RESEARCH ARTICLE

Dalbergia sissoo seedlings Inoculated to Arbuscular Mycorrhizal Fungi, an Effective Candidate to Arsenic Tolerance

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Abstract

Arbuscular mycorrhizal fungi (AMF), as biofertilizers and natural root symbionts, may increase plants' resistance to heavy metal stress and supply host plants with vital inorganic nutrients, thereby enhancing growth and production in both unstressed and stressed conditions. The present study aimed to determine the impact of two AMF species (*Glomus macrocarpum* and *G. fasciculatum*), on the development and biomass of *Dalbergia sissoo* seedlings exposed to soil arsenic stress (0, 25, 50, 100 mg/kg soil) for three months in a nursery environment. This symbiotic relationship caused inoculated plants to thrive and produce more biomass under all concentrations of arsenic stress than uninoculated plants did. Therefore, this study concludes that the AM Fungi and *Dalbergia sissoo* combination can survive under arsenic stress in soil.

Highlights:

- Both the AMF species (*Glomus macrocarpum* and *Glomus fasciculatum*) shown resistance to arsenic heavy metal.
- AMF represents the good symbiotic relationship with the roots of *Dalbergia sissoo*.
- AMF inoculation with *D. sissoo* enhances the growth and biomass of the seedlings.
- Increasing arsenic presence in soil affected the non-AMF seedlings more compared to AMF ones.

Keywords: Arbuscular mycorrhizal fungi, *Dalbergia sissoo, Glomus macrocarpum, Glomus fasciculatum.*

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Introduction

Arsenic (As) has been perceived as a cancer-causing substance given its chemical and physical structures as well as concentration and length of exposure and represents a serious risk to human wellbeing because its transport into the food chain. It exists normally in a wide range of the covering rocks, particularly orpiment, realgar, and different mine (Alam *et al.*, 2019a; Singh *et al.*, 2015). As contamination has been accounted for around the world, especially in Argentina, Australia, Bangladesh, Chile, China, Hungary, Mexico, Peru, Thailand, and Vietnam. The most serious pollution to humans, water, and land is at this point in Asia, particularly Bangladesh, West Bengal (India) (Alam *et al.*, 2019a). Although incidents of arsenic poisoning in groundwater are well known worldwide, the public, especially those living in affected nations, is still largely unaware of the consequences of soil pollution (Shrivastava *et al.*, 2015a).

Naturally, arsenic exists as a regular form and inorganic form/species. The key presence of As are arsenic tri-sulfide (As_2S_3) and arsenopyrite or ferrous arsenic sulfide ($FeAsS_2$), arsenic sulfide (As_2S_2). Inorganic arsenic has two main oxidative states, i.e., pentavalent arsenate (As (V)) and trivalent arsenite (As (III). In As adulterate soil, the inorganic forms of As (V) and As (III) are frequently found. As (III), which oxidizes to As (V), is a component of insecticides and herbicides. Arsenite is sometimes 60 times more poisonous than arsenate (Cubadda *et al.*, 2010). Both As (V) and As (III) impair plant metabolism, although through different methods (Finnegan and Chen, 2012). In highly impacted soils mainly found arsenic is arsenate. Because in it imitates phosphorus (P) and can be taken up by leguminous plants and AMF by a regular Pi (phosphate) pathway (Toulouze *et al.*, 2012). The arbuscular mycorrhiza plays a very important role in Pi absorption from the soul where, arsenite (V) hampers due to its analogous nature to phosphate.

Depending on plant species, metal aggregation in the soil, AM growth species, and isolation, the effect of AMF on component take-up might vary significantly (Orlowska *et al.*, 2012a). The extra-radical network, which stretches out into soil interstices from the root surface, out of reach to roots, and the arrangement of points of interaction (arbuscules and intercellular hyphae) by the intra-radical mycelium, thus exchange supplement between symbionts sites, are two techniques that make sense of how the mycorrhizal impact work (Catska *et al.*, 1997).

Poisonous, arsenic is not necessary for plants. The first tissues exposed to arsenic are the roots, where the metalloid prevents the growth and enlargement of the roots. As it may essentially

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hamper plant advancement anytime it is transported to the shoot by diminishing biomass development and extension, as well as by impairing plant regeneration capability through reductions in fruit output, fertility, and yield (Azizur Rahman *et al*., 2007; Garg *et al*., 2011; Shri *et al*., 2009).

By altering the root system, such as by increasing the length of the roots and their branches, AMF enhances mineral feeding and facilitates the root system's absorption of nutrients (Padilla *et al*., 2005).

Different plants store arsenic in various locations and absorb different amounts of it from the soil. While some plants absorb and store arsenic primarily in their roots, others transport it from the roots to the leaves. Arsenic mostly enters biological systems through plant roots. Depending on their species and habitat, plants absorb and accumulate different amounts of As. Compared to terrestrial plants, wetland plants such as *Pteris vittata* fern and Chinese brake are better in absorbing As from the soil (Farooq *et al*., 2016).

