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Research Paper

Effect of *Azotobacter chroococcum* and Different Rates of Nitrogen Fertilizer on Coneflower (*Echinacea purpurea* L. Moench) Yield and Phytochemical Properties

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Abstract

The effects of biological and chemical fertilizers were studied on quantitative and qualitative yields of coneflower in a factorial experiment carried out at the agricultural research farm in Parsabad, Ardebil. The experiment was based on a randomized complete block design with four replications. The experimental factors included N fertilizer (N0= 0, N1= 75 and N2= 150 kg ha^{-1}) and *Azotobacter* (inoculation with *A. chroococcum* bacteria SW22 strain = B1 and non–inoculation = B0). The morphological traits such as plant height, number of lateral shoots, shoot fresh and dry weight, root fresh and dry weight, number of flowers per plant, and phenol, nitrogen (N), and phosphorus (P) concentration were measured. The results showed the significant effects of the treatments on the growth parameters. Inoculation with *Azotobacter* + 75 kg N ha^{-1} improved important parameters, such as shoot dry weight (40.42%), root dry weight (60.02%), and the number of flower plant^{-1} (65.68%). Additionally, phenol, N, and P concentration in the plants treated with *Azotobacter* + 75 kg N ha^{-1} were 25.11%, 34.6%, and 39.8% higher than those of the control plants, respectively. The results indicate that the use of biological fertilizers is a good choice to reduce the use of chemical fertilizers as an important tool to contribute to sustainable agriculture.

Keywords: Biofertilizer, Biological yield, Chemical fertilizers, Nitrogen, Ornamental plant.

INTRODUCTION

Coneflower (*Echinacea purpurea* L. Moench) is a small genus of the Asteraceae family used as an ornamental and medicinal plant (Chen, 2016). This species is extensively used as a landscape plant that is resistant to wind and salt stress (Araim *et al*., 2009; Dehestani-Ardakani *et al.,* 2020). *Echinacea* is much valued as a cut flower. Medicinal preparations from different parts of this species, e.g., flowers and leaves, are used worldwide for their healing properties. The dried root is used in modern herbal medicines, skin creams, and shampoos (Senica *et al*., 2019).

The objective of commercial medicinal plant production is to produce high biomass yields per hectare with higher marker compound content. The fertilizer requirements for production are a major aspect that influences the yield of all horticultural and agronomic crops (Lu *et al.*, 2016). Marker compounds in medicinal plants may also be affected by fertilizer positively or negatively (Chrysargyris *et al*., 2016). Nitrogen fertilizers are effective in increasing yield and improving the vegetative characteristics of medicinal plants, such as coneflower (Lu *et al.,* 2016). Although nitrogen plays a key role in enhancing the yield of medicinal plants, its inappropriate use poses causes ecological and human health risk, results in the depletion of nonrenewable resources, and reduces plant resistance to pests and diseases (Hassan *et al*., 2009; Brandt, 2008). Since product quality is preferred to product quantity in sustainable farming systems, the production of medicinal plants whose quality is highly important is desirable in these systems (Anwar *et al*., 2005). Also, one of the main purposes of these systems is to eliminate or reduce the use of chemical inputs and replace them with organic and biofertilizers to overcome environmental problems and improve the health of agricultural products (Vessey, 2003; Arora *et al*., 2020).

Currently, biofertilizers have been proposed as an alternative option for chemical fertilizers, such as nitrogen, to increase soil fertility in sustainable agricultural production. In recent decades, a broad spectrum of soil bacteria in the rhizosphere was identified which can improve the growth of most medicinal plants. Some of these bacterial species, which are useful to plants, belong to the genera *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Bacillus* (Tilak *et al*., 2004). Biofertilizers contain a variety of free-living microorganisms (Vessey, 2003) that can fix atmospheric N through the process of biological nitrogen fixation (BNF), solubilize plant nutrients like phosphates, and stimulate plant growth through the synthesis of growth-promoting substances, and have a C:N ratio of 20:1, indicating the stability of the biofertilizer (Sable *et al*., 2016).

