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Ffects of Mycorhiza and Plant Growth Promoting Rhizobacteria on Growth, Nutrients Uptake and Physiological Characteristics in *Calendula officinalis* L.

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Abstract: *Calendula officinalis* Linn. (*Asteraceae*) is used medicinally in Europe, China and India amongst several places in the world. It is also known as "African marigold". It has been a subject of several chemical and pharmacological studies. Effects of biological Fertilizers were analyzed on growth parameters, nutrients uptake and psychological characteristics of marigold (*Calendula officinalis* L.) in Completely Randomized Design (CRD) with eight treatments and four replicates. Inoculation treatments were included strains of *Azotobacter*, *Pseudomonas*, *Azospirillum*, Arbescular Mychoriza (AM) and the combined composition of them. The results showed that marigold seeds inoculated with Plant Growth Promoting Rhizobacteria (PGPR) extremely increased the growth parameters such as the growth dry weights of shoot and root, photosynthetic pigments, the concentration of nitrogen, phosphorus and potassium in leaves and roots.

Key words: *Calendula officinalis* Linn. Growth · Nutrients uptake · Physiological characteristics · Mycorrhiza · Plant Growth Promoting Rhizobacteria

INTRODUCTION

Medicinal plants, as the sources of chemical or combination of active phytochemicals are known for their natural characteristics which are widely used in the world [1].

Marigold (*Calendula officinalis* L.) is an annual herbaceous plant belongs to the *Asteraceae* family [2]. This medicinal plant has several activities that can be point out for its anti-HIV [3], antioxidant activity [4], anti-inflammatory [5], protecting the liver [6], intermittent spasms [7] and anti-cancer activities [8]. Also, for a long time, it has been used in food, pharmaceutical and cosmetics industries.

In recent decades, Plant Growth Promoting Rhizobacteria (PGPR) bacteria and mycorrhiza have been considered as a biological fertilizer, a cheap alternative, environment compatible biosolid or bioorganics for the replacement of expensive chemical fertilizers [9].

Creative technology development and sustainable agriculture development such as application of *Arbescular Mychoriza* (AM) fungi is an important economical indicator in agriculture crop growth. It have been reported that certain micro-organisms improve growth (PGPRs), promote fungi mycorrhiza activity and growth behavior [10, 11]. Thus, inoculation of specific bacteria may enhance and bring a good health and fertility to the soil that contributes and leads to a higher value sustainable products and quality [12]. However, useful information about the use of micro-organisms in plants is very limited.

This paper analyzes the effect of plant growth-promoting *Rhizobacteria* and *Mycorrhiza* in growth, nutrients uptake and some physiological characteristics of *Calendula officinalis* L.

MATERIALS AND METHODS

Experiments were carried out in pot culture conditions with Clay loamy soil in Agriculture and Natural Resources Research Center of Mazandaran province. Before any plantation, combined soil samples were collected and their physicochemical properties were analyzed based on standard methods.

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The experiments were conducted in Completely Randomized Design with eight treatments and four replicates in 2010. Treatments include: 1. control (uninoculated sample pot), 2. *Azotobacter chroococcum* 5 (AZ), 3. *Pseudomonas fluorescens* 168 (PS), 4. mycorrhiza (MY), 5. *Azospirillum lipoferum* (AZ.L), 6. coinoculation of *Azotobacter chroococcum* 5 + *Pseudomonas fluorescens* 168 (AZ + PS), 7. *Azotobacter chroococcum* 5 + mycorrhiza (AZ+MY) and 8. *Azotobacter chroococcum* 5 + *Azospirillum lipoferum* (AZ + AZ.L).

In these experiments inoculants were provided from Agricultural Research Center of Mazandaran and the seed was inoculated by different treatments. For this purpose, 1 g of calendula seeds was inoculated with 3 ml of the suspension of inoculums with 10^8 cfu (Colony Forming Unit) for 8 hours. For *Mycorrhiza*, each gram of the seed was inoculated with 20 grams of powdered fungi and for both bacteria and fungi, the mentioned ratio was applied.

