{b}$ | 193.28$^{c}$ | 47.82$^{b}$ |
| | | 0 | 75.58$^{c}$ | 328.52$^{c}$ | 191.37$^{c}$ | 172.25$^{d}$ | 44.07$^{c}$ |
| | 0 | 10 | 75.32$^{c}$ | 330.67$^{c}$ | 187.28$^{c}$ | 174.46$^{d}$ | 43.86$^{c}$ |
| | | 0 | 72.11$^{d}$ | 331.84$^{c}$ | 179.37$^{d}$ | 162.57$^{e}$ | 40.01$^{d}$ |
| 50% F.C. | 20 | 10 | 74.98$^{c}$ | 332.28$^{c}$ | 192.53$^{c}$ | 159.34$^{ef}$ | 51.42$^{a}$ |
| | | 0 | 72.35$^{d}$ | 318.04$^{d}$ | 190.43$^{c}$ | 160.81$^{ef}$ | 35.12$^{d}$ |
| | 10 | 10 | 71.05$^{de}$ | 321.55$^{d}$ | 191.67$^{c}$ | 161.73$^{ef}$ | 34.63$^{de}$ |
| | | 0 | 69.25$^{e}$ | 319.35$^{d}$ | 159.45$^{e}$ | 150.56$^{f}$ | 31.72$^{e}$ |
| | 0 | 10 | 69.28$^{e}$ | 315.67$^{de}$ | 177.28$^{d}$ | 140.39$^{g}$ | 31.86$^{e}$ |
| | | 0 | 65.45$^{f}$ | 308.43$^{e}$ | 158.45$^{e}$ | 129.43$^{h}$ | 28.11$^{f}$ |
| 70% F.C. | 20 | 10 | 65.56$^{f}$ | 314.52$^{de}$ | 157.76$^{e}$ | 128.56$^{h}$ | 28.01$^{f}$ |
| | | 0 | 63.79$^{fg}$ | 307.71$^{e}$ | 141.12$^{f}$ | 119.68$^{i}$ | 26.81$^{fg}$ |
| | 10 | 10 | 63.28$^{fg}$ | 289.67$^{f}$ | 156.42$^{e}$ | 119.24$^{i}$ | 27.37$^{fg}$ |
| | | 0 | 62.53$^{g}$ | 286.28$^{f}$ | 138.68$^{f}$ | 118.97$^{i}$ | 24.16$^{g}$ |
| | 0 | 10 | 60.32$^{h}$ | 273.16$^{g}$ | 140.72$^{f}$ | 95.89$^{j}$ | 20.33$^{h}$ |
| | | 0 | 58.04$^{i}$ | 241.53$^{h}$ | 117.24$^{g}$ | 84.13$^{k}$ | 17.08$^{i}$ |

Dissimilar letters indicate significant differences according to Duncan's test at the 5% level.

**Table 6.** Effect of drought stress, biochar and mycorrhiza on flower number in plant, main stem diameter, essential oil percentage and total phenols of coneflower (*Echinacea purpurea* L.)

| Treatments | Flower number in plant | Main stem diameter (cm) | Essential oil percentage (%) | Total phenols ($mg.g^{-1}$) |
|---|---|---|---|---|
| Drought Stress | | | | |
| Normal irrigation (control) | 11.07$^{a}$ | 0.39$^{a}$ | 0.62$^{a}$ | 40.21$^{b}$ |
| 50% F.C. | 8.33$^{b}$ | 0.31$^{b}$ | 0.51$^{b}$ | 51.32$^{a}$ |
| 70% F.C. | 5.67$^{c}$ | 0.22$^{c}$ | 0.38$^{c}$ | 29.56$^{c}$ |
| Biochar ($t.ha^{-1}$) | | | | |
| 20 | 15.43$^{a}$ | 0.41$^{a}$ | 0.79$^{a}$ | 54.83$^{a}$ |
| 10 | 11.33$^{b}$ | 0.33$^{b}$ | 0.64$^{b}$ | 48.72$^{b}$ |
| 0 (control) | 9.02$^{c}$ | 0.27$^{c}$ | 0.53$^{c}$ | 37.24$^{c}$ |
| Mycorrhiza (10 g $seedling^{-1}$) | | | | |
| 10 | 16.64$^{a}$ | 0.45$^{a}$ | 0.89$^{a}$ | 55.95$^{a}$ |
| 0 (control) | 11.32$^{b}$ | 0.31$^{b}$ | 0.62$^{b}$ | 38.72$^{b}$ |

