

Original Research Article

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Mycorrhizal Inoculation affects the Biochemical Composition of Echinacea

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ABSTRACT

Medicinal plants contain a wide variety of bioactive compounds. Echinacea (Asteraceae) is a North American native plant that is well-known for its use in the treatment of a wide variety of ailments. Medicinal herbs such as Echinacea have been used to treat a wide variety of diseases since ancient times. Here we studied the effect of arbuscular mycorrhizal fungi (AMF) inoculation on physiological and mycorrhization characteristics of Echinacea. The physiological and mycorrhization characteristics (Root essential oil, ABTS Antioxidant, Ferric-Reducing Antioxidant, Total phenolic, AM Spore No., AM Root Colonization) both are higher under mycorrhizal association than the control plants of different echinacea species. *E. purpurea* showed greater results than the *E. angustifolia* and *E. pallida*. Physiological and mycorrhizal traits were significantly higher under *E. purpurea* than the other species. *E. purpurea* showed higher AM Spore No., AM Root Colonization 231.30, 78.70% respectively. Lowest physiological and mycorrhization characteristics were found under *E. pallida*. The result of mycorrhizal association was very effective for plant growth and increased bio-physicochemical properties than the control plants.

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Introduction

Medicinal plants are a source of a wide range of bioactive compounds (1–3). In this direction, Echinacea (Heliantheae, Asteraceae) is a North American native herb that is well-known for its use in treating a wide variety of diseases(4). Medicinal plants like Echinacea

that are used on daily basis are employed to treat a wide variety of illnesses from a long time (5, 6). In Europe and North America, various preparations of Echinacea are widely used as herbal medicines to prevent and treat the common cold, the flu, upper respiratory tract infections, and a variety of other diseases and conditions (7).

In Europe, America, and Canada, the plants are cultivated for phytomedicine, nutritional supplements, and natural health products and even new approaches are being adapted to increase the content of bioactive compounds of in the already cultivated popular plants (8, 9). *Echinacea purpurea* (L.) Moench, *Echinacea pallidavar. pallida* (Nutt.), and *Echinacea angustifolia* (DC.) are three Echinacea species that are used in commercial medicinal formulations. *Echinacea purpurea* (L.) Moench, *Echinacea pallida var. pallida* (Nutt.), and *Echinacea angustifolia* () are believed to possess a variety of other pharmacological properties, including anti-inflammatory efficacy (10). Echinacea species contain diverse active ingredients, including lipophilic compounds (alkamides, ketoalkenes, and ketoalkynes), and phenolic acids (phenolic acids) glycoproteins, and polysaccharides that is also affected by cultivation methods (11–13).

When it comes to the bioactive chemicals found in Echinacea species, alkamides are the most thoroughly studied (14). The roots of *Echinacea purpurea* and *Echinacea angustifolia* contain the highest concentrations of alkamides. The aerial parts of these plants, as well as the roots of *E. pallida*, have been shown to contain significantly less of this chemical (10). Bio-inoculants such as arbuscular mycorrhizal fungi may remain a viable method of increasing plant growth and production while also increasing plant tolerance to biotic and abiotic stresses (15–18). It is critical to remember that shifting dynamics, a complex of their development, and a heterogeneous mix of components in the finished device can all contribute to a bio-success inoculant's by providing a unique sequence of activities (19).

The issue is further complicated by the long life of plants, the use of bio-inoculants, and the presence of a diverse range of components

in the spectrum of growing bio-inoculants. Any bio-inoculant results in the synergistic action of numerous large bioactive compounds and mineral nutrients (20). When used properly, these techniques work to develop a defensive reaction alongside matter in response to environmental changes that have the potential to impair plant development irreversibly (21, 22). The possible trade-off between growth and acclimation metabolisms results in a form of physical exercise for plant life, as energy and nutrients normally devoted to development are redirected to stress-responsive systems, and newspapers are generated to support these activities.

Abiotic stresses have evolved over time as a result of farmers' experiences, technological advancement, climate change, and scientific knowledge. Agronomic management has resulted in increased resilience to abiotic stressors. The quantity of the appropriate cultivar, the optimal acquisition time, the sowing density, and, of course, the amount of water and fertilizer used are all critical considerations (23–25). Crop production in a protected environment is a method of farming that protects plants from harmful environmental conditions (26). In light of this overview, this study makes recommendations regarding the use of bio-inoculants, their benefits for Echinacea.

