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EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGAL INOCULATION ON SOIL NUTRIENT UPTAKE IN *Tecomella undulata*

Monika Jangra[1*], Virender Dalal[1], Karan Singh Ahlawat[1], K.K Bhardwaj[2], Mamta Khaiper[3], Sunitha Fogat[4] and Anu Sharma[5]

[1]Department of Forestry, CCS Haryana Agricultural University, Hisar 125004, India.
[2]Department of Soil Science, CCS Haryana Agricultural University, Hisar 125004, India.
[3]Department of Forestry, Dr. Rajendra Prasad Central Agricultural University, Samastipur - 848125, India
[4]Department of Agriculture, GDC Memorial College, Bahal, 127028, India
[5]School of Agriculture, Sterling Global University, Hisar 125004, India.
*Corresponding author Email: monikajb247@gmail.com
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ABSTRACT

Tecomella undulata (Rohida) is a valuable native tree species of the arid and semi-arid regions of north-western India, playing an important role in ecological stability and traditional forestry systems. Although arbuscular mycorrhizal fungi (AMF) are well known for enhancing plant nutrient uptake in low-fertility soils, their role in improving nutrient acquisition and soil properties in *T. undulata* has received limited attention. This study evaluated the effect of four AMF species-*Glomus intraradices*, *G. mosseae*, *G. fasiculatum*, and *G. hoi*-on macro- and micronutrient uptake in T. undulata seedlings raised under nursery conditions at CCS HAU, Hisar, during 2021-22 and 2022-23. AMF inoculation significantly enhanced nutrient concentrations in seedlings compared with uninoculated controls. Among the treatments, *G. fasiculatum* consistently produced the highest levels of nitrogen (3.88–3.67%), phosphorus (0.46-0.40%), and potassium (0.58-0.52%) at 180 days after sowing. Micronutrient uptake was also notably improved, with *G. fasiculatum* recording maximum iron (35.63-33.67 ppm), zinc (1.88-1.80 ppm), copper (0.31-0.29 ppm), and manganese (1.28-1.23 ppm) concentrations over both years. Soil fertility showed parallel improvements, as *G. fasiculatum* increased available soil N (115.99 kg/ha), P (10.91 kg/ha), and K (219.56 kg/ha), along with higher DTPA-extractable micronutrients. Overall, *G. fasiculatum* proved most effective in enhancing plant nutrition and soil nutrient availability, highlighting its potential for improving nursery performance and supporting arid-zone afforestation of *T. undulata*.

Key words: Arbuscular mycorrhizal fungi, *G. fasiculatum*, Nitrogen, Micronutrients, *T. undulata*, Treatments

Introduction

Tecomella undulata (Rohida), a member of the family Bignoniaceae, is a characteristic tree species of arid ecosystems, with its natural distribution largely confined to the western parts of Rajasthan, while its occurrence in other Indian states is scattered and relatively rare. As the state flower of Rajasthan, *T. undulata* is naturally distributed across districts such as Barmer, Jaisalmer, Jodhpur, Pali, Ajmer, Nagaur, Bikaner, Churu, and Sikar (Nadkarni, 2000). Vesicular arbuscular mycorrhizal (VAM) associations provide substantial benefits to host plants by improving the uptake of phosphorus and other soil-borne nutrients through well-developed fungal hyphal networks (Harrier and Watson, 2003). Although numerous arbuscular mycorrhizal fungi (AMF) species have been reported worldwide, commonly studied model species include *Funneliformis mosseae* (formerly *Glomus mosseae*), *Gigaspora rosea*, *G. margarita*, *G. gigantea*, and Rhizophagus *irregularis* (previously *Glomus intraradices* and *G. irregulare*)

