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Effects of Different Vesicular arbuscular Mycorrhizal (VAM) Fungi on the Seedling Growth of *Tecomella undulata*

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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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Original Research Article

ABSTRACT

Tecomella undulata (Sm.) Seem. (family Bignoniaceae) is a tree found in desert parts of India, and Arabia. The cultivation of high-quality seedlings is vital for establishing a successful plantation and the contribution of arbuscular mycorrhizal fungi (AMF) in the soil plays a vital role in the nursery phase. A study conducted during the periods of 2021-22 and 2022-23 scrutinised the effect of four distinct species of *Glomus* spp. (*G. mosseae, G. intraradices, G. fasciculatum* and *Glomus hoi*) on *Tecomella undulata* seedlings in AMF-inoculated soil. The inoculation involved applying of 400-500 sporocarps/kg of soil during sowing and the evaluation encompassed growth and survival parameters. Results revealed that among the 4 *Glomus* species, soil inoculated with *Glomus*

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fasiculatum at 180 days after sowing, significantly increased the root colonization percentage with (50.69 and 46.71 %) in year (2021-22) and (2022-23) compared to the uninoculated control. Additionally, seedlings exhibited significantly higher no. of spores per 100g of soil, when treated with (*Glomus fasiculatum*) inoculated soil, which was statistically at par with soil inoculated with (*Glomus intraradices*) at (103.67 and 98.65). During both the experimental year, in terms of germination percentage, root length and root: shoot ratio were found non- significantly with (44.45 %), root length at 360 DAS, (26.67 and 25.74) respectively, root shoot ratio at 360 days after sowing. While, plant survival percentage (30.63 and 25.41%) at 90 DAS. Whereas, at 360 DAS, collar diameter (6.14 and 10.07 mm), shoot length (33.36 and 32.19 cm), plant biomass (8.31 and 8.05 g), leaf area (196.29 and 185.46 cm ²) was observed significantly higher in treatment T3 (*Glomus fasiculatum*) which was statistically at par with treatment T1 (*G. intraradices*). It is concluded from study that *Glomus fasiculatum* were found best for growth and survival of seedlings followed by *Glomus intraradices* as compare to control.

Keywords: Seedlings; Glomus fasiculatum; Arbuscular mycorrhizal fungi; G. intraradices.

1. INTRODUCTION

Tecomella undulata (Sm.) Seem., belonging to the Bignoniaceae family, is a tree of significant economic and pharmaceutical importance found in the arid regions. The plant is renowned for its diverse therapeutic effects. includina hepatoprotective, antibacterial, antimicrobial, antifungal and anti-inflammatory activities. Symbiotic microorganisms in the mycorrhiza play a vital role in plant adaptation and survival. Mycorrhizal interactions enhance the overall absorption capacity of plant roots through morphological and physiological modifications. These modifications involve an increase in the absorption surface area, exploration of a larger soil area (as the fungus acts as an extension of the root), prolonged lifespan of absorbing roots, improved utilization of low-availability nutrients, and enhanced retention and storage of soluble nutrients. [1]. It reduces interactions with soil colloids and minimizes leaching losses [2]. AM fungi confer a distinct advantage to their host compared to non-host species by offering nutrients and defending against various pathogenic organisms [3]. Among these, the primary significance lies in nutrient absorption, preventing nutrient leaching, contributing to soil structure, and serving as hyphal highways for the dispersion of bacteria [4]. Mycorrhizal fungi play a role in inhibiting the growth of pathogens. However, the establishment of mycorrhizal associations is influenced by various factors such as soil moisture, soil pH, organic carbon content, soil phosphorus, and soil nitrogen. These factors affect both root colonization by mycorrhizal fungi and the density of spores. Additionally, seasonal variations impact mycorrhizal associations, while the use of modern pesticides and foliar reduce the incidence fungicides can of

mycorrhizal presence. The natural occurrence of (vesicular-arbuscular VAM mvcorrhizal) association in desert plants is crucial in arid zone forestry. These associations play a vital role by offering not only additional mineral support to the plants but also aiding in their survival under challenging climatic conditions. Prasad and Mertia [5], proposed that the presence of VAM fungi associated with tree roots could serve as criteria for selecting agroforestry trees in arid ecosvstems. Limited research has been conducted on the application of arbuscular mycorrhizal fungi (AMF) in cultivating seedlings of Rohida. Consequently, the current study was designed with the aim of identifying a suitable AMF that can be artificially introduced into the soil prior to sowing.