Materials and Methods

Experimental Setup

The current experiment took place between April and August of 2020 using a completely randomized block design. Ten days later, the plants were transplanted. It was grown in soil containing AMF, *Glomus mosseae* (which contained 82–86% colonized root pieces and 620–630 AM spores per 100 g), and Gigantean spores (which contained 620–630 AM spores per 100 g) (having 70-74 percent

colonized root pieces and 500-520 AM spores per 100 g). Each AMF was mass replicated in greenhouse conditions for 90 days using a sterile sand soil mixture (1:3) and Maize as the host plant. To ensure their survival as an obligatory symbiont, AMF is propagated as endomycorrhizal species. The native density of mycorrhizal spores in the experimental location was 24.57% 10g soil, as determined by the gridline intersect method applied to soil samples collected(27, 28). *Bacillus subtilis* (MTCC 1305) and *Pseudomonas fluorescens*. After 48 hours of culture in a nutritious broth medium.

Field preparation

Each species has a separate control flowerbed that is kept inoculum-free. On the other hand, other regions were treated with consortium therapy, as it has been demonstrated that consortium therapy stimulates flowering plant growth and development. At a 65-67% rate, *G. mosseae* colonized maize roots (1 cm) and spores (610-630 spores per 100 g of corn rhizospheric soil). *G. gigantea*-colonized maize roots (1 cm) and 580–600 spores (per 100 g of Maize rhizospheric soil) were used in the inoculation, along with *B. subtilis* and *P. fluorescens*.

The initial bacterial infection occurred during seedling planting in the flowerbed by simply dipping the seedlings' roots in the appropriate broth medium for ten minutes.

After 10-13 days, when the plantlets had formed their roots (approximately 10 mm), a second treatment with *G. mosseae* and *G. gigantea* was applied by burying additional Maize rhizospheric soil around the roots to ensure that the inoculum was established effectively. Similarly, the second treatment of *B. subtilis* and *P. fluorescens* was carried out similarly, with media being sprinkled around the bacteria's roots.

Characteristics

Ten randomly selected plants from each plot were evaluated 90 days after inoculation (DOI), out of a total of fifteen plants in each plot. The ferric-reducing antioxidant was determined using a modified version of the Farhat *et al.*, method(29) (22). Wang *et al.*, (23) the method was used to determine the total phenolic content. The antioxidant activity of ABTS, on the other hand, was determined using the Re *et al.*, (30) method. The Mazza and Cottrell hydrodistillation method was used to determine the root's total essential oil content(31). Using Trypan blue staining, we developed and quantified mycorrhization 65–70 days after inoculation. Additionally, the amount of AM spores produced and the percentage of roots colonized were determined using previously described and established procedures(32).

Statistical analyses

To ascertain differences in the means of each treatment, an analysis of variance (ANOVA) and a one-way ANOVA were conducted using the SPSS (11.5 version) software program. The results of the experiment were compared between control and microbial-inoculated plants using the least significant difference (LSD) (P0.05) method to determine the significance of the differences between the two groups.

Results and Discussion

Biophysical traits (Root essential oil, ABTS Antioxidant, ferric- reducing antioxidant and total phenolic) and mycorrhization pattern (AM spore, AM root colonization) were higher under consortium than the control plant (Table 1). Root essential oil was higher under *E. purpurea* (0.61 ml g⁻¹) followed by *E. angustifolia* (0.511ml g⁻¹) and lowest in *E. pallia* (0.47 ml g⁻¹) (Table 1). From Table 1 it

is clear that biophysical attributes were higher under consortium than the control plant and AM spore no. and Am root colonization were also higher under mycorrhizal treatment than the control (Table 1). Among echinacea species, biophysical traits and mycorrhization pattern were higher in *E. purpurea* followed by *E. Angustifolia* lowest under *E. pallida* (Table 1). ABTS antioxidant was highest

under *E. purpurea* (24.98 mm ml⁻¹) and followed by *E. angustifolia* and *E. purpurea* (23.16 mm ml⁻¹) (Table 2). *E. purpurea* showed the highest ferric-reducing antioxidant (0.99 nmol μl⁻¹) and total phenolic (24.16 mEAG mL⁻¹) (Table 2). *E. angustifolia* and *E. pallida* showed ferric-reducing antioxidants 0.90 nmol μl⁻¹, 0.85nmol μl⁻¹ respectively.

Table.1 Changes in the physiological and mycorrhization characteristics of 3 different Echinacea species with/without mycorrhizal inoculation.

