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Effect of Arbuscular Mycorrhizal fungi in *Trigonella foenum - graecum* L. under salinity stress

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Abstract

In plant breeding crops for salt tolerance, recent emphasis is on exploring the possible role of plant-microbe interactions in amelioration of salt stress in plants. It is now well established that arbuscular mycorrhizal (AM) symbiosis alleviates the effect of salt stress in plants. Further, available evidences suggested that AM symbiosis regulates many of the plant's salt stress mechanisms. The effect of Arbuscular Mycorrhizal (AM) fungi in *Trigonella foenum- graecum* L. under salinity were studied. The plants were inoculated with different mycorrhizal species either individually or in a mixed mycorrhizal inoculation. The experiments were divided into three salt treatments: 1.5 dS/m, 3.5 dS/m and 0 dS/m electrical conductivity (tap water) as the control. In these experiments plant height was reduced by salinity stress . At the 3.5 dS/m salinity level, plant height decreased in comparison with that at lower salinity levels Leaf number was significantly affected by salinity; the leaf number at 1.5 dS/m was greater than that at the higher salinity level, as at 3.5 dS/m the number of leaves started to decrease. Inoculation with *G. pubescens* showed reduced leaf production. The final recorded leaf number was lower after the addition of *G. pubescens* at a low salinity level. Salinity significantly enhanced the growth of second-generation seedlings. Low salinity stress (1.5 dS/m) increased seed germination and seedling growth more than in the no-salt treatment or high salinity stress At the highest salinity level (3.5 dS/m), the colonization rate of the hyphae was dramatically lower with all species of mycorrhizas used. Colonization of hyphae by *G. facciculatum* was highest at 0 dS/m and decreased with increasing salinity levels. Colonisation by vesicles and arbuscules was generally low at all salinity levels. Field experiments showed that mycorrhizas did not actually help the plant to overcome salinity at higher stress.

Keywords: Arbuscular mycorrhizal fungi, Saline soil, Salt stress, *Trigonella foenum- graecum* L.

Introduction

AM fungi are obligate symbiotic fungi the hyphae of which develop mycelium, arbuscules and in most fungal genera vesicles in root and is ubiquitous in distribution. These hyphae can explore an area around the root for exceed that available to root hairs. These extrametrical hyphae are more efficient in nutrient uptake than root hairs (Allen, 2007, Mehboob and Anil Vyas, 2013).

However, little information is available on the colonization potential of these fungi in plants grown in the soil amended with chemical fertilizers, fungicides and other amendments *viz.* non–cultivated soils. Studies were conducted to know, if the soil amendments have any effect on the percent mycorrhizal infection and root volume colonization by AM fungi in non–cultivated and cultivated soil.

The production and application of AMF biological fertilizers may be effective in the improvement of plant production. It may be the most effective method to improve the quality and quantity of the medicinal materials obtained in the non-fertile soils. Arbuscular mycorrhizal fungi can increase plant growth, photosynthesis, nutrients storage, metabolites and beneficial chemical compounds and decrease soil borne plant diseases by inhibition of fungal pathogen (Ratti *et al*., 2010, Oliveira *et al*., 2013, Ali Akbar Safari Sinegani and Masoumeh ElyasiYeganch, 2017). Hence, there is an upcoming demand for research in improving the quality and quantity of plants in relatively less time with application of AM fungi (Karthikeyan *et al.*, 2009). Hyphae of AM fungi have been shown to play an important role in soil stabilization through formation of soil aggregates. Additionally, the extra radical hyphae are generally believed to be important to the plants for acquisition of phosphorus (P) and other mineral nutrients (Read and Perez-Moreno, 2003).
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Materials and Methods

Trigonella foenum- graecum L. seeds were germinated in a controlled environment room. After reaching the four-leaf stage, the seedlings were transplanted into 11-cm square pots filled with commercial sterilized compost. The plants were inoculated with different mycorrhizal species either individually or in a mixed mycorrhizal inoculation. Plants were thus inoculated with *G. facciculatum*, *G. pubescens* and a mixed inoculation of both species, in addition to the control. The mycorrhizal-treated plants were maintained for two weeks before the salinity treatments were applied, to ensure that the mycorrhizas were established in the root system.

The experiments were divided into three salt treatments: 1.5 dS/m, 3.5 dS/m and 0 dS/m electrical conductivity (tap water) as the control. Each week, 100 mL of the salt solutions were applied to each of the plants in each treatment group to maintain the desired salinity level in the pot constant. Nutrient solutions were added to the plants at two-week intervals. The salt type used in this experiment was mixed salt.

The experiment was done for four months in controlled room conditions and at the end plant height, leaf number, inflorescence number and length were counted. The weight of inflorescence was also measured, to indicate the weight of the seeds they contained. Initial shoot biomass and final dry shoot weight were taken for each plant, to calculate the final shoot biomass for each treatment. The roots for each plant were cleaned and stained for Mycorrhizal visualization. The stained roots were prepared on glass slides for AM fungal quantification and identification of different parts of mycorrhizas such as vesicles, hyphae and arbuscules.

The seeds produced from F1 plants in each treatment were used for the germination of F2 generation. From each plant, 15 healthy seeds were selected for the germination test. Petri dishes of 90 mm diameter with filter paper inside were used for the germination test at constant room temperature (26°C). The seeds of each plant were sub-divided into three Petri dishes, and five seeds were placed in each Petri dish, making a total of 15 seeds for each plant. The seeds were watered daily with distilled water and seedling germination was recorded for the seven days of the experiment, together with the final total shoot and root length for each successfully germinated seedling.