2. MATERIALS AND METHODS

The investigation carried out in the nursery of the Forestry Department, CCS Haryana Agricultural University, located in Hisar (latitude 29.10° N, longitude 75.46° E, altitude 215 meters above sea level) in the semi-arid region of North-Western India. The soil employed in the experiment was of sandy loam composition. Hisar, situated in Haryana, experiences a semiarid climate characterized by intense heat and dry, desiccating winds, often accompanied by high-velocity dust storms in the summer. Pure cultures of four different AMF species, namely Glomus intraradices. Glomus mosseae. Glomus fasciculatum and Glomus hoi with control. The samples of four different AM fungi of Glomus spp. were obtained from the Department of Plant Pathology, CCS Harvana Agricultural University, Hisar. These samples were then multiplied on pearl millet (Pennisetum typhoides) and wheat (Triticum aestivum) under controlled conditions

within a screen house. The soil and rootlets from the root horizon of plants inoculated with G. intraradices, G. mosseae, G. fasciculatum and Glomus hoi were utilized in various treatments before sowing the seeds, as outlined below. The seeds were planted at a depth of 2-3 cm in sterile sandy soil individually inoculated with G intraradices, G. mosseae, G. fasciculatum and Glomus hoi at a rate of 450-500 sporocarps/kg of soil. This was carried out in polythene bags measuring 22.5 x 12.5 cm in the months of august. A control was also included by sowing seeds in uninoculated soil. Each treatment being replicated five times. Fifteen seedlings per replication were raised and maintained.

Growth parameters of the seedlings, mycorrhizal colonization in roots, and sporocarp numbers in the soil were assessed at 90 and 180 days after sowing (DAS). Mycorrhizal colonization was determined using the method outlined by Phillips and Hayman [6] and sporocarps were quantified following the procedure of Gerdeman and Nicolson [7]. The study was conducted at the Forestry Nursery of CCS, Haryana Agricultural University, Hisar, during the experimental year, (2021-22 and 2022-23). The treatments for soil inoculation with *Glomus spp.* before sowing and the control (uninoculated) AMF were as follows:

- T1: Glomus intraradices
- T2: Glomus mosseae
- T3: Glomus fasciculatum
- T4: Glomus hoi
- T5: Control (uninoculated)

2.1 Statistical Analysis

Growth parameters and analytical data obtained in the study were subjected to statistical analysis to determine the effects due to treatments. The tool used for statistical analysis were OPSTAT software. This experiment was carried out with completely randomized design and each treatment having five replication.

3. RESULTS AND DISCUSSION

3.1 Effect of Different AMF on Seed Germination and Seedling Growth Parameters

The information provided in Tables 1 to 7. discloses the growth parameters, including plant survival %, collar diameter (mm), root length (cm), shoot length (cm), plant biomass (g), leaf area (cm 2)/ plant and root : shoot ratio for *Tecomella undulata* seedlings at 90, 180, 270,

