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Impact of Different *Glomus* Species on Growth and Survival of *Acacia nilotica* Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Acacia nilotica (L.) Willd. ex Del, commonly known as babul, kikar belongs to family Leguminosae and is recognized as a multipurpose tree. It helps in improving soil fertility, increasing the activity of symbiotic microorganisms and providing various commercial benefits. The raising of high quality kikar seedlings is necessary to establish a good plantation and the role of arbuscular mycorrhizal fungi (AMF) in soil becomes imminent in raising of seedlings under nursery. The effect of three different species of *Glomus* spp. (*G. mosseae, G. intraradices* and *G. fasciculatum*) of AMF inoculated soil with *Acacia nilotica* seeds were observed in the nursery during 2018-2019 and 2019-2020. These AMF were applied at 400-500 sporocarp/kg of soil at sowing time and evaluated for their performance on the growth parameters, survival percentage, root colonization (%) and number of sporocarps. The experiment was laid out as completely randomized design, replicated three times with twenty seedlings per replication. The results revealed that among three *Glomus* species, the shoot length of 60.75 (2018-2019) and 59.80 cm (2019-2020) was the significantly high in soils inoculated with *G. intraradices* as compared to check (uninoculated). The root length of seedlings was also significantly higher when seeds were sown in soil inoculated with *G. intraradices* followed by *G. fasciculatum* inoculated soil. The plant biomass was recorded significantly high when seeds were sown in soil infested with *G. intraradices* (23.77 g) followed by *G. fasciculatum* (21.57 g) and minimum in the *G. mosseae* (20.20 g) among *Glomus* spp. during 2019-2020. The seedlings

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survival was 81.53 and 85.79 % in soils inoculated with *G. intraradices* followed by 74.65 and 79.01 % in *G. fasciculatum* during 2018-2019 and 2019-2020, respectively and all the treatments differed significantly as compared to check. A significant higher root colonization (20.35 and 19.16 %) and number of sporocarps (28.0 and 26.51 /per 100 g of soil) at 150 DAS were recorded in soils inoculated with *G. intraradices* followed by *G. fasciculatum*.

Keywords: Acacia nilotica; G. fasciculatum; G. intraradices. G. mosseae; Sporocarps.

1. INTRODUCTION

Forests, as the key element of the terrestrial ecosystem are crucial for the well being of humanity. They provide foundations for life on earth through ecological functions, by regulating climate and water resources and by serving as habitats for plants and animals. They also furnish a wide range of essential goods such as food, fodder, fuel and medicines, in addition to opportunities for recreation, spiritual renewal, includes the amelioration of soil chemical and physical properties, the reduction of soil erosion and improved weed control. *Acacia nilotica* plays a significant role under these circumstances. It is commonly known as babul or kikar belonging to the family Leguminosae [1]. It has been well recognized as a multipurpose tree globally. This versatile tree is generally found growing in forest areas, farmlands, tank foreshores, agricultural fields, village pasture lands, wastelands, bunds and along highways and railway lines. It is a drought-resistant multipurpose legume tree capable of biological nitrogen fixation which helps in providing various ecological as well as commercial benefits such as fodder, timber etc [2]. It contains a huge number of alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, hormones, tannins, and terpenes that have the property to avoid, alleviate, or cure various diseases and conditions [3]. Among the various microorganisms, the arbuscular mycorrhizal fungi (AMF) present in soils contribute substantially to trees growth and survival. The AMF are soil microorganisms composing the essential components of the sustainable soil-plant system. The AMF form extensive extraradical mycelia which increase accessible soil volume for the plant to absorb phosphorus and water [4]. The AMF belonging to the phylum *Glomeromycota*, are obligate symbiotic fungi forming mutualistic associations with the roots of most land plants. The increased access to low-mobility soil mineral nutrients has been considered to be the main beneficial effect of AMF on their host plants [5]. The importance of AMF in nursery management and in its re-vegetation in various land types has been realized now and it has become an integral part of afforestation programs. The other benefits of AMF in soil to trees is the production of plant growth hormones, protection of roots from pathogens, uptake of heavy metals, tolerance to salinity, radionuclides uptake and protection of trees from radioactivity [6]. The use of AMF provides an effective alternative to improve the growth and nutrient status of plants. The high quality seedlings to establish a good plantation are necessary. The apparent results of these AMF may not be evident under all natural conditions because of their insufficient population occurring naturally in the soil [7]. There are many reports which have shown that plants raised in soil having AMF increased nodulation in various legumes resulting into higher fixation of atmospheric nitrogen in roots [8]. A limited work has been done on the use of AMF in raising of healthy seedlings of *A. nilotica*, therefore, the present study was planned with an objective to find out an appropriate AMF which may be inoculated artificially in soil before sowing and may provide healthy seedlings of high vigor for better establishment under biotic and abiotic stresses in field conditions.

