The Effect of Cyanobacterial Bioelicitors on Total Phenolic Content of *Echinacea purpurea* L.

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Abstract

Cyanobacteria have the ability to nitrogen fixation, and the production of plant growth-stimulating substances, which increases plant growth and productivity as biological elicitors. Considering the medicinal value of *Echinacea purpurea* (L.) Monch, the effect of two species of heterocystous cyanobacteria, *Nostoc punctiforme* Hariotand, and *Nostoc calcicola* Brébisson ex Bornet & Flahault, on growth factors and phenolic content of this medicinal plant were evaluated. For this purpose, four pots were considered for each treatment and four others for the control plants. *E. purpurea* cultivated in a randomized complete block design in an experimental greenhouse condition. Then the treated plants were irrigated with 120 ml of 0.2% cyanobacterial suspensions at 20-day intervals. After 60 days, plants were collected and dried under shade and at room temperature. The total phenolic content of the aerial part and root of treated and control plants were evaluated using the Folin-Ciocaltiu method. The results showed that the total phenolic content of cyanobacterial-treated plants, especially plants treated with *N. calcicola*, significantly increased. This metabolite improvement was observed both in the aerial part and the root system of the *N. calcicola* treated plants. The results also showed that plant growth parameters such as root and stem length, as well as number of leaves in both treatments increased significantly compared to the control. As a result of this study, cyanobacteria can be improving the phenolic content and growth indices of the medicinal plant *E. purpurea*.

Keywords: Cyanobacteria, *Echinacea purpurea,* Secondary metabolite, Bioelicitor, Nitrogen fixation

Introduction

Echinacea purpurea (L.) Moenchis is an herbaceous and perennial plant that belongs to the Asteraceae family and its origin is reported in North America. All plant parts contain valuable substances, including caffeic acids (cicuric acids, chlorogenic acids, cynarine), alkamides, polysaccharides, polyphenols, proteoglycans, lipophilic alkylamides, and other phenolic compounds (Attarzadeh et al., 2020). This medicinal herb has immunostimulatory and anti-inflammatory properties, especially to

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alleviate cold symptoms. The plant chemical compounds have several pharmacological properties such as anti-cancer, antianxiety, antidepression, and antimutagenicity (Manayi et al., 2015). The antioxidant activity of this plant, as well as antimicrobial, antifungal, and antiviral properties also have been reported in several studies (Thygesen et al., 2007; Sharma et al., 2010; Manayi et al., 2015).

Phenolic compounds are one of the most important chemical constituents of this medicinal plant that stimulate and strengthen the body's immune system (Noori et al., 2017). Various biological functions have been proposed for phenolic compounds, the most important of which are: antioxidant, antimicrobial, anti-inflammatory, and anti-cancer effects (Fazeli-Nasab et al., 2018; Noori et al., 2017). Phenolic compounds with antioxidant and free radical scavenging properties can be crucial in preserving food products and maintaining human health (Noori et al., 2017). The antioxidant activity of Echinacea extract has been reported in previous studies (Sloley et al., 2001).

One of the methods for the elevation of the plant's secondary metabolite content is the use of elicitors. The direct and indirect effects of elicitors on plant metabolic pathways can amplify the production of some secondary metabolites and could serve as a defense mechanism for the plant (Kokate et al., 2002). Cyanobacteria or blue-green algae are the most primitive group of photosynthetic organisms that played an important role in the evolution of Early Earth and the biosphere (Demoulin et al., 2019). Today, this group of photosynthetic organisms plays a specific role in biotechnological research and can produce a wide range of valuable organic compounds (Soleimani et al., 2022). Cyanobacteria are found in various habitats such as terrestrial ecosystems (Gademann and Portmann, 2008). Soil is the habitat of some cyanobacteria species that improve soil fertility by fixing atmospheric nitrogen, helping to maintain moisture, preventing soil erosion, and producing hormonal and non-hormonal plant growth-promoting substances (Shariatmadari et al., 2013; Abinandan et al., 2019). Therefore, soil microflora plays a significant role in the carbon and nitrogen cycle of terrestrial ecosystems. For this reason, this group of photosynthetic microorganisms can act as a suitable stimulus in the pathway of plant growth (Johnson et al., 2013; Miralles et al., 2011; Parikh, 2006).

