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Effect of Biofertilizers (rhizobacteria and mycorrhizal fungi) on Growth Characteristics of *Zygophyllum eurypterum* L.

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ABSTRACT

Chemical fertilizers have a devastating impact on soil and the environment when used in seedling production and planting. Conversely, biofertilizers can enhance soil structure and fertility while mitigating the harmful effects of chemical fertilizers on the environment. This study aimed to identify an appropriate biofertilizer for Zygophyllum eurypterum, a species that is particularly amenable to arid area restoration. To this end, we conducted an experiments using six different biofertilizer treatments (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Flavobacterium* F-40, *Bacillus megaterium*, *Pseudomonas fluorescens*, and *Rhizophagus irregularis*) and fertilizer-free control in a completely randomized design by cultivation of the plants in the seedling bags with 15 replications. This was done in the spring of 2018, in the research farm of Semnan University. Vegetative growth parameters such as root length, fresh and dry weight of roots and shoots, number of leaves, shoot diameter, and total chlorophyll were measured three months after planting. The percentage of root colonization with mycorrhizal fungi was measured at three and six months of age of seedlings. In this context, the maximum length of root (33.40 cm) and shoot (18.20 cm), height (51.30 cm), weight of root (99.94 g) and shoot (473.90 g), number of leaves (58.00), shoot diameter (3.32 mm) and total chlorophyll (74.96) were observed in the treatment by *Pseudomonas fluorescens*. Symbiotic mycorrhizal fungi was confirmed and it increased root length and plant height. The percentage of root colonization increased over time. Root to shoot ratio was increased by application of *Azospirillum lipoferum* fertilizer. The results showed that the use of biofertilizers *Pseudomonas fluorescens*, *Azospirillum lipoferum*, and *Rhizophagus irregularis* can be recommended in the production of *Zygophyllum eurypterum* seedlings.

Keywords: *Pseudomonas fluorescens***,** Growth improvement, Symbiotic Mycorrhiza, Arid area restoration.

INTRODUCTION

Zygophyllum is a native, perennial nutritive fodder shrub and medicinal plant that is adapted to stressful conditions of the desert, arid, and semi-arid regions (Shawky *et al*., 2019; Ranjbar-Fordoei, 2018). It is suitable for soil conservation and sand-dune fixation (Moghimi, 2006) and useful in arid and semi-arid region's ecosystem rehabilitation projects. Plants growth need three macronutrients (nitrogen, potassium, and phosphorus) and 13 micronutrients, which are mainly provided by chemical fertilizers and a very small amount of organic fertilizers (Borkar, 2015). Chemical fertilizers (especially phosphorus and nitrogen) are used universally in the production of seedlings and growing plants in the open environment, and may causeg soil pollution. One of the best alternatives to chemical fertilizers is the use of biofertilizers (Gupta *et al*., 2015).

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Biofertilizers are a low-cost source of nutrients (Cordero *et al*., 2018), environmentally friendly as well as complementary to chemical fertilizers (Borkar, 2015). These fertilizers improve soil structure and fertility, suitably decompose organic matter, dissolve mineral nutrients, produce regulators necessary for plant growth, stimulate root growth, increase vegetation yield (Sivasakhti *et al*., 2014), and reduce destructive effect of chemical fertilizers on the environment (Pérez-Montaño *et al*., 2014). Biofertilizers such as growth-promoting bacteria, endo-mycorrhizal and ectomycorrhizal, cyanobacteria, and many beneficial microscopic organisms improve material uptake, plant growth, and yield, and increase plant tolerance to biological and abiotic stresses (Bhardwaj *et al*., 2014), and modify soil fertility and restore vegetation (Qiu *et al*., 2019).

The exchange of nutrients between the plant and the mycorrhizal fungus begins with the symbiosis and penetration of the host root that cause physiological and morphological changes in the host plant (Varma, 2008). The selection of biofertilizers to improve plant growth conditions requires identification of the fertilizers appropriate for the plant species. Many researchers have tried to achieve this goal, which can be mentioned as follows.