2. MATERIALS AND METHODS

The study was carried out in the nursery of Forestry Department, CCS Haryana Agricultural University, Hisar (20^0 10’ N lat.,75^0 46’ E long., alt. 215 m msl), situated in the semi-arid region of North-Western India. The soil type used in experimentation was sandy loam. The climate of Hisar (Haryana) is semi-arid with hot and dry desiccating winds accompanied by frequent dust storms with high velocity in summer months, severe cold during in winter months and humid warm during monsoon rainy season. The mean monthly maximum and minimum temperature sometimes exceed 48^0 C on hot summer days. The relative humidity varies from 5 to 100 %, while temperature below freezing point accompanied by frost in winter is usually experienced in this region. The pure cultures of three AMF *viz. Glomus intraradices, Glomus* *mosseae* and *Glomus fasciculatum,* were collected from the Department of Plant Pathology, CCS Haryana Agricultural University, Hisar and were multiplied on pearl millet (*Pennisetum typhoides*) and wheat (*Triticum aestivum*) under screen house conditions. The soil and rootlets from the root horizon of *G. intraradices, G. mosseae* and *G. fasciculatum* inoculated wheat and pearl millet plants were used in different treatments before sowing of seeds as per detail given below. The seeds were sown 2-3 cm deep in sterile sandy soil inoculated individually with *G. intraradices, G. mosseae* and *G. fasciculatum* at 450-500 sporocarps/kg soil in polythene bags of 22.5 x 12.5 cm size in the month of July, 2019 and 2020. The sowing of seeds in uninoculated soil served as a check. This experiment was carried out under a completely randomized design, each treatment was replicated three times and twenty seedlings per replication were raised and maintained. The growth parameters of seedlings, mycorrhizal colonization in roots and sporocarp numbers in soil were determined at 60, 90, 120 and 150 DAS. The mycorrhizal colonization was calculated by using the method given by Phillips and Hayman (1970) and sporocarps were calculated as per the method given by Gerdemann and Nicolson [9]. The present study was conducted in Forestry Nursery at CCS Haryana Agricultural University, Hisar during 2018-2019 and 2019-2020. There were three treatments of soil inoculation with *Glomus* spp. inoculated before sowing and control (uninoculated AMF) as shown below:-

T_1 - Soil inoculated with *G. intraradices*
T_2 - Soil inoculated with *G. mosseae*
T_3 - Soil inoculated with *G. fasciculatum*
T_4 - Control (un-inoculated)