The operation of sowing was performed in the pots. After the relative growth was obtained in each pot, 4 plants were kept for testing. Operations, such as irrigation and spraying for pests control were successfully carried out.

Plant growth parameters such as; fresh weight of shoot and root, dry weight of shoot and root, some physiological parameters including Chlorophyll content based on the Bruinsma's method were evaluated [13]. In addition, the amount of carotenoids (defined by Jensen) [14] were measured. In the flowering stage, sampling was performed in order to measure the nutrient elements; nitrogen, phosphorus and potassium. P-value with Olsen method [15], nitrogen with kjeltec method [16], potassium with flame photometer [17] were determined.

The obtained data were analyzed and statistical calculated by SPSS and Excel software.

RESULTS

Chlorophyll A: Analysis of variance showed that the treatments caused a statistically significant at five percent level based on Duncan test with control (Figure 1). In this figure, AZ + MY treatment showed the best effect on chlorophyll a content, also MY inoculation had no significant increase in compare to control.

Chlorophyll B: Analysis of variance also showed that the treatments caused a statistically significant at five percent level based on Duncan test with control (Figure 2). In this figure, AZ + MY treatment showed the best effect on chlorophyll a content, also MY inoculation had no significant increase in compare to control.

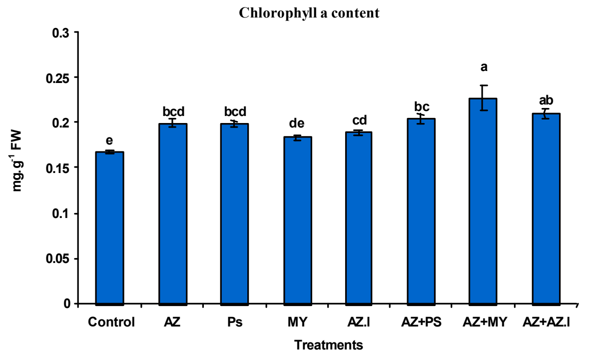

Fig. 1: Effect of different treatments on Chlorophyll a content

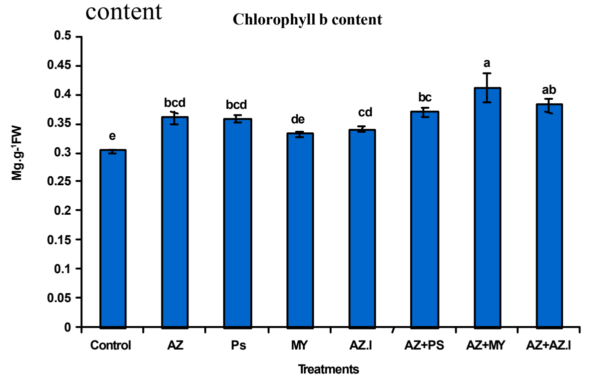

Fig. 2: Effect of different treatments on Chlorophyll b content

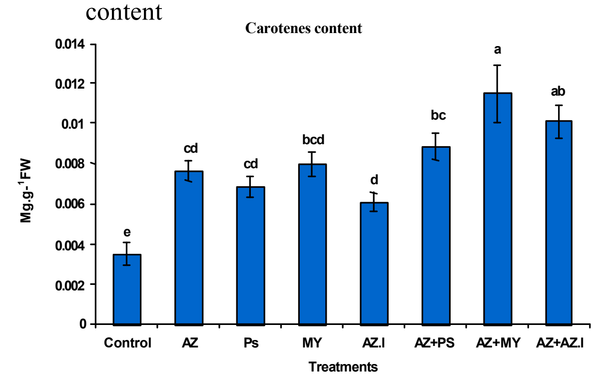

Fig. 3: Effect of different treatments on carotenes content

Carotene: Results of variance analysis also showed, based on Duncan (α=5%). There were no significant differences between treatments and control in carotene amount of leaves (Figure 3). Carotenoid content analysis of variance showed that the treatments caused a statistically significant at five percent level test with control (Figure 2). In this figure, AZ + MY treatment showed the best effect on chlorophyll a content, also MY inoculation had no significant increase in compared with control. AZ + MY and AZ.L inoculants had the highest and lowest rates of carotene amounts in compare to control.