The experiment comprised six replicates per plants, three levels of salinity and four different treatments of mycorrhiza.

Statistical analysis

Data from this experiment were tested for normality prior to subjecting the data to factorial ANOVA, employing salt and AM colonization as the main effects, and Tukey's test was used for means separation. The statistical software package was used to analyze data. The results of the Analysis of Variance are summarized in tables and only the comparisons that showed statistically significant differences between subgroups are represented graphically.

Results

Plant height was reduced by salinity stress (Table -1). At the 3.5 dS/m salinity level, plant height decreased in comparison with that at lower salinity levels (Figure -1). With regard to the addition of different mycorrhiza species, *G.pubescens e*nhanced plant height (Table -2). At 1.5 dS/m stress, *G.pubescens* caused an increase in plant height in comparison with other mycorrhizas (Figure -1); however, inoculation with *G. facciculatum* mycorrhizas did not affect plant height (Table -1).

Table: 1: Summary of the results of Analysis of Variance testing for the effect of different salinity levels and mycorrhizal inoculation on various vegetation parameters of *Trigonella foenum- graecum L*. (GP= *Glomus pubescens* and, *Gf= Glomus facciculatum*). Degree of freedom of salinity levels = 2, 50; Gp=1.50;Gf=1.50

	Plant height (cm)		Leaf number		Shoot dry biomass (g)	
	F- value	P- value	F- value	P- value	F- value	P- value
Salinity	22.0	<0.001	3.2	<0.04	7.6	<0.001
Gp	4.01	<0.05	5.86	<0.01	2.4	0.12
Gf	0.46	0.50	0.1	0.7	0.54	0.46
Salinity x Gp	1.8	0.18	1.8	0.18	0.20	0.65
Salinity x Gf	1.6	0.21	2.7	0.10	4.6	0.03
Gp x Gf	1.6	0.21	1.10	0.29	0.14	0.7
Salinity x Gp x Gf	0.64	0.42	1.2	0.27	0.26	0.61

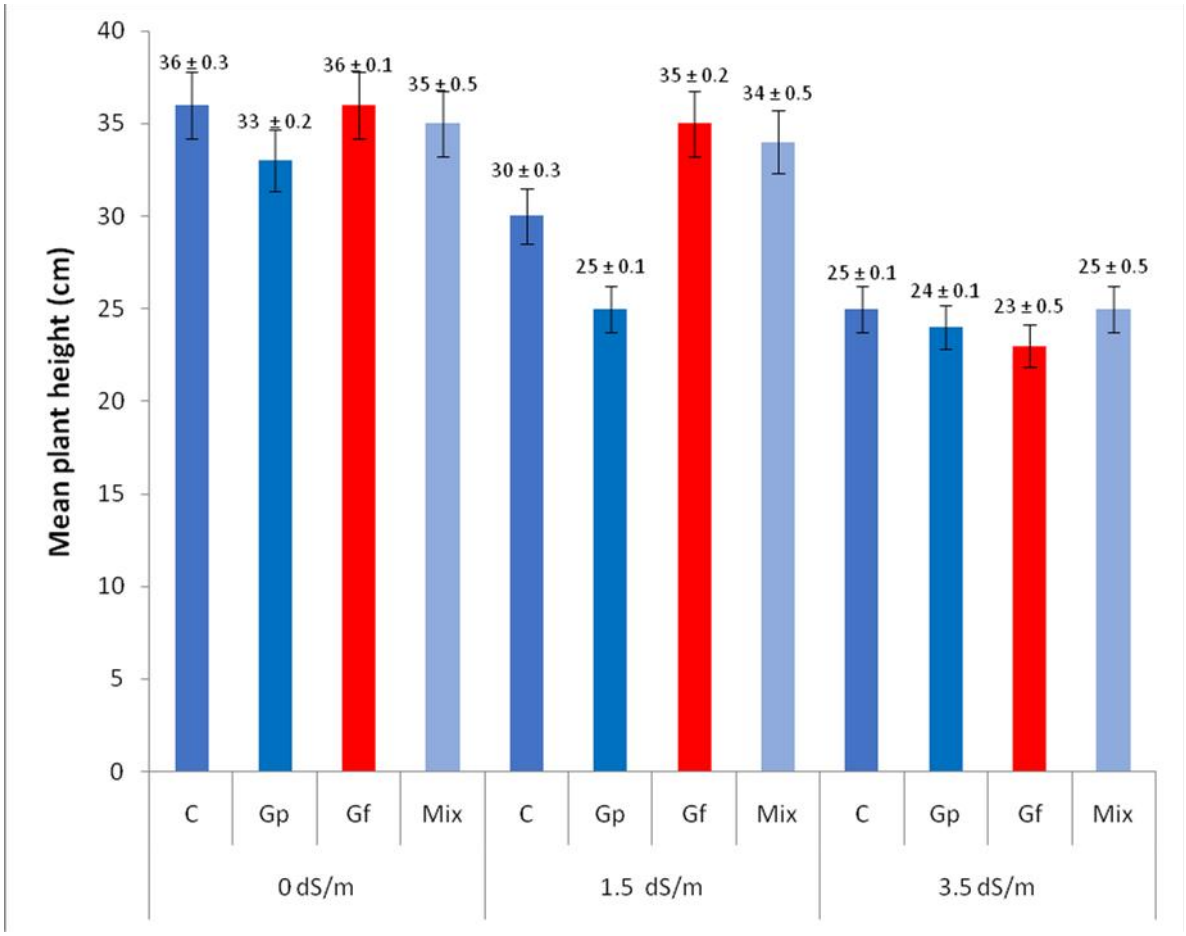

Figure -1: Mean plant height (cm) produced after inoculation with different mycorrhizal species under different salinity levels (dS/m). C (no mycorrhizas added), Gf (*Glomus facciculatum*), Gp (*Glomus pubescens*) and Mix (*G. facciculatum*+ *G. pubescens*). An asterisk shows significant differences with respect to the AM effect within the groups.

