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Effect of the salinity stress and arbuscular mycorhizal fungi (AMF) on the growth and nutrition of the *Marigold (Calendula officinalis*)

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Abstract

In order to evaluate the effect of the Arbuscular mycorhizal fungi (AMF) on Calendula officinalis, an experimental has been carried out in term of salinity stress in totally randomized Factorials in shahrood. The samples were cultivated in tested sandy loam soil. This experiment were conducted in hub seedling (pot) method with four salinity treatment in 1.5-3.5-5.5-7.5 (dSm^{-1}) concentration, two level with and without mycorrhiza in 4 trial. The Fl Calendula seed were cultivated with mycorhizal fungi simultaneously in which the salinity treatment was applied in leaf stage four. The measured characteristics included dry weight, root and shoots, leaf area, number of flowering branches. The result has implied that utilizing the different mycorrhiza salinity stress has a meaningful effect on measured characteristics that is on the dry weight of shoots, the leaf area on 5%, and the number of flowering branches Chlorophyll a and b on the 1%.

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Introduction

Soil salinity affects the establishment, growth, and development of plants, causing important yield losses Salinity negatively affects three aspects of plant physiology *(Evelin et al., 2007)*.

Salinity is one of the significant growth reduction factors of the plants in different part of the world. It decreases the water potential of the root area, so that it lowers the ability of the plant water absorption. Besides, increasing the Salinity around the roots area could reduce the absorption and up taking of the toxic ions into the tissue to which it lower the essential minerals absorption, ionic balance disturbance and toxicity due to the Na^+ and Cl^- accumulation *(Rangasamy and Olsson, 1991)*.

Arbuscular mycorrhizae fungi are the most significant microorganism existing in majority of the indegraded soils. So that it is estimated that about 70% of the soils' microbic biomass are fungi's Mycelium *(Mukerji and Chamola, 2003)*. In most cases studied, the association between an AM fungus and a plant makes the host plant more tolerant to abiotic stresses *(Dodd and Ruíz-Lozano, 2012)*. In addition, AM fungi can be found under extreme saline conditions, and they can be adapted to these conditions *(Wilde et al., 2009)*.

The literature about the effects of salinity on AM fungi and their capacity to colonize plants is somewhat controversial. Some studies state that salt inhibits germination of spores or other fungal propagules, colonization of the plant roots, and sporulation of AM fungi *(Juniper and Abbott, 2006; Giri et al., 2007)*. However, several publications report that AM fungi in saline soils can increase plant salt tolerance, decreasing plant yield losses *(Ruíz-Lozano et al., 2011)*. These studies have suggested some mechanisms to explain the enhanced salt tolerance of AM plants (e.g. better ability for nutrient and water uptake due to an extended explored soil surface by fungal hyphae, greater root hydraulic conductivity and osmotic adjustment, maintenance of enhanced K^+/Na^+ ratios, and lower accumulation of sodium in the shoots of the host plants).

Calendula officinalis originally is from Asteracea family which scientifically named calendula officinalis; it has some medical characteristics and is used for majority of skin illnesses. Regarding the mentioned issues, the propose of this paper is to investigate the effect of using Arbuscularmycorhizal fungi for medical and decorate officinalis to preserve and enhance the growth of field crop under the environmental stress circumstances like salt, especially in desert portals.

Materials and methods

This experiment was carried out in pot condition. The soil type was sandy- loam. The officinalis seeds were FL type and Arbuscularmycorhizal fungi type was Glomus moseea. This plan was conducted with factorial design in four trial and 4 salinity treatment and two level with and without mycorrhiza in pots; the salt concentration was 1.5-3.5-5.5-7.5 (dSm^{-1}). The salinity treatment was conducted at 4 leaf stage. The plant has grown in 200 days and as soon as it reaches to the flowering period the mentioned characteristics are measured. First of all the plant has taken out of soil smoothly; the roots are washed. Then, they have been laid on a piece of paper for a few minutes. After that, they are cut from the collar to measure the wet weight of roots, and then leaf, with digital weighing machine. After all, the leaf and roots are dried by a mortar in order to measure the dry weights of both.

Measuring the leaf Chlorophyll (Arnon method, 1967)

1- 0.25 g of wet plant is grinded finely in a ceramic mortar 2-10 ml of acetone is added to the sample, and then put it in to a Centrifuge with 4000 rpm. The extracted which is derived from mentioned centrifuge is transformed to the Erlenmeyer Bulbs.

3-Some of the sample is spilled into the cuvette Spectrophotometerand then the amount of absorption is read at Wavelength of 663 Nm for

Chlorophyll 'a' and 645 nm for Chlorophyll 'b' separately. The acetone witness is 80%.

4- Finally the concentration of Chlorophyll is measured via statement below:

Total Chlorophyll (g/l) = (90.0202×645 OD) + (0.00802×663OD)

)OD) – (0/0127 × 663OD(0/002690 × 645 Chlorophyll 'a' (g/l)= (*OD*) – (0/00468 × 663*OD* × 645 (0/02290 Chlorophyll 'b' (g/l) = Where 663OD and 645OD are the amount of absorption at 663 and 645 wavelength respectively.

The statistical analysis of experiment data was performed via SAS and MSTATC software. The diagrams were draw via Excel and the comparison between averages was made via least significant difference (LSD) method at probable level of 5 and 1%.