Traits	Control	Consortium (<i>Glomus mosseae</i> + <i>Giga sporagigantean</i> + <i>Pseudomonas fluorescence</i> + <i>Bacillus subtilis</i>)	ANOVA (F2, 27)	LSD (P≤0.05)
<i>Echinacea angustifolia</i>				
Root essential oil (ml g ⁻¹ FW)	0.166±0.064 ^{b‡}	0.511±0.051 ^a	253.753	0.438
ABTS Antioxidant (mm ml ⁻¹)	15.343±1.711 ^b	23.164±1.425 ^a	791.887	1.214
Ferric-Reducing Antioxidant (nmol μl ⁻¹)	0.429±0.125 ^b	0.909±0.128 ^a	181.673	0.901
Total phenolic (mEAG mL ⁻¹)	15.383±1.043 ^b	21.644±0.896 ^a	1,799.164	0.707
AM Spore No.	38.600±8.275 ^b	221.300±8.069 ^a	552.277	4.754
AM Root Colonization %	21.400±6.186 ^b	74.200±6.442 ^a	2,758.162	6.576
<i>Echinacea purpurea</i>				
Root essential oil (ml g ⁻¹ FW)	0.1852±0.093 ^{b‡}	0.613±0.123 ^a	207.758	0.082
ABTS Antioxidant (mm ml ⁻¹)	14.903±2.061 ^b	24.982±2.129 ^a	495.176	1.571
Ferric-Reducing Antioxidant (nmol μl ⁻¹)	14.033±2.494 ^b	0.999±0.174 ^a	426.688	1.631
Total phenolic (mEAGmL ⁻¹)	0.504±0.092 ^b	24.167±1.805 ^a	62.778	0.104
AM Spore No.	37.600±8.154 ^b	231.300±9.849 ^a	997.800	11.368
AM Root Colonization %	22.400±4.97 ^{1b}	78.700±5.618 ^a	858.682	3.974
<i>Echinacea pallida</i>				
Root essential oil (ml g ⁻¹ FW)	0.153±0.068 ^{b‡}	0.4711±0.064 ^a	613.859	0.051
ABTS Antioxidant (mm ml ⁻¹)	13.603±1.719 ^b	22.055±1.778 ^a	550.412	1.310
Ferric-Reducing Antioxidant (nmol μl ⁻¹)	13.528±1.114 ^b	0.851±0.125 ^a	984.040	0.884
Total phenolic (mEAGmL ⁻¹)	0.339±0.137 ^b	19.765±1.241 ^a	104.020	0.098
AM Spore No.	33.900±5.665 ^b	195.200±7.871 ^a	3,445.503	5.138
AM Root Colonization %	19.600±4.501 ^b	69.600±3.949 ^a	1,052.777	3.179

±- Standard deviation; ‡- values in column followed by the same letter are not significantly different; AM- Arbuscular Mycorrhizal; p≤0.05- LSD (least significant difference test); FW- Fresh Weight; mEAG mL⁻¹- Equivalent mg of gallic acid per milliliter of extract; mm ml⁻¹- millimolar per milliliter of extract (=Trolox Equivalent Antioxidant Capacity); ABTS-2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); nmol μl⁻¹= mM Fe²⁺ equivalents.

The total phenolic compound was lowest under *E. pallida* (19.76mEAG mL⁻¹) (Table 1). The differences between the AM spore no., AM root colonization (%) are illustrated in (Table 1).

Both the parameters were found significantly higher under consortium than the control. AM spore no. was found highest in *E. purpurea* than the other species of echinacea (Table 1). AM spore no. *E. angustifolia* and *E. purpurea* were 221.30 and 231.30 respectively. AM root colonization is also higher under *E. purpurea* than the other echinacea species. AM root colonization was higher under *E. purpurea* depicted in (Table 1). *E. angustifolia* and *E. pallida* showed AM root colonization 74.2% and 69.6%, respectively (Table 1).

Fertilizers were used to improve mycorrhizal inoculum and shoot biochemical composition. Echinacea species differed significantly in biochemical composition. Mycorrhizal fungi have been shown to benefit a variety of echinacea species (33).

Mycorrhizal fungus colonization of *E. purpurea* roots was comparable to other plants (34). Growing root colonization with biofertilizers may help plant roots contain higher levels of phenolic compounds like cynarin, cichoric acid, and caftaric acid. Biochemical fertilizers such as AMF and *Pseudomonas* species have increased plant growth and yield components.

AMF and phosphate solubilizing bacteria (PSB) outperformed other treatments in increasing plant nutrients, leaf relative water content, and reducing ion leakage (23, 35).

Medicinal values of any plant can be altered, this can also increase the economy of the country and even application of chemical fertilizers can be reduced. The result of

mycorrhizal association was very effective for plant growth and increased biophysicochemical properties than the control plants. Out of the current tests, it might be recognized that the phytohormones are changed by bio inoculants, water uptake success in addition to photosynthetic activity.

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