3. RESULTS AND DISCUSSION

3.1 Impact of Soil Application of Different *Glomus* spp. on Growth Parameters

The data presented in Table 1 and Table 2 revealed the growth parameters viz. shoot length (cm), root length (cm), collar diameter (mm), plant biomass (g) and plant survival (%) of *Acacia nilotica* seedlings at 60, 90, 120 and 150 DAS in soil treated with different *Glomus* spp. during 2018-2019 and 2019-2020, respectively. The shoot length of *A. nilotica* seedlings was significantly more in all the treatments having soil inoculated with *Glomus* sp. as compared with control (uninoculated). The maximum shoot length of 60.75 and 59.80 cm was recorded in treatment when *G. intraradices* was inoculated in soil before sowing which was significantly better than all the treatments. Singh and Chugh [10] also observed a maximum shoot length in *Dalbergia sissoo*, *Eucalyptus tereticornis*, *Azadirachta indica* and *Ailanthus excela* trees when sown in soils incorporated with *G. mosseae* as compared to the non-inoculated soil. However, G. *mosseae* also increased shoot length in present study but it was less effective than the soil incorporation of *G. intraradices*. It may be due to the differences in inherent character of AMF. The minimum shoot length of 50.75 cm and 50.00 cm was observed in soils treated with *G. mosseae* during 2018-2019 and 2019-2020, respectively. Amongst all treatments, the shoot length was better in all seedlings grown under three *Glomus* spp. inoculated soil as compared to control (uninoculated). The root length of 45.78 and 42.33 cm in *A. nilotica* seedlings was significantly highest during 2018-2019 and 2019-2020, respectively when seeds were sown in soil inoculated with *G. intraradices* and it was followed by root length of 42.52 and 39.58 cm during 2018-2019 and 2019-2020, respectively in soil inoculated with *G. fasciculatum.* The root length of 38.91 and 36.47 cm was significantly low in seedlings grown under *G. mosseae* inoculated soil during 2018-2019 and 2019-2020, respectively as compared to other *Glomus* spp. used in the experiment. However, the root length was better in all seedlings grown under three *Glomus* spp. inoculated soil as compared to control (uninoculated). While comparing the thickness at the collar region of *A. nilotica* seedlings, the maximum collar diameter of 5.95 and 5.77 mm was found in seedlings grown under soil inoculated with *G. intraradices* followed by *G. fasciculatum* (5.27 and 5.08 mm) and *G. mosseae* (4.83 and 4.53 mm) during 2018-2019 and 2019-2020, respectively. The application of *Glomus* spp. in soil significantly increased the collar diameter as compared to control. It was significantly as low as 4.12 and 4.10 mm during both years in control (uninoculated). The biomass production was 21.52 and 23.77 g in seedlings grown in soils inoculated with *G. intraradices* followed 19.66 and 21.57 g in *G. fasciculatum* treated soil and 18.94 and 20.20 g in soil incorporated with *G. mosseae* at 150 days after sowing during 2018-2019 and 2019-2020, respectively. The inoculation of *Glomus* spp. in soil significantly increased plant biomass production as compared to control (14.44 and 15.97 g during 2018-2019 and 2019-2020). The survival percentage of seedlings of *A. nilotica* was significantly high in soil inoculated with *G. intraradices* at the rate of 81.53 and 85.79 % during 2018-2019 and 2019-2020, respectively as compared with control as well as soil inoculated with *G. mosseae* and *G. fasciculatum*. Among *Glomus* spp. the maximum survival percentage was 74.65% and 79.01 % during 2018-2019 and 2019-2020, respectively in soil treated with *G. fasciculatum* treatment and minimum (73.89% and 75.35% during 2018-2019 and 2019-2020, respectively) in *G. mosseae*. Since there are many studies on effect of soil application of AMF in different field crops and different forest trees but there is a very limited study in the selection of an appropriate AMF which may be inoculated artificially in soil before sowing and may provide healthy seedlings of high vigor for better establishment under biotic and abiotic stresses in field conditions. The observations in this study are in accordance with the findings of Singh and Chugh [10] who observed an improvement in survival percentage, root length, shoot length, total biomass, mycorrhizal colonization and sporocarp number in *Dalbergia sissoo*, *Eucalyptus tereticornis*, *Azadirachta indica* and *Ailanthus excela* trees when sown in soils having mycorrhiza as compared to the non-inoculated soil. Similarly, the AMF have been found to enhance various growth parameters in *Glycine max* L. and helped the plants in drought tolerance [11,12] reported that the AMF after establishing symbiosis produce extensive underground extraradical mycelia ranging from the maize roots up to the surrounding rhizosphere, thereby helped in improving the uptake of nutrients. The plant growth parameters in wheat (*Triticum aestivum*) were increased in the presence of *G. mosseae* and *G. fasciculatum* [13]. Giri *et al.* [14] found increased fresh root weight in *Cassia siamea* when AMF was inoculated in soil over control. Kaushik *et al.* [15] showed that *Acacia nilotica* and *Dalbergia sisso* seedlings had significantly higher biomass, root and shoot length in soils inoculated with *G. mosseae* treatment than un-inoculated treatments, however, in the present study *G. mosseae* inoculated artificially in soil was also effective in promoting growth parameters as compared to control but not as effective as *G. intraradices* application. Gupta and Rahangdale [16] also found the increased fresh root weight in *Dalbergia sissoo* and *Albizzia lebbek* with dual inoculation of AMF and *Rhizobium* in soil. An improved germination of seeds with application of bio-inoculants has also been reported by various workers on different forest plants [17, 18, 19, 20]. Since AMF have been reported to produce plant growth hormones and help in uptake of available and non-available form of nutrients by plants, it might have resulted in increased plant growth parameters in *Acacia nilotica*. Similarly, plant survival (%) was also high in soil treated with AMF, it may be due to protection of seedlings from destructive pathogens by *Glomus* sp. The plant strength also increases by the presence of AMF in soil and it might have resulted in better plant survival of *A. nilotica*. The modification of the soil environment by AMF surrounding the roots may also be the reason of better growth of seedlings, high survival rate, better root colonization and multiplication.