Results and discussion

The results of statistical analysis are given in (Table 1) and (Table 2). The analyzed characteristics include dry weight of roots, dry weight of shoots, leaf area, number of flowering branches, Chlorophyll 'a' and 'b'.

Table 1. Variance analysis of measured characteristics.

Source of changes	Degrees of freedom	Dry weight of roots	Dry weight of shoots	Leaf area	number of flowering branches	Chlorophyll 'a'	Chlorophyll 'b'
fungi	1	0/004*	3/61*	3/638*	19/547**	38/720**	20/145**
salinity	3	0/003**	2/64**	0/714^{ns}	0/512^{ns}	30/536**	10/016**
Fungi+salt	3	0/002^{ns}	0/06^{ns}	0/305^{ns}	1/791^{ns}	0/773^{ns}	0/538^{ns}
Error	24	0/010	0/307	0/507	1/233	3/594	1/480
CV%		17/34	18/22	17/34	34/90	16/02	24/62

*, **, ns: significant at 0.05, 0.01 probability level and no significant respectively.

Dry weight of roots

The results of variance analysis (Table 1) show that effect of funguses on the dry weight of roots is meaningful at 5% level while effect of salinity concentration was meaningful at 1% level. The comparison of averages represented that dry weight of roots in shrub and different stages of using funguses and various salinity concentrations, categorized into different groups (Table 2).

Table 2. Comparison of measured characteristics average.

Salinity	Dry weight of roots (g/plant)	Dry weight of shoots (g/plant)	Chlorophyll 'a' (Mg/g)	Chlorophyll 'b' (Mg/g)
No	a0/14	a3/79	a14/41	a6/24
N1	b0/12	b2/13	b12/11	ab5/39
N2	a0/11	bc2/80	bc11/02	bc4/46
N3	c0/09	c2/24	c9/81	c3/66

The results of averages' comparison, however, imply that the maximum amount of dry weight of roots at different concentration of salinity was (0.14a g/plant) and minimum average was (0.09b g/plant).

It is reported that in Ocimum basilicum the stress cause meaningful decreasing in wet and dry weight of radical and shoots *(AlaviPanah, 1992)*.

Dry weight of shoot

The results of variance analysis (Table 1) has implies that effect of fungi on dry weight of shoots at 5% level

and effect of salinity concentration at 1% level was meaningful. The comparison of the averages shows that dry weight of shoots in shrubs and different stage of using fungi and different concentration of salinity categorized into different groups (Table 2). The results of calculating the average, however, showed that the dry weight of shoot at concentration 'a' was 3.79 (g/plant) and at 'b' was 2.44 (g/plant).

Utilizing the mycorhizal fungi accelerate the speed of plant growing and affect the adoption and transformation of nutrition between roots and stem so that increase the nutrition absorption and facilitate the up taking of them and consequently increase the dry weight of shoot.

Leaf area

The results of variance analysis (Table 1) has implies that effect of fungi on leaf area was meaningful at 5% level.

Most of the differences in calculating the Net Primary Production of plants cause by differences in calculating the amount of leaf and proposed algorithms applied to calculate the leaf area of plant. The difference in number of leafs can lead to difference in production of dry material and consequently the difference in performance of the plant inside a farm

Number of flowering branches in shrub

The results of variance analysis (Table 1) has implies that effect of fungi on Number of flowering branches in shrub was meaningful at 5% level Evaluating the effect of salt stress on Dwarf bunt presented that applying salinity stress on fertile spikelet; reduce the performance of seed in Dwarf bunt *(Goh et al., 1997)*.

Chlorophyll 'a' and 'b' in leaf

The results of variance analysis (Table 1) has implied that effect of fungi on a Chlorophyll 'a' content was meaningful at 1% level and effect of salinity concentrations on Chlorophyll 'a' content was also meaningful at the same level as well. The results of averages' comparison, however, imply that the maximum Chlorophyll content at different concentration of salt was (14.41a mg/ml) and minimum content was (9.8b mg/ml) (Table 2). The results of variance analysis (Table 1) has implied that effect of fungi on a Chlorophyll 'b' content was meaningful at 1% level and effect of salinity concentrations on Chlorophyll 'b' content was also meaningful at the same level as well.

Regarding the results of the experiment carried out on 4 different types of Iranian Medicago sativa in term of salinity it was reported that mean level of salinity (7 dSm^{-1}) decrease the concentration of the chlorophyll in leaves while in the higher level of salinity (12 DSm^{-1}) this concentration increases up to the witness plant and even more than them. *Valentine et al. [2006]* found that grapevine plants inoculated with an AM fungus and subjected to drought had higher water-use efficiency and Rubisco activity than non-AM plants.

Conclusion

Calendula officinalis is more resistance with Arbuscular mycorhizal fungi coexistence supports in term of salinity. This fungi increase the nutrition absorption such as Phosphorus in term of salinity in Calendula officinalis. Regarding the disadvantages of chemical fertilizers, specifically phosphate fertilizers, and the pollutions related to the cadmium, as long as their high cost and environmental pollutions, the scientist looking forward to using biologic and organic fertilizers instead of chemical ones to naturally provide enough nutrition for Calendula officinalis as a medical plant.

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