Applying *Azotobacter chroococcum* isolated from the soil rhizosphere of *Triticum aestivum* as fertilizer under greenhouse conditions increased the root mass of this plant (Narula *et al*., 2000). *Azotobacter chroococcum* improved vegetative growth (especially root mass) and seed yield of three cultivars of *Triticum beautivum* (Kumar *et al*., 2001). Application of *Pseudomonas fluorescens* biofertilizer under drought stress on *Catharanthus roseus* could reduce the effect of drought and increase vegetative parameters such as the fresh and dry weight of the plant (Jaleel *et al*., 2007). Use of *Azotobacter chroococcum* and *Glomus mosseae* produced the highest growth, seedling establishment, plant height, and fruit yield of *Punic agranatum* (Aseri *et al*., 2008).

According to the results of Adesemoye *et al*. (2009), biofertilizers of *Bacillus pumilus* T4, *Bacillus amyloliquefaciens* IN937a, and *Rhizophagus irregularis* greatly increased the yield and growth of *Solanum lycopersicum.* Biofertilizers of the *Azotobacter* sp., *Azospirillum* sp., *Phosphobacter* sp., and *Rhizobacter* sp. significantly increased the yield and growth of *Helianthus annuus* (Dhanasekar and Dhandapani, 2012). Use of *Bacillus* sp. bacterium in *Vigna unguiculata* laboratory and field conditions indicated an increase in seed germination percentage, root length, fresh and dry weight of roots and leaves, number of pods, and seeds of this plant in bags (Nain *et al.*, 2012). Application of three different strains of *Bacillus megaterium* increased shoot and root dry weight, shoot diameter, leaf area, seedling height, gibberellic acid, salicylic acid, and indole acetic acid (IAA) in *Brassica oleracea* seedlings (Turan *et al*., 2014).

Use of *Azospirillum* sp. and *Azotobacter* sp. increased production and yield of vitamin C in tomatoes (Meena *et al*., 2017). According to the results (Mathivanan *et al*., 2017), inoculation of *Arachis hypogaea* with *Rhizobium* sp., *Pseudomonas* sp., and *Bacillus* sp. increased photosynthetic pigments (chlorophyll and carotenoids) and protein. The effect of biofertilizers such as *Azotobacter chroococcum, Azospirillum lipoferum, Pseudomonas fluorescens, Acetobacter diazotrophicus*, and *Trichoderma aviride* increased grain yield, nutrient uptake, and grain quality of *Pennisetum glaucum* (Singh *et al*., 2018). In the study of Chu *et al*. (2018), use of *Bacillus megaterium* in soil with high nitrate concentration increased root length and improved plant growth in Zea mays. *Azotobacter chroococcum* and *Pseudomonas fluorescens* biofertilizers increased leaf number, leaf area, and crown of *Brassica oleracea* compared to the control (Salim *et*

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al., 2018). *Glomus mosseae* increased growth factors of *Triticum durum* in phosphorus-deficient soil (Di Martino *et al*., 20018).

Use of *Bacillıs megaterium*, *Bacillus subtilis, Paenibacillus polymyxa, Pseudomonas putida,* and *Pseudomonas fluorescens* increased plant height, canopy diameter, leaf chlorophyll, essential oil, and yield of *Origanum onites* (Kutlu and *et al*., 2019). According to a report by Gabra *et al*. (2019), Protein, total carbohydrates, dry weight of aerial and underground biomass, and pigment content of *Helianthus annuus* and *Zea mays* were increased by Bacillus sp. growth, and yield *Capsicum frutescens* were increased by *Azotobacter* sp., *Azospirillum* sp., *Bacillus* sp., *Pseudomonas* sp., and *Cytophaga* sp. (Al Habib *et al*., 2020). Rhizobacteria can be used as biofertilizers to increase soil fertility and plant productivity in the Eastern region of Saudi Arabia (Al Ali *et al*., 2021).

In the present study, we aimed to investigate the effect of different biofertilizers (Azotobacter chroococcum, Azospirillum lipoferum, FlavobacteriumF-40, Bacillus megaterium, Pseudomonas fluorescens, and Rhizophagus irregularis) on Zygophyllum eurypterum seedlings to determine the best biofertilizer.