(Schüßler and Walker, 2011). AMF are known to establish symbiotic associations with most terrestrial plant species, enhancing nutrient absorption and water availability, particularly under stress-prone environments (Raghuwanshi and Upadhyay, 2014; Parihar *et al.*,2020). In arid regions, AMF also play an important ecological role by stabilizing soil aggregates, reducing wind erosion, and aiding the fixation of moving sand dunes (Kumawat *et al.*, 2012). Being obligate symbionts, AMF depend on host plants for survival while significantly improving plant growth by enhancing the uptake of essential nutrients such as nitrogen, phosphorus, and potassium through efficient mycelial networks (Anderson *et al.*, 2018). Additionally, AMF improve soil properties by enhancing aggregation, nutrient availability, microbial activity, water retention, and biogeochemical cycling of nitrogen, carbon, and phosphorus, while also moderating soil acidity (Parihar *et al.*, 2020; Lambers *et al.*, 2008). The Macronutrients like nitrogen (N), phosphorus (P) and (K) potassium are essential elements for crops growth and development (Rashid *et al.*, 2016; Razaq *et al.*, 2017).

Mycorrhizal symbiosis also facilitates the uptake of relatively immobile micronutrients such as potassium, calcium, magnesium, copper, zinc, iron, manganese, and cobalt (Garcia *et al.*, 2016; Hashem *et al.*, 2018). Under dryland conditions, co-inoculation of AMF with beneficial microbes such as Rhizobium has been shown to improve nutrient acquisition, soil enzyme activity, and microbial diversity (Calderon and Dangi, 2024). Meta-analytical studies further confirm that AMF significantly enhance biomass production, root development, and uptake of macro- and micronutrients under drought stress (Chandrasekaran, 2022). Hence, successful establishment of Rohida in arid and semi-arid regions requires appropriate pre-planting mycorrhizal inoculation to enhance nutrient solubilization, absorption, and overall plant performance (Ahmad and Ayub, 2003; Krishna and Bagyaraj, 1984). Nonetheless, few studies have been done to isolate and select effective AM fungi for inoculation in *T. undulata* under nursery conditions. Keeping in view of all the present study was planned to find out the effect of different AM fungi (*Glomus sp.*) on the nutrient uptake of *T. undulata* seedling.

Materials and Methods

The study was conducted during 2021 at the Forestry Nursery of CCS Haryana Agricultural University, Hisar. Soil inoculation with four *Glomus* species was carried out before sowing, along with a non-inoculated control treatment, as detailed below:

T_1 - Soil inoculated with *Glomus intraradices*

T_2 - Soil inoculated with *Glomus mosseae*

T_3 - Soil inoculated with *Glomus fasiculatum*

T_4 - Soil inoculated with *Glomus hoi*

T_5- Control (un-inoculated)

Inoculum of Fungi

Pure cultures of *Glomus intraradices*, *G. mosseae*, *G. hoi*, and *G. fasiculatum* were procured from the Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, and subsequently mass-multiplied on wheat (*Triticum aestivum*) and pearl millet (*Pennisetum typhoides*) under screen-house conditions. Seedlings of *Tecomella undulata* were inoculated using the soil-root mixture collected from the rhizosphere of wheat plants previously inoculated with *G. mosseae*, *G. fasiculatum*, and *G. intraradices*. During July, Rohida seeds were sown at a depth of 2–3 cm in sterilized sandy soil and each seedling was inoculated individually with *G. intraradices*, *G. mosseae*, *or G. fasiculatum* at a concentration of 450–500 sporocarps per kilogram of soil in 22.5 × 12.5 cm polybags. An uninoculated treatment was also maintained to serve as a control.

Plant and Soil Analysis

The soil samples were collected before and after harvesting of *Tecomella undulata* seedlings. These samples were air-dried and passed through 2 mm sieve before determining macro and micronutrients.

Macro-nutrients in Plant Analysis

Analysis of Nitrogen - Total N in plant samples was determined using the colorimetric Nessler's method as outlined by Lindner (1944).

Analysis of *Phosphorus*- Plant P content was analysed with the vanado-molybdate yellow colour method (Koenig & Johnson, 1942).

Analysis of Potassium- Potassium in acid-digested plant extracts was measured using a flame photometer, and K concentration was quantified using a standard curve (Jackson, 1973).

