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Impact of Different Arbuscular Mycorrhizal Fungi on Nutrient Status and Their Uptake by *Melia azedarach* Seedling

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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

Melia azedarach belongs to the family *Meliaceae*, is a deciduous tree with a short bole and a spreading crown and one of the most important trees in arid and semi-arid area. The arbuscular mycorrhizal fungi (AMF) are soil microorganisms composing the essential components of the sustainable soil-plant system. These AMF form extensive extraradical mycelia which increases accessible soil volume for the plant to absorb phosphorus and water. The effect of three different species of *Glomus* (*G. mosseae, G. intraradices* and *G. fasciculatum*) of AM fungi inoculated with *Melia azedarach* drupes was observed in the nursery of the Forestry Department, CCS Haryana Agricultural University, Hisar during 2019. The results revealed that application of three *Glomus* species in combination produced synergistic increase in soil macro and micro nutrient contents after harvest i.e. N = 119.86 kg ha^{-1}, P = 16.91 kg ha^{-1}, K = 260.86 kg ha^{-1}, Fe = 5.95 ppm, Zn = 0.66 ppm, Cu =0.42 ppm and Mn = 1.63 ppm. Similarly, there was significantly higher macro and micronutrient (N = 1.39 %, P = 0.27 %, K = 0.63 %, Fe = 90.43 ppm, Zn = 32.24 ppm, Cu = 9.96 ppm and Mn = 31.28 ppm) concentrations in leaves after 180 days in the treatments when soil was

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inoculated with all three *Glomus* species. The seedlings uptake of macro and micronutrient was also found highest when three *Glomus* species were inoculated artificially and simultaneously in soil. Inoculation of the AM fungi improved soil fertility, nutrient content and uptake in *Melia azedarach*.

Keywords: AMF; G. mosseae; G. intradices; G. fasciculatum; macro and micro nutrient.

1. INTRODUCTION

India is the seventh-largest country globally, with a total area of 7, 67,419 square km under the tree. The country's total forest area is 24.62 per cent of its geographical area comprising 21.71 per cent of the forest cover and 2.91 per cent tree cover and most growing states are Madhya Pradesh, Arunachal Pradesh, Maharashtra, Orrisa, Chhattisgarh and Haryana. In Haryana, forests cover shares only 3.63 per cent of the total geographical area [1]. *Melia azedarach* (family Meliaceae) is a moderate deciduous tree with a short bole and a spreading crown, bi-pinnate or tri-pinnate leaves and a dark grey bark with shallow longitudinal furrows [2]. This plant has a reasonably coarse root system with very few root hairs, which are common characteristics of plants that are responsive to arbuscular mycorrhizal symbiosis. The arbuscular mycorrhizal fungi (AMF) are soil microorganisms having extensive extraradical mycelia which increases phosphorus and water absorption [3]. These fungi provide their host with various benefits including higher phosphorus absorption (Gossus and Mohammad, 2009), defending root against diseases [4], plant growth-promoting hormone [5] and boosting plant growth and productivity [6]. The mycorrhizal fungi are naturally occurring species that form symbiotic relationships with almost all plants inside the roots. This also provides a perfect ecological niche that is necessary for fungal growth and development including the completion of the sexual cycle [7]. The arbuscules are branched hyphae, within the root cells and this is a connection for nutrient exchange between fungi and the host plant [8]. Fungus improves nutrition, growth, drought resistance and provides nutrients and water to the host plant [9]. Besides the direct effects of AM fungi on the cycling of nutrients and plant performance, there are several indirect effects of AMF for improving soil fertility via enmeshment of soil particles for better soil aggregation ([10] and nutrient maintenance [11]. Therefore, farmers' field management through a greater abundance of AM fungi could have improved nutrient accessibility and availability [12]. However, sustaining AM fungi especially mycelia, needs a supply of plentiful carbohydrates from host plants [13]. In Haryana, agroforestry is distributed throughout arid and semi-arid regions [14], which could act as perennial AM fungi hosts and thus satisfy AM fungi continuous carbohydrate demand [15]. Some trees are exhaustive for nutrients but not all so, it depends on tree species for their effect on soil nutrient status, and differences in their relative availability which may further affect the AM fungi symbiosis relationship functioning [16] and AM fungi growth strategies [17]. Furthermore, it is vital to contemplate the overall effect of tree species with AM fungi inoculation on nutrients trade-off ultimately affecting soil fertility status, tree growth, and biomass production, including potential trade-offs regarding competition for light, water, and nutrients [18,19]. Globally, it remains an open question whether trees grown with AM fungi can rejuvenate and preserve soil fertility and, thus, boost crop performance. However, few studies have been made to isolate and select effective AM fungi for inoculation in *Melia azedarach* in nursery conditions*.* Keeping in view of all these factors the present study was planned to find out the effect of different AM fungi (*Glomus sp.*) on the growth and nutrient uptake of *Melia azedarach* seedling.

