

Effect of Arbuscular Mycorrhizal Fungi on Growth of *Salvadora persica* L. Seedlings under the Nursery Condition

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ABSTRACT: Salt affected soil is a major challenge for vegetation conservation and restoration in many districts of Rajasthan. The high salt content in the soil affects its fertility and this type of soil cannot support vegetation and plantation activities. Plants face toxicity of ions and physiological drought due to presence of excessive salt in the soil. Arbuscular mycorrhizal fungi (AMF) have been proved to support plant growth under salt stress. Though, the property of these fungi promoting growth in salt affected areas is not widely used in the afforestation programme. The application of AMF technology can contribute to the rehabilitation of this problem soil. *Salvadora persica* is an important tree species that survive in salt affected soils, but is not widely used in plantation activities due to slow-growing nature. In this study, a nursery experiment was conducted to test the effects of AMF on *S. persica*, a tree species with significant potential for afforestation of salt affected soil. *S. persica* seedlings inoculated with two AMF strains (*Glomus fasciculatum* and *Glomus aggregatum*) were maintained under nursery conditions. The results of this experiment indicated that the AMF technique can enhance plant growth in *S. persica*, especially *G. fasciculatum* under the nursery conditions.

Keywords: AM fungi, Rehabilitation, salt affected soil, afforestation.

INTRODUCTION

Salt affected soils are a major problem at the present, and it is increasing day by day. India is also affected by this serious problem (Giri *et al.*, 2003). Five percent of the world's agricultural land is affected by excessive salt content (Sheng *et al.*, 2008). In India, approximately 7 MHA lands are salt affected. The high salt content in the soil affects its fertility and this type of soil cannot support vegetation and plantation activities. In Rajasthan, water table is decreasing drastically but due to the availability of electricity irrigation water is used from these deep wells. This saline irrigation water also causes the salt to build up in the soil. Along with natural salt occurrence in the soil, the secondary salinity also developed due to saline water irrigation make the problem worse in arid areas of Rajasthan and makes a big hindrance for food and fodder availability. The properties of these soils are also different and require specific treatments for their rehabilitation and management to maintain their long-term productivity. AM fungi are famous for making symbiotic associations with most plants (Raghuwanshi and Upadhyay 2010). These fungi support the host plants in enhancing nutrition and water availability (Parihar *et al.*, 2019). These fungi also protect host

plant from various microbial diseases, enhance plant growth by producing growth-promoting substances, and participate in improving soil quality (Berta *et al.*, 1995; Leifheit *et al.*, 2014; Frosi *et al.*, 201; Campo *et al.*, 2020; Bona *et al.*, 2016; Hashem *et al.*, 2016; Faghinihnia *et al.*, 2020). AM fungi are also responsible for host plant survival in stress environments like drought, extreme temperature, salinity, and alkalinity (Abed and Esfandiari 2017); Chang *et al.*, 2018; Chen *et al.*, 2017; Zhang *et al.*, 2018; Rydlova and Püschel 2020; Mathur and Jajoo 2020). Due to the above qualities of AM fungi, these are used for the establishment of vegetation in problematic soils such as alkaline, acidic, alkali-saline, calcareous soils (Yue *et al.*, 2019). The salt content in the soil beyond the permissible limit makes soil unsuitable for the survival and growth of any plant (Mathur *et al.*, 2007). Many physiological processes essential for plant life are affected badly by elevated salt content in the soil. High salt is responsible for the reduction of the osmotic potential of soil and increases toxicity of Na⁺ and Cl⁻ which is harmful to the essential living process of the plant (Adiku *et al.*, 2001). Occurrence of AM fungi reported in diverse kinds of environment. These are also found in salt affected soils naturally (Aliasgharzadeh *et al.*, 2001, Yang *et al.*, 2020). The

density of AM fungi in salt affected soils may vary from low (Barrow *et al.*, 1997) to high density (Landwehr *et al.*, 2002). *Glomus* sp. is the most dominant AM fungi reported in salt affected soil (Khare and Rai 2012).

Halophytes are also having an association with these fungi (Mason 1928; Brown and Bledsoe 1996; Marcum 2002). The importance of AM fungi in the rehabilitation of challenging sites has been recognized (Chandra and Kehri 1994). AM fungi also help in the transport of phosphorous in comparison to sodium ions and ultimately in the rehabilitation of salt affected soil. Behl (1990) also reported the use of AM fungi in nurseries of fuel wood for alkaline soil sites. AM Fungi have been proved to support plant growth under salt stress (Asghari *et al.*, 2008). Though, the property of these fungi promoting growth in salt affected areas is not widely used in the afforestation programme. The application of AMF technology can contribute to the rehabilitation of this type of problem soil (Paymaneh *et al.*, 2019). *S. persica* is an important tree species that survive in salt affected soils, but not widely used in plantation activities due to slow-growing nature. In this study, a nursery experiment was conducted to test the effects of AMF on *S. persica*, a tree species with significant potential for afforestation of salt affected soil.