In addition, cyanobacteria can increase soil phosphate due to the production of organic acids. Phosphorus is the second essential element in agriculture, which is needed for the growth and development of plants (Zahra et al., 2020).

According to available reports, the proper use of biofertilizers can increase crop production in many plants by 20-30%, and significantly reduce the occurrence of plant pests (Ananya and Ahmad, 2014). In general, biofertilizers, such as fertilizers derived from cyanobacteria, are the more suitable alternative for agricultural purposes due to limited production costs and energy consumption compared with synthetic fertilizers such as urea (Zahra et al., 2020). The results of the previous studies showed the improvement of growth indices, as well as quantitative and qualitative improvement of the essential oil and metabolites in cyanobacteria-treated plants (Chookalaii et al., 2020).

Considering the economic value of *E. purpurea*, it is necessary to find ways to improve the chemical composition of this plant. Therefore, the present study aimed to investigate the effect of native cyanobacteria as biological elicitors in increasing the plant biomass and total phenolic content of *E. purpurea* as widely used and an economically crucial medicinal plant in Iran.

Materials and methods

Isolation, purification, and identification of cyanobacteria

For the isolation of cyanobacterial species employed in this study, soil samples were collected from several central fields of Iran under the cultivation of medicinal plants. The purification of the cyanobacteria was done through repeated subculturing of the colonies on the nitrate free BG11 solid medium (Andersen, 2005). The cyanobacteria were cultured under controlled laboratory conditions and artificial light illumination (74 µmol photons/m^2 s), with a 16:8 hours light-dark cycle, and 25 ± 2 ºC temperature. Finally, purified taxa were identified by optical microscope (Olympus, Model BH-2) based on valid identification key books (Komárek, 2013).

Cyanobacterial suspension preparation

The suspension of two isolated and purified cyanobacterial species, *Nostoc punctiforme* Hariot and *Nostoc calcicola* Brébisson ex Bornet & Flahault, were prepared through homogenizing 2 g of cyanobacterial biomass, after four weeks of culturing, in one liter of sterilized distilled water (0.2% cyanobacterial suspensions).

Pot culture and growing condition

E. purpurea plant was received from Karaj Medicinal Plants Research Institute. Healthy and similar plants were grown in pots containing 40% soil, 40% sand, and 20% perlite for 60 days. The experiment was performed in a randomized complete block design in an experimental greenhouse. All pots of treated and control plants were irrigated similarly for 60 days and for cyanobacterial treatments, 125 ml of cyanobacterial suspensions (2 g biomass in one-liter distilled water) was added to each treated pot on the first day of planting and every 20 days thereafter. The control plants were irrigated only with distilled water.

Total phenolic assay

To evaluate the total phenolic content (TPC), the dried leaves and roots were extracted with methanol for 24 hours with three-time repetitions. The TPC of the total extracts was measured using the Folin-Ciocaltiu method. To analyze the content of total phenolic content, Folin reagent 10% in the amount of 200 µl, and $NaHCO_3$ in the amount of 800 µl were added to 100 µl of methanolic extract (three replicates of each sample) and placed at ambient temperature for three hours then absorption was recorded at 765 nm. From the calibration curve, the total amount of phenolic compounds (mg. ml^{-1}) was calculated and determined with gallic acid equivalent (mg GA dry matter/g) (Karimi et al., 2018; Ashouri Sheikhi et al., 2016; Kamtekar et al., 2014; Singleton et al., 1999).

Statistical analysis

Statistical analyses were conducted using SAS version 9.1. Analyses of variance (ANOVA) were performed to compare the data. All determinations were done at least in triplicate, and all were averaged. The confidence limits used in this study were based on 95% ($p \leq 0.05$).

Results

Considering the medicinal importance of phenolic compounds in this medicinal plant, the total phenol content of the treated and control plants was measured and compared. The results showed that the TPC of seedlings treated with *N. calcicola* significantly increased compared to the control plants. Seedlings treated with this strain (*N. calcicola*) showed a significant increase of TPC in the root and aerial parts (Figure 1). It should be noted that this increase was not the same in both sections of plants, and the TPC in the root section was more than the aerial part of treated plants.

Also, the growth parameters such as root length and number of leaves have increased significantly in both treatments compared to the control (Figure 2, 3). However, the leaf area was significantly different only in the *N. calcicola* treated plants compared to the control plants (Figure 4).