Leaf number was significantly affected by salinity (Table-1); the leaf number at 1.5 dS/m was greater than that at the higher salinity level, as at 3.5 dS/m the number of leaves started to decrease (Figure -2). Inoculation with *G.pubescens* showed reduced leaf production (Table -1). The final recorded leaf number was lower after the addition of *G. pubescens* at a low salinity level (Figure -2). However, *G. facciculatum* showed a significant interaction with salt, because fungal addition reduced the leaf number when no salt was added but had no effect or a small positive effect when salt was present (Table -1; Figure -2).

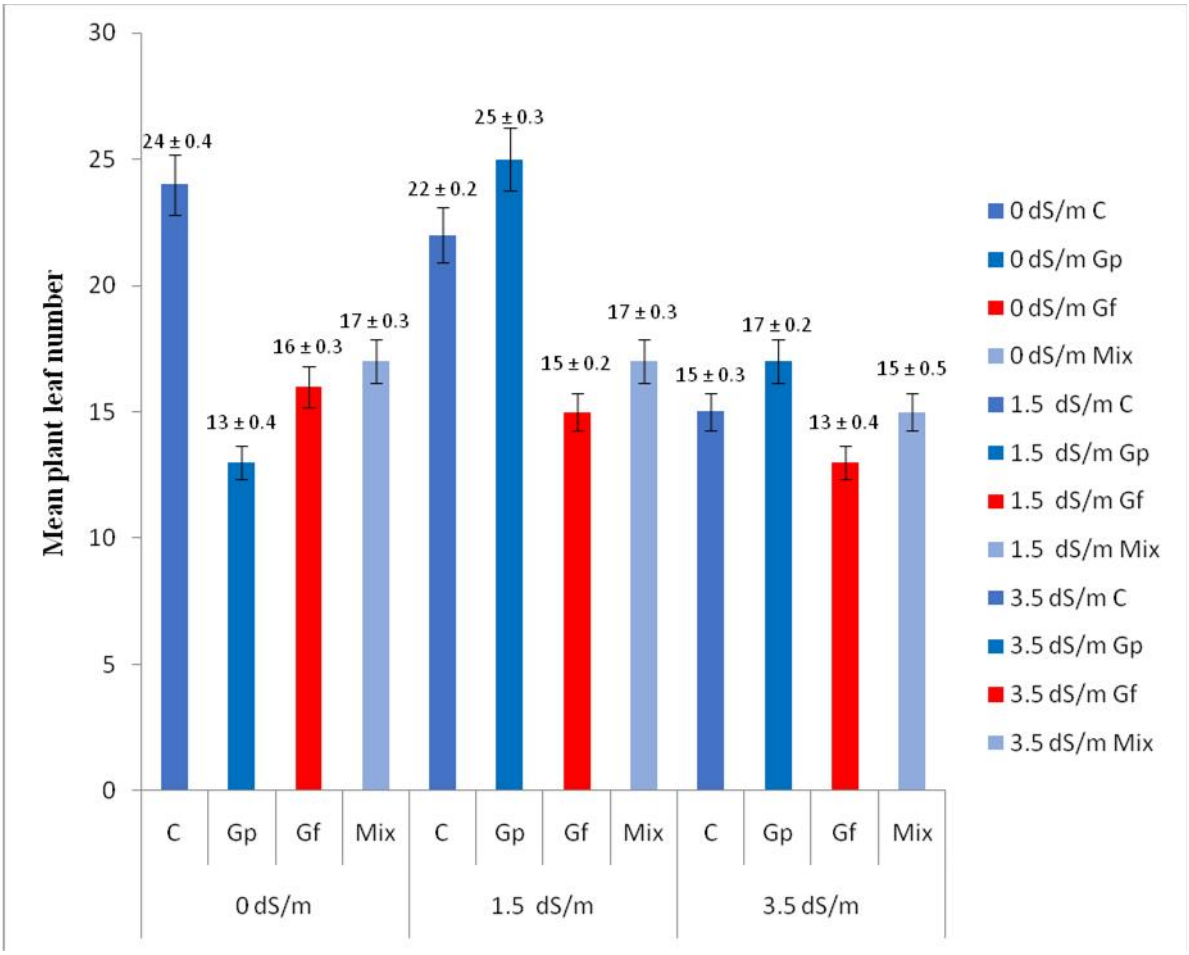

Figure -2: Mean plant leaf number produced under different salinity levels (dS/m) following inoculation with different mycorrhizal species. C (no mycorrhizas added), Gf (*Glomus facciculatum*), Gp (*Glomus pubescens*) and Mix (*G. facciculatum+ G. pubescens*). An asterisk shows significant differences with respect to the AM effect within the groups.

Plant exposure to salinity considerably reduced final biomass production (Table -1). At the highest salinity level (3.5 dS/m), mean dry biomass was much lower than in the presence of lower salinity levels or no salt (Figure-3). Similar to leaf number, plant biomass showed an interaction between the addition of *G. facciculatum* and salinity stress (Table -1), as fungal addition reduced plant size when no salt was added, but had a small positive effect when salt was present in any concentration (Figure -3).