METHODOLOGY

The study was carried out at the Arid Forest Research Institute Jodhpur, Rajasthan. The following steps were included in the experiment:

A. Collection of rhizosphere soil and root samples

In the field, healthy mature trees were selected for the study. Samples were collected from various districts viz., Barmar, Bikaner, Jodhpur, Jaisalmer, Nagaur, and Pali district and analyzed in the laboratory by adopting standard procedure. Laboratory investigations were carried out for detailed AM fungal study and physico-chemical properties of soils.

Fifteen rhizosphere soil samples were collected from each site. Randomly five trees were selected at each site and three samples were collected at the base of each selected tree. All samples were taken in sealed polythene bags. The topsoil was scraped off at the time of sample collection to remove litter and other unwanted foreign particles and. All soil samples were sieved with a 0.5 cm diameter mesh width to remove stones, garbles, and coarse roots. The roots were separated from collected soil samples and suitably processed to investigate the mode of infection by AM fungi, development of vesicle, arbuscules, spores, and hyphae.

B. Isolation of AM fungal propagules from soils

The collected soil samples were passed through a 2 mm sieve for removing foreign debris such as leaves and stones. The standard wet sieving and decanting technique of Gerdemann and Nicolson (1963) was used

for AM fungal propagates isolation from the soil samples.

C. Inoculum culture and Mass Multiplication

Starter culture was prepared with the help of mycorrhizal endophytes, collected from the rhizosphere soil of *S. persica* trees. The pure culture of *Glomus aggregatum* and *Glomus fasciculatum* were maintained. A stereo zoom microscope was used for the examination of collected and sieved spores. Surface sterilization was done with the help of Chloramine-T (0.2%) and 200 ppm streptomycin for 15 minutes and further washed in sterile distilled water 3 to 4 times. Approx five hundred spores were surface sterilized by the above procedure. The soil funnel technique suggested by Nicolson (1967) was used for the preparation of starter culture. Autoclaved Soil and sand were filled in equal amount in 3/4th part of the funnel and the end of the funnel was closed with help of glass wool. The funnel was kept over a conical flask filled with sterilized water to touch the end of the funnel. Spores suspension was spread over and covered with a thin layer of soil. *Cenchrus ciliaris* seeds were sown and covered with soil. Roots were evaluated for infection of arbuscular mycorrhiza after 20-25 days of inoculation procedure. Inoculum developed by the above procedure was shifted into small plastic pots contained filled sterilized sand and soil in 1:2 ratios. In these plastic pots, the seeds of *Cenchrus ciliaris* were sown. This process of inoculum production was performed in the AFRI model nursery, Jodhpur. For further maintenance, all pots were kept in the greenhouse. The watering schedule fixed for these pots was every alternate day. Inoculum transferred in bigger pots follow the above process. It takes 60-90 days for inoculum preparation for further experimentation work. Preservation of this inoculum required 4°C temperature with 30-40 percent humidity. For the above condition, it is stored in the refrigerator and used whenever required.

D. Inoculation of *S. persica* seedlings grown in polybags under nursery conditions

Seeds of *S. persica* were collected from mature trees of forest area. These selected seeds were immersed in water overnight and after the depulping process sown in polythene bags. Two seeds were sown in each polybag. After germination, only one seedling was allowed to grow in each polybag. The side banding procedure was used to inoculate the seedlings. In this method, 5-6 inches deep (4 to 5) holes were made around the seedlings, and holes were filled with the inoculum, and light watering was done.

Four treatments including control (un-inoculated) were taken up. These were as under:

- (i) T₁ - *G. fasciculatum*;
- (ii) T₂ - *G. aggregatum*;
- (iii) T₃ - *G. fasciculatum* + *G. aggregatum*;
- (iv) T₄ - Control or un-inoculated

Table 1.