The highest root length was recorded for plants treated with *N. punctiforme* with 84 % increase compared to the control. The *N. calcicola* treated plants also showed a 32 % increase in the root length compared to the control.

The highest increase in the length of aerial parts of plants was also recorded for *N. punctiforme* treated plants with a 21 % increase compared to the control plants, followed by those inoculated with *N. calcicola* (Figure 2).

The highest leaf area was recorded for

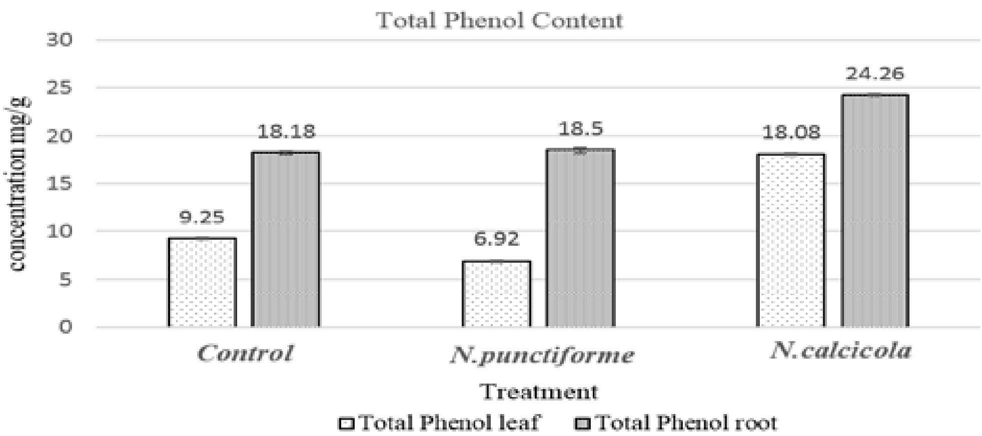

Fig. 1. The total phenol content of *Echinacea purpurea* in control and Nostoc-treated plants

plants treated with *N. calcicola* with a 155% increase compared to the control. Plants treated with *N. punctiforme* showed an 22% increase compared to the control.

Discussion

In this study, the potential of two

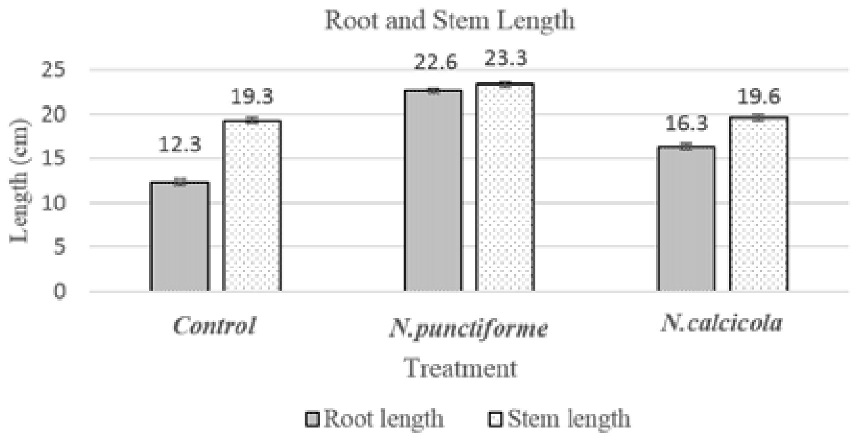

Fig. 2. The length of the root and stem of *Echinacea purpurea* in

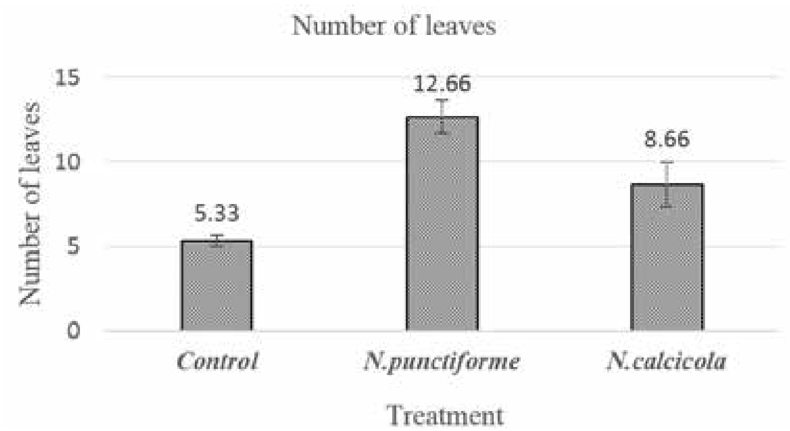

Fig. 3. The number of *Echinacea purpura* leaves in control and Nostoc-treated plants