Micro-nutrients in Plant (Fe, Cu, Zn, Mn) - Micronutrient concentrations (Fe, Cu, Zn, Mn) in plant digests were quantified by Atomic Absorption Spectrophotometry (AAS) following Elwell and Gridley (1967).

Macro-nutrients in Soil (N, P, K) Analysis–

Available Nitrogen - Soil available N was estimated by the alkaline $KMnO_4$ distillation method proposed by Subbiah and Asija (1956).

Available *Phosphorus*- Available P was extracted using 0.5 M $NaHCO_3$ (Olsen method) and quantified

Table 1: Nitrogen, Phosphorus and Potassium content (%) in plant.

Treatment	180 DAS 2021-22			180 DAS 2022-23		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
Glomus intraradices	3.63	0.45	0.55	3.57	0.37	0.50
Glomus mosseae	2.97	0.42	0.50	2.21	0.30	0.43
Glomus fasiculatum	3.88	0.46	0.58	3.67	0.40	0.52
Glomus hoi	3.12	0.44	0.52	2.82	0.33	0.47
Control (uninoculated)	2.71	0.40	0.49	2.00	0.28	0.40
CD at 5%	0.29	0.03	0.04	0.25	0.02	0.03

calorimetrically at 660 nm (Olsen *et al*., 1954).

Available Potassium- Exchangeable K was extracted with neutral normal NH_4OAc and determined using a flame photometer (Hanway & Heidel, 1952).

Micro-nutrients in Soil (Fe, Cu, Zn, Mn)

Available micronutrients (Fe, Cu, Zn, Mn) were extracted with DTPA solution (Lindsay & Norvell, 1978). Transfer 10 g of soil in a 125 ml polyethylene bottle and add 20 ml of DTPA extracting solution and close the mouth with stopper. Shake continuously for 2 hrs on a shaker and filter through Whatman No.42 filter paper. Determine the content of these elements (Zn, Mn, Cu and Fe) in the DTPA extract of the soil by Atomic Absorption Spectrophotometer using respective cathode lamps.

Statistical Analysis

The experimental data were statistically analysed using OPSTAT software developed by CCS Haryana Agricultural University, Hisar. The experiment was laid out in a Completely Randomized Design (CRD) and all the values have been presented as the mean of five replicates. Analysis of variance (ANOVA) were performed to determine the significance of treatment effects. The significance of differences among treatment means was tested using the F-test, and comparisons were made at the 5% level of significance ($P \leq 0.05$). When the treatment effects were found significant, the means were separated using the Critical Difference (CD) test at the same probability level. The critical difference (CD) values were calculated to assess the variability and precision of the experimental data.

Results

Effect of AM fungi on plant and soil nutrients

Nitrogen, Phosphorus and Potassium content (%) in plant:

The N, P and K content in plant at 180 DAS were higher in mycorrhizal treatments as compared to control are shown in Table 1. The symbiotic association of mycorrhizal fungi improve mineral nutrient acquirement by the plant, in N, P and K. This resulted in significantly higher N, P and K content in treatment T_3 *G. fasiculatum* -inoculated seedlings at 180 DAS N (3.88%, 3.67%), P (0.46%, 0.40%) and K (0.58% , 0.52% respectively) contents during both the years (2021-22 and 2022-23) as compare to control with N (2.71%, 2.00%), P (0.40% and 0.28%) and K (0.49% and 0.40%) at 180 days after sowing. On the other hand, plants inoculated with *G. intraradices* also showed outstanding enhance in nutrient uptake, although this was comparatively less than in *G. fasiculatum* inoculated plants.