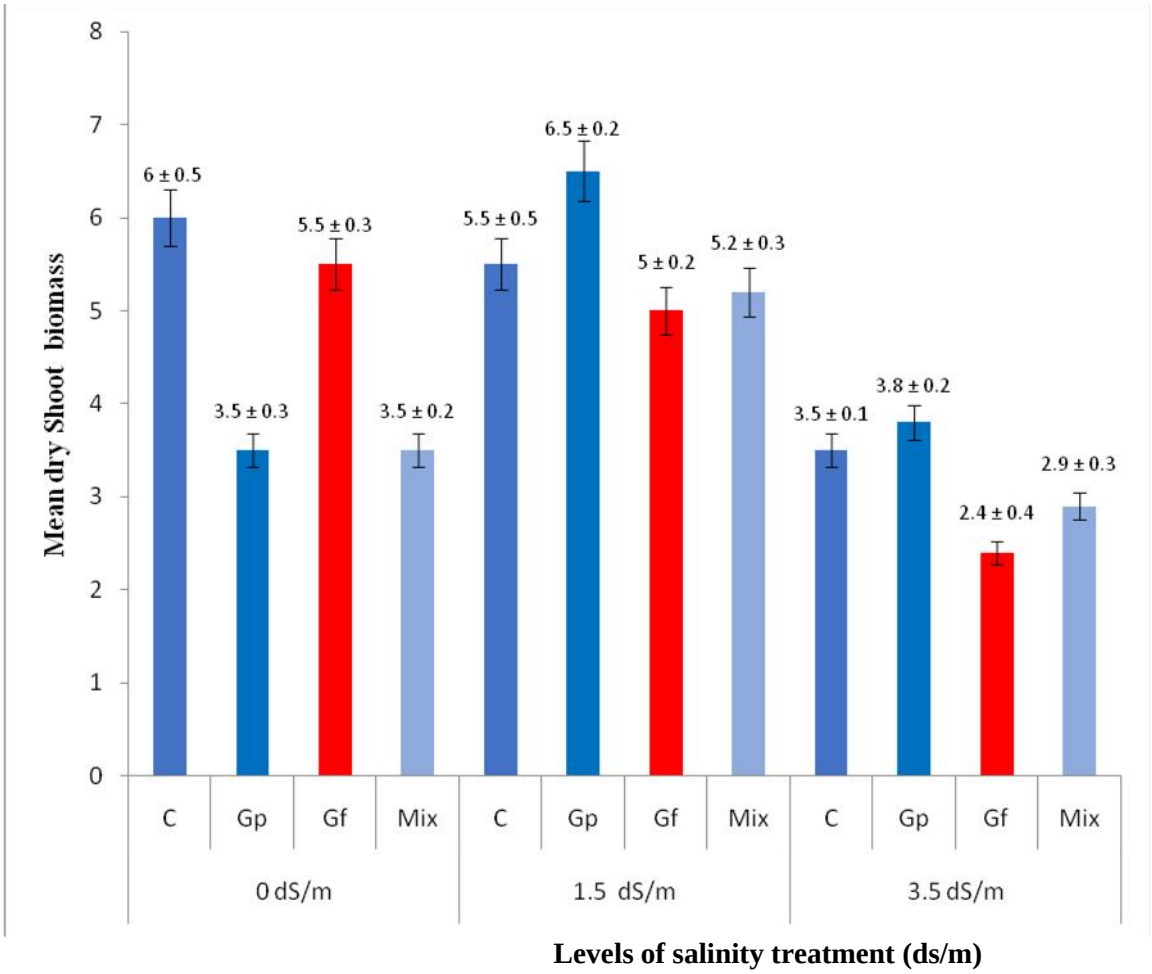

Figure - 3: Mean plant final dry shoot biomass (g) after treatment with different salinity levels (dS/m) and mycorrhizas. C (no mycorrhizas added), Gf (*Glomus facciculatum*), Gp (*Glomus pubescens*) and Mix (*G. facciculatum*+ *G. pubescens*). An asterisk shows significant differences with respect to the AM effect within the groups.

With respect to plant reproduction, salinity significantly increased the number of inflorescences produced by the plants under certain stress levels (Table -2). Exposure to the 1.5 dS/m level of salinity in particular resulted in a greater number of inflorescences than in the no salt or the higher salinity treatments (Figure -4). There was no overall effect of the addition of either mycorrhizal species (Table-2; Figure- 4). However, addition of *G. facciculatum* increased flower production at the high level of salt and when no salt was added, but not at the low level of salt, leading to a significant interaction term (Table-2). Furthermore, although addition of either mycorrhiza increased flower production, no further increase was observed when both fungi were added, again leading to a significant interaction between the fungi (Table - 2; Figure-4). A similar increase in inflorescence head length was observed in the presence of 1.5 dS/m salinity stress than in the absence of salinity or in higher salinity stress (Table -2; Figure -4). However, the combined addition of *G. pubescens* and *G. facciculatum* resulted in a sharp decrease in the head-length parameter in 0 dS/m and 1.5 dS/m saline treatments in comparison with the addition of either mycorrhizal fungus alone, producing a highly significant interaction term between the fungi (Figure - 5).

