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Effect of arbuscular mycorrhizal fungi on offspring quality of *Trigonellafoenum- graecum* L.

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Abstract

Fenugreek (*Trigonellafoenum-graecum* L.) belonging to the Leguminosae (Fabacecae family) is an important leguminous seed spice and well known aromatic and medicinal crop. *Trigonellafoenum- graecum* L. seeds were germinated in a glasshouse. At the four-leaf stage, 40 healthy plants were transferred to individual pots filled with compost. Twenty plants were treated with a commercial mix of AM fungi at root level and the other twenty *Trigonellafoenum- graecum* L. plants were grown without AM inoculation as an experimental control. The duration of the experiment was 2 months. Growth parameters from both the first and the second-generation plants were recorded, including leaf number, plant height (to tip of shoot, leaf height not included), shoot water content and final shoot dry biomass. The roots of first generation *Trigonellafoenum- graecum* L. were cleared for staining and the extent of AM fungal root colonisation was quantified.

Keywords: Fenugreek, *Trigonellafoenum- graecum* L. mycrorrhiza, AM fungal.

Introduction

Fenugreek (*Trigonellafoenum- graecum* L.) is a well known aromatic and medicinal herb. It is a native of Southeastern Europe and Western Asia and broadly cultivated in India which harbor great diversity of fenugreek. In agricultural crop production systems, nitrogen is often the most limiting nutrient that dictates crop production. Despite its presence in large quantities in the atmosphere, plants cannot utilize nitrogen since it is inert. Legume – *Rhizobium* symbiosis is an important facet of symbiotic nitrogen fixation which is exploited to benefit agriculture and its sustainability (AnshuKarel. *et al.*, 2016).

VAM is the most abundant kind of mycorrhiza described as ‘a universal plant symbiosis’ The mycorrhizae are the feeder root of plant growing in natural world and are beneficial to their host plant.

The plant’s root with zone of powerful microbial metabolic activity occurring where, there is a high concentration of carbon is called the rhizosphere (Barea, *et al*., 2005). This is in contrast to the antagonistic interactions of plants and pathogenic fungi, with defense mechanism of Arbuscularmycorrhizal fungal relationship with plants which can increase the growth of plants by enhancing phosphate uptake mainly and perhaps the other minerals such as K, Fe, Cu, Ca and Zn (Lakshman, 2009).

These fungi play vital role in transformation of plant nutrients from unavailable to available forms. They improve the host plant growth by better uptake of water and other minerals especially the uptake of phosphorus (P), which is present in fixed form in soil

(Yamawaki,*et al.* 2013). A lot of work has been done on the effect of these fungi on the improvement of growth of plants (Cavagnaro, *et al*. 2012, Kaur, *et al.* 2014 and Aminifar & Sirousmehr, 2014).

Although plants could adapt a number of strategies to alleviate/ overcome stress effects, mycorrhizal association is emerging as one of the efficient ways to combat stress effects and hence makes the plants to grow better under drought environment (Gianinazzi,*et al*. 2010). In fact, several studies have shown the importance of use of microbial inoculants, especially the AMF in alleviating drought stress in crop plants (Zoppellari,*et al*. 2014).Fenugreek (*Trigonellafoenum-graecum* L.) belonging to the Leguminosae (Fabacecae family) is an important leguminous seed spice and well known aromatic and medicinal crop for improving the crop. In the present study in this plant AMF studies are carried out.

Materials and Methods

Trigonellafoenum- graecum L.(Fenugreek) seed were collected from Arya Farm products Pvt, Ltd. Bangalore. At the four-leaf stage, 40 healthy plants were transferred to individual pots filled with compost (organic potting mix from nutria-gro). Twenty plants were treated with a commercial mix (Mycotonemycorrhiza from amazon. in) of AM fungi at root level and the other twenty *Trigonellafoenum-graecum* L. plants were grown without AM inoculation as an experimental control. The duration of the experiment was 2 months. All the experiments were carried out under controlled conditions in a glass house (GH) at Department of Botany Government Arts College Ariyalur, Tamilnadu, India.