Among these bacteria, *Azotobacter* has high efficiency in host root colonization and plant growth metabolite production (Wani *et al*., 2013). *Azotobacter* fixes about 10 mg nitrogen g^{-1} of carbon source under *in vitro* conditions. They are cheaper, low capital-intensive, and eco-friendly (Vessey, 2003).

A study by Govedarica *et al*. (1993) on the production of growth substances by nine *Azotobacter chroococcum* strains isolated from a chernozem soil showed that these strains could produce auxins, gibberellins, and phenols. They could also increase the height, mass, and nitrogen content of tomato plants. *A. chroococcum* produces an antibiotic that inhibits the growth of several pathogenic fungi in the rhizosphere, thereby hindering seedling mortality (Subba Rao, 2001). Single inoculants of *A. chroococcum* were found to enhance the growth of bamboo shoots and maize plants by phosphate solubilization and phytohormone production (Dhamangaonkar, 2009). Under greenhouse conditions, the inoculation of *A. chroococcum* recorded a significant N and P uptake in both seed and stover in brown sarson over the control (Wani, 2012). The present work aimed at studying the effect of different levels of N on the yield and phytochemical properties of coneflower (*Echinacea purpurea* L. Moench) seeds inoculated with *A. chroococcum*.

MATERIALS AND METHODS

The experiment was carried out at a research farm in Parsabad, Ardebil, Iran in the 2017-

2018 cropping season (39°23′ N, 48°22′ E, with an elevation of 78 m from sea level). The climate of the site is considered to be semi-temperate with an average annual precipitation of 390-420 mm based on the 30-yr weather station data mainly as snowfall in winters.

Soil analysis

The soil texture of the site was silty-loam with 51%, 25%, and 24% of loam, clay, and sand, respectively. Also, the soil pH was about 8.2 and an EC of 1.61 dS m^{-1}. The content of the available nitrogen was 0.16% and phosphorous and potassium rates were 20 and 340 ppm, respectively. Saturation percent was 46% and the organic carbon was measured at 1.71%.

Experimental design

The study was carried out as a factorial experiment based on a randomized complete block design (RCBD) with 4 replications. The experimental factors included N fertilizer (N0 = 0, N1 = 75 and N2 = 150 kg ha^{-1}) and *Azotobacter* (inoculation with *A. chroococcum* bacteria SW22 strain = B1 and non–inoculation = B0). After land preparation, including plowing, disking, and ridging, coneflowers were sowing in late-May of 2017. Each plot had five rows with 50 cm inter-row spacing and 20 cm between-plant spacing in each row. The seeds were supplied by the Seed and Plant Improvement Institute (SPII), Karaj, Iran. Three to four seeds per hole were placed at 1-2 cm planting depth. The plants were thinned to one at the 3-4 leaf stage.

The N fertilizer (150 kg ha^{-1} as urea) was applied in three stages (at planting time, four-leaf stage, and pre-flowering). The applied bio-fertilizer was *A. chroococcum.* These bacterial strains, which were originally isolated from farm soils in Iran, were obtained from Iranian Soil and Water Research Institute. Inoculants that contained 10^7 active and alive bacteria per gram were used for seed incubation. Maximum care was taken to avoid contamination and the mixing of bacterial inoculations during sowing. Irrigation and nutrition were performed based on local practices.

After they flowered in late June, plants with their roots were harvested from 1 m^2 and one fully-expanded leaf, stem, flower, and root sample was prepared from each plant. The samples were separately put in plastic bags and transported to the laboratory. Then, the maximum length of all samples was measured by a ruler. The samples were then oven-dried at 80°C for 24 h to measure their dry weight. The dry weights of the plants were measured to the nearest 0.001 g. Also, the harvest index or HI was calculated by the following formula (Omidi *et al*, 2009). It should be noted that HI is a measure of the efficiency of plants in producing economical parts. It is defined as the ratio of economical yield to total aboveground biomass.

$$HI = \frac{\text{Flower yield}}{\text{Biological yield}} \times 100$$

To determine the total phenolic content, 250 mg of the medicinal herbs of each replication was ground and dissolved in 10 ml of 80% acetone. The sample extracts were rotated for 1 hour in the darkness and centrifuged at 5000 rpm for 10 minutes. The amounts of total phenols in the extracts were determined with the Folin-Ciocalteu reagent using the method of Javanmardi *et al*. (2003). To 100 µl of each sample, 2.5 ml of 1/10 dilution of Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (7.5% w/v) were added, and it was incubated at 45°C for 15 minutes. The absorbance of the samples was read at 765 nm using a Perkin Elmer UV-vis spectrophotometer. The results were expressed in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