and 360 days after sowing (DAS). These parameters were observed in soil treated with various Glomus spp. during the years 2021-2022 and 2022-2023, respectively. The Plant survival Tecomella undulata seedlings was % of significantly higher in all the treatments having soil inoculated with Glomus sp. as compared with control (uninoculated). The maximum plant survival percentage at (23.36 and 22.68 %) was recorded in treatment T3 (G. fasiculatum) was inoculated in soil before sowing which was significantly higher than all the treatments. The minimum plant survival % were recorded at (17.22 and 15.68%) when soils treated with G. fasiculatum during 2021-22 and 2022-23, respectively. Amongst all treatments, the plant survival percentage were better in all seedlings grown under different Glomus spp. inoculated soil as compared to control (uninoculated). Ahlawat et al. [8] also observed a maximum plant survival% in Acacia nilotica tree when sown in soils incorporated with different Glomus spp. as compared to the non-inoculated soil. However, G. intraradices also increased plant survival % in present study but it was less effective than the soil incorporation of G. mosseae. It may be due to the differences in inherent character of AMF. While comparing the thickness at the collar region of seedlings, maximum collar diameter was found in seedlings grown under soil inoculated with G. fasciculatum followed by G. intraradices (10.44 and 10.07 mm) and (10.09 and 9.38 mm) during 2021-22 and 2022-23 respectively. The application of Glomus spp. in soil significantly increased the collar diameter as compared to control. The root length at (26.67 and 25.74 cm) in seedlings was in treatment T3 found non-significant during 2021-22 and 2022-23, respectively. It was followed by G. fasciculatum (25.84 and 24.08 cm) in soil inoculated. However, the root length was better in all seedlings grown under three Glomus spp. inoculated soil as compared to control (uninoculated). The shoot length at (33.36 and 32.19 cm) in seedlings was in treatment T3 G. fasiculatum significantly highest during 2021-22 and 2022-23, respectively. It was followed by G. intraradices (32.67 and 30.38 cm) in soil inoculated. The root : shoot ratio was found nonsignificant. The Plant biomass production was recorded significantly increase when treated with G. fasciculatum at (8.31 and 8.07 g) in seedlings which was followed by T1 with (7.58 and 7.41 g). While minimum were recorded in control at (4.97 and 3.93 g) respectively at 360 days after sowing during both the experimental year respectively. The leaf area (cm²) per plant was recorded significantly increase when treated with *G*. *fasciculatum* at (196.29 and 185.46 cm²) per plant in seedlings which was followed by T1 with (193.29 and 182.32 cm²) at 360 days after sowing during both the experimental year respectively. While minimum were recorded in control at (4.97 and 3.93 g) respectively.

There is a paucity of studies focusing on the selection of a suitable arbuscular mycorrhizal fungus (AMF) for artificial soil inoculation before sowing, especially for the production of robust with high vigour to seedlings enhance establishment under various biotic and abiotic stresses in field conditions. Khaiper et al [9]. examined that among of all the treatments, Glomus mosseae was found effective for enhancing plant growth parameters and maximizing the biomass of Melia azedarach seedlings. Similar observations were observed by Singh and Chugh reported that enhanced survival percentage, root length, shoot length, total biomass, in trees like Dalbergia sissoo, Eucalyptus tereticornis, Azadirachta indica, and Ailanthus excela when sown in soils containing mycorrhiza compared to non-inoculated soil. Similarly, studies by Gulab et al [10]. demonstrated that significantly higher plant growth parameters survival percentage, root length, shoot length and plant biomass in Melia azedarach in the presence of G. fasciculatum as compared to control. Giri et al [11]. reported that to enhancing the survival rate, the inoculation of arbuscular mycorrhizal (AM) fungi resulted in improved seedling growth, including plant height and collar diameter. Among the AM fungi treated, Glomus macrocarpum exhibited higher efficacy compared to *G. fasciculatum*, both in the nursery seedlings. Additionally, Kaushik et al [12]. revealed that Acacia nilotica and Dalbergia sissoo seedlings exhibited significantly higher biomass, root and shoot length when inoculated with G. mosseae compared to the control. Singh et al [13]. investigated that percentage of plant survival was elevated in soil treated to arbuscular mycorrhizal fungi (AMF) treatment, potentially attributed to the safeguarding of seedlings from detrimental pathogens facilitated by *Glomus* spp. Khaiper et al [14]. reported that the interaction between arbuscular mycorrhizal fungi (AMF) and the roots of trees, which benefits the plant for better germination and growth. dormancy of seeds or a delay in growth can be identified as a disadvantage in a plantation program. Hamidi et [15]. investigated the impact al of endomycorrhiza, specifically Glomus etunicatum, on cork oak plant (Quercus suber) seedlings.