Seed production by first generation *Trigonellafoenum-graecum* L. plants from both the AM and the control treatments were collected at the end of the growth cycle and kept separately. Seeds produced from this first generation of *Trigonellafoenum- graecum* L. plants were propagated to produce 40 individual seedlings, 20 from the AM colonised parent plants and 20 from the control plants. All 40 seedlings were transferred to individual pots containing the same compost. Thus, a total of 40 *Trigonellafoenum-graecum* L. plants were tested for the effect of AM colonisation on the parental plants (first generation) and their effect on offspring (second generation) under the same growing conditions in the GH. However, in the second-generation seedling test, AM fungal inoculum was not added to the roots, thereby forcing reliance on seed reserves from the first generation inoculation. The second-generation seedlings were allowed to grow for 4 months just as the first generation.

Growth parameters from both the first and the second-generation plants were recorded, including leaf number, plant height (to tip of shoot, leaf height not included), shoot water content and final shoot dry biomass. The roots of first generation *Trigonellafoenum- graecum* L. were cleared for staining and the extent of AM fungal root colonisation was quantified. The data obtained were tested for normality and then analysed by one factor Analysis of Variance (ANOVA).

Results

First generation Trigonellafoenum- graecum L. *harvest.*

Among the different parameter of *Trigonellafoenum-graecum* L. harvest in first generation the dry shoot biomass was significantly higher (approximately 20%) for control plants compared with plants treated with AM fungi (Table 1; Figure 1). After three months of growth, the mean final leaf number slightly differ between plants treated with AM fungi and the non-treated controls (Figure 2). A similar result was observed for mean plant height; the application of the AM fungal inoculum had no effect on mean plant height at the end of the three-month growing period (Table 1; Figure 3).

Table 1 : Summary of results from Analysis of Variance of different plant growth parameters for *Trigonellafoenum-graecum* L. plants either treated with a commercial arbuscularmycorrhizal fungi mix or not (control) at the first generation stage. All degrees of freedom = 1,48.

Parameters	AM	
	F- value	P-value
Dry shoot biomass	5.5	<0.01”
Final leaf number	0.03	0.8
Plant height	0.49	0.48
Shoot Moisture content	0.68	0.41