The leaf and shoot P contents were determined by the vanadomolybdo-phosphoric acid method and the absorbance of the solution was recorded at 430 nm using a spectrophotometer as described by Skroch *et al*. (1999). The N concentration was determined using the micro-Kjeldahl

method following salicylic-H_2SO_4 digestion (Yamakawa, 1993).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using SAS 9.2 software. When the F-test indicated statistical significance at $P < 0.05$, the least significant difference (LSD) was used to separate the means.

RESULTS

Plant height

The analysis of variance showed that the main effects of N and *Azotobacter* were significant on the number of flowers and lateral branches, the dry weight of the flowers, roots, stems, and leaves, biological yield, harvest index, and leaf N, P, and phenol contents, but they had no significant effect on plant height (Table 1). Means comparison showed that *Azotobacter* inoculation increased plant height by 13.29% (Table 2) and N fertilization up to 75 kg ha^{-1} increased plant height by 24.39%, but a further increase in N use from 75 to 150 kg ha^{-1} did not significantly change plant height (Table 2).

Table 1. Analysis of variance for the effects of nitrogen fertilizer and *A. chroococcum* on plant height, lateral branches, number of flowers, and flower yield.

S.o.V	df	MS			
		Plant height	Lateral branches plant^{-1}	Flowers plant^{-1}	Flower dry weight
Replication	3	224*	0.58^{ns}	13.9^{ns}	279315^{ns}
Nitrogen (N)	2	419**	46.35^{ns}	191*	21856063**
Bacteria (B)	2	230*	81.0*	155^{ns}	4101802^{ns}
N × B	4	41^{ns}	94.0*	211*	7047515**
Error	12	97.24	27.84	84.3	3219006
CV (%)		4.43	9.82	7.7	8.7

*, ** and ^{ns}: Significant at P<0.05, P<0.01 and no significant, respectively.

Table 2. Means comparison for the main effects of nitrogen fertilizer and *A. chroococcum* on plant height, lateral branches, number of flowers, and flower yield.

Bacteria	Plant height (cm)	Lateral branches plant^{-1}	Flowers plant^{-1}	Flower dry weight (kg ha^{-1})
Non-inoculation	40.98^{b}	20.37^{b}	8.81^{b}	3491^{b}
A.chroococcum	45.58^{a}	23.13^{a}	10.78^{a}	3960^{a}
Nitrogen (kg ha^{-1})				
0	38.00^{b}	19.24^{b}	7.97^{b}	3315^{b}
75	44.08^{a}	22.68^{a}	10.50^{a}	3969^{a}
150	47.77^{a}	23.35^{a}	10.91^{a}	3893^{a}

*In each column, means with a similar letter are not significantly different ($P < 0.05$) using the LSD test.

Lateral branches, flowers per plant, and dry weight of flowers

The comparison of the means for the main effect of *Azotobacter* showed that *Azotobacter* significantly increased the number of lateral branches, the number of flowers plant^{-1} and the dry weight of flowers compared to non-inoculated plants. N fertilization up to 75 kg ha^{-1} significantly increased the number of flowers per plant and the dry weight of flowers but no significant difference was observed between 75 and 100 kg N ha^{-1} (Table 2). The comparison of means for the interactive effects of N × *Azotobacter* on the number of lateral branches, the number of flowers per plant, and flower dry weight revealed that these traits were significantly higher in plants inoculated with *Azotobacter* and fertilized with N up to 75 kg ha^{-1}, but with a further increase in N level from 75 to 150 kg N ha^{-1}, no significant change was observed in the means of these traits (Table 3).

Table 3. Means comparison for the interactive effect of nitrogen fertilizer and *A.chroococcum* on plant height, lateral branches, number of flowers and flower dry weight.