Dissimilar letters indicate significant differences at the 5% level according to Duncan's test.

## 3. Results

### *3.1. Yield and yield compounds*

The analysis of variances presented that the plant height, dry matter yield and flower dry weight were influenced significantly by the main effects of drought stress, mycorrhiza, and biochar, also the interactions between drought stress ×biochar, drought stress × mycorrhiza, biochar× mycorrhiza, as well as the triple interactions between drought stress ×biochar × mycorrhiza ($p$ < 0.01) (Table 4). The flower number in plant and the main stem diameter were influenced significantly by the main effects of drought stress, mycorrhiza, and biochar ($p$< 0.01) (Table 4). In both years, with increased drought stress intensity, yield and yield compounds of coneflower decreased. In addition, biochar and mycorrhiza treatments improved yield and yield compounds of coneflower. Interaction treatment of normal irrigation (control) ×biochar 20 (ton.ha$^{-1}$) × mycorrhiza improved plant height (41.67%), dry matter yield (60.36%), and flower dry weight (98.45%) compared to the drought stress 70% F.C. without biochar and mycorrhiza (lowest one) (Table 5). In drought stress treatments, the normal irrigation (control) increased flower number in plant (95.23%), and the main stem diameter (77.27%) compared to the drought stress 70% F.C. In biochar treatments, biochar 20 (ton.ha$^{-1}$) raised flower number in plant (71.06%), and the main stem diameter (51.85%) compared to the control. In mycorrhiza treatments, usage of mycorrhizal improved flower number in plant (46.99%), and the main stem diameter (45.16%) compared to the control (Table 6).

### *3.2. Essential oil percentage and yield*

The essential oil percentage was influenced significantly by the main effects of drought stress, mycorrhiza, and biochar ($p$<0.01) (Table 4). Also the essential oil yield was influenced significantly by the main effects of drought stress, mycorrhiza, and biochar, also the interactions between drought stress ×biochar, drought stress × mycorrhiza, biochar× mycorrhiza, as well as the triple interactions between drought stress ×biochar × mycorrhiza were significant ($p$< 0.01) (Table 4). In both years, with increased drought stress intensity, essential oil percentage and yield of coneflower decreased. In addition, biochar and mycorrhiza treatments raised essential oil percentage and yield of coneflower. In drought stress treatments, the normal irrigation (control) increased essential oil percentage (63.15%) compared to the drought stress 70% F.C. In biochar treatments, biochar 20 (t.ha$^{-1}$) raised essential oil percentage (49.05%) compared to the control. In mycorrhiza treatments, usage of mycorrhiza improved essential oil percentage (43.54%) compared to the control (Table 6). Interaction treatment of normal irrigation (control) ×biochar 20 (t.ha$^{-1}$) × mycorrhiza improved essential oil yield (156.66%) compared to the drought stress 70% F.C. without biochar and mycorrhiza (lowest one) (Table 5).