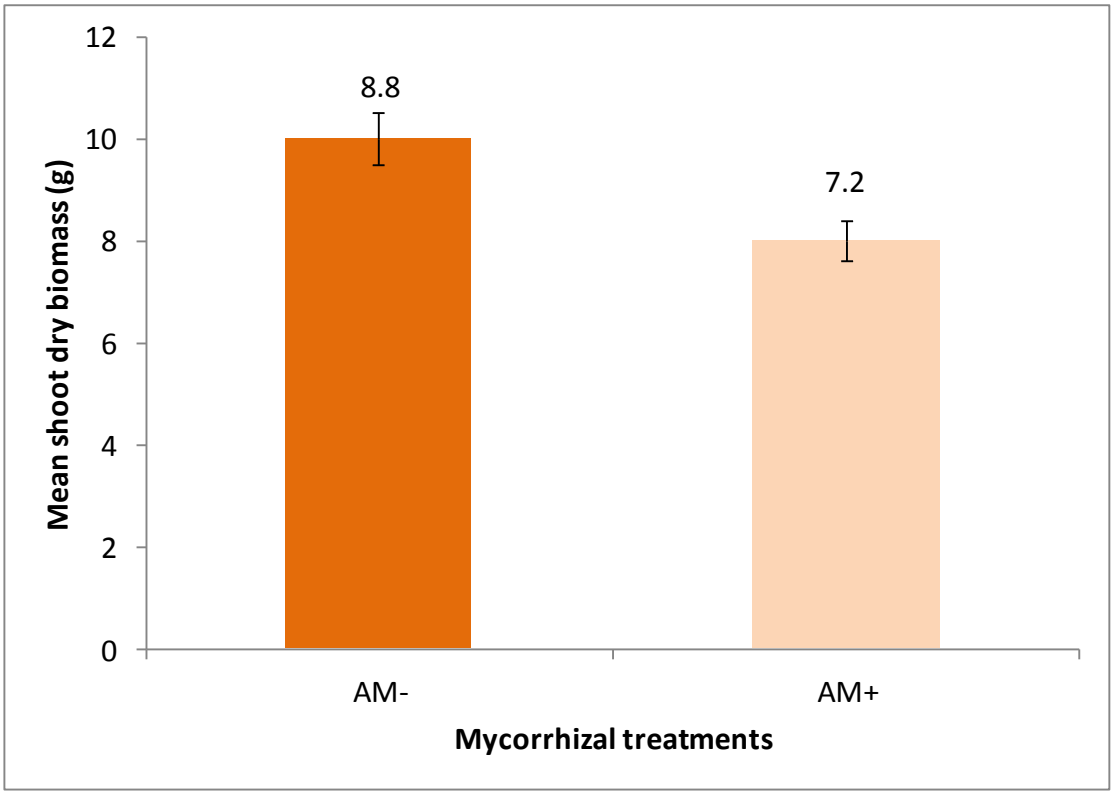

Figure - 1: Final shoot dry biomass of *Trigonellafoenum- graecum* L. plants colonised with the commercial arbuscularmycorrhizal fungi mix (AM+) and of control plants (AM-). Bars represent mean values (± SE), n = 20.

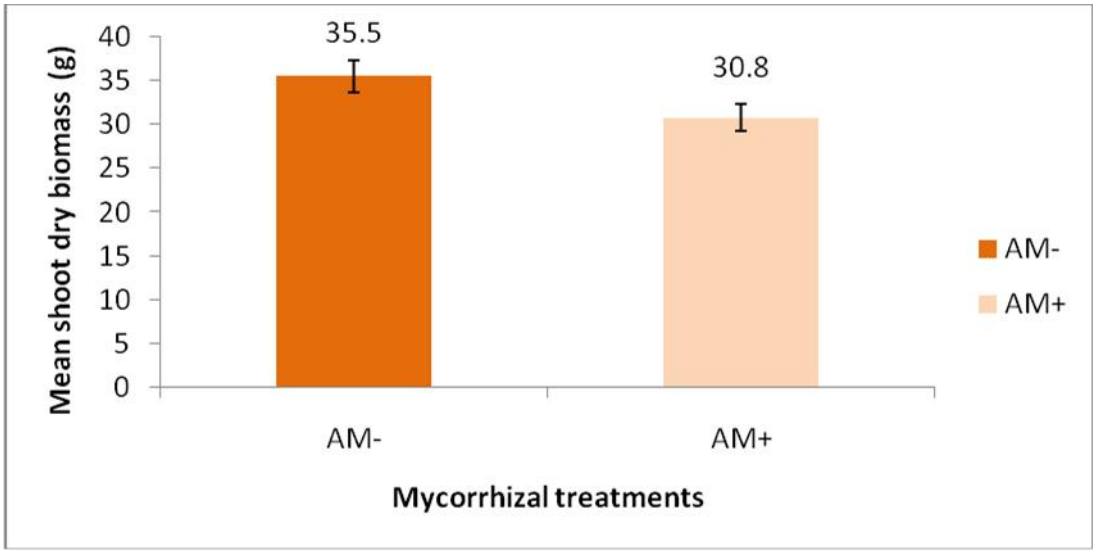

Figure- 2: Final leaf number for *Trigonellafoenum- graecum* L. plants colonised with the commercial arbuscular mycorrhizal fungi mix (AM+) and for control plants (AM-). Bars represent mean values (± SE), n = 20.

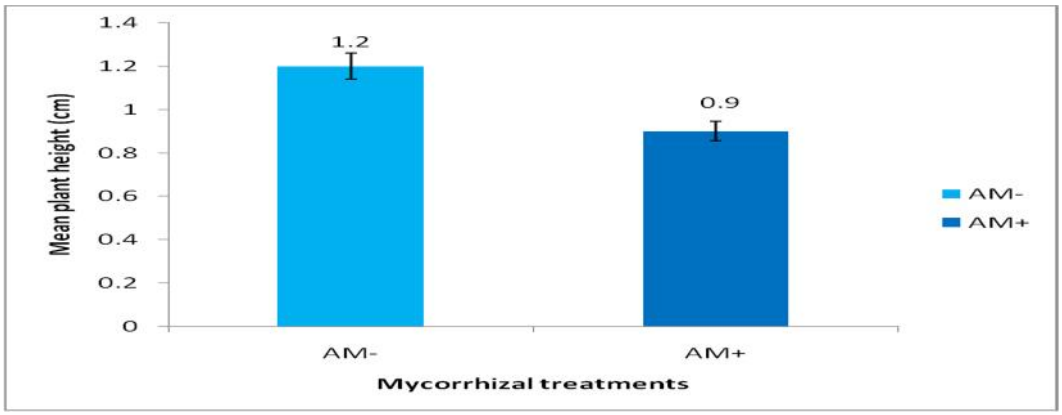

Figure -3: The height of *Trigonellafoenum- graecum* L. plants colonised with the commercial arbuscular mycorrhizal fungi mix (AM+) and of control plants (AM-). Bars represent mean values (± SE), n = 20.