Table: 2 Summary of the results of Analysis of Variance testing for the effect of different salinity stress levels and mycorrhizal inoculation on the reproductive output of plants. *Gp=Glomus pubescens and Gf= Glomus facciculatum*. Degrees of freedom of salinity levels = 2.50; Gp=1, 50; Gf=1.50

	Inflorescence number		Inflorescence head length (cm)	
	F- value	**P- value**	**F- value**	**P- value**
Salinity	5.4	<0.007	4.00	0.02
GP	0.1	0.75	0.32	0.57
Gf	1.5	0.22	0.96	0.33
Salinity x Gp	4.2	0.04	1.5	0.22
Salinity x Gf	1.1	0.29	1.1	0.29
Gp x Gf	4.2	0.04	8.2	<0.005
Salinity x Gp x Gf	4.0	<0.05	0.50	0.48

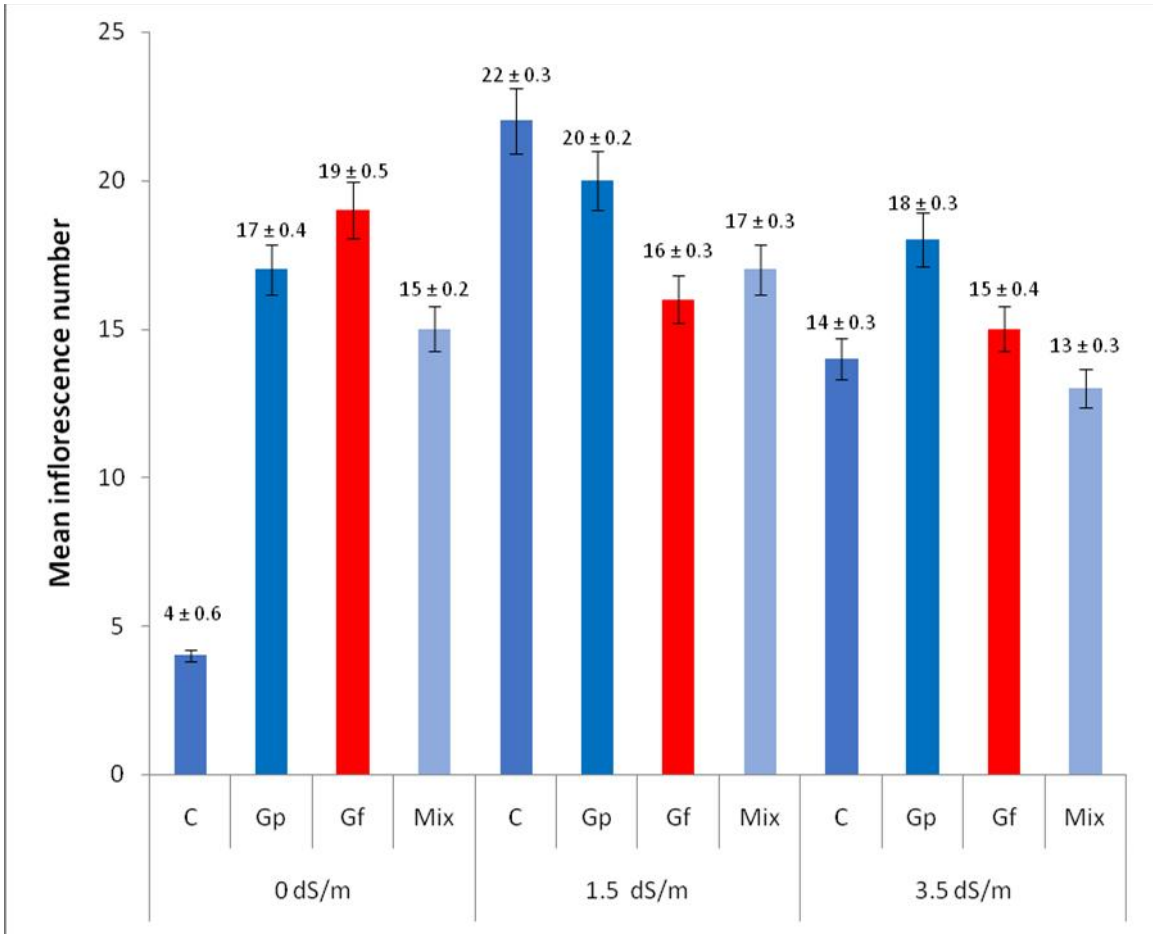

Figure- 4: Mean number of inflorescences produced by plants under different salinity stresses (0, 1.5 and 3.5 dS/m) and different species of mycorrhizas. C (no mycorrhizas added), Gf (*Glomus facciculatum*), Gp (*Glomus pubescens*) and Mix (*G. facciculatum*+ *G. pubescens*). An asterisk shows significant differences with respect to the AM effect within the groups.