3.2 Impact of Soil Application of Different *Glomus* sp. on Root Colonization and Sporocarp Numbers

The data presented in Table 3 and Table 4 depicts root colonization (%) and number of sporocarps/100 g of soil in *Acacia nilotica* seedlings at 60, 90, 120 and 150 DAS in soil treated with different *Glomus* spp. during 2018-2019 and 2019-2020, respectively. Amongst the three *Glomus* species inoculated singly in soil before sowing of *A. nilotica* during 2018-2019 and 2019-2020, a significant higher root colonization of 20.35 and 19.16 % was achieved in seedlings at 150 DAS during 2018-2019 and 2019-2020, respectively when *G. intraradices* was applied to the soil before sowing and no root colonization was seen in control (uninoculated). It was followed by 18.55 and 17.83 % root colonization 150 DAS during 2018-2019 and 2019-2020, respectively in *G. fasciculatum* treated soil and significantly low root colonization of 17.87 and 16.93 % was observed in soil treated with *G. mosseae*. The number of sporocarps was also significantly high at 150 DAS (28.0 and 26.51 /per 100 g of soil during 2018-2019 and 2019-2020, respectively) in soil treated with *G. intraradices.* It was followed by *G. fasciculatum* (25.67 and 24.15 sporocarps per 100 g soil during 2018-2019 and 2019-2020, respectively at 150 DAS. The number of sporocarps per 100g soil was found to be minimum (23.45 and 20.70 during 2018-2019 and 2019-2020 in *G. mosseae* treated soil. Similar observations on increase in sporocarp numbers in soil and root colonization in *Dalbergia sissoo* have also been reported by Kumar, *et al.* [21]. The presence of *G.*

Table 1. Effect of soil application of different *Glomus* spp. on growth parameters of *Acacia nilotica* seedlings during 2018-2019

Treatments	Shoot length (cm)				Root length (cm)				Collar diameter (mm)				Plant biomass (g)				Plant survival (%)
	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS	
G. intraradices	23.8	35.6	46.5	60.8	12.5	22.9	34.5	45.8	2.11	3.86	4.56	5.95	4.9	10.6	16.6	21.5	81.5
G. mosseae	21.5	30.5	40.4	50.8	12.1	20.1	30.5	38.9	2.00	3.45	4.00	4.83	4.2	09.1	14.1	18.9	73.9
G. fasciculatum	22.6	32.6	42.7	55.5	12.3	21.1	32.9	42.5	2.09	3.76	4.44	5.27	4.8	10.0	15.1	19.7	74.7
Control (un-inoculated)	20.6	28.3	35.3	48.3	12.0	16.8	21.6	30.6	1.95	2.88	3.47	4.12	4.0	07.4	10.6	14.4	70.5
Mean	22.11	31.8	41.2	53.8	12.2	20.2	29.9	39.5	2.04	3.49	4.12	5.04	4.5	09.3	14.1	18.6	75.1
CD at 5%	1.94	2.79	3.62	4.73	NS	1.78	2.65	3.49	NS	0.30	0.36	0.44	0.39	0.82	1.25	1.64	6.59

Table 2. Effect of soil application of different *Glomus* spp. on growth parameters of *Acacia nilotica* seedlings during 2019-2020