MATERIALS AND METHODS

Seeds of *Zygophyllum eurypterum* were collected in late spring 2018 from a rangeland located in the east of Semnan City. The experiment was conducted in the form of a completely randomized design with biological fertilizer treatments (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Flavobacterium* F-40, *Bacillus megaterium, Pseudomonas fluorescens,* and *Rhizophagus irregularis*). The seeds were separated from the wings and were stored in the refrigerator (15°C) until the start of the experiment. Then, the seeds were sterilized with 70% ethanol solution for 30 seconds and 2% sodium hypochlorite for 15 minutes. Anti-UV black seedling bags, 35 cm high and 9 cm in diameter, were prepared and sterilized with sodium hypochlorite. The bags were filled with a combination of 50% agricultural soil, 40% sand, and 10% animal manure. Five seeds were planted in each bag and covered with a 1 cm layer of sand. After germination and when seeding growth reached three leaves, the seedlings were weeded. At this stage, liquid bacterial biofertilizers *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Flavobacterium* F-40, *Bacillus megaterium,* and *Pseudomonas fluorescens* (produced by the Soil and Water Research Institute of Iran) were added to the soil of the bag with 5×10^7 CFU. Solid inoculation of *Rhizophagus irregularis* was applied before sowing. Fifteen replications were considered for each treatment. Samples were irrigated with water with a salinity of 371 μmho cm^{-1} for six months. The vegetative growth was measured in five replications after three months.

The percentage of root colonization was measured in three replications after three and six months. Root coloration was performed by the method of Phillips and Hayman (1970) and the percentage of root colonization (symbiosis) was calculated by the intersection method (Norris *et al*., 1991). Vegetative characteristics of root and shoot length and plant growth (total root and shoot length) of seedlings were measured by a ruler (in cm). The Root-to-shoot length ratio

Table 1. Treatments coding guide.

Cod	Treatment	Cod	Treatment
Co	Control	Ba	*Bacillus megaterium*
AZ	*Azotobacter chroococcum*	So	*Pseudomonas fluorescens*
AS	*Azospirillum lipoferum*	Mi	*Rhizophagus irregularis*
Fl	*Flavobacterium sp.*		

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in treatments was also determined. The fresh and dry weights of roots, shoots, and leaves were measured by balance (accuracy of 0.001 grams). The shoot diameter was measured using a digital caliper (in mm). Estimation of total chlorophyll was performed with Chlorophyll Meter (Spad-502) 3 months after planting.

Statistical Analysis

Data analysis was carried out using SAS software version 9.4 and the means were compared by Tukey test.

RESULTS

The analysis of variance (Table 2) showed that biofertilizers significantly affected most of the growth parameters, including plant height, root length, and shoot and root biomass.

Root Symbiosis

The study of the symbiosis of *Rhizophagus irregularis* in three treatments (control, 3-month- and 6-month-old seedlings) indicated the symbiosis of this plant with the fungi (Figure 2). The increasing attendance of fungus in the root zone caused a significant increase in fungal symbiosis. Symbiosis was 19.73% in 3 months and 79.09% in 6 months (Figure 3).

Underground Biomass

According to Table 2, there was a significant difference between the studied treatments in root length and root fresh weight ($P < 0.05$) and the number of leaves ($P < 0.01$); while root dry weight was not significant. The highest root length (33.40 cm) and (35.60 cm) in *Zygophyllum eurypterum* seedlings were due to the use of *Pseudomonas fluorescens* and *Rhizophagus irregularis*, respectively. Root fresh weight (99.94 g) was higher than the other treatments in *Pseudomonas fluorescens*. There was no statistically significant difference in root dry weight between treatments. *Pseudomonas fluorescens* led to the highest number of leaves (57.60) in seedlings [Figure 4 (A-C), and Table 2].

Plant Height and Root-to-Shoot Ratio

Significant differences in plant height and root-to-shoot ratio were observed ($P < 0.01$) (Table 2). *Pseudomonas fluorescens* and *Rhizophagus irregularis* caused the highest plant height 51.30 and 52.40 cm, respectively. The highest root-to-shoot ratio (3.602) was developed by *Azospirillum*

(a) (b) (C)

Figure 1. Stages of the project. a: Treatments with rhizobacteria, b: Plant roots in seedling bag, c: Estimating chlorophyll of plants.