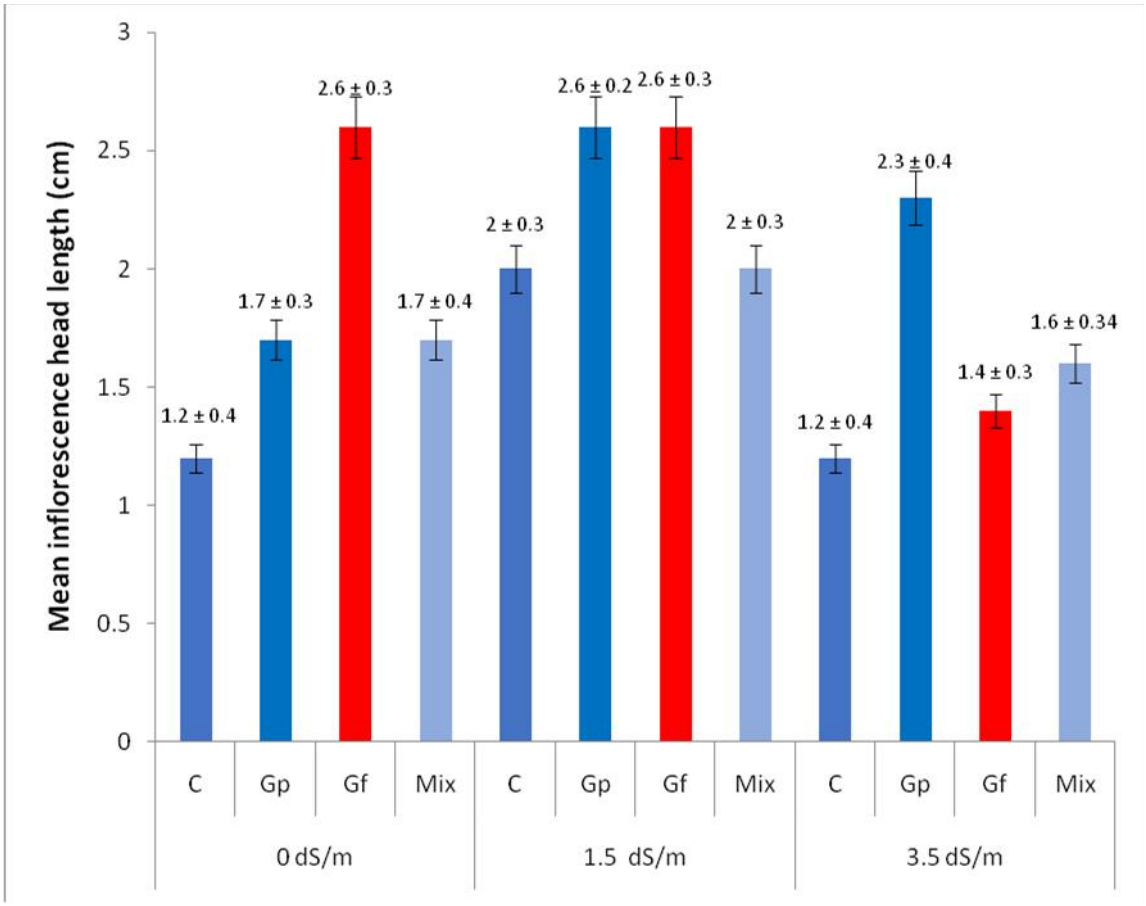

Figure -5: Mean inflorescence head length (cm) for *Trigonella foenum- graecum* L .inoculated with different species of mycorrhizas under different salinity levels (0, 1.5 and 3.5 dS/m). C (no mycorrhizas added), Gf (*Glomus facciculatum*), Gp (*Glomus pubescens*) and Mix (*G. facciculatum*+ *G. pubescens*). An asterisk shows significant differences with respect to the AM effect within the groups.

Salinity significantly enhanced the growth of second-generation seedlings (Table-3). Low salinity stress (1.5 dS/m) increased seed germination and seedling growth more than in the no-salt treatment or high salinity stress (Figures -6 and 7). The addition of *G. pubescens* had no overall effect on seedling performance, but a significant interaction was found between its addition and salinity (Table -3). *G. pubescens* tends to increase both seed germination and seedling growth in the absence of salt stress, but no effect on these variables was observed with salt stress and the effect of this fungus on seedling growth even appeared to be negative on occasions (Figures -6 and 7). Inoculation of parent plants with *G. facciculatum* had no effect on the growth rate and showed no interaction with salinity (Table 3).

Table: 3 Summary of the results of Analysis of Variance testing for the effect of different salinity stress levels and mycorrhizal inoculation on second Gf generation plant offspring *Gp= Glomus pubescens, Gf= Glomus facciculatum.* Degrees of freedom of salinity levels = 2.50; Gp=1, 50; Gf=1.50.

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	Seed Gf germination rate		Seedling length (cm)	
	F- value	**P- value**	**F- value**	**P- value**
Salinity	9.1	<0.001	11.1	<0.0001
GP	0.05	0.82	0.38	0.54
Gf	0.05	0.82	0.26	0.61
Salinity x Gp	4.01	<0.05	3.2	<0.05
Salinity x Gf	0.56	0.45	1.2	0.45
Gp x Gf	1.1	0.29	0.072	0.78
Salinity x Gp x Gf	0.44	0.51	0.76	0.38