Available Fe, Cu, Zn and Mn contents (ppm) in plants:

The concentrations of Fe, Cu, Zn and Mn in *Tecomella undulata* (rohida) plants measured at 180 days after sowing during the years 2021-22 and 2022-23 under different arbuscular mycorrhiza (AM) fungi treatments are presented in Table 2. During both years, inoculation with *Glomus fasiculatum* consistently resulted in the highest micronutrient contents among all treatments. In 2021–22, plants inoculated with *G. fasiculatum* recorded the maximum Fe (35.63 ppm) and Cu (0.31 ppm) and Zn (1.88 ppm) and Mn (1.28 ppm) compared to other AM fungi. Similarly, in 2022-23, the highest Fe (33.67 ppm), Cu (0.29 ppm), Zn (1.80 ppm) and Mn (1.23 ppm) was found in *G. fasiculatum* as compare to control. Other AM fungi, such as *Glomus intraradices*, *G. mosseae* and *G. hoi*, improved micronutrient uptake relative to the control but to a lesser extent than *G. fasiculatum*. The control treatment consistently exhibited the lowest Fe, Cu, Zn and Mn concentrations in both years, indicating

Table 2: Micronutrient content in Tecomella undulata plant at 180 days after sowing.

Treatments	2021-22				2022-23			
	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
T_1 *Glomus intraradices*	35.12	0.30	1.76	1.13	32.39	0.28	1.73	1.10
T_2 *Glomus mosseae*	34.26	0.28	1.45	0.85	30.21	0.24	1.42	0.81
T_3 *Glomus fasiculatum*	35.63	0.31	1.88	1.28	33.67	0.29	1.80	1.23
T_4 *Glomus hoi*	34.89	0.29	1.63	0.89	32.03	0.26	1.58	0.86
T_5 Control (un-inoculated)	33.88	0.26	1.24	0.81	29.15	0.23	1.21	0.77
CD at 5%	N. S	N. S	0.14	0.09	2.80	0.03	0.13	0.08

Table 3: Available nitrogen, phosphorus, and potassium content (kg/ha) before sowing and after harvest of seedlings.

Treatments	Before sowing (kg/ha)			After harvest (kg/ha)		
	N	P	K	N	P	K
T_1 *Glomus intraradices*	113.47	10.12	217.89	114.86	10.39	218.97
T_2 *Glomus mosseae*	113.00	9.33	217.33	114.39	9.67	218.74
T_3 *Glomus fasiculatum*	114.56	10.80	218.78	115.99	10.91	219.56
T_4 *Glomus hoi*	113.11	9.86	217.67	114.76	9.93	218.81
T_5 Control (un-inoculated)	112.97	8.93	217.06	113.12	9.00	218.33
CD at 5%	N. S	0.87	N. S	N. S	0.89	N. S

the beneficial role of AM fungi in enhancing micronutrient acquisition. During 2021-22, Fe and Cu were not significantly influenced (N.S.) by AM fungal inoculation, whereas Zn and Mn showed significant differences between treatments. In 2022-23, all four micronutrients exhibited significant variation among treatments, with *G. fasiculatum* outperforming the other fungi.

Available N, P and K contents (kg/ha) in soil:

The response of soil macronutrient availability (N, P, K) to different species of arbuscular mycorrhizal fungi (AMF) inoculated in rohida seedlings are demonstrated in Table 3. Across all AMF treatments, a marginal improvement in available N, P, and K after harvest was observed when compared to the pre-sowing status. This trend is consistent with earlier evidence that AMF enhance nutrient turnover by increasing root surface area and stimulating microbial mineralization processes in the rhizosphere. Among the evaluated fungi, *Glomus fasiculatum* (T_3) recorded the highest post-harvest nutrient levels (N 115.99 kg/ha, P 10.91 kg/ha, K 219.56 kg/ha). This reflects the species' superior colonization efficiency and its well-documented role in mobilizing both nitrogen and phosphorus through improved enzymatic activity and hyphal extension. Other inoculated treatments (T_1, T_2, T_4) also exhibited slight increases relative to the uninoculated control (T_5), confirming the positive influence of AMF on enhancing nutrient uptake and release. Phosphorus showed statistically significant variation whereas nitrogen and potassium remained non-significant. This aligns with typical AMF-mediated responses, since P is the nutrient most strongly influenced by mycorrhizal symbiosis due to its low mobility in soil. The improvement in nutrient levels even after harvest suggests better nutrient cycling and reduced depletion of soil fertility under AMF-assisted systems. Overall, the table indicates that AMF inoculation particularly *Glomus fasiculatum*- supports improved soil nutrient availability in rohida cultivation, thereby sustaining soil fertility and promoting more efficient nutrient use.