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 types were Typic Ustochrept with sandy loam texture. 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 48^0C on hot summer days. The relative humidity varies from 5 to 100 per cent, while temperature below freezing point accompanied by frost in winter is usually experienced in this region. The

characteristics of mother plant i.e. plant height; DBH and clear bole height were 9.1 m, 0.4 m and 4.2 m, respectively. An average of 100 drupes were used for the experiment and traits such as fruit length (15 mm), fruit breadth (10 mm), fruit weight (0.32 g), fruit thickness (1.7 mm) and test weight (24 g) were recorded. The soil and rootlets from the root horizon of *Glomus mosseae*, *Glomus fasciculatum* and *Glomus intraradices* inoculated wheat plants were used to inoculate soil before sowing of drupes of *Melia azedarach* in seven treatments [20]. The experimental soil was collected from Balsamand Research Farm, CCS HAU, Hisar. The soil was drenched with formaldehyde, and immediately covered with an air-tight polythene sheet for 5 days. The drupes of *Melia azedarach* were collected from plus tree at Hisar and were sown at about 2-3 cm deep in polybags of one kg soil capacity inoculated with different *Glomus* species as per detail given below. This experiment was carried out with completely randomized design and each treatment having three replications.

There were seven treatments of soil inoculation of three different *Glomus* sp. inoculated singly as well as in combination before sowing and control (uninoculated AM fungi) as shown below:-

T_1 - Treated soil +*G. mosseae* (450-500 sporocarps kg^{-1} soil)
T_2 - Treated soil +*G. intraradices* (450-500 sporocarps kg^{-1} soil)
T_3 - Treated soil +*G. fasciculatum* (450-500 sporocarps kg^{-1} soil)
T_4 - Treated soil + *G. mosseae* + *G. intraradices* (225-250 sporocarps of each AM fungi kg^{-1} soil)
T_5 - Treated soil +*G. mosseae* + *G. Fasciculatum* (225-250 sporocarps of each AM fungi kg^{-1} soil)
T_6 - Treated soil +*G. intraradices* + *G. Fasciculatum* (225-250 sporocarps of each AM fungi kg^{-1} soil)
T_7 - Treated soil +*G. mosseae* + *G. intraradices* + *G. fasciculatum* (150-165 sporocarps of each AM fungi kg^{-1} soil)
T_8 - Control (un-inoculated)

The soil samples were collected before and after harvesting of *M. azedarach* seedlings. These samples were air-dried and passed through a 2 mm sieve before determining macro and micronutrients [21]. The alkaline permanganate method described by Subbiah and Asija [22] was used to determine available nitrogen in soil. The available phosphorus was determined spectrophotometrically by Olsen's method [23] and blue colour intensity was measured at 660 nm wavelength using a red filter. Neutral normal NH_4OAC solution was used for estimation of available Potassium on flame photometer [24]. DTPA-TEA buffer (0.005 M DTPA + 0.001 M $CaCl_2$ + 0.1 M TEA, pH 7.3) as proposed by Lindsay and Norvell [25] was used for the determination of available micronutrients (Fe, Cu, Zn and Mn) concentration using atomic absorption spectrophotometer. The plant leaf samples were ground and digested with the di-acid mixture (nitric acid and perchloric acid). The colorimetric method proposed by Lindner [26] was used to estimate total nitrogen in the plant. Blue filter at 440 nm wavelength was used to read the intensity of orange colour on spectrophotometer. In the plant sample, total phosphorus was determined by Vanado-molybdophosphoric yellow colour method proposed by Koenig and Johnson [27]. The potassium was determined by a Flame photometer using the standard curve [28]. The micronutrient concentration in aliquot was determined spectrophotometrically at most sensitive wavelengths (Fe-248.7 nm, Zn-213.7 nm, Cu-324.6 nm and Mn-279.5 nm), Elwell and Gridley, [29].