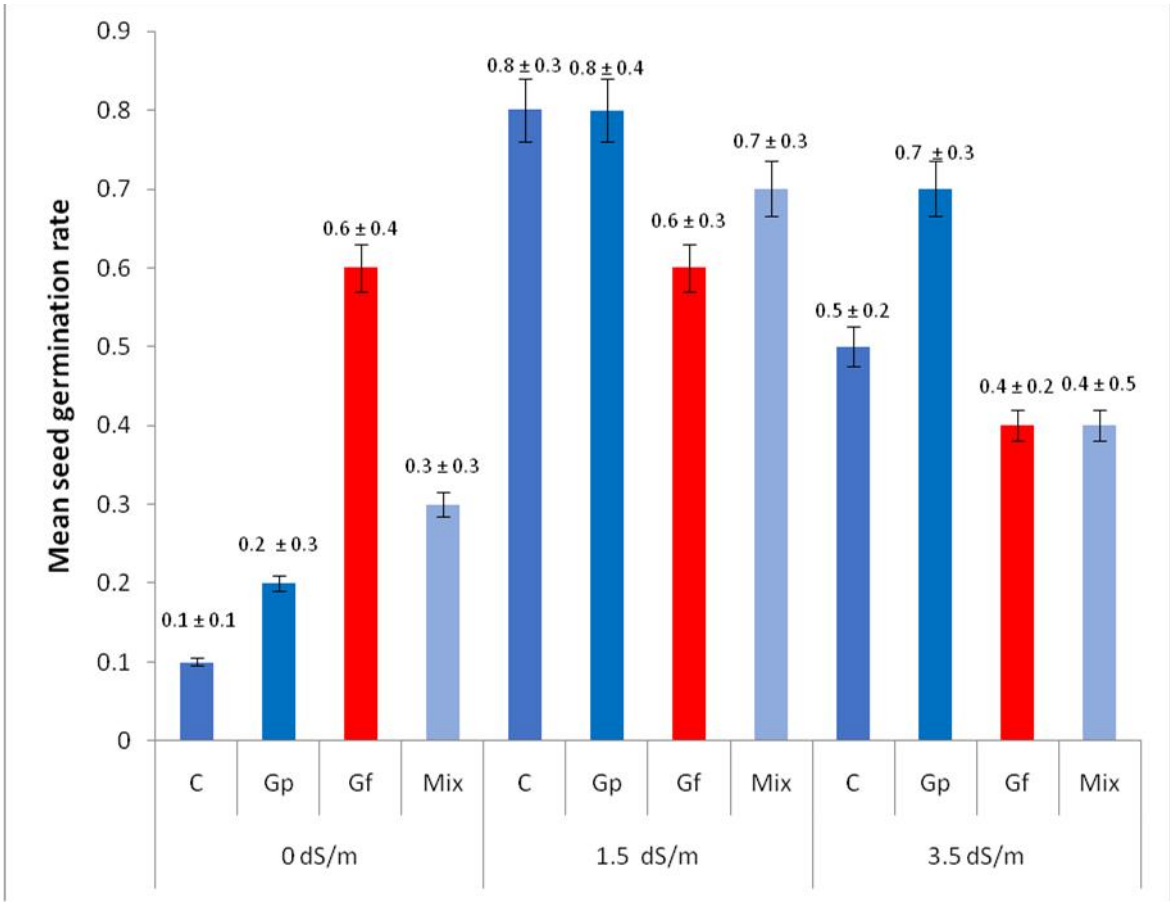

Figure -6: Mean offspring seed germination rate for *Trigonella foenum- graecum L.*when the parental plants were inoculated with different species of mycorrhizas under different salinity levels (0, 1.5 and 3.5 dS/m). C (no mycorrhizas added), Gf (*Glomus facciculatum)*, Gp (*Glomus pubescens*) and Mix (*G. facciculatum+ G. pubescens*). An asterisk shows significant differences with respect to the AM effect within the groups.