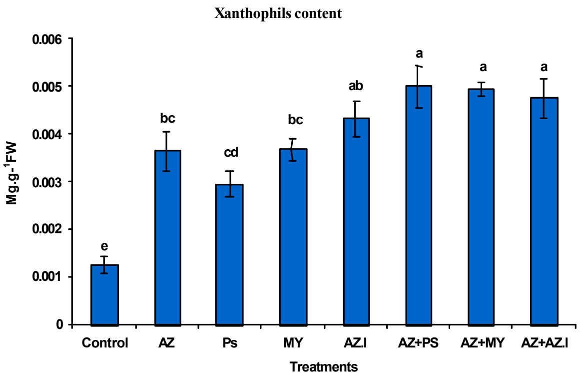

Fig. 4: Effect of different treatments on Xanthophylls content

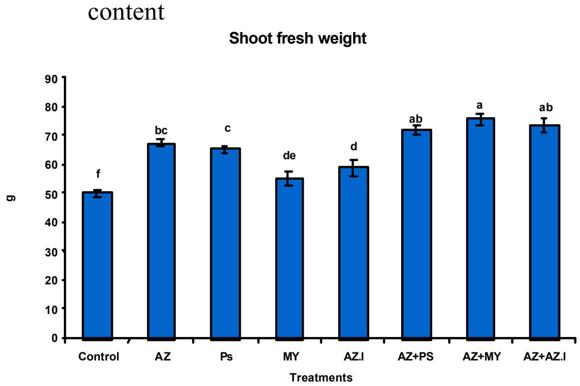

Fig. 5: Effect of different treatments on fresh shoot weight

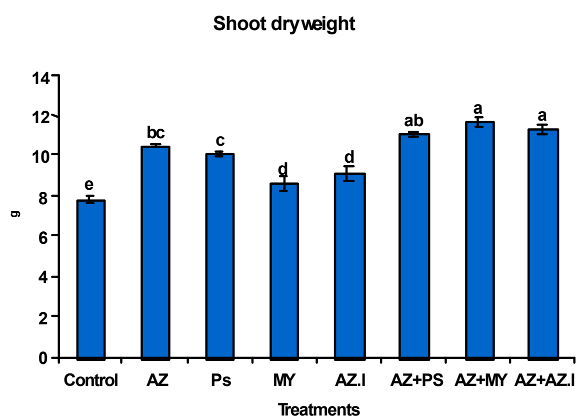

Fig. 6: Effect of different treatments on dry shoot weight

Xanthophylls: Results of Xanthophylls amount in leaves showed a significant difference between treatments and control based on Duncan test at five percent level (Figure 4). In this study, co-inoculation of AZ + AZ.L, AZ + PS and AZ + MY had maximum rates of treatment showed the highest impact on chlorophyll a content, while MY inoculation had no significant increase in compared with control. AZ + MY and AZ.L inoculants had the highest and lowest rates of Xanthophylls in leaves.