Macronutrient uptake (g $plant^{-1}$) = [Macronutrient content in plant (%) × total dry weight of corresponding plant (g)] / 100

Micronutrient uptake (mg $plant^{-1}$) = [Micronutrient content in plant (ppm) × total dry weight of corresponding plant (g)] / 10^3]

Hyphae contribution (HC) was calculated using the following formula [30]:

> **HC =** (X uptake of the mycorrhizal plants - X uptake of the non-mycorrhizal plants) / (X uptake of the mycorrhizal plants × 100)

Where, X= respective nutrient (P/Zn)

3. RESULTS AND DISCUSSION

The data presented in Fig. 1 showed that available N, P and K contents were found maximum 119.86, 16.91 and 260.86 kg ha^{-1}, respectively in treatment T_7 followed by treatment (T_5) when *G. mosseae* and *G. fasciculatum* were applied in soil together i.e. 118.99, 15.81 and 258.87 kg ha^{-1}, respectively after harvest of seedlings. However, the nutrient content of N and K were found non-significant, when drupes were inoculated individually with *G. mosseae*

(T_1), *G. intraradices* (T_2) and *G. fasciculatum* (T_3). The phosphorus was also non-significant when drupes were inoculated singly with *G. mosseae* (T_1) and *G. fasciculatum* (T_3). The maximum Fe, Cu, Zn and Mn contents were recorded in T_7 (5.95, 0.42, 0.66 and 1.63 ppm, respectively) followed by treatment of *G. mosseae* + *G. fasciculatum* (5.66, 0.39, 0.63 and 1.58 ppm, respectively) and minimum in control (un-inoculated). The iron (Fe) and manganese (Mn) were found non-significant after harvest in soils treated with *G. mosseae* (T_1), *G. intraradices* (T_2) and *G. fasciculatum* (T_3) whereas, *G. mosseae* and *G. fasciculatum* treatment were found non-significant for Cu and Zn content in the soil after harvest of *Melia azedarach* seedlings (Fig. 2). There was significantly higher N, P and K (%) content in six months old seedlings in T_7 (1.39, 0.27 and 0.63 %, respectively) and minimum in control (T_8) with 1.27, 0.10 and 0.51 %, respectively (Table 1). However, the nutrient content of N and K (%) was found non-significant in seedlings after harvest in treatments of *G. mosseae, G. intraradices* and *G. fasciculatum* whereas, *G. mosseae* and *G. fasciculatum* treatments were non-significant with each other for P (%) content. Among all the treatments, Fe, Cu, Zn and Mn (ppm) contents were recorded maximum in T_7 (90.43, 9.96, 32.24 and 31.28 ppm, respectively) whereas, minimum in control (77.79, 5.58, 15.45 and 25.12 ppm, respectively). However, in treatments with *G. mosseae, G. intraradices* and *G. fasciculatum* the content of Fe and Mn (85.56, 85.23, 85.43 ppm and 27.61, 27.45, 27.52 ppm, respectively) were found non-significant in seedlings after harvest, whereas, Cu and Zn content (7.15, 7.11 ppm and 21.87, 21.71 ppm, respectively) were found non-significant in seedlings of *M. azedarach* after harvest in *G. mosseae* and *G. fasciculatum* treatment. Basumatary et al. [31] reported a significant higher nutrient concentration of N, P, K and Carbon in soil when the soil was infested with *Acaulospora* sp. and *Glomous* sp. over control. The mycorrhizae play an important role and helps in nutrient cycling to establish plants [32]. It helps in better availability of nutrients to the plants mainly N and K with AM fungi inoculation in star fruits [33].