### *3.3. Total flavonoids and total phenols*

The analysis of variances showed that the total flavonoids was influenced significantly by the main effects of drought stress, mycorrhiza, and biochar, also the interactions between drought stress ×biochar, drought stress × mycorrhiza, biochar× mycorrhiza, as well as the triple interactions between drought stress ×biochar × mycorrhiza were significant ($p$<0.01) (Table 7). The total phenols was influenced significantly by the main effects of drought stress, mycorrhiza, and biochar ($p$< 0.01) (Table 7). In both years, with increased drought stress intensity (50% F.C.), total flavonoids and total phenols of coneflower increased, but in 70% F.C. flavonoids and total phenols contents decreased. In addition, biochar and mycorrhiza treatments improved total flavonoids and total phenols of coneflower. Interaction treatment of 50% F.C. ×biochar 20 (t.ha$^{-1}$) × mycorrhiza improved total flavonoids (201.05%) compared to the drought stress 70% F.C. without biochar and mycorrhiza (lowest one) (Table 5). In drought stress treatments, the 50% F.C. increased total phenols (73.61%) compared to the drought stress 70% F.C. In biochar treatments, biochar 20 (t.ha$^{-1}$) raised total phenols (47.23%) compared to the control. In mycorrhiza treatments, usage of mycorrhizal improved total phenols (45.67%) compared to the control (Table 6).

### *3.4. Photosynthesis pigments*

The analysis of variances illustrated that the chlorophyll *a*, *b*, total chlorophyll, carotenoids and flower carotenoids were influenced significantly by the main effects of drought stress, mycorrhiza, and biochar, also the interactions between drought stress ×biochar, drought stress × mycorrhiza, biochar× mycorrhiza, as well as the triple interactions between drought stress ×biochar × mycorrhiza were significant ($p$< 0.01) (Table 7). In both years, with increased drought stress intensity, photosynthesis pigments of coneflower decreased. In addition, biochar and mycorrhiza treatments increased Photosynthesis pigments of coneflower. Interaction treatment of normal irrigation (control) ×biochar 20 (t.ha$^{-1}$) × mycorrhiza improved chlorophyll *a* (67.51%), chlorophyll *b* (94.4%), total chlorophyll (76.88%), carotenoids (60.27%), and flower carotenoids (99.42%) compared to the drought stress 70% F.C. without biochar and mycorrhiza (lowest one) (Table 8).

**Table 7.** ANOVA for total flavonoids, total phenols and photosynthesis pigments of coneflower (*Echinacea purpurea* L.)