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Table 2. Effect of different biofertilizer treatments on seed vegetative traits of *Zygophyllum eurypterum*.[A]

Treatments	Underground biomass(Root)						Aerial biomass(Shoot)				
Biofertilizers	Length (cm)	Fresh weight (g)	Dry weight (g)	Number of leaves	Plant height (cm)	Ratio of root to shoot	Length (cm)	Fresh Weight (g)	Dry Weight (g)	Shoot diameter (cm)	Total chlorophyll
Control	30.80b	74.64ab	27.06a	25.80c	41.60c	2.94ab	10.80c	222.8c	81.46a	3.28a	53.76b
Azotobacterchroococcum	33.00ab	93.22ab	34.56a	31.00bc	45.70bc	2.71ab	12.70bc	352.2abc	97.18a	3.82a	60.10b
Azospirillumlipoferum	33.80ab	67.72b	28.10a	37.00bc	43.62bc	3.60a	9.82c	308.3abc	138.40a	3.40a	57.04b
Flavobacterium F-40	33.00ab	92.04ab	31.28a	28.80bc	46.50bc	2.47ab	13.50bc	441.4ab	119.50a	3.76a	63.90ab
Bacillus megaterium	30.50ab	92.22ab	37.04a	40.60b	47.80ab	2.11ab	16.90ab	384.5abc	132.50a	3.30a	54.24b
Pseudomonas fluorescens	33.40a	99.94a	32.46a	57.60a	51.30a	1.99b	18.20a	473.9a	119.80a	3.32a	74.96a
Rhizophagusirregularis	35.60a	90.22ab	34.98a	39.60bc	52.40a	2.67ab	17.90ab	240.9bc	123.60a	4.00a	66.34ab
Pr≤ F	P< 0.05	P< 0.05	P> 0.05	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P< 0.01	P> 0.05	P> 0.05	P< 0.01

[a] P< 0.05 and P< 0.01 indicate that means of treatments are different in each column in the significant level of 0.05 and 0.01 according to Tukey's Test. P> 0.05 indicate that there is no significant difference between the treatment in the column according to Tukey's Test. Different letters (a, b, c) indicate significant differences in means.

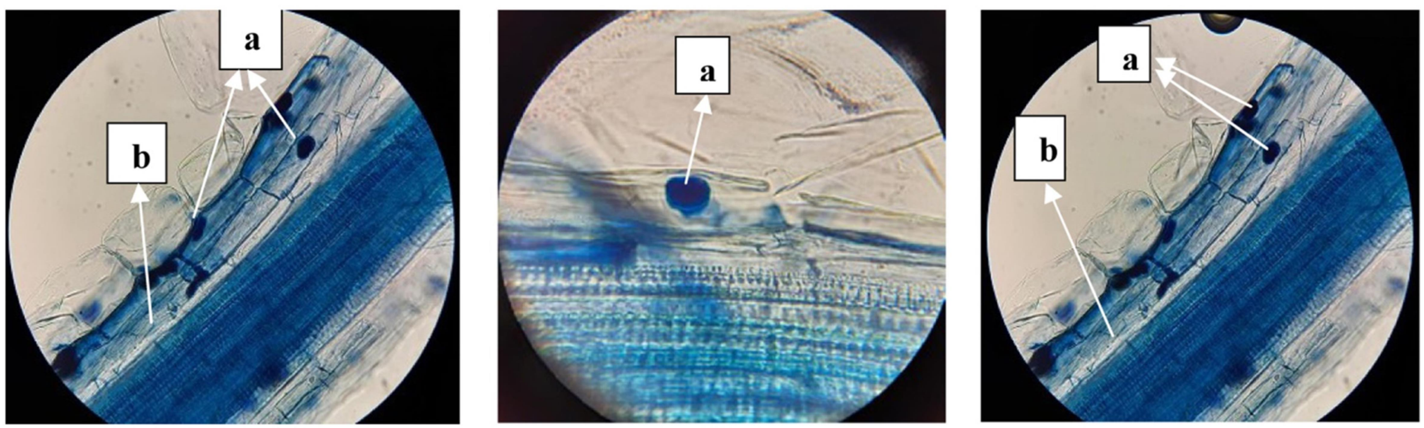

Figure 2. Formation of *Rhizophagus irregularis* organs due to symbiosis in the *Zygophyllum eurypterum* roots of inoculated: (**a**) Fungi vesicle in roots of the plant (*Zygophyllum eurypterum*) and (**b**) Fungi hyphae in roots of the plant (*Zygophyllum eurypterum*).