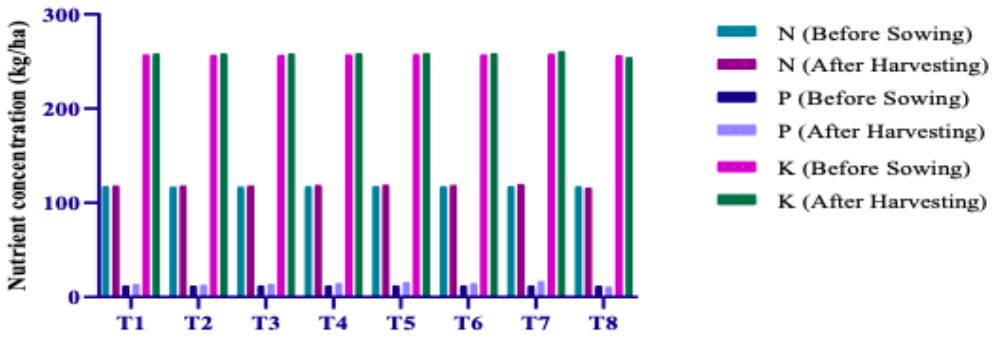

Fig. 1. Available nitrogen, phosphorus and potassium in soil before sowing and after harvest of *M. azedarach* seedlings

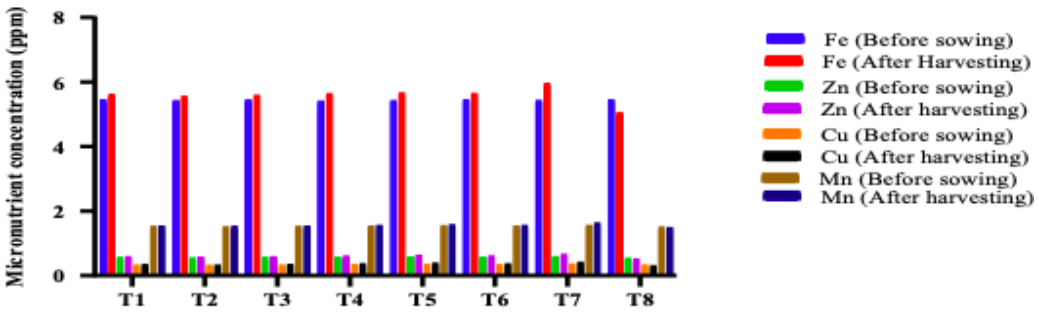

Fig. 2. Soil DTPA extractable Fe, Zn, Cu and Mn content before sowing and after harvest of *Melia azedarach* seedlings

Table 1. Macro and micronutrient content in *Melia azedarach* seedlings after 180 days of sowing

Treatments		Nutrients content						
		N (%)	P (%)	K (%)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
T_1	*Glomus mosseae*	1.31	0.17	0.55	85.56	7.15	21.87	27.61
T_2	*G. intraradices*	1.30	0.14	0.54	85.23	6.45	18.67	27.45
T_3	*G. fasciculatum*	1.31	0.17	0.54	85.43	7.11	21.71	27.52
T_4	*G. mosseae + G. intraradices*	1.34	0.20	0.58	86.81	7.82	24.97	28.83
T_5	*G. mosseae +G. fasciculatum*	1.35	0.23	0.59	87.02	8.77	27.91	28.99
T_6	*G. intraradices + G. fasciculatum*	1.34	0.20	0.58	86.27	7.78	24.91	28.76
T_7	*G. mosseae + G. intraradices + G. fasciculatum*	1.39	0.27	0.63	90.43	9.96	32.24	31.28
T_8	Control (un-inoculated)	1.27	0.10	0.51	77.79	5.58	15.45	25.12
CD at 5%		0.02	0.02	0.02	2.13	0.46	2.25	1.45