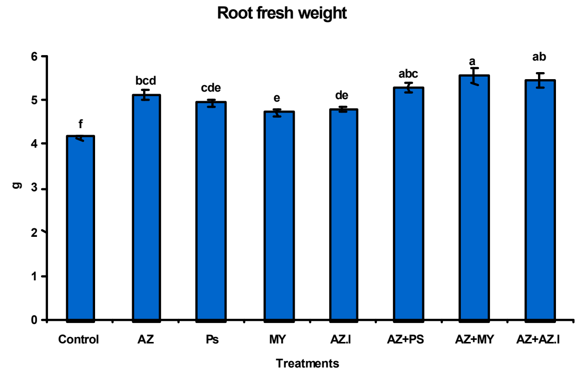

Fig. 7: Effect of different treatments on fresh root weight

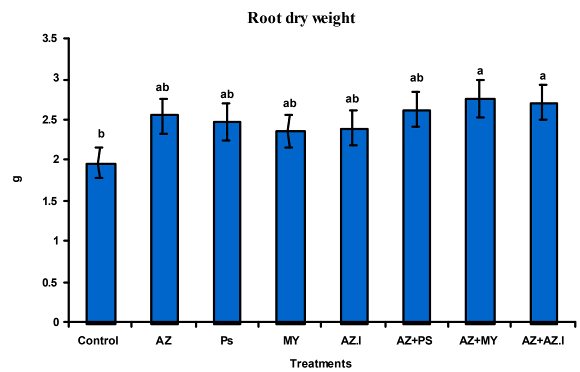

Fig. 8: Effect of different treatments on dry root weight

Fresh Weight of Shoot: Analysis of variance showed that the treatments caused a statistically significant at five percent level based on Duncan test with control (Figure 5). In this figure, AZ + MY, AZ + PS, AZ + AZ.L had shown the greatest increase in compare to control.

Dry Weight of Shoot: Analysis of variance showed that the treatments caused a statistically significant at five percent. Comparison results of mean shoot dry weight showed that the differences between the mean stem dry weight in all treated and control plants were significant (Figure 6). AZ.L treatment showed the lowest increase in combined treatments and AZ + PS, AZ + MY, AZ + AZ.L also showed that maximum increase in compare to the control.

Fresh Weight of Root: Analysis of variance of fresh root weight showed that the treatments caused a statistically significant at five percent level based on Duncan test with control (Figure 7). The results showed that among treatments, combined treatments of AZ + AZ.L, AZ + MY, AZ + PS had the highest rate of fresh root weight.