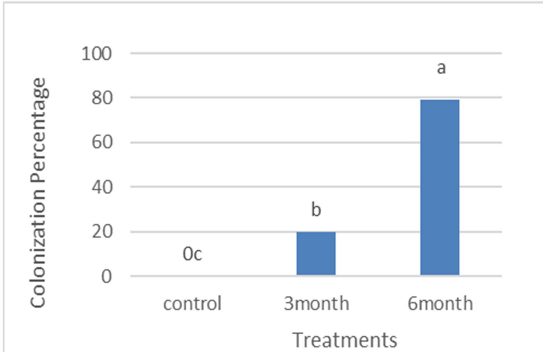

Figure 3. Percentage of fungal colonization by *Zygophyllum eurypterum* root in the 3- and 6-months period compared to the control (no fungus).

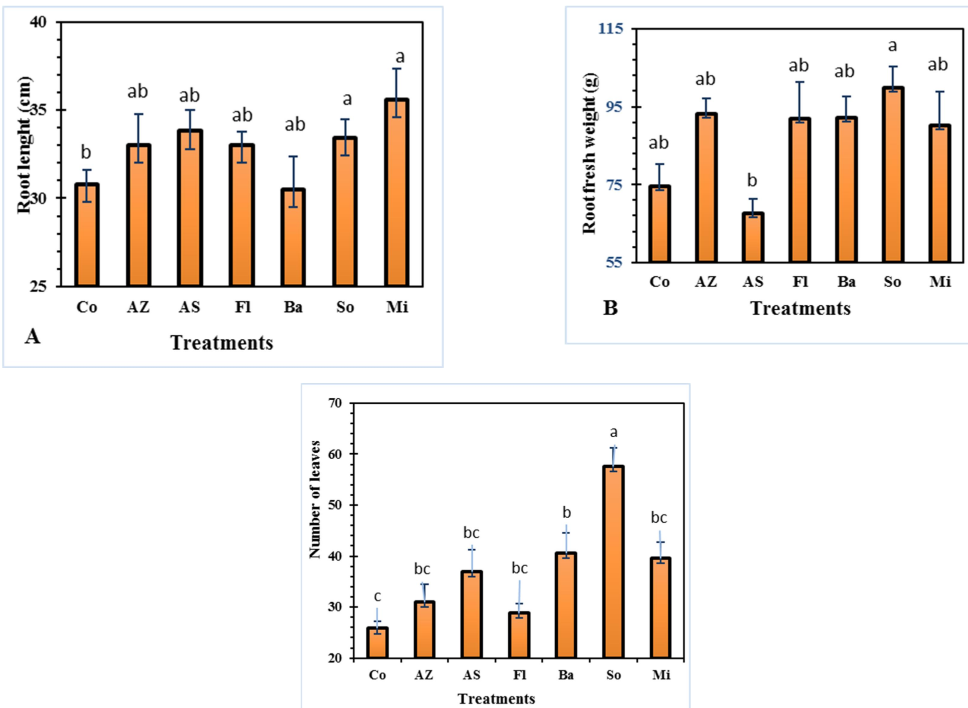

Figure 4. Effect of different biofertilizer treatments on underground biomass traits of *Zygophyllum eurypterum*. Different letters (a, b, c) indicate significant differences in means.

lipoferum [Figure 5 (A-B), and Table 2].

Aerial Biomass

The shoot length and fresh weight were affected by the treatments (P< 0.01), but the shoot fresh and dry weights were not significantly different (Table 2). Among the treatments, the maximum length of the shoot (18.20 cm) and shoot fresh weight (473.9 g) was observed in the use of *Pseudomonas fluorescens*. No significant difference was observed in shoot dry weight and shoot diameter due to the use of biofertilizers [Figure 6 (A-B) and Table 2].