Table 2. Macro and micronutrient uptake in *Melia azedarach* after 180 days of sowing

Treatments		Nutrients uptake						
		g/plant			mg/plant			
		N	P	K	Fe	Cu	Zn	Mn
T_1	*G. mosseae*	0.26	0.04	0.10	1.70	0.14	0.43	0.55
T_2	*G. intraradices*	0.23	0.02	0.09	1.52	0.12	0.33	0.49
T_3	*G. fasciculatum*	0.25	0.04	0.10	1.64	0.14	0.42	0.54
T_4	*G. mosseae + G. intraradices*	0.30	0.06	0.13	1.91	0.17	0.55	0.60
T_5	*G. mosseae +G. fasciculatum*	0.33	0.08	0.16	2.01	0.22	0.69	0.67
T_6	*G. intraradices + G. fasciculatum*	0.29	0.06	0.13	1.88	0.17	0.54	0.63
T_7	*G. mosseae + G. intraradices + G. fasciculatum*	0.37	0.11	0.19	2.41	0.27	0.86	0.83
T_8	Control (un-inoculated)	0.19	0.01	0.08	1.22	0.09	0.24	0.39
CD at 5%		0.03	0.01	0.02	0.21	0.01	0.05	0.07

Table 3. Hyphal contribution (%) of AM fungi for Phosphorus and Zinc

Treatments		180 DAS	
		HC (%) for P	HC (%) for Zn
T_1	*G. mosseae*	75.0	44.18
T_2	*G. intraradices*	50.0	27.27
T_3	*G. fasciculatum*	70.0	42.85
T_4	*G. mosseae + G. intraradices*	83.3	56.36
T_5	*G. mosseae + G. fasciculatum*	87.5	65.21
T_6	*G. intraradices + G. fasciculatum*	83.3	55.55
T_7	*G. mosseae + G. intraradices + G. fasciculatum*	90.9	72.09
T_8	Control (un-inoculated)	00.0	0

A significantly higher N, P and K uptake was recorded in six months old seedlings in T_7 (0.370, 0.11 and 0.19 (g plant^{-1}), respectively and minimum in control with 0.19, 0.01 and 0.08 (g plant^{-1}), respectively (Table 2). Caglar and Akgun [34] reported that uptake of nitrogen in the leaf of *Pistacia* seedlings was enhanced upon inoculation with *Glomus* species. Among all the treatments Fe, Cu, Zn and Mn uptake were recorded maximum in interactive treatments of all species (T_7) (2.41, 0.27, 0.86 and 0.83 mg plant^{-1}, respectively) whereas, minimum was recorded

in control (1.22, 0.09, 0.24 and 0.39 mg plant^{-1}, respectively). These result are in corroboration with the findings of many researchers *viz.,* Jamaluddin and Shukla [35], Muthukumar et al*.* [36], Mohan and Sandeep [37] and Ortiz et al*.* [38] who reported an increase in nutrient uptake in different plant species with the application of different AM fungi species. The maximum Hyphal contribution (%) for P and Zn was found in T_7 followed by T_5 while under control there was no HC recorded (Table 3).

4. CONCLUSION

The drupes of *M. azedarach* treated concomitantly with *G. mosseae, G. intraradices, G. fasciculatum* enhanced the soil nutrients especially P and Zn. The nutrients content in *M. azedarach* seedlings such as N (1.39 %), P (0.27 %), K (0.63 %), Fe (90.43 ppm), Cu (9.96 ppm), Zn (32.24 ppm) and Mn (31.28 ppm) were also found significantly higher in drupes sown in soil concomitantly treated with *G. mosseae, G. intraradices* and *G. fasciculatum.* The macro and micronutrient uptake was also found significantly higher in drupes sown in soil treated with all three *Glomus* species as cited above*.* Thus, it is concluded that the concomitant application of all three *Glomus* species have the potential to improve soil fertility, nutrient content and uptake of *M. azedarach.*

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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