N × B	Nitrogen (kg ha^{-1})	Plant height (cm)	Lateral branches number plant^{-1}	Flowers number plant^{-1}	Flower dry weight (kg ha^{-1})
Non-inoculation	0	36.43^{a}	16.70^{c}	6.16^{d}	3292.3^{b}
	75	41.15^{a}	21.48^{b}	9.59^{c}	3549.6^{b}
	150	45.38^{a}	22.95^{ab}	10.69^{ab}	3632.0^{b}
A.chroococcum	0	39.58^{a}	21.78^{b}	9.79^{bc}	3337.6^{b}
	75	47.01^{a}	23.88^{a}	11. 42^{a}	4389.4^{a}
	150	50.16^{a}	23.75^{a}	11.13^{a}	4155.2^{a}

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the LSD test.

Table 4. Analysis of variance for the effects of nitrogen fertilizer and *A.chroococcum* on root dry weight, leaf dry weight, stem dry weight, biological yield, and harvest index.

S.o.V	df	MS				
		Root dry weight	Leaf dry weight	Stem dry weight	Biological yield	Harvest index
Replication	3	236321^{ns}	14800^{ns}	46114^{ns}	76337^{ns}	0.001^{ns}
Nitrogen (N)	2	14070036**	3150299*	3619234*	25766052**	0.293**
Bacteria (B)	2	9230478*	23031609**	6340211*	85201814**	0.047*
N×B	4	13910207**	4331979*	10588358**	26482938**	0. 44**
Error	12	4805996	1016058	2169239	6248734	0.015
CV (%)		12.15	4.38	9.82	4.2	6.42

*, ** and ns: Significant at P<0.05, P<0.01 and non-significant, respectively.

Dry weight of roots, stems, leaves, biological yield, and harvest index

Analysis of variance showed that the main effects of N and *Azotobacter* and the interactive effects of N × *Azotobacter* were significant on dry weight of root, stem, and leaf, biological yield, and harvest index (Table 4). The comparison of means for the main effect of *Azotobacter* showed that inoculation increased stem dry weight, but had no statistically significant effect on leaf dry weight, biological yield, and harvest index. The main effect of N also showed that with increasing

N up to 75 kg ha^{-1}, leaf, stem, and root dry weight and biological yield were significantly increased (Table 5). Means comparison for N × *Azotobacter* interaction also revealed that root, stem and leaf dry weight were increased significantly with N fertilizer application, while biological yield was increased with N up to 75 kg ha^{-1}. But, a further increase in N rate from 75 to 150 kg N ha^{-1} had no significant effect on this trait (Table 6).

But in inoculated plants fertilized with 75 kg N ha^{-1}, significant increases were observed in stem, leaf and root dry weight and biological yield, but 150 kg N ha^{-1} not only had no effect on increasing these traits, but it also decreased leaf dry weight and biological yield.

Also, harvest index was significantly decreased with increasing N application in both inoculation and non-inoculation conditions so that the highest harvest index was obtained from non-inoculation + non-use of N (Table 6). The harvest index, which is influenced by flower yield (flower dry weight) and biological yield (total dry weight of the plant), was decreased with increasing N use, which may be due to the effect of N on stimulating the vegetative growth of flowers, thereby contributing vegetative components to current photosynthesis. Fig. 1 shows that flower dry weight was increased with increasing N rate, but biological yield increased to a greater extent than flower yield did, especially in the absence of *Azotobacter*.

Leaf and shoot N contents

The results showed that although the concentration of N was higher in the non-inoculated plants than in the inoculated plants, this difference was not statistically significant. N fertilizer application significantly increased leaf and stem N concentrations although there was no significant difference between 75 and 150 kg N ha^{-1} (Table 8). The N × *Azotobacter* interaction also showed that the highest leaf and stem N contents were obtained from *Azotobacter* + 75 kg N ha^{-1} (Table 9).