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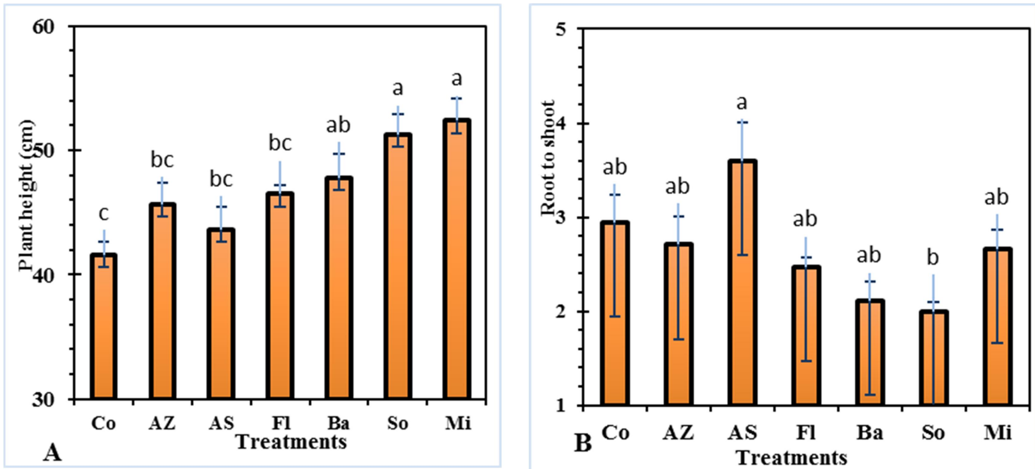

Figure 5. Effect of different biofertilizer treatments on plant height and the root-to-shoot ratio of *Zygophyllum eurypterum.* Different letters indicate (a, b, c) significant differences in means.

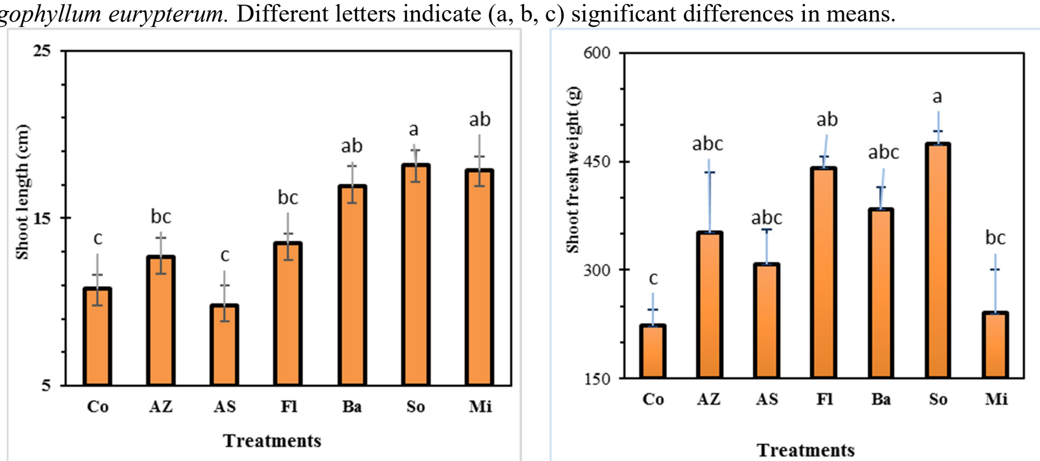

Figure 6. Effect of different biofertilizer treatments on aerial biomass of *Zygophyllum eurypterum.* Different letters indicate (a, b, c) significant differences in means.

Total Chlorophyll

Different treatments of biofertilizer caused significant differences (P< 0.01) in total chlorophyll (Table 2). The highest total chlorophyll (74.96) was observed in the *Pseudomonas fluorescens* treatment (Figure 7 and Table 2). Also, results indicated no significant difference in total chlorophyll content between *Rhizophagus irregularis*, and *Flavobacterium sp.* treatments with So treatment.