Treatments	Shoot length (cm)				Root length (cm)				Collar diameter (mm)				Plant biomass (g)				Plant survival (%)
	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS	
G. intraradices	22.2	33.9	46.4	59.8	12.3	21.8	30.9	42.3	2.03	3.82	4.75	5.77	4.9	10.9	18.5	23.8	85.8
G. mosseae	19.1	28.6	40.5	50.0	12.1	20.5	27.0	36.5	1.97	3.43	4.03	4.53	4.2	9.3	15.0	20.2	75.4
G. fasciculatum	20.2	30.3	42.0	53.1	12.0	20.0	28.9	39.6	2.00	3.80	4.65	5.08	4.9	10.5	16.4	21.6	79.0
Control (un-inoculated)	18.1	26.5	31.8	47.5	11.8	15.6	20.5	29.5	2.96	2.41	3.12	4.10	3.8	7.9	11.4	15.9	72.1
Mean	19.9	29.8	40.2	52.6	12.1	18.9	26.8	37.0	1.99	3.37	4.14	4.87	4.4	09.7	15.3	20.4	78.1
CD at 5%	2.02	3.43	3.40	4.00	NS	1.94	3.24	3.76	NS	0.35	0.54	0.46	0.65	1.06	1.49	2.00	6.76

Table 3. Effect of different *Glomus* spp. treated soils on *Acacia nilotica* seedlings root colonization and sporocarps number in soil during 2018-2019

Treatments	Root colonization (%)				No. of sporocarps /100 g of soil			
	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS
G. intraradices	10.0	14.6	18.8	20.4	18.3	21.7	23. 6	28.0
G. mosseae	08.2	12.2	16.3	17.9	16.7	18.4	20.8	23.5
G. fasciculatum	09.3	14.1	17.0	18.6	17.5	20.8	21.6	25.7
Control (un-inoculated)	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
Mean	06.9	10.2	13.0	14.2	13.1	15.2	16.5	19.3
CD at 5%	0.69	1.03	1.32	1.43	1.32	1.54	1.67	1.95

Table 4. Effect of different *Glomus* spp. treated soils on *Acacia nilotica* seedlings root colonization and sporocarps number in soil during 2019-2020

Treatments	Root colonization (%)				No. of sporocarps /100 g of soil			
	60 DAS	90 DAS	120 DAS	150 DAS	60 DAS	90 DAS	120 DAS	150 DAS
G. intraradices	10.0	14.1	18.1	19.2	16.5	19.3	21.1	26.5
G. mosseae	07.7	11.2	15.7	16.9	14.7	16.2	17.7	20.7
G. fasciculatum	08.0	13.6	16.6	17.8	15.9	18.9	19.0	24.1
Control (un-inoculated)	00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
Mean	06.4	09.7	12.6	13.5	11.8	13.6	14.5	17.8
CD at 5%	1.10	0.95	1.00	1.28	1.67	1.32	1.71	1.87

intraradices in soil was more effective in root colonization of seedlings of *A. nilotica* and have better multiplication in the rhizosphere of *A. nilotica* as compared to *G. fasciculatum* and *G. mossease*. The higher root colonization and better multiplication of *G. intraradices* in the rhizosphere of *A. nilotica* seedlings may be due to the inherent character of this *G. intraradices* which might have stimulated nutrient uptake in seedlings and hence better growth. The AMF root colonization and spore density in soil is an important parameter to determine the status of the association and studies have shown that higher colonization rates are often positively correlated with plant growth and nutrition. The root exudates of seedlings may also have influenced the better root colonization, faster multiplication of AMF and hence better growth and survival of seedlings [22].

4. CONCLUSION

Glomus species inoculated individually in soil before sowing of seed were helpful in promoting growth characters (shoot length, root length, collar diameter, plant biomass) and plant survival of *Acacia nilotica* seedlings but *G. intraradices* was found to be more effective amongst all three *Glomus* spp. Similarly, root colonization of seedlings was highest in *G. intraradices* soil treatments followed by *G. fasciculatum* and *G. mosseae* soil treatments. The multiplication of *G. intraradices* was also found to be high followed by *G. fasciculatum* and *G. mosseae*. It is concluded that *G. intraradices* used as a soil application before sowing of seeds of *A. nilotica* is highly effective in establishment of seedlings under nursery conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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