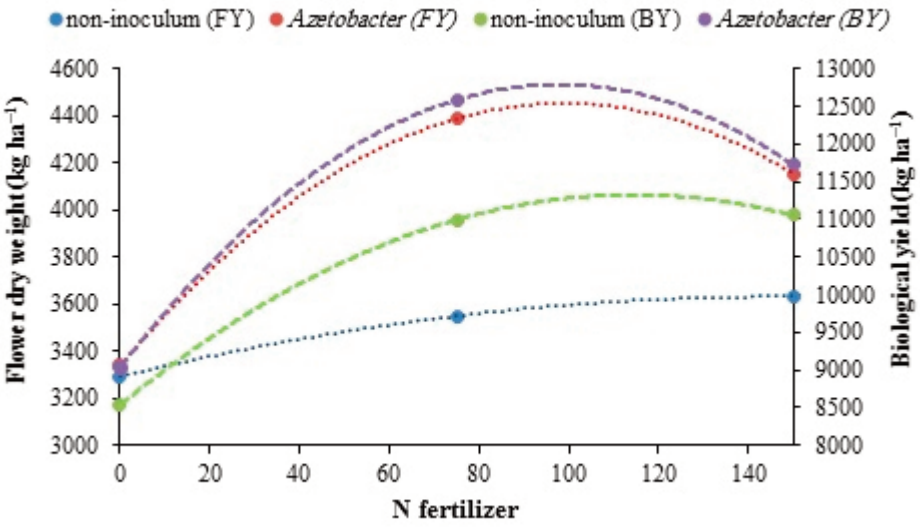

Fig. 1. The interactive effect of N × *Azotobacter* on flower dry weight and biological yield of coneflowers.

Leaf and stem P contents

The results indicated that *Azotobacter* inoculation significantly increased leaf and stem P concentration. N fertilizer increased leaf and stem P concentration to 75 kg ha^{-1}, whereas 150 kg N ha^{-1} had no effect on shoot P concentration and significantly decreased leaf P concentration (Table 8). Based on the results for N × *Azotobacter* interaction, *Azotobacter* exhibited the highest P concentration at both leaf and stem levels at 0 and 75 kg ha^{-1}, but at 75 kg N ha^{-1}, it showed the highest P concentration in the non-inoculated plants. However, this difference was not statistically significant for 150 kg N ha^{-1} (Table 9).

Table 5. Means comparison for the main effect of nitrogen fertilizer and *A.chroococcum* on root dry weight, leaf dry weight, stem dry weight, biological yield, and harvest index.

Bacteria	Stem dry weight	Leaf dryweight (kg ha^{-1})	Root dry weight (kg ha^{-1})	Biological yield	Harvest index (%)
Non-inoculum	2461^{b}	3944^{a}	3000^{b}	10194^{a}	35.33^{a}
A.chroococcum	3009^{a}	4457^{a}	3583^{a}	11117^{a}	34.00^{a}
Nitrogen (kg ha^{-1})					
0	2227^{b}	3256^{b}	2822^{b}	8786^{b}	37.50^{a}
75	2932^{a}	4897^{a}	3356^{a}	11791^{a}	33.50^{b}
150	3047^{a}	4450^{a}	3697^{a}	11390^{a}	33.00^{b}

*In each column, means with a similar letter are not significantly different (P < 0.05) using the LSD test.

Table 6. Means comparison for the interactive effect of nitrogen fertilizer and *A.chroococcum* on root dry weight, leaf dry weight, stem dry weight, biological yield, and harvest index.

N×B	Nitrogen (kg ha^{-1})	Stem dry weight (kg ha^{-1})	Leaf dry weight (kg ha^{-1})	Root dry Weight (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (%)
Non-inoculation	0	1721.2^{d}	3068.7^{e}	2577.4^{c}	8534.5^{c}	39^{a}
	75	2677.0^{c}	4745.7^{b}	2836.9^{c}	10995.4^{b}	32^{c}
	150	2985.6^{ab}	4020.4^{c}	3587.6^{ab}	11052.4^{b}	35^{b}
A.chroococcum	0	2732.9^{bc}	3443.5^{d}	3066.6^{bc}	9037.8^{c}	36^{b}
	75	3187.9^{a}	5048.6^{a}	3875.0^{a}	12588.1^{a}	34^{bc}
	150	3108.6^{a}	4879.8^{ab}	3807.4^{a}	11727.7^{ab}	32^{c}

*In each column, means with the similar letters are not significantly different (P < 0.05) using the LSD test.