that plants inoculated Thev noted with mycorrhizae exhibited a stimulative influence on the aerial part, aerial part weight, average root length, average fresh weight, and leaf count compared to the control group. Sumana and Bagyaraj carried out an experiment utilizing eight different VAM (vesicular-arbuscular mycorrhizal) fungi [16]. They aimed to identify the most suitable symbiont for Dalbergia latifolia and found that plants inoculated with these fungi displayed increased plant height, stem girth, dry weight, and phosphorus content in comparison to noninoculated plants. Among the VAM fungi, Glomus fasciculatum were particularly effective in enhancing the growth of D. latifolia. The presence of AMF in the soil contributes to enhanced survival rates for rohida. The alteration of the soil environment by AMF enveloping the roots could also be a factor contributing to the improved seedling growth, survival rates, more effective root colonization. and increased multiplication. Diverse workers in various forest plants [17,18,19], have observed that Arbuscular Mycorrhizal Fungi (AMF) play a crucial role in producing plant growth hormones and facilitating the absorption of both available and unavailable forms of nutrients by plants. Consequently, this likely contributes to enhanced plant growth parameters in Tecomella undulata. Furthermore, the percentage of plant survival was notably high in soil treated with AMF, potentially attributed to the safeguarding of seedlings from harmful pathogens by Glomus sp. The presence of AMF in the soil also fortifies the strength of the plants, potentially leading to improved survival rates for Tecomella undulata.

3.2 Impact of Soil Application of Different *Glomus* spp. on root colonization and Numbers of Sporocarp Per 100 g of Soil

The information provided in Table 8. and Table 9. illustrates the root colonization percentage and the number of sporocarp per 100 g of soil in Tecomella undulata seedlings at 90, 180 days after sowing (DAS) in soil treated with various Glomus spp. in the periods of 2021-2022 and 2022-2023, respectively. In both years, when each of the 4 Glomus species was individually inoculated into the soil before sowing seedlings G fasciculatum consistently resulted in significantly higher root colonization at 180 DAS, with (50.69% and 46.71%) respectively. were closely followed by G. intraradices treated soil exhibited root colonization percentages with (42.71% and 39.66 %) at 180 DAS during 202122 and 2022-23, respectively. In contrast, no root colonization was observed in the control (uninoculated) soil. Regarding the number of sporocarps per 100 grams of soil, *G. fasciculatum* treated soil exhibited significantly higher values at 180 DAS (103.67 and 98.65)

sporocarps per 100 g of soil during 2021-2022 and 2022-2023, respectively. which was followed by *G. intraradices*, treated soil recorded at (94.33 and 90.45) sporocarps per 100 g of soil during 2021-2022 and 2022- 2023, respectively at 180 DAS.

Table 1. Effect of arbuscular mycorrhiza fungi on plant survival % of rohida in nur

Trea	tments				Plant s	survival 9	6				
			2021-2022				2022-2023				
		90 DAS	180 DAS	270 DAS	360 DAS	90 DAS	180 DAS	270 DAS	360 DAS		
T1	Glomus intraradices	28.41	25.14	23.12	22.23	26.45	23.25	21.89	19.82		
T2	Glomus mosseae	25.28	22.98	21.78	19.64	23.21	20.20	18.63	16.20		
Т3	Glomus fasiculatum	30.63	27.09	25.84	23.36	28.55	25.41	24.64	22.68		
T4	Glomus hoi	27.57	23.12	22.31	20.24	24.49	22.35	19.21	18.31		
T5	Control	24.12	20.85	19.29	17.22	22.56	19.12	17.56	15.68		
	C.D at 5 %	2.44	2.15	2.03	1.86	2.24	1.98	1.84	1.67		

Table 2. Effect of arbuscular mycorrhiza fungi on Collar diameter of rohida in nursery

Trea	atments	Collar diameter (mm)								
		2021-2022				2022-2023				
		90	180	270	360	90	180	270	360	
		DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	
T1	Glomus intraradices	5.96	7.29	9.35	10.09	4.98	6.67	8.70	9.38	
T2	Glomus mosseae	4.71	5.98	7.45	7.91	3.67	5.03	7.04	7.46	
Т3	Glomus fasiculatum	6.14	7.96	9.61	10.44	5.93	7.68	9.02	10.07	
Τ4	Glomus hoi	5.21	6.46	8.33	8.92	4.10	6.06	7.96	8.39	
T5	Control un-inoculated)	4.09	4.88	6.76	6.93	3.03	4.34	6.33	6.49	
	C.D at 5 %	0.47	0.60	0.75	0.80	0.40	0.55	0.70	0.76	