DTPA extractable micronutrients (Zn, Fe, Mn, and Cu) content in soil:

The micronutrient concentrations in response to inoculation with different AMF species are illustrated in Table 4 and Fig. 3. The data show a consistent increase in Fe, Cu, Zn, and Mn contents at harvest compared to pre-sowing levels across all inoculated treatments. This reflects the fundamental role of AMF hyphae in enhancing micronutrient mobilization, particularly for elements with limited mobility such as Fe and Zn. Among the treatments, *Glomus fasiculatum* (T_3) found the highest concentrations of all measured micronutrients after harvest (Fe- 4.67 ppm, Cu- 0.95 ppm, Zn-0.91 ppm, Mn-10.76 ppm). The superior performance indicates that this species establishes a more effective symbiotic interface capable of improving root absorption efficiency and activating biochemical pathways involved in micronutrient transport. Compared with the uninoculated control (T_5), increases of 10-25% in micronutrient levels were recorded in AMF-treated seedlings. This improvement is consistent with the recognized ability of AMF to solubilize and mobilize bound micronutrient complexes through the secretion of organic acids, siderophores, and enhanced

Table 4: Effect of different arbuscular mycorrhiza fungi on DTPA extractable micronutrients (Zn, Fe, Mn, and Cu) content in leaves of rohida before sowing and after harvest.

Treatments	Before sowing (kg/ha)				After harvest (kg/ha)			
	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
T_1 *Glomus intraradices*	4.06	0.76	0.81	9.89	4.33	0.83	0.86	10.54
T_2 *Glomus mosseae*	34.26	0.28	1.45	0.85	30.21	0.24	1.42	0.81
T_3 *Glomus fasiculatum*	3.78	0.69	0.74	9.12	3.92	0.72	0.79	9.89
T_4 *Glomus hoi*	4.00	0.71	0.78	9.68	4.18	0.75	0.82	10.41
T_5 Control (un-inoculated)	3.66	0.62	0.71	9.03	3.80	0.66	0.73	9.24
CD at 5%	0.35	0.06	0.07	0.85	0.38	0.07	0.09	0.93

rhizosphere enzymatic activity. The observed differences were statistically significant for all micronutrients for both pre-sowing and post-harvest stages.

Discussion

The nutrient status of Rohida seedlings was assessed six months after sowing under different treatments during the experimental years 2021–22 and 2022–23. Macronutrient analysis revealed that nitrogen, phosphorus, and potassium contents at 180 days after sowing were higher in AMF-inoculated treatments. The maximum values of N (3.88 and 3.67%), P (0.46 and 0.40%), and K (0.58 and 0.52%) were recorded in seedlings inoculated with *Glomus fasiculatum* during the first and second year, respectively. With respect to micronutrients, iron and copper concentrations in plant tissues were non-significant during the first year; however, during the second year, Fe and Cu contents were significantly higher, recording values of 33.67 ppm and 0.31 ppm, respectively. Zinc and manganese contents were consistently significant across both years, with maximum values of Zn (1.88 and 1.80 ppm) and Mn (1.28 and 1.23 ppm) observed in treatments receiving *Glomus fasiculatum*, followed by *Glomus intraradices*. Soil nutrient analysis before sowing and after harvest indicated significantly higher nitrogen and potassium levels under *Glomus fasiculatum* treatment, recording N (114.56 and 115.99 kg ha^{-1}) and K (218.78 and 219.56 kg ha^{-1}), followed by *Glomus intraradices* after 180 days of sowing. Available phosphorus content showed non-significant variation between treatments, with values ranging from 10.80 to 10.91 kg ha^{-1}. However, DTPA-extractable micronutrients Fe, Cu, Zn, and Mn were significantly enhanced in *Glomus fasiculatum* -inoculated soils, with respective values of Fe (4.20 and 4.67 ppm), Cu (0.80 and 0.95 ppm), Zn (0.84 and 0.91 ppm), and Mn (10.23 and 10.76 ppm). Similar results have been reported earlier by Chen *et al.*, (2017), who observed enhanced phosphorus and potassium accumulation in plants inoculated with *Glomus mosseae*. Increased nitrogen uptake following inoculation with *Glomus* species was also reported in *Pistacia* seedlings (Caglar and Akgun, 2006). Several studies have documented improved nutrient uptake in diverse plant species following AMF inoculation (Haselwandter and Bowen, 1996; Jamaluddin and Shukla, 2012; Muthukumar *et al.*, 2012; Mohan and Sandeep, 2015; Ortiz *et al.*, 2017). Basumatary *et al*., (2014) observed significantly higher soil N, P, and K contents in soils inoculated with Glomus species, emphasizing the role of AMF in nutrient enrichment. Dhar and Mridha (2012) further highlighted the importance of mycorrhizae in nutrient cycling during plant establishment. Enhanced nutrient availability, particularly nitrogen, potassium, and calcium due to AMF inoculation, was also reported in star fruit by Filho *et al.*, (2017). Positive effects of *Glomus mosseae* and *Glomus fasiculatum* on nutrient content in both plants and soil were documented by Youpensuk *et al.*, (2005).