Table 7. Analysis of variance for the effects of nitrogen fertilizer and *A. chroococcum* on leaf N, stem N, leaf P, stem P, shoot phenol, root phenol and total phenol.

S.o.V	df	MS						
		Leaf N	Stem N	Leaf P	Stem P	Shoot phenol	Root phenol	Total phenol
Replication	3	0.005^{ns}	0.018**	0.013^{ns}	0.65^{ns}	0.71**	0.002^{ns}	0.62**
Nitrogen (N)	1	0.078**	0.016**	1.34**	5.16*	0.047^{ns}	0.0084*	4.07**
Bacteria (B)	2	0.341**	0.028**	2.09**	5.85*	1.68**	1.926**	3.11**
N × B	2	0.093**	0.008*	1.09**	6.94*	4.51**	0.662**	1.89**
Error	30	0.041	0.001	0.071	3.65	0.0598	0.0047	0.05
CV (%)		8.55	5.08	13.5	9.17	10.25	13.38	7.79

*, ** and ns: Significant at P<0.05, P<0.01 and no significant, respectively.

Phenol concentrations of root and leaf and total

Phenol concentrations in roots and leaves were affected by N fertilizer and *Azotobacter* (Table 7). The results showed that in non-inoculated plants the highest amount of root and shoot phenol were obtained from 75 kg ha^{-1} and total phenol from 150 kg ha^{-1}. In inoculated plants, root phenol content increased with increasing N consumption, but in aerial parts 75 kg N showed the highest amount of phenol. The highest total phenol content was obtained from *Azotobacter* + 75 kg N, which increased it by 20.39% compared to the control (non-inoculation + non N use) (Table 9). It should be noted that there was a significant relationship between N and P in and phenol content of the shoot. However, the shoot N concentration was more correlated with shoot P than the shoot P concentration (Figs. 2 and 3).

Table 8. Means comparison for the main effects of nitrogen fertilizer and *A. chroococcum* on leaf N, stem N, leaf P, stem P, shoot phenol, root phenol and total phenol.

Bacteria	Leaf N	Stem N	Leaf P	Stem P	Shoot phenol	Root phenol	Total phenol
	(%)				(mg g^{-1} DW)		
Non-inoculum	0.815^{a}	0.858^{a}	0.246^{b}	0.228^{b}	4.26^{a}	1.028^{a}	5.29^{a}
A.chroococcum	0.883^{a}	0.885^{a}	0.410^{a}	0.278^{a}	4.55^{a}	1.059^{a}	5.68^{a}
Nitrogen (kg ha^{-1})							
0	0.700^{b}	0.833^{b}	0.317^{ab}	0.235^{b}	3.97^{b}	1.023^{a}	4.99^{c}
75	0.995^{a}	0.893^{a}	0.364^{a}	0.307^{a}	4.63^{a}	1.038^{a}	5.97^{a}
150	0.852^{a}	0.889^{a}	0.302^{b}	0.217^{b}	4.73^{a}	1.069^{a}	5.50^{b}

*In each column, means with similar letters are not significantly different ($P < 0.05$) using the LSD test.

Table 9. Means comparison for the interactive effects of nitrogen fertilizer and *A. chroococcum* on leaf N, stem N, leaf P, stem P, shoot phenol, root phenol and total phenol.

N × B	Nitrogen (kg ha^{-1})	Leaf N	Stem N	Leaf P	Stem P	Shoot phenol	Root phenol	Total phenol
		(%)				(mg g^{-1} DW)		
Non-inoculation	0	0.704^{d}	0.898^{b}	0.187^{d}	0.187^{b}	3.889^{d}	0.995^{c}	4.884^{d}
	75	0.886^{b}	0.811^{c}	0.293^{c}	0.311^{a}	4.354^{c}	1.061^{ab}	5.415^{bc}
	150	0.819^{c}	0.866^{b}	0.256^{c}	0.221^{b}	4.565^{bc}	1.029^{bc}	5.594^{b}
A.chroococcum	0	0.696^{d}	0.768^{c}	0.448^{a}	0.282^{a}	4.059^{d}	1.052^{b}	5.111^{c}
	75	1.068^{a}	0.976^{a}	0.434^{a}	0.337^{a}	4.914^{a}	1.016^{ab}	6.13^{a}
	150	0.922^{b}	0.912^{b}	0.347^{b}	0.214^{b}	4.701^{ab}	1.110^{a}	5.811^{ab}

*In each column, means with similar letters are not significantly different ($P < 0.05$) using the LSD test.