The analysis of shoot moisture showed that AM fungal inoculation had no effect on the mean water content of the plants (Table 1). The controls had slightly higher mean moisture content (24%) than the AM colonised plants (22%), but the difference did not reach significance (Figure 4). Root staining data revealed successful in the colonisation by AM fungi in the treated plants (Table 2). On average, roots showed 36% colonisation by hyphae, 8% by vesicles and 2% by arbuscules. The control plants showed no result of AM fungal colonisation.

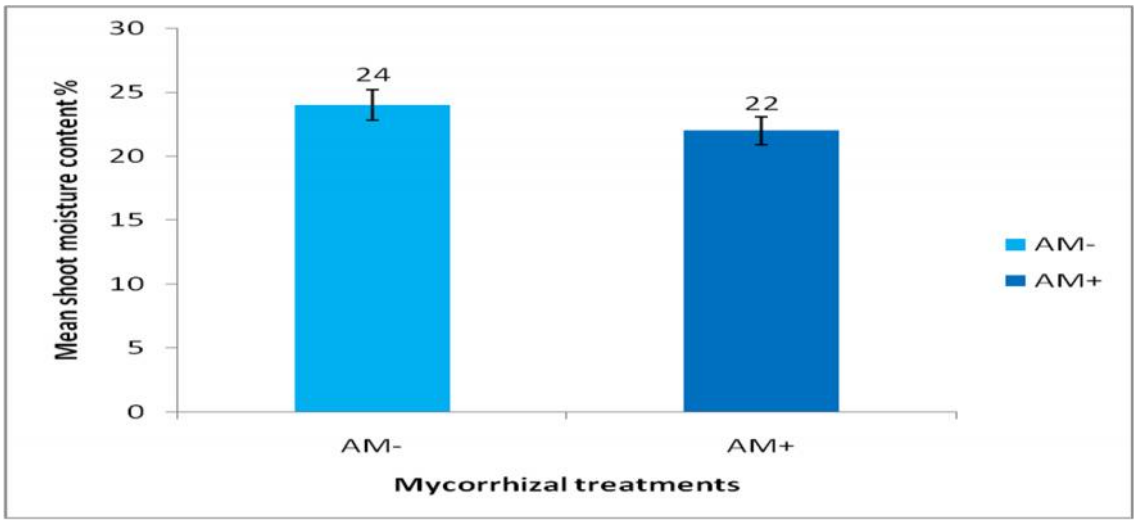

Figure -4: Shoot moisture content (%) of *Trigonellafoenum- graecum* L. colonised with the commercial arbuscularmycorrhizal fungi mix (AM+) and of control plants (AM-). Bars represent mean values (± SE), n = 20.

Table 2: Percent root length colonization in *Trigonellafoenum- graecum* L. plants treated with commercial arbuscularmycorrhizal fungi, or not treated (control) at the first generation stage.

	Hyphae		Vesicles		Arbuscules	
	Control	AM	Control	AM	Control	AM
Root colonization (%)	0	36	0	8	0	2

Second generation Trigonellafoenum- graecum L. harvest

Among the dry shoot biomass, interestingly there was no significant difference between dry shoot biomass in offspring generated from AM colonised plants and the offspring from control parental plants. Control treatment offspring had a mean shoot biomass just 3% higher than offspring from AM colonised parents (Table 3; Figure 5). It should be indicated that there was a large amount of variation between plants, demonstrated by the large standard errors (Figure 5).