Sr. No.	Treatment	Seedling height increment in cm. (mean)	Shoot dry weight in gm. (mean)	Root dry weight in gm. (mean)	Seedling Biomass in gm. (mean)
1.	T-1	53.14±0.82	15.82±0.31	7.32±0.17	23.14±0.48
2.	T ₂	49.79±0.865	11.48±0.28	5.23±0.19	16.72±0.44
3.	T ₃	60.76±0.89	20.08±0.45	9.37±0.24	29.44±0.69
4.	T ₄	41.15±0.88	5.86±0.26	2.53±0.11	8.38±0.35
6.	SD	10.98	5.43	2.63	8.064

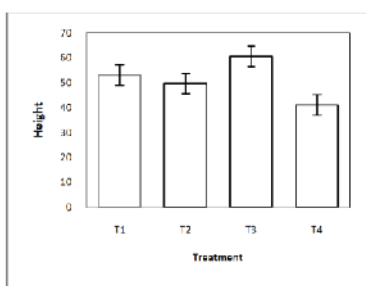
T₁- *G. fasciculatum*, T₂- *G. aggregatum*
 T₃- *G. fasciculatum* + *G. aggregatum*, T₄- Control

The experiment was laid out in Completely Randomized Design (CRD) with three replications; there were thirty-two plants for each replication. The experiment was conducted in a shade house at AFRI nursery.

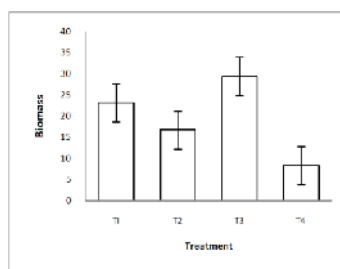
Shoot Height (cm) of seedlings was taken from the collar to tip with a measuring scale bimonthly. The initial height of seedlings was not uniform, therefore height increment was noted and used in the analysis of data. After 240 DAI (days after inoculum) of the experiments, the shoots were cut near the base, and roots of the harvested plants were excavated. Root

length was recorded in (cm). For estimation of the dry weight of shoot and root, the samples were oven-dried at 70°C for 72 hrs. For biomass estimation, dry weights of leaves + shoot + root were taken. The shoot height increment was recorded bimonthly and the final following observations were recorded (a). Shoot/plant height increment (cm); (b). Shoot dry weight (g); (c). Root dry weight (g); (d). Total biomass (g).

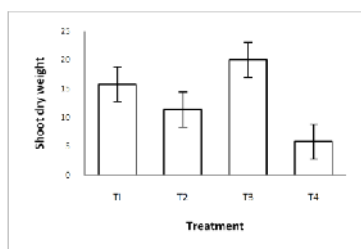
To compare the effects of treatments one-way analysis of variance (ANOVA) was employed, and the treatment's means were evaluated by Duncan's multiple range test ($p < 0.05$). SPSS was used to analyze all data.



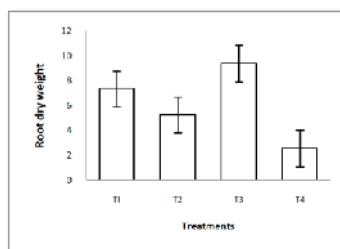
(a) Shoot height increment ($p < 0.01$)



(b) Plant biomass ($p < 0.01$)



(c) Shoot dry weight ($p < 0.01$)



(d) Root dry weight ($p < 0.01$)

Graph with error bar represents significant differences between three treatments and control (T4)

RESULT AND DISCUSSION

This study proved that *G. fasciculatum* and *G. aggregatum* increased height, dry weight of shoot, dry weight of root, and Biomass of *S. persica* seedlings under the nursery conditions as shown in table 1.1 and graph. Regarding the enhancement of plant growth, *G. fasciculatum* was found to be superior to *G. aggregatum*. *G. fasciculatum* significantly increased shoot height alone and with *G. aggregatum* also. In comparison to control *G. fasciculatum* treated plants showed significantly higher shoot height increment, shoot dry weight, root dry weight, and plant biomass. Best result obtained in Sharma et al.,

T₃ treatment (*G. fasciculatum* + *G. aggregatum*). The difference in values of Shoot height increment, shoot dry weight, root dry weight, and plant biomass was highly significant in treatments over the control plants (as $p < .01$). All inoculated seedlings showed colonization in their roots under experimental conditions, but this enhancement was not found in control/uninoculated seedlings. It is a well-known fact that AM fungi are responsible for plant growth enhancement. In agreement with our result, uninoculated seedlings of *M. paniculatus* and *A. saman* (Dewi et al., 2014) and *Z. serrata* roots (Wang et al., 2019) didn't show any AM colonization. Under the