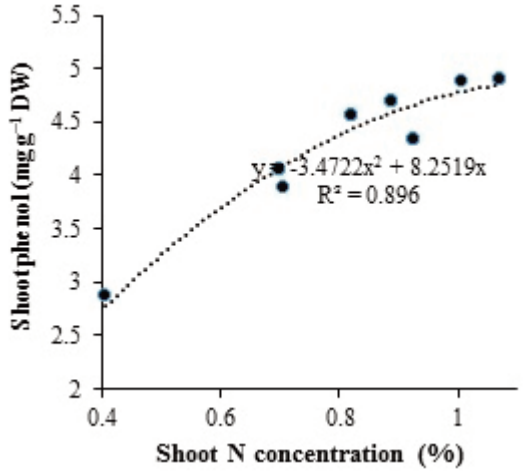

Fig. 2. The interactive effects of N × *A. chroococcum* on flower dry weight and biological yield of coneflower.

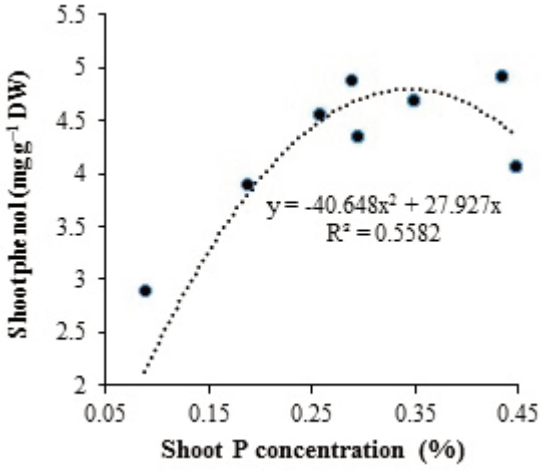

Fig. 3. The interactive effects of N × *A.chroococcum* on flower dry weight and biological yield of coneflower.

DISCUSSION

Based on the results, the application of *Azotobacter* resulted in an increased number of lateral branches, more flowers per plant, and higher flower dry weight at different levels of N fertilizer. Manafi *et al.* (2013) reported that N fertilizer had a significant effect on the number of coniferous branches in the purple coneflower, and with the increase in N fertilizer, the number of branches and the number of flowers per plant were increased. Increasing N fertilizer increased shoot and root growth of plants, which can be attributed to the role of N in increasing vegetative growth and increasing chlorophyll accumulation (Omidi *et al*., 2009) whereas Shaalan (2005) also showed that the application of biofertilizers such as *Azotobacter*, *Azospirillum*, and *Pseudomonas* led to an increase in the number of lateral branches and the number of capsules in *Nigella sativa*. Lu *et al*. (2016) also showed that in conifer culture, nitrogen utilization can be reduced through the application of N_2-fixing bacteria such as *Azotobacter* and *Azospirillum*. In addition to stabilizing air N_2 and balancing the uptake of nutrients, especially P and micronutrients, *Azotobacter* also secretes amino acids and antibiotics, hydrogen cyanide, and siderophore and promotes root and shoot growth and development (Tilak *et al*., 2004; Sable *et al*., 2016).

In this respect, one should not overlook the role of P in flowering. Any factor that significantly increases soil P is effective in flowering (Milani and Anthofer, 2008). Therefore, increasing P by increasing reproductive organs can increase the number of flowers. On the other hand, due to the poor soil nitrogen content, the addition of *Azotobacter* bacteria along with nitrogen improves soil physical and biological conditions and increases moisture retention and cation exchange capacity (CEC), gradual and sustained nutrient supply, and growth enzymes and hormones. Due to the stimulation of vegetative growth and repeated transfer of material from older leaves to younger leaves and as a result of the later emergence of the signs of aging, dry matter accumulation especially flower dry weight increases (Wani *et al*., 2013). Thus, this bacterium can be effective in increasing plant height, leaf number, dry weight of different parts of the plant, thereby producing more crops. In a study on the effect of biofertilizers on hyssop, Seghatoleslami (2013) confirm this part of our results. The maximum biological yield (dry weight of stem + leaf + root) was obtained from *Azotobacter* + 75 kg N ha^{-1}.