| S.O.V. | DF | Total flavonoids | Total phenols | Chl. *a* | Chl. *b* | Total Chl. | Carotenoids | Flower carotenoids |
|---|---|---|---|---|---|---|---|---|
| Year | 1 | 3.54$^{ns}$ | 10.49$^{ns}$ | 12.73$^{ns}$ | 23.98$^{ns}$ | 31.72$^{ns}$ | 17.06$^{ns}$ | 24.61$^{ns}$ |
| Replication (year) | 4 | 2.35 | 13.66 | 19.26 | 34.73 | 42.51 | 33.52 | 31.48 |
| Drought stress (D) | 2 | 89.21$^{**}$ | 97.82$^{**}$ | 206.54$^{**}$ | 198.61$^{**}$ | 337.95$^{**}$ | 189.43$^{**}$ | 156.75$^{**}$ |
| Year × D | 2 | 3.56$^{ns}$ | 11.74$^{ns}$ | 21.39$^{ns}$ | 16.52$^{ns}$ | 18.49$^{ns}$ | 16.73$^{ns}$ | 15.66$^{ns}$ |
| Replication (year × D) | 8 | 15.73 | 10.29 | 22.41 | 19.86 | 34.22 | 23.94 | 24.31 |
| Biochar (B) | 2 | 101.98$^{**}$ | 104.65$^{**}$ | 173.58$^{**}$ | 102.45$^{**}$ | 537.54$^{**}$ | 205.32$^{**}$ | 189.85$^{**}$ |
| Year × B | 2 | 10.49$^{ns}$ | 13.47$^{ns}$ | 16.94$^{ns}$ | 13.28$^{ns}$ | 10.38$^{ns}$ | 18.73$^{ns}$ | 17.26$^{ns}$ |
| Mycorrhiza (M) | 1 | 97.65$^{**}$ | 98.24$^{**}$ | 189.25$^{**}$ | 124.07$^{**}$ | 449.73$^{**}$ | 183.65$^{**}$ | 204.32$^{**}$ |
| Year × M | 1 | 12.51$^{ns}$ | 15.33$^{ns}$ | 16.05$^{ns}$ | 18.39$^{ns}$ | 32.21$^{ns}$ | 20.45$^{ns}$ | 16.79$^{ns}$ |
| D × B | 4 | 81.36$^{**}$ | 14.65$^{ns}$ | 216.23$^{**}$ | 161.24$^{**}$ | 504.59$^{**}$ | 172.38$^{**}$ | 186.53$^{**}$ |
| Year × D × B | 4 | 11.43$^{ns}$ | 13.08$^{ns}$ | 18.46$^{ns}$ | 15.31$^{ns}$ | 21.85$^{ns}$ | 16.24$^{ns}$ | 14.32$^{ns}$ |
| D × M | 2 | 107.23$^{**}$ | 11.94$^{ns}$ | 305.17$^{**}$ | 249.75$^{**}$ | 473.67$^{**}$ | 189.73$^{**}$ | 174.95$^{**}$ |
| Year × D × M | 2 | 11.49$^{ns}$ | 15.29$^{ns}$ | 21.35$^{ns}$ | 18.46$^{ns}$ | 32.76$^{ns}$ | 11.44$^{ns}$ | 10.35$^{ns}$ |
| B × M | 2 | 123.56$^{**}$ | 8.73$^{ns}$ | 243.69$^{**}$ | 201.39$^{**}$ | 511.28$^{**}$ | 155.26$^{**}$ | 165.82$^{**}$ |
| Year × B× M | 2 | 19.64$^{ns}$ | 10.36$^{ns}$ | 23.75$^{ns}$ | 18.55$^{ns}$ | 27.04$^{ns}$ | 18.69$^{ns}$ | 11.06$^{ns}$ |
| D × B× M | 4 | 133.28$^{**}$ | 9.54$^{ns}$ | 207.11$^{**}$ | 183.94$^{**}$ | 481.65$^{**}$ | 204.45$^{**}$ | 193.79$^{**}$ |
| Year × D × B × M | 4 | 12.95$^{ns}$ | 11.32$^{ns}$ | 16.52$^{ns}$ | 12.73$^{ns}$ | 19.72$^{ns}$ | 9.83$^{ns}$ | 10.33$^{ns}$ |
| Error | 60 | 21.38 | 19.26 | 20.43 | 23.89 | 32.81 | 22.57 | 18.75 |
| C.V (%) | | 10.46 | 8.73 | 9.11 | 10.57 | 12.06 | 9.83 | 10.89 |

**Significant at $p < 0.01$. ns, no significance (at $p < 0.05$)

### *3.5. Correlation*

The results of the correlation of traits indicate that the highest positive and significant correlation occurred between flower dry weight and essential oil yield (0.961) and also flower dry weight and dry matter yield (0.957). Positive and significant correlation was observed between flower yield traits and plant yield components. Also, there was a positive and significant correlation between plant yield, yield components, essential oil content and photosynthetic pigments. Flower dry weight had a positive and significant correlation with total flavonoid (0.746) and total phenol (0.698) traits. However, the correlation between flower dry weight and two traits, phenol and total flavonoids, was lower than other physiological traits. The dry matter yield trait had a positive and significant correlation with all traits except total phenols and total flavonoids (Table 9).