Table 3 : Summary of results of Analysis of Variance of different plant growth parameters for the offspring of *Trigonellafoenum- graecum* L. in relation to their parental plant and either treated with the commercial arbuscularmycorrhizal fungi mix or not (Control). All degrees of freedom= 1,48

Parameters	AM	
	F-value	P-value
Dry shoot biomass	2.89	0.08
Final leaf number	0.16	0.16
Plant height	0.6	0.44
Shoot moisture content	0.69	0.41

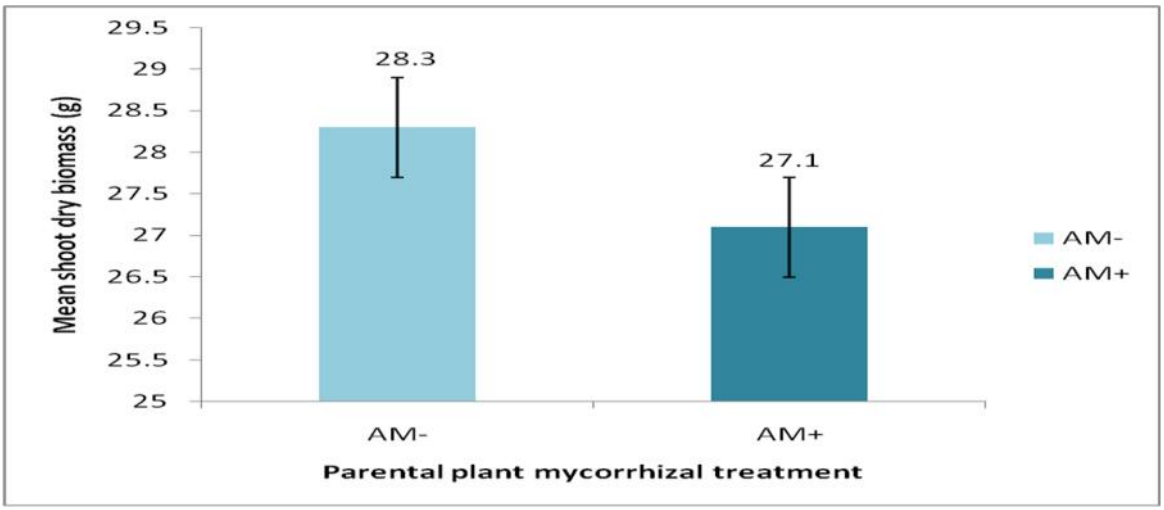

Figure -5: Dry shoot biomass of the offspring of *Trigonellafoenum- graecum* L. plants whose parents were either colonised with the commercial arbuscularmycorrhizal fungi mix (AM+) or were control plants (AM-). Bars represent mean values (± SE), n = 20.

A similar result was observed for total leaf number, which revealed that the offspring of parental plants associated with mycorrhizas had comparable mean leaf counts to the control (Table 3; Figure 6). Similar results obtained by the other growth parameters, mean plant height did not differ between plants grown from mycorrhizal or non-mycorrhizal parents (Table -3), even though the offspring of non-AM (control) parent plants produced slightly more offspring (10% higher) than the offspring of AM colonised parental plants (Figure-7).

Although the control plants contained 2% higher mean moisture content (Figure 8), the mean shoot moisture content in the offspring of the control parents was not significantly different from that recorded in the offspring of AM colonised parents (Table-3).