Regarding the mechanisms by which PGPR influences plant characteristics, it seems that these bacteria are effective in photosynthesis and accumulation of plant growth hormones as well

as biological stabilization of N and solubilization of P and other elements. It affects the dry matter of the plant, including increasing the dry weight of the whole plant. It should be noted that in inoculated plants, the N level of 75 kg ha^{-1} had a higher mean for most traits than the N level of 150 kg ha^{-1}, which could be due to the effect of *Azotobacter* in partially supplying the nitrogen requirement of the plants. However, the use of more than 75 kg N ha^{-1} not only had no effect on increasing average traits, but it also decreased some traits. The decrease in *Azotobacter* efficiency with increasing N fertilizer has been reported by Martin *et al*. (2011), Nosheen *et al*. (2016), and Zhang *et al*. (2018), too.

The higher amount of phenol in the plants treated with 75 kg N ha^{-1} + *Azotobacter* may be due to the N supply in the nutrient system, which resulted in a higher N (150 kg ha^{-1}) reduction in total phenol content in the inoculated plants. Similarly, Mudau *et al*. (2007) reported that an increase in the amount of N and P increased phenolic compounds in *Athrixia phylicoides*. In another report, increased levels of nitrogen increased the yield and phenolic compounds of hop bush shrubs inoculated with bacteria compared to the control levels (Yousefi *et al*., 2017). Therefore, increasing nitrogen uptake can also increase the phenol content of the bush tea (Mudau *et al*., 2007), which shows a high relationship ($R^2 = 0.896$) between shoot N and total phenol content.

Phosphorus, on the other hand, is one of the elements that play a major role in increasing the phenol content of the plant (Hajagha *et al*., 2017). Also, in this experiment, there was a significant relationship between P concentration and total phenol ($R^2 = 0.558$). *Azotobacter* is capable of solubilizing inorganic phosphate by producing organic acids, thereby converting insoluble P into plant-absorbable P (Nagananda *et al*., 2010). The inoculated plants showed the highest P concentration at the N level of 75 kg ha^{-1}, but 150 kg ha^{-1} nitrogen decreased plant P concentration, which could be due to the decreased activity of *Azotobacter* because the activity of *Azotobacter* in the rhizosphere has effects on the amount of organic matter, moisture, N, pH, and EC of the soil (Vessey, 2003). Overall, the results of this experiment showed that biofertilizers are promising for improving the quantitative and qualitative performance of medicinal herbs as has been confirmed by studies on medicinal plants. Arora *et al*. (2020) found that IAA production by different strains of the genus *Azotobacter* and Fulchieri *et al*. (1993) found that production of auxin and gibberellic acid by *Azospirillum* was responsible for the marked increase in root and shoot growth of corn. Gibberellins increase the elongation of stems, and auxins enhance cell division (Vessey, 2003), thereby increasing plant height, stem diameter, flower number, leaf number, and dry weight of different parts of the plants. In a similar study by Hajagha *et al*. (2017), who investigated the effect of *Azospirillum* and phosphate solubilizing bacteria on the coneflower plant, PGPR could be used instead of N and P inorganic fertilizers to reduce production costs. The use of these fertilizers can prevent damage to the environment, especially N in the form of nitrates.

CONCLUSION

Based on the results, 75 kg N ha^{-1} + *Azotobacter* treatment can be recommended as the best treatment in this experiment. Influenced by this recommendation, the coneflowers produced the tallest stem, highest number of lateral branches, highest leaf and stem dry weight, flower yield, and biological yield. On the other hand, the whole vegetable body of the coniferous can be used for the extraction of extracts and pharmaceuticals. The reduction of nitrogen fertilizer use in this treatment contributes to long-term soil stability and ecosystem health. Therefore, it is justified to choose this regime as the best treatment.

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