## 4. Discussion

Hydrodistillation Drought stress is a major environmental stress for farming. Losses in agricultural production due to drought are likely the most consequential, with the intensity and length of the stress being decisive factors (Shanthi et al. 2023). In this study, drought stress reduced the yield and yield compound of coneflower due to a significant decrease in photosynthesis. With increasing drought stress intensity, although we saw a decrease in yield and yield compound in treatments with and without biochar and mycorrhiza application. The intensity of the decrease was much greater in the treatment without biochar and mycorrhiza application. The first sign of drought stress in a plant is the rapid inhibition of aerial growth, especially photosynthetic organs (stems and leaves), with a decrease in turgidity. In addition, cell growth and development reduced. In other words, the first noticeable effect of water shortage on plants can be recognized from the smaller size of leaves or plant height. In addition, in water shortage conditions, the absorption of nutrients and elements is also reduced, thus limiting the growth and development of leaves (Khalid et al. 2023; Rahman et al. 2023).

The use of biological fertilizers that provide optimal conditions for nutrient cycling, optimal use of resources, and increasing the quantity and quality of these plants in the long term will be necessary. Combination of biochar and mycorrhiza improved the plant weight probably by improving the qualitative characteristics of the soil, which can be said to be due to the availability of essential elements like phosphorus in mycorrhiza and promoted plant access to this element through biochar (Merlin et al. 2020; Mumivand et al. 2023).

Biochar and mycorrhiza improved essential oil of coneflower. The use of biofertilizers along with biochar can provide suitable conditions for beneficial microbial activities in the soil by maintaining appropriate moisture for the plant and providing the elements needed by the plant. These factors create synergistic and intensifying effects between bacteria and soil elements, and as a result, increase the amount of plant essential oils. Since the essential oil yield is obtained from the product of the dry matter yield and the amount of essential oil produced by the plant and has a direct relationship with the two mentioned indicators, any factor that leads to an increase in the two mentioned indicators will also lead to an increase in the essential oil yield. Since terpenoids are the main components of essential oils, the biosynthesis of their constituent units (isopernoids) depends on acetyl-coenzyme A, ATP, NADPH, and the concentration of inorganic phosphorus in the plant.

Nitrogen also indirectly provides ATP and the substrate required for the synthesis of isoprenoids by increasing the rate of electron transport and leaf photosynthesis. Similarly, researchers confirmed that mycorrhiza usage increased coarse mint (*Plectranthus amboinicus Lour*.) essential oil percentage (Merlin et al. 2020).

Biochar increased total phenols and flavonoids of coneflower by holding the nutrient and provide nutrient for plants better than control. This increases the plant's access to more mineral elements and increases the plant's absorption of nutrients, which improves photosynthesis and increases the production of essential oils, phenols, and total flavonoids in the plant (Anteh et al. 2023). Antioxidant defense systems, including enzymatic and non-enzymatic antioxidants, have evolved to protect plants against damage from reactive oxygen species. In a dynamic equilibrium, the synergistic effect of antioxidants causes the production and quenching of reactive oxygen species within the plant, thus reducing the negative effects of stress and facilitating plant adaptation to environmental stresses (Yang et al. 2021). Increasing the concentration of total phenols and total flavonoids under environmental stress (50% F.C.) is one of the strategies to increase stress tolerance and reduce their negative effects. Phenolic and flavonoid compounds are a group of non-enzymatic antioxidants that are activated in plants under environmental stress and reduce the destructive effects of stress on plants. Since phenolic compounds in plants, play a more defensive role, when plants are exposed to some abiotic stresses such as drought, various phenolic compounds increase in the plant and the non-enzymatic antioxidant system is strengthened in them. However, in the present study, the plant was able to cope with drought stress up to 50% F.C. and the amount of phenol increased. At 70% F.C., the plant's defense system decreased and the amount of phenol and flavonoids decreased. Reactive oxygen species can increase the production of antioxidants and secondary plant metabolites such as phenolic compounds; these compounds play a very important role in inhibiting reactive oxygen species (Wagay et al. 2023; Martinez et al. 2016). It seems that the lower content of total phenol and flavonoids in the plant under normal irrigation conditions compared to mild drought stress conditions (50% F.C.) is also related to this.