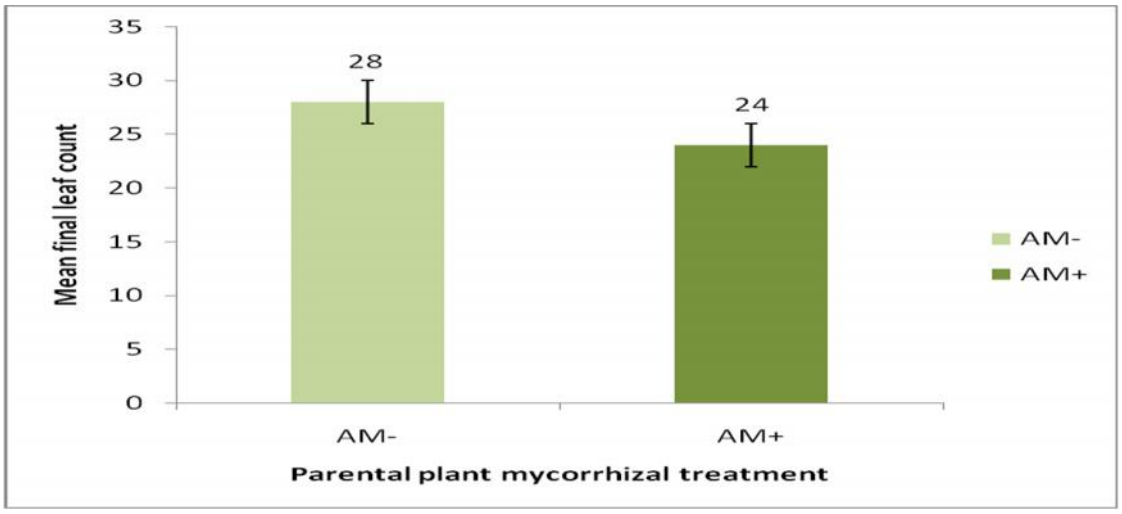

Figure- 6: The final leaf number produced by second generation *Trigonellafoenum- graecum* L. plants whose parents were either colonised with the commercial arbuscularmycorrhizal fungi mix (AM+) or were control plants (AM-). Bars represent mean values (± SE), n = 20.

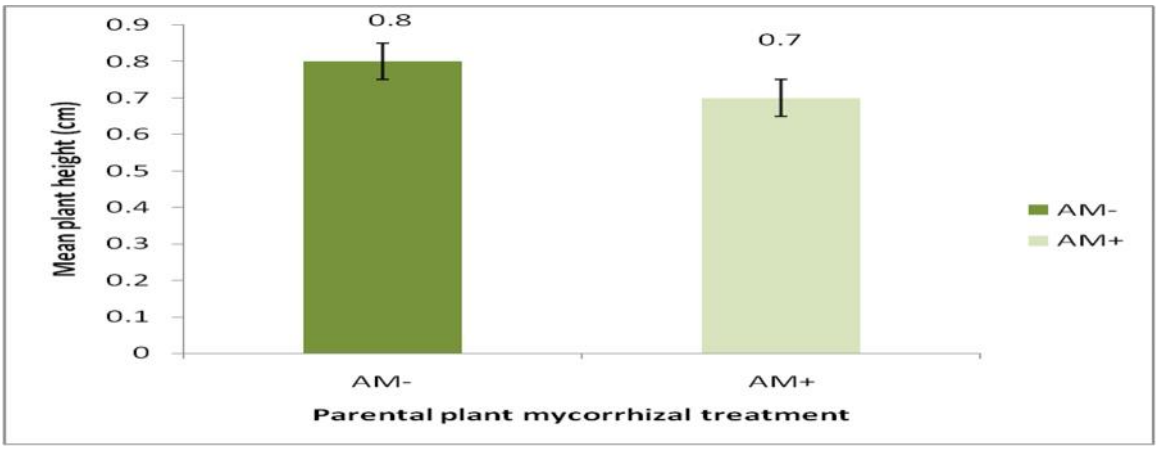

Figure -7: The height of second-generation *Trigonellafoenum- graecum* L. offspring plants whose parents were either colonised with the commercial arbuscularmycorrhizal fungi mix (AM+) or were control plants (AM-). Bars represent mean values (± SE), n = 20.