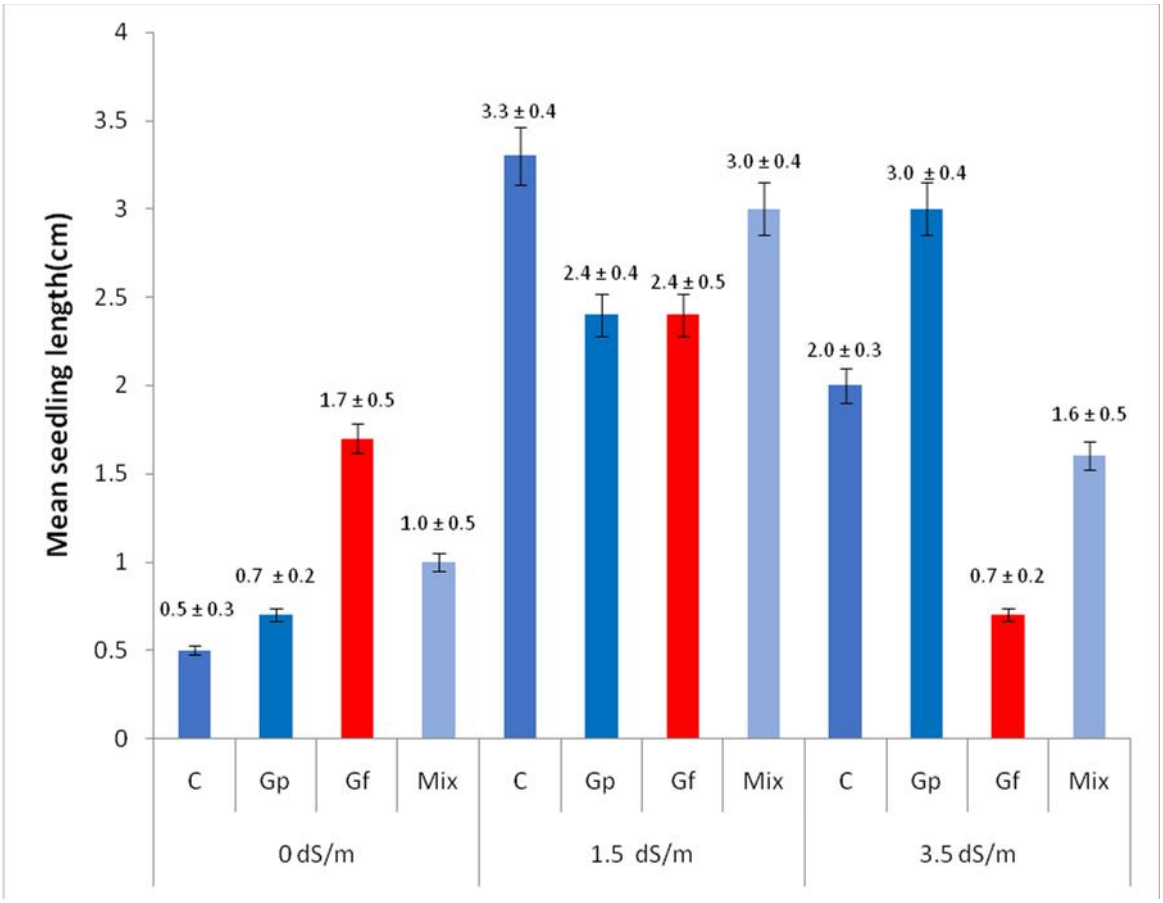

Figure -7: Mean offspring seedling length growth for *Trigonella foenum- graecum L.*when the parental plants were inoculated with different species of mycorrhizas under different salinity levels (0, 1.5 and 3.5 dS/m). C (no mycorrhizas added), Gf (*Glomus facciculatum*), Gp (*Glomus pubescens*) and Mix (*G. facciculatum+ G. pubescens*). An asterisk shows significant differences with respect to the AM effect within the groups.

Staining and visualization of mycorrhizas in plant roots revealed different degrees of colonization (Table-4).

Table 4: The percentages root mycorrhizal colonization of the different physiological parts. Treatments include salinity stress (0, 1.5 and 3.5 dS/m EC) and Mycorrhizal Species Gf (*Glomus facciculatum*), Gp (*Glomus pubescens*) and Mix (*G. facciculatum. pubescens*).

Treatments	Hyphae %	Vesicles %	Arbuscules %
0 EC+ Gp	28	0	1
0 EC+ Gf	24	0	0
0 EC+ Mix	16	0	1
1.5 EC+ Gp	41.5	1	2
1.5 EC+ Gf	20	0	1
1.5 EC+ Mix	26	1	1
3.5 EC+ Gp	12	1	0
3.5 EC+ Gf	8	0	0
3.5 EC+ Mix	5	2	1

In the salinity control, *G. pubescens* showed the highest hyphal colonization (28%) in comparison with other mycorrhizal inoculants. At the lowest salinity level (1.5 dS/m), the hyphal colonization by *G. pubescens* and the mix was approximately double that of plants with no salt addition (Table-4). At the highest salinity level (3.5 dS/m), the colonization rate of the hyphae was dramatically lower with all species of mycorrhizas used. Colonization of hyphae by *G. facciculatum* was highest at 0 dS/m and decreased with increasing salinity levels. Colonisation by vesicles and arbuscules was generally low at all salinity levels (Table-4).

Discussion

Similar finding that the mixed addition of individual mycorrhizal species (*G. mosseae* and *G. etunicatum*) reduced the inflorescence head length (compared to that of plants exposed to either individual fungus) is consistent with previous reports that the addition of individual mycorrhizal species had a positive impact on vegetative growth (Ciftci *et al.*, 2010).Inoculation of *Glomus* specieswith *Campanula rotundifolia* L. produced seeds with a higher phosphorus content and faster germination rate (Nuortila *etal.*, 2004).

Furthermore, there is again as certain mycorrhizal inoculate can have a positive influence, whereas others show no response at all (Buwalda&Goh 1982). For example, mycorrhizalinoculation neither affected seed production in *Lycopersicon esculentum* Mill. Plants (Bryla & Koide 1990), nor accelerated the germination rate of *Avena fatua* L. seeds, which displayed similar characteristics to the non-mycorrhizal treatment controls (Koide & Lu 1992).

The expected, mycorrhizal colonization decreased as salinity stress increased. Similarly, using different species of mycorrhizas, (Juniper & Abbott, 2006) observed a decreased colonization of roots with increasing salinity stress. Several experiments on *Archaeo sporatrappei*, *Gigaspora decipiens* and *Scutellospora calospora* showed a reduction in root colonization with an increasing addition of NaCl.

A similar result was obtained with *Scutellospora calospora* (WUM 12-2), which showed an increased colonization of plant roots at 13 dS/m salinity stress compared with the control (Juniper & Abbot, 2006).This might be because the species of AM used were not from a saline habitat; according to previous studies, the habitat of origin of mycorrhizas is important in determining the outcome of any interactions with salt stress (Bentivenga *et al.*, 1997; Smith & Smith 1997; Bago *et al.*, 1998). It was shown that mycorrhizas originating from a saline habitat more effectively help plants tolerate salinity stress. The presence of mycorrhizas of saline origin reduced the Cl^- content of tomato leaves to help it tolerate soil salinity more than non-saline AM strains did (Copeman *et al.*, 1996).

The presence of *G. mosseae* originating from a saline habitat helped cotton plants to tolerate high salinity stress and increased the concentration of phosphorus in plant tissues (Tian *et al.*, 2004).

Conclusion

The results obtained from both field experiments showed that mycorrhizas did not actually help the plant overcome salinity at higher stress. Also, different levels of salinity and different salt types influenced mycorrhizal species interaction with plants in different ways. In the first field experiment with higher salinity levels and mixed commercial mycorrhizas, the results were impressive regarding the plant offspring quality. In the second experiment with reduced levels of salinity and the addition of individual mycorrhizal species, the results were not conclusive.

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