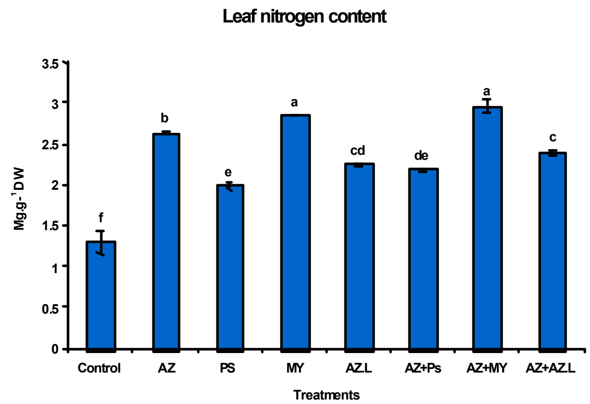

Fig. 9: Effect of different treatments on nitrogen concentration in plant

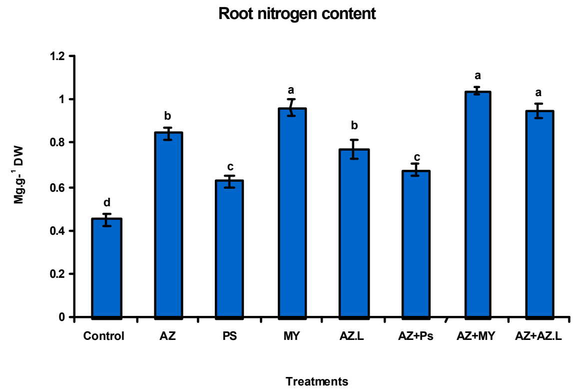

Fig. 12: Effect of different treatments on nitrogen concentration in root

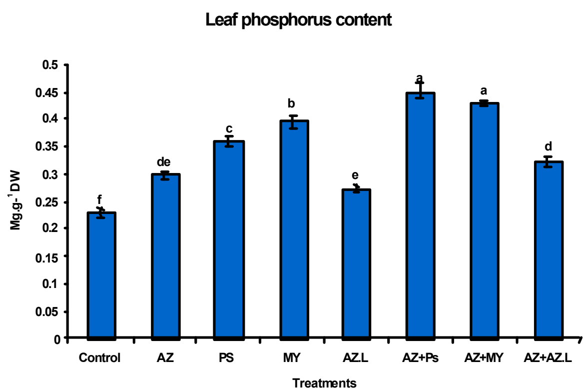

Fig. 10: Effect of different treatments on phosphorus concentration in plant

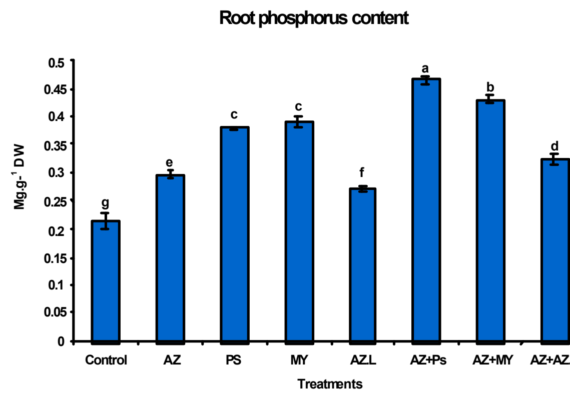

Fig. 13: Effect of different treatments on phosphorus concentration in root

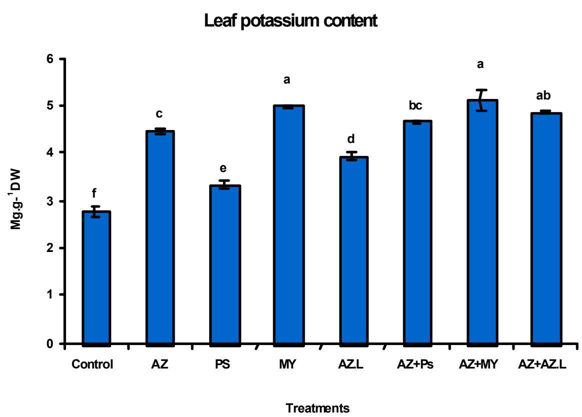

Fig. 11: Effect of different treatments on potassium concentration in plant

Dry Weight of Root: The results indicated that only treatments AZ + MY, AZ + AZ.L showed significant differences to control and other treatments. there were no significantly differences of the dry weight of root (Figure 8).

Effect of MY and Different PGPR's Inoculation on Nutrients Uptake by Plant: Inoculation of plant growth promoting *Rhizoorganisms* includes bacteria and fungi can help nutrients uptake in plant, so this matter caused improvement of plant growth. In this experiment, nitrogen, phosphorus and potassium uptake in plant increased to control. Analysis of variance showed that the effect of different treatments was statistically significant in α=5% (Figure 9). MY and AZ + MY treatments showed the highest rate in compare to control. Co-inoculation of AZ + PS and PS treatments showed the lowest rate of nitrogen uptake in plants. Changes of phosphate uptake affected by inoculation of various microorganisms showed in Figure 10. The AZ + PS, MY + AZ inoculants showed the highest rate of phosphate uptake in plant in compare to control. Maximum potassium uptake was obtained by inoculation of AZ + MY and MY and minimum it's uptake was by Ps inoculation (Figure 11).

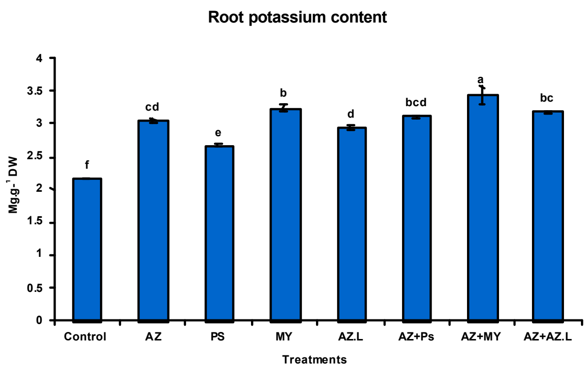

Fig. 14: Effect of different treatments on potassium concentration in root

Effect of MY and Different PGPR's Inoculation on Nutrients Uptake by Root: According to Duncan test ($p \leq 0.05$), N, P and K uptakes were significantly affected by different strains. Results of N uptake in roots of plants inoculated with MY and AZ + MY and AZ + AZ.L, according to Duncan test ($P \leq 0.05$), had statistically different significant in compare to control (Figure 12). Highest rate of P uptake was obtained by inoculation of AZ + PS and AZ + MY (Figure13). Maximum rate of K uptake was obtained in plant roots which was inoculated with AZ + MY and MY treatments (Figure 14).