Photosynthetic pigments are an essential factor in plant growth. Chlorophyll is the main pigment involved in photosynthesis, which is used to absorb, transfer and convert light energy and is closely related to photosynthesis and plant performance; in other words, chlorophyll can reflect the growth status of plants and the degree of stress. The amount of chlorophyll decreases under environmental stress, and the ratio of chlorophyll *a*, *b*, total and carotenoids changes, causing changes in photosynthetic performance. Chlorophyll concentration is known as a means of detecting stress-related changes Chlorophyll content is an essential component of photosynthesis that is affected by drought stress. The photosystem, electron transport component, oxidative phosphate ATPase, stomatal closure, and chloroplast ultrastructure were also among the genes that discovered to have differential expression (Hu et al. 2023). Drought stress prevents the expression of key enzymes involved in chlorophyll synthesis or increases the activity of enzymes effective in its degradation (Bhusal et al. 2018). The higher production of carotenoids in biochar and mycorrhiza treatments under drought conditions suggests that this approach facilitated sustained photosynthesis and helped mitigate the negative effects of decreased chlorophyll levels. Researchers suggested that application of mycorrhiza improved *Satureja Macrantha* L. essentioal oil. In addition, mycorrhizal treatments had higher chlorophyll (Chl) $a + b$ and total flavonoids and total phenolic content in comparison with no amendment application (Abkenar et al. 2024).

Considering the correlation of physiological traits, it can be stated that yield was affected by all the evaluated traits and changes in these traits caused yield changes under drought stress conditions. The correlation between morphological indicators and percentage can be evaluated as positive and effective in increasing the amount of essential oil of the coneflower plant. Investigating correlations between traits is of particular importance, because when selecting for a trait, the effect of that trait on other traits should be considered. The existence of a high and positive correlation between essential oil percentage, dry matter yield, and flower dry weight and essential oil yield was very justifiable and expected, because with increasing flower dry weight, due to the plant's proper access to nutrients and the creation of suitable environmental conditions for plant growth, especially better transfer of organic matter from roots to shoots and the expansion of flower formation, the essential oil in the flower also increases. Positive correlation physiologically indicates positive changes (increases) in one trait in raising the value of another trait. It seems that the reason for the low correlation of flower dry weight with the two traits phenol and total flavonoids, compared to other physiological traits, can be explained as follows, these traits had their highest values under drought stress conditions (50% F.C.), while flower dry weight decreased in this treatment. However, in other biochar and mycorrhiza treatments, with an increase in flower dry weight, the amount of phenol and flavonoids also increased. In addition, it was determined that total dry weight and essential oil percentage have an effect on essential oil yield (Begum et al. 2021; Wagay et al. 2023). Similarly, other studies reported the positive and significant correlation occurred between flower dry weight, morphological traits and essential oil (Mugano, 2024).

## 5. Conclusion

Considering the global challenge of drought, the utilization of compounds that enhance plants' ability to tolerate water stress will play a crucial role in water management. The results of this study illustrate that biochar as a soil amendment and mycorrhiza as a biological and organic fertilizer improved yield, yield compound, essential oil and quality of coneflower compared to the control. This treatment reduced drought stress effect by holding the water and also provide the nutrient and minerals for plants. The use of organic fertilizer in the organic system and the sustainable management of the soil are of great importance. In addition, organic fertilizers cause less pollution in the environment due to the gradual release of nutrients. In general, drought stress especially 70% F.C. is not suitable for coneflower cultivation. However, in arid and semi-arid regions, the use of mycorrhiza and biochar is recommended to reduce damage caused by drought stress. Also, enhance plant tolerance to drought stress and also to improve the plant yield. Further researches are needed to fully explore the potential benefits and mechanisms of inoculating coneflower with mycorrhizal fungi and biochar under drought stress conditions. However, these approaches show promise in mitigating the adverse effects of water stress and improving the overall health and productivity of plants.

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