Table 3. Effect of arbuscular mycorrhiza fungi on Root length of rohida in nursery

Root length (cm)									
			202	1-2022	2022-2023				
Treatments		90	180	270	360	90	180	270	360
		DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS
T1	Glomus intraradices	15.67	18.67	22.51	25.84	14.57	17.36	21.45	24.08
T2	Glomus mosseae	14.12	17.59	21.79	24.91	13.11	16.62	19.67	23.11
Т3	Glomus fasiculatum	16.50	19.33	23.41	26.67	15.92	18.65	22.59	25.74
T4	Glomus hoi	14.33	18.03	22.02	25.13	13.69	17.22	21.03	23.78
T5	Control(uninoculated)	13.89	16.69	20.15	23.79	12.76	16.03	19.13	22.67
	C.D at 5 %	1.33	1.61	1.96	N. S	1.25	1.53	1.85	N. S

Table 4. Effect of arbuscular mycorrhiza fungi on Shoot length of rohida in nursery

Trea	atments		Shoot length (cm)								
		2021-2022				2022-2023					
		90	180	270	360	90	180	270	360		
		DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS		
T1	Glomus intraradices	23.10	26.33	28.53	32.67	22.17	24.45	26.53	30.38		
T2	Glomus mosseae	19.45	22.95	24.65	30.12	19.21	21.69	23.29	28.46		
Т3	Glomus fasiculatum	25.00	27.12	29.12	33.36	24.13	26.17	28.10	32.19		
T4	Glomus hoi	21.20	25.19	27.39	30.55	20.89	22.33	25.45	29.18		
T5	Control	18.89	21.55	23.83	29.55	18.16	20.67	22.16	28.33		
	C.D at 5 %	1.93	2.20	2.38	2.77	1.88	2.07	2.25	2.64		

Similar observations on increase in sporocarp numbers in soil and root colonization in Dalbergia sissoo have also been reported by Kumar, et al [20]. 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. Tapwal et al [21]. reported maximum growth and germination in Shorea robusta on inoculation with arbuscularmvcorrhiza fungi. 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. Improved phosphorus absorption and a decrease in the

presence of detrimental microorganisms in the root zone of seedlings, as a result of inoculation with arbuscular mycorrhizal (AM) fungi, could contribute to the enhanced growth and survival of seedlings. The increase of plant growth and survival of seedlings by AM fungi result from the Glomus fasiculatum best mycorrhizal association as compared to others and enabling the plant root system to effectively utilize a larger soil volume through (a) the extension of the root zone, (b) accessing smaller soil pores that are not reachable by root hairs, and (c) obtaining organic phosphate via the secretion of extracellular acid phosphatases. Similar results were reported by Zolfaghari et al [22]. in Ocimum basilicum L.

	Root: shoot ratio								
		202	1-2022		2022-2023				
Treatments	90	180	270	360	90	180	270	360	
	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	
T1 Glomus intraradices	0.68	0.71	0.79	0.79	0.66	0.71	0.81	0.79	
T2 Glomus mosseae	0.73	0.77	0.88	0.83	0.68	0.77	0.84	0.81	
T3 Glomus fasiculatum	0.66	0.71	0.80	0.80	0.66	0.71	0.80	0.80	
T4 Glomus hoi	0.68	0.72	0.80	0.82	0.66	0.77	0.83	0.81	
T5 Control (un-inoculated)	0.74	0.77	0.85	0.81	0.70	0.78	0.86	0.80	
C.D at 5 %	N. S	N. S	N. S	N. S	N. S	N. S	N. S	N. S	

Table 6. Effect of arbuscular mycorrhiza fungi on Plant biomass of rohida in nursery