nursery conditions, seedlings inoculated with AM fungi showed better plant growth. Results of the above experiment are also in agreement with another research that showed *Acacia collie* seedlings treated with different AM fungi showed an increase in biomass production (Mathur and Vyas 2016). It is also proved that *G. fasciculatum*; *Sclerocystis dussi*, *Acaulospora laevis*, and *Gigaspora margarita* increased shoot growth in half year old *Phyllanthus emblica* (Byatanal and Lakshman 2011) seedlings under greenhouse conditions. Nursery experiment also performed in four-month-old *Tamarindus indica* seedlings by inoculation of *Glomus aggregatum* and proved better growth and nutrient uptake in inoculated seedlings (Guisso, 2009). Another study showed a positive growth response in *Senna spectabilis* inoculated with *G. etunicatum* and *Glomus macrocarpum* (Kung'u *et al.*, 2008). Inoculation by *G. decipiens* and *G. clarum* increased shoot P and N uptake, shoot height, shoot dry weight, and leaf number of *M. paniculatus* and *A. saman* seedlings (Dewi *et al.*, 2014). Our results proved that arbuscular mycorrhizal fungi are important for enhancing the growth and biomass of *S. persica* seedlings under nursery conditions. Growth and yield of Sorghum due to AM fungi was studied was studied by Cahyani *et al.*, (2019). Garg and Saroy (2020) established positive effect of AM fungi on plant biomass in *Cajanus cajan*. Effects of AM fungi colonization in rice were studied by Campo *et al.*, (2020) and observed positive effect on growth, productivity, and resistance from blast disease. Mathur and Jajoo (2020) told role of AM fungi in protection of Maize from high temperature environment. Gogoi (2011) studied effect of AM fungi on growth of *Piper longum*. Many researcher evaluated role of AM fungi in nutrient uptake and growth of host plant like green asparagus, winter wheat, barley and faba bean (Conversa *et al.*, 2019; Ingrassia *et al.*, 2019; Luo *et al.*, 2019). Wu *et al.*, (2011) studied the effect of AM fungi inoculation on growth of *Prunus persica* seedlings. *Eucalyptus pellita* and *Acacia crassicarpa* seedlings raised by micropropagation inoculated by AM fungi and effect of AM fungi on seedling height, stem diameter, and root dry weight were studied in both species (Agustini *et al.*, 2020). The effect of inoculation of *Rhizobium* and (*G. fasciculatum*) was tested by Sengupta and Chaudhuri (2002) on the growth response of *Sesbania grandiflora* in Sundarbans area of W. Bengal. Both single and dual inoculations showed the better result. Sidhu and Behl (1995) worked on the effect of three AM fungi *G. fasciculatum*, *G. macrocarpum*, and *G. mosseae*, on the growth of *Prosopis juliflora* and achieved promising results on plant growth in inoculated plants. The effect of *G. mosseae*, *G. fasciculatum*, and *G. aggregatum*, was studied in Chamomile and found enhancement of growth and productivity in alkaline soil by Janardhanan and Abdul-Khaliq (1995).

Saralabai and Vivekanandan (1995) proved the capabilities of AM fungi to enhance the survival of host plants in stress environment like high pH and EC of the soil. A lot of research work was done on the role of AM fungi in the rehabilitation of salt affected soil by Giri *et al.*, (1999) including effect of the *G. macrocarpum* on the growth of *Sesbania aegyptiaca*. Giri and Mukerji (2003) also investigated the performance of *G. macrocarpum* inoculated seedlings of *Sesbania grandiflora* in saline soil and found encouraging results, for growth enhancement of mycorrhizal plants successful colonization by AM fungi is a prime requirement. In this study AM fungal inoculum was successfully prepared with high percentage colonization in *S. persica* seedlings to attain growth enhancement. The results showed that a good inoculum preparation of appropriate AM fungi, such as *G. fasciculatum* and *G. aggregatum*, is effective in promoting the growth of *S. persica* under the nursery condition. Although it is proved that AM fungi promote growth of seedlings, but exact mechanism by which AM fungi regulate growth of plants should be primary focus of upcoming research in this field. Efforts in direction of responsible gene identification of AM fungi will be prove helpful in enhancement of host plant productivity. More AMF strains should be explored to enhance use of these fungal strains as a bio-fertilizer in forestry sector.

CONCLUSION

The study of mycorrhizal associations of *S. persica* is critical to access their dependence on mycorrhizal fungi as well as selecting out candidate species of endomycorrhizal fungi for mass multiplication. Inoculation by *G. fasciculatum* and *G. aggregatum* increased, shoot height and biomass of *S. persica* seedlings. Regarding the enhancement of plant growth, *G. fasciculatum* was found superior as compare to *G. aggregatum*. Selected AM fungi may be used for fortifying seedlings in the nursery before taking them at plantation sites. Further field trials are required to evaluate the impact on growth and survival of *S. persica* inoculated by AM fungi under the field condition as the present results were obtained under the nursery conditions. We can conclude our experiment with the recommendation that the inoculation with AM fungi especially *G. fasciculatum* had a strong impact on seedling growth of *S. persica*.

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Conflict of interest. Nil.

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