Lalitha (2015) reported a significant increase in plant biomass and soil nutrient content with dual inoculation of *Glomus fasiculatum* and *Rhizobium*. Khaiper *et al.*, (2021) demonstrated improved seed germination in *Melia azedarach* when seeds were treated with cow dung slurry and grown in *Glomus mosseae*-inoculated soil. Similarly, Gulab *et al.*, (2022) reported significantly higher macro- and micronutrient concentrations in leaves following AMF inoculation. Jamaluddin and Shukla (2012) also confirmed that combined application of AMF and root exudates resulted in maximum colonization, phosphatase activity, phosphorus uptake, and leaf protein content compared to individual treatments. Similarly, Jangra *et al.*, (2024) reported that inoculation with *Glomus fasciculatum* significantly enhanced root colonization, collar diameter, shoot length, biomass and leaf area of *Tecomella undulata* seedlings compared with the uninoculated control.

Conclusion

The study clearly demonstrated that inoculation with arbuscular mycorrhizal fungi (AMF) significantly improved nutrient uptake in *Tecomella undulata* seedlings as well as soil nutrient status. Among the evaluated treatments, *Glomus fasiculatum* (T_3) consistently recorded the highest concentrations of macronutrients (N, P, and K) and micronutrients (Fe, Cu, Zn, and Mn) in plant tissues during both experimental years (2021–22 and 2022–23). In addition, soils receiving *G. fasiculatum* showed notably higher available nitrogen and potassium levels before sowing and after harvest, indicating its strong capacity to mobilize, retain, and recycle nutrients throughout the 180-day growth period. Although available soil phosphorus did not differ significantly among treatments, the overall enhancement of nutrient availability-especially DTPA-extractable micronutrients-clearly reflected the superior performance of *G. fasiculatum* compared with *Glomus intraradices*. The improved nutrient dynamics observed under AMF inoculation can be attributed to enhanced hyphal development, improved rhizosphere activity, and efficient nutrient translocation to the host plant. Overall, the findings confirm that AMF application, particularly with *Glomus fasiculatum*, plays a crucial role in enhancing nutrient acquisition, improving soil fertility, and creating favorable growth conditions for *Tecomella undulata*. Therefore, future studies should explore the strong potential of *G.*

fasiculatum as an eco-friendly biofertilizer, especially for improving plant establishment and productivity in nutrient-deficient arid and semi-arid ecosystems.

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Authors' contributions

MJ participated in analysing results of experiments carried out the field experiment and original draft writing. The conceptualization and supervision of the research was carried out by V.D. and K.K. in finalization data and taught me how to use software. MK and SF helps in data analysis and technical support with laboratory analysis. Plant and Soil analysis done by M.J. M.K. and A.S. helps in writing and review editing. All the authors studied and approved the final manuscript.

Conflict of interest: The authors declare no conflict of interest

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