		Plant Biomass (g)								
			202	1-2022		2022-2023				
Treatments		90	180	270	360	90	180	270	360	
		DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	
T1	Glomus intraradices	2.15	4.36	6.12	7.58	2.00	4.20	6.00	7.41	
T2	Glomus mosseae	1.53	3.92	4.97	5.08	1.15	3.17	4.46	4.93	
Т3	Glomus fasiculatum	3.26	5.67	7.65	8.31	3.11	5.34	7.27	8.07	
T4	Glomus hoi	1.85	4.00	5.71	6.21	1.32	3.95	5.33	6.11	
T5	Control (un-inoculated)	1.25	2.58	3.67	4.97	1.10	2.28	3.53	3.93	
	C.D at 5 %	0.39	0.48	0.54	0.59	0.35	0.43	0.46	0.57	

Table 7. Effect of arbuscular mycorrhiza fungi on Leaf area (cm²) per plant of rohida in nursery

			Leaf area (cm ²) / plant									
Trea	atments		2021	-2022		2022-2023						
		90	180	270	360	90	180	270	360			
		DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS			
T1	Glomusintraradices	112.63	153.13	183.76	193.29	108.53	150.37	178.27	182.32			
T2	Glomus mosseae	107.56	150.36	177.43	188.45	100.67	146.63	170.36	178.51			
Т3	Glomus fasiculatum	115.67	157.49	184.79	196.29	112.15	152.39	181.33	185.46			
T4	Glomus hoi	110.15	152.27	180.35	191.37	105.46	149.21	173.29	180.67			
T5	Control	103.19	148.75	175.13	186.65	97.31	142.16	168.61	172.79			
	C.D at 5 %	2.18	2.47	2.82	3.05	2.03	2.41	2.72	2.96			

Trea	atments	Root colonization %						
		2	2021-2022	2	2022-2023			
		90 DAS	180 DAS	90 DAS	180 DAS			
T1	Glomus intraradices	36.27	42.71	32.48	39.66			
T2	Glomus mosseae	22.45	26.33	18.61	22.49			
T3	Glomus fasiculatum	43.56	50.69	40.37	46.71			
Τ4	Glomus hoi	27.67	35.27	24.53	32.33			
T5	Control(uninoculated)	00.00	00.00	00.00	00.00			
	C.D at 5 %	2.84	3.38	2.54	3.07			

Table 8. Effect of different arbuscular mycorrhiza fungi on root colonization % of rohida

Table 9. Effect of arbuscular mycorrhiza fungi on number of spores of Rohida in nursery

		No. of spores per 100 gm of soil							
Treatme	nts	2021-2022		2022-2023					
		90 DAS	180 DAS	90 DAS	180 DAS				
T1	Glomus intraradices	82.47	94.33	78.66	90.57				
T2	Glomus mosseae	70.39	81.54	64.51	76.51				
Т3	Glomus fasiculatum	95.61	103.67	91.37	98.65				
T4	Glomus hoi	76.55	87.45	72.33	84.36				
T5	Control (un-inoculated)	00.00	00.00	00.00	00.00				
	C.D at 5 %	5.63	5.75	4.09	5.38				

4. CONCLUSION

The effect of different Glomus spp. (Glomus intraradices, Glomus mosseae. Glomus fasiculatum and Glomus hoi) on the growth characteristics (plant survival %, shoot length, root length, collar diameter, plant biomass, leaf area) and survival of Tecomella undulata seedlings was investigated. The results indicated that G. fasiculatum exhibited the highest effectiveness compared to the other species. Additionally, G. fasiculatum treatment resulted in the highest root colonization of seedlings followed by G. intraradices and G.hoi treatments. The multiplication rate of G. fasiculatum was also found to be the highest, followed by G. intraradices and Glomus hoi. When soil phosphorus levels are low, AM fungi induce a enhancement in the notable uptake of phosphorus, leading to a remarkable surge in plant and survival of seedlings. In conclusion, AM fungi. particularly the Glomus fasiculatum association, enhance plant growth and seedling survival by expanding the root zone and accessing smaller, unreachable soil pores, allowing the plant's root system to effectively utilize a larger soil volume. By applying G. fasiculatum to the soil before sowing Tecomella undulata seeds proved to be highly effective for seedling establishment in nursery conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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