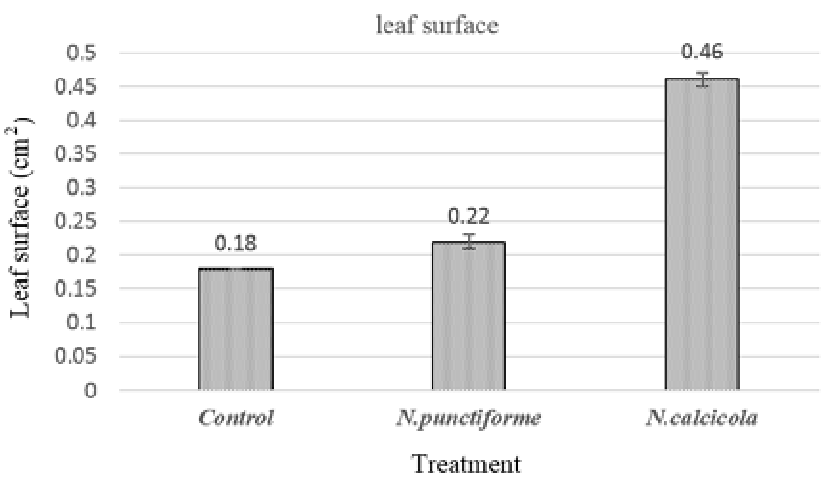

Fig. 4. Leaf surface of *Echinacea purpura* in control and Nostoc-treated plants

cyanobacterial strains as biological stimulants in *E. purpura* was investigated. As a result, treatment of this medicinal plant with heterocystous cyanobacterium *Nostoc calcicola*, has increased the amount of phenol content in the root and aerial parts of the plant. Phenolic compounds are one of the most important compounds that stimulate the immune system, and these compounds are found in the root and aerial part of this medicinal plant and are considered a metabolite that stimulates the immune system (Noori et al., 2017).

The results of the present study showed that the cyanobacterium *N. calcicola* significantly enhances the amount of total phenol in the roots and shoots of *E. purpurea*. The results also showed that several species of cyanobacteria do not have the same ability, and the cyanobacterial suspension of *N. punctiforme* has not shown a similar and positive performance about the level of TPC. In the previous studies, the improvement of plant growth and phenolic content of *Vicia faba* by using some biofertilizers was reported. Another study also showed that the treatment of bean plants with biofertilizers increases the phenolic compounds of treated plants (Ragaa at el., 2013). Previous studies have also shown that cyanobacterial biostimulants increased the flavonoid content of *Plantago major* L. and improved the medicinal properties of this plant (Chookalaii et al., 2020).

Some researchers believe that biological factors can increase the production of phenolic compounds in plants by stimulating the secretion of organic acids and the production of growth regulators (Attarzadeh et al., 2020). Several studies have shown that phenols and flavonoids increase as a result of plant interactions with the stimulating agents such as plant pathogens (Pusztahelyi et al., 2015). Researchers also reported that biofertilizers improve plant growth and increase phenolic compounds in plants by increasing the absorption of nutrients and biosynthesis of phytohormones (Atsami et al., 2018), which findings are consistent with the results of the present study.

Based on the studies conducted in this field, it can be concluded that cyanobacteria can be used as natural and effective agents to increase the quality and quantity of valuable metabolites of medicinal plants and can be used purposefully to increase the efficiency of medicinal plant production. As a result, the *N. calcicola* strain can increase phenol production in *E. purpurea* plant. In summary, the selected cyanobacteria, isolated domestically, can act as potential biofertilizer candidates to promote the production of *E. purpurea* as a medicinal plant.

Considering the ability of this taxa to fix nitrogen and produce growth-stimulating compounds, these cyanobacteria are suitable biological stimulating agents for the quantitative and qualitative improvement of the phenolic content of medicinal plants such as *E. purpurea*. It suggested that further studies be conducted to determine the effects of cyanobacteria on other metabolites of *E. purpurea*.

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