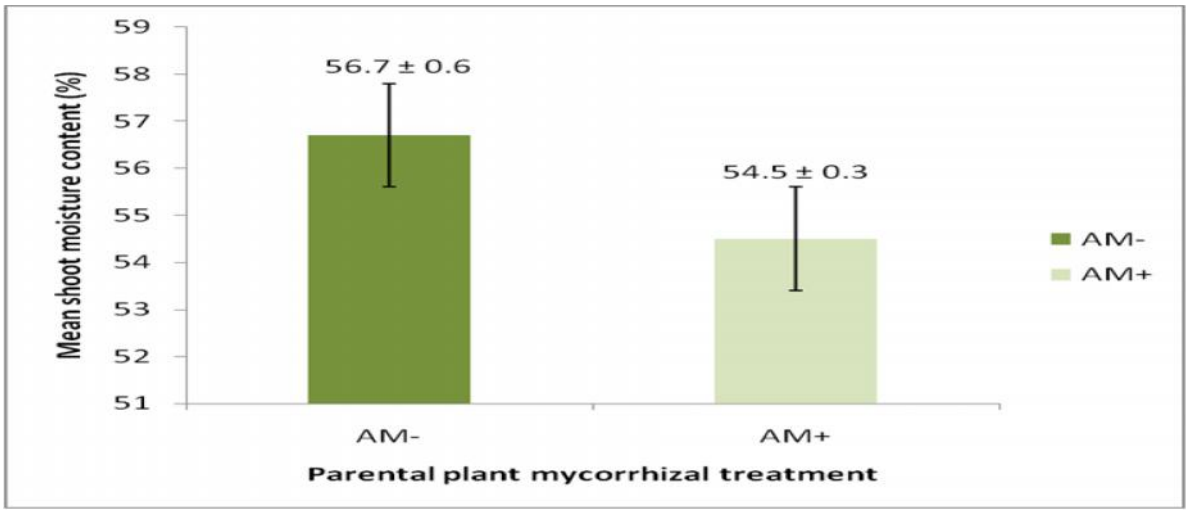

Figure -8: The moisture content (%) of second generation *Trigonellafoenum- graecum* L. plants whose parents were either colonised with the commercial arbuscularmycorrhizal fungi mix (AM+) or were control plants (AM-). Bars represent mean values (± SE), n = 20.

Plant second generation morphology.

After allowing two weeks of seedling propagation for the second-generation plants (F2), the leaves of offspring plants not treated with mycorrhizas started to turn yellowish as a sign of nutrient deficiency. On the other hand, seedlings of AM colonised parents did not exhibit any sign of stress or change in leaf color.

Discussion

The results contributed variation in spore density and colonization of AM associated with different host plant species may be generated by a variety of mechanisms, including variation in host species and their phenology, mycorrhizal dependency, host mediated alterations of the soil microenvironment or other host plant traits (Eom,*et al*., 2000; Lorgio,*et al.*, 1999). The Significance of AM in plant ecology is based on its widespread occurrence in natural ecosystems (Kavitha and Nelson, 2013). The similar results hyphae of AM fungi play an important role in the formation and stability of soil aggregates and contribute to the composition of plant community structures (Smith and Read, 1997).The possible reason for the predominance of *Glomus* is known to be more common in natural and slightly alkaline soils (Mukerji,*et al*. 2002).

Our results to germination of AM fungal spores and the initial growth of hyphal germ tubes can occur in the absence of the plant. Experimental evidence indicates that the quality and source of exudates playan important role in triggering germination and AM fungi respond to host exudates with extensive hyphal growth and branching (Kaur,*et al.*, 2014).Earlier workers have revealed that AM fungi assist plants in accumulating higher concentration of phosphorus (Simard and Durall, 2004) which in turn have a positive effect on both nodulation and nitrogen fixation by *Rhizobium* inoculants. Mycorrhizal association improving salinity tolerance of agronomically important crop plants has been reported by earlier workers in crops like wheat, soybean, onion, capsicum, maize, barley, cotton, etc. (Beltrano,*et al.*, 2013, Maya and Mastbara, 2013). Improved growth and development of mycorrhizal plants especially in stressful environment is partly attributed to better water status of the leaf tissues (Colla,*et al.*, 2008), improved abilities to absorb nutrients from soil, higher root hydraulic conductivity and high photosynthetic rates of mycorrhizal plants (Yang,*et al*., 2014).

Conclusion

The current findings reflect the complexity of the AM-plant association and the wide spectrum of effects emerging from this symbiosis. In *Trigonellafoenum-graecum* L. association with mycorrhizas produced no stimulatory effect on the growth of the parental plants and their offspring. However, the morphological appearance of the second generation plants might point toward a beneficial effect of AM colonisation, possibly by accumulating more phosphorous and other nutrients in *Trigonellafoenum- graecum* L. and their offspring. Further future studies are needed to confirm this investigation. For the next experiments, it was decided to use a mycotrophic plant species that has the ability to exhibit positive results from AM associations during the first generation. This would allow for the examination of results on the offspring. Also, it may be better to monitor and record the first five days of seed germination of the next generation. This would result in a better understanding of the negative or positive effect of AM fungi on seed germination in the next generation of plants.

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