DISCUSSION

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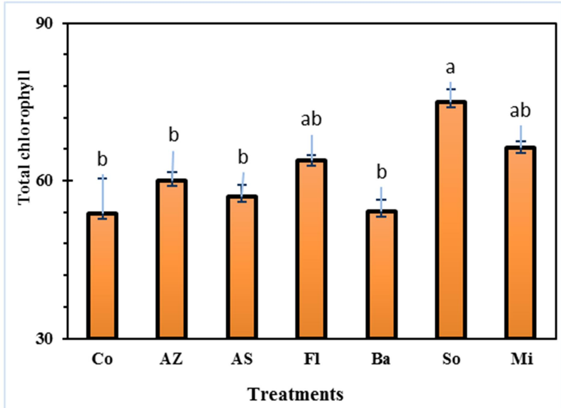

Figure 7. Effect of different biofertilizer treatments on total chlorophyll of *Zygophyllum eurypterum.* Different letters indicate (a, b, ...) significant differences in means.

Based on the results of this study, use of *Pseudomonas fluorescens* increased root length, shoot length, plant height, fresh weight of roots and shoots, number of leaves, shoot diameter, and total chlorophyll. These results are similar to the results of other researchers (Katiyar and Goel, 2003; Kochar *et al*., 2011; Alemu and Alemu, 2015; Jamil *et al*., 2018; Prabhukarthikeyan *et al*., 2018)). The root-to-shoot ratio increased by *Azospirillum lipoferum*, which is similar to the results of Dhanasekar and Dhandapani (2012), Singh *et al*.(2018), and Meena *et al*. (2017). The increase of aerial and underground biomass was significant with the use of bacterial biological fertilizers, especially *Pseudomonas fluorescens*. Improving plant growth by using different strains of PGPR (plant-growth-promoting rhizobacteria) can be due to the increased production of phytohormones, iron-chelating siderophores, and the production of amino cyclopropane and carboxylate deaminase (Ashraf *et al*., 2013), osmotic pressure regulation, production of antioxidants, and physiological support to the plant (Abbas *et al*., 2019), and the impact of pathogens, especially pathogenic fungi (Hernández-León *et al*., 2015). Growth-promoting bacteria, by producing IAA (Indole Acetic Acid), increased root spread (increase the number of capillary and lateral roots) and shoot growth. The ability of bacteria to produce IAA in the rhizosphere depends on the primary materials available and the use of tryptophan from root secretions and carious cells (Alemu and Alemu, 2015). Increased total chlorophyll in plants inoculated with bacteria indicates increased carbon uptake and photosynthetic activity (Cappellari *et al*., 2015). Many bacteria, especially *Pseudomonas* sp., increase photosynthetic pigments and chlorophyll (Mathivanan *et al*., 2017). *Pseudomonas fluorescens* increase plant growth by exudating growth-promoting compounds such as 13-Tetradecadien-1-ol, 2-butanone, and 2-Methyl-n-1-tridecene (Park *et al*., 2015). Also, *Pseudomonas* sp. strains increase the uptake of potassium, calcium, iron, magnesium, and manganese by plants and improve their growth (Esitken *et al*., 2006).

The symbiosis of this plant with mycorrhiza was observed and confirmed. Increasing the time of mycorrhizal fungus near the root of *Zygophyllum eurypterum* increased the percentage of colonization and symbiosis of the fungus with the root. The mycorrhizal symbiosis between fungi and roots plants increased root length and plant height (Shankarappa *et al*., 2017; Tian *et al*., 2019; Chenchouni *et al*., 2020; Liu *et al*.,

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2020; Asghari *et al*., 2021). Applying mycorrhizal fungi as biofertilizer improves plant yield, sustainability, and evolution of biodiversity, sustainability, and productivity of ecosystems by providing mineral nutrients and water, reducing the impact of environmental stresses, and also protecting against pathogens (Hijri and Boi, 2018), specialty the fungi (Khaosaad *et al*., 2007). Also, plant symbiosis with mycorrhizal fungi increases the growth of other rhizosphere microorganisms and enhances plant growth. These changes root colonization and coexistence, release root exudates, and produce the enzyme phosphatase in the rhizosphere. Phosphatases produced by extracellular hyphae can hydrolyze extracellular phosphate bonds and ultimately increase available phosphorus for plants and improve plant growth (Swamy *et al*., 2016). It also increases the chances of plants obtaining micronutrients by increasing the solubility of heavy metals (Bhattacharyya and Jha, 2012). The use of mycorrhizal fungi in seedling production improves plant establishment (Jiménez-Moreno *et al*., 2018). Mycorrhizal fungi Arbuscular mycorrhizal fungi enhance the reestablishment of *Leymus chinensis.* Survival, growth and asexual reproduction of plants indicate that the plant-AM fungi mutualism could improve the vegetation reestablishment in bare and saline-alkaline soils (Zhang *et al*., 2011).