DISCUSSION

The main objective of present investigation was to screen the efficient plant growth promoting rhizobacteria, mychorriza and their co-inoculations on *Calendula officinalis* L. The isolates used in our pot experiments showed positive effects on growth, physiological parameters and nutrients uptake in plant and root. The experiments envisaged in this work indicated that the benefits of plant inoculation with growth stimulating bacteria, the elected isolates of PGPR improved the growth parameters, nutrients uptake and physiological characteristics of the targeted plants. Increase of growth parameters affected by selected isolates were due to in part to the ability of isolates to promote the plant growth such as Nitrogen fixation, phosphate solubility and sidrophore production or other properties [18].

Importance of PGPR's was also reported by other research scientists. Inoculation of wheat with mycorrhizal fungi in the medium contained heavy metals significantly increased the chlorophyll content. Azotobacter has increased chlorophyll content through increasing the growth activity in nitrogen uptake [19, 20]. Inoculation of basil (*Ocimum Basilicum* L.) with *Mycorrhizal* fungi (*Glomus fasciculatum*) has also increased the amount of chlorophyll, vegetative growth parameters of shoot and root dry weight in compared to the control [1]. Inoculation of Corn and bean with *Mycorrhizal* fungi showed a significant increase in the amount of carotenoids [21]. Results of present investigation showed that the impact and importance of the selected isolates on colonization, adaptive and competitive capacities in the rhizophere of marigold. In other earlier investigations, these PGPR's were affected on yield, growth parameter and physiological properties. Inoculation of *Driganum vulgarel L.* (hirtum) with *Mycorrhizal* fungi caused an increase in the parameters such as leaf surface, fresh and dry weight of roots [22, 23].

In the present research, investigation was carried out effectiveness of some isolates on nutrients uptake. The obtained results were confirmed that PGPR had triggered an increase in root surface area of the plant. It was resulted in an increase in mineral uptake and in turn has enhanced shoot biomass accumulation. Uptake refers to a function, namely the transport of nutrients through the plasma member of root cells, i.e. the net flux from the cultivation medium to the plant, should theoretically be expressed per unit of absorbing surface. Because of the difficulties exist in measuring the surface; it is usually expressed per unit of root weight. Therefore, if an increase in the amount of nutrients taken up from the medium by PGPR- inoculated plants were simply dye to an extension of the root surface, the effect of PGPR is entirely dependent on root development and not on the uptake function. Nutrients uptake, particularly N uptake, is known to be tightly regulated by the plant's nutrient demand [24], in which changes normally resulted in opposite variation of rates of the root growth and nutrients uptake. Besides that, the possible direct effect of PGPR on nutrients uptake, the indirect effect via an increase in the root surface area due to the stimulation of the root development has to be considered. An enhanced plant 'nutrient uptake' owing to PGPR was extensively described in the literatures [1, 22, 25-28].

The cause of an increase in nutrients uptake in *Mycorrhizal* coexist plants may be due to the *Mycorrhizal hayf* that grow in a greater volume of soil and consequently find more resources of the elements [29]. Developed *Hayf Arbuscular Mycorrhizal* fungi were able to penetrate the pores of the soil which the cords and capillary roots were unable to penetrate. As a result, the absorption area of non-moving elements such as phosphorus and potassium has increased [29].

CONCLUSION

In this study, effects of *Mycorhiza* (AM) and plant growth promoting *Rhizobacteria* (PGPR's) on growth, nutrients uptake and physiological characteristics of *Calendula officinalis* L. were investigated. Based on obtained results, it was concluded that these bacteria had significant potential for the practical use of the sources for biological preparations. In addition, substitution of chemical fertilizers had improved the physiological characteristics and medicine properties of the *Calendula officinalis* Linn. (*Asteraceae*).

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