CONCLUSIONS

The effect of bacterial and mycorrhizal biological fertilizers on the growth of biomass of *Zygophyllum eurypterum* was evaluated. The use of *Pseudomonas fluorescens* and *Azospirillum lipoferum* bacteria increased plant growth. Mycorrhizal fungi symbiosis with the plant improved growth characteristics. The rhizobacteria *Pseudomonas fluorescens*, *Azospirillum lipoferum,* and *Rhizophagus irregularis* are useful treatments for seedling production and planting of *Zygophyllum eurypterum*.

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تاثیر کودهای زیستی (باکتریایی و قارچ مایکوریز) بر ویژگی‌های رویشی *Zygophyllum eurypterum* L.

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چکیده

استفاده از کودهای شیمیایی در تولید و کاشت نهال تاثیر مخربی بر خاک و محیط زیست دارد. جایگزینی کودهای شیمیایی با کودهای زیستی متناسب اثرات مثبتی دارد. استفاده از کودهای زیستی، سبب بهبود ساختمان و باروری خاک، تجزیه مناسب مواد آلی، حل کردن مواد مغذی معدنی، تولید تنظیم کننده‌های لازم برای رشد گیاهان، تحریک رشد ریشه و افزایش عملکرد پوشش گیاهی و کاهش تاثیر مخرب کودهای شیمیایی بر محیط زیست می‌شوند. در این پژوهش شناسایی کود زیستی متناسب با گیاه Zygophyllum eurypterumبه عنوان گونه‌ای مستعد در احیائ مناطق خشک، مورد توجه قرار گرفت. آزمایشی با شش تیمار کود زیستی (باکتری‌های Azotobacter chroococcum، Azospirillum lipoferum ، Flavobacterium F-40، Bacillus megaterium ، Pseudomonas flourescens و قارچ Rhizophagus irregularisو (شاهد فاقد کود) با ۱۵ تکرار طراحی و در قالب طرح کاملا تصادفی با کشت گیاه در کیسه نشا، اجرا شد. بعد از سه ماه از کاشت، صفات رویشی (طول و وزن تر و خشک ریشه و ساقه و تعداد برگ و قطر یقه و کلروفیل کل) اندازه‌گیری شد. اندازه‌گیری درصد کلونیزاسیون ریشه با قارچ مایکوریز در دو زمان سه و شش ماهه شدن نهال‌ها انجام شد. بیشترین طول ریشه (۳۳/۴۰ سانتی‌متر) و ساقه (۱۸/۲۰ سانتی‌متر)، ارتفاع گیاه (۵۱/۳۰ سانتی‌متر)، وزن تر ریشه (۹۹/۹۴ میلی‌گرم) و ساقه (۴۷۳/۹ و ۱۱۹/۸ میلی‌گرم)، تعداد برگ (۵۸)، قطر یقه (۳/۳۲۰ میلی‌متر) و کلروفیل کل (۷۴/۹۶) در اثر استفاده از باکتری P. fluorescens، ایجاد شد. همزیستی گونه با قارچ مایکوریز تایید شد و تیمار مایکوریز سبب افزایش طول ریشه و رشد گیاه شد. افزایش زمان قرارگیری کود در مجاور ریشه گیاه، سبب افزایش درصد کلونیزاسیون شد. نسبت ریشه به ساقه با کاربرد کود A. lipoferum افزایش یافت. نتایج نشان داد استفاده از کودهای زیستی (باکتری‌هایP. fluorescens ، A. lipoferumو قارچ Rhizophagus irregularisدر تولید نهال Z. eurypterumقابل توصیه است.