Phytoremediation can be used to remediate polluted soil, depending on the level of pollution, the characteristics of the soil, and the rate of plant growth. Soil microorganisms have been successfully utilized in recent years to enhance plants' resistance to, removal of, or degradation of contaminants (Yang *et al*., 2020). Mycorrhizal symbiosis increased phosphorus absorption more than non-mycorrhizal plants in 90% of higher plants (Smith *et al*., 2010a). Arbuscular mycorrhizal fungi (AMF) are ubiquitous root-symbiotic soil microorganisms in the phylum Glomeromycota. Over their 400 million years of life, these endophytes finally developed mutualistic relationships with 80% of terrestrial higher plants (Zhang *et al*., 2020).

It is commonly recognised that arbuscular mycorrhizal fungi (AMF) affect how plants respond to heavy metal stress. The arsenic (As) resistance system of AMF-immunized woody legumes stays indistinct (Zhang *et al*., 2020). In order to cope with the negative effects of heavy metals, plants evolve a series of mechanisms (Bilal *et al*., 2019; Dhalaria *et al*., 2020). The majority of terrestrial plants have arbuscular mycorrhizal fungi (AMF), which are common symbiotic fungi that play a critical role in the transformation and transit of arsenic (As) in the plant or soil continuum and have a major effect on plants' ability to withstand arsenic (Li *et al*., 2018).

The present work, we attempted to add to greater utilization of this strategy in the field. For this reason, we focused on the As remediation proficiency of AMF-inoculated Dalbergia sissoo over a 3-month period in the nursery environment.

Methodology

The wet sieving and decanting technique given by (Gerdemann and Nicolson, 1963) was used to isolate AM fungus spores from soil. Spores have a somewhat lower specific gravity than soil particles. Plastic beakers of 500 mL are filled with ten grams of dried soil sample. AM fungus spores are frequently abundant in rhizosphere soils. Approximately 500 ml of tap water is used to float the soil. The top layer of the soil suspension is poured into the sieve after the soil particles have settled down. Suspended particles were poured into a sieve and washed in tap water. The fungal spores were sieved through fine mesh (200μm) to collect the spores in a small beaker and filtered using Whatman filter paper 1. The isolated mycorrhizal fungal spores were further grown through pot culture with *Trigonella foenum graecum* (Methi) plant for 3 months. The infectivity of the roots was tested and 100% colonization was observed. The roots of the plant were chopped into approximately 1cm sections and mixed with the soil, to used as inoculum for the seedlings of *D. sissoo*.

The experiment took place at the nursery of the Department of Forestry and Natural Resources at H.N.B. Garhwal University in Uttarakhand, India (30.225° N and 78.804° E), under greenhouse condition of 20–35°C Temperature and 75-90% Humidity during the period of 2021-2022. Seeds of *D. sissoo* were collected from the university campus during March –June, water soaked for 24 hours to seeds before sowing. To eliminate non-soil elements, the soil was sieved using a 2 mm mesh screen before being used to fill poly bags or pots for seeding. Soils for growing *D. sissoo* seeds were mixed with sand in the ratio of 1:4 (soil: fine sand), and sterilized by fumigation. Each pot was filled with roughly 2 kg of screened soil, leaving the top 10 cm of the pots empty to facilitate watering.

Sodium Arsenate ($Na_2HAsO_4.H_2O$) was used to for arsenic treatments, four concentrations of arsenic (0, 25, 50, 100mg As/kg soil) were prepared. Arsenic was dissolved in 50 mL of water and mixed thoroughly with soil for homogeneous distribution. The pots were left for One-month to equilibrate with repeated application of distilled water and air drying.

Each experimental pot was filled with about 15 g of mycorrhizal inoculum, and then a 1 cm soil layer was put on top of the inoculum layer. In each container, two seeds were sowed at a 1-cm soil depth. Only one healthy seedling remained after the treatment, and the other seedlings were removed from the container. Plants were inoculated with the most dominant Arbuscular mycorrhiza Fungus *Glomus macrocarpum* and *Glomus fasciculatum*. Both the AM inoculums were cultivated separately to examine its effect on Arsenic concentration and plant growth and development.

There was a total of twelve treatments (4×3), with four arsenic concentrations (0, 25, 50, 100 mg As/kg soil) and three AMF treatments (control, *Glomus fasciculatum*, *Glomus macrocarpum*). All the treatments replicated five times.

Shoots and roots were collected separately. All the parts above the soil were considered as shoot, and below the soil as root. Fragments of roots were collected by sieving soil. To get rid of adhering soil particles, samples were gently rinsed in deionized water. After drying in an oven at 60°C until weight remained constant, the dry weights of the shoot and root were recorded.

During the study, the growth reciprocations of *D. sissoo* inoculated with *Glomus fasciculatum* and *G. macrocarpum* were measured on parameters listed ahead: root length, shoot height, number of leaves and number of nodules and collar diameter of seedlings after 3 months of growth.

After three months of development, the following characteristics were assessed for *D. sissoo* infected with *G. macrocarpum* and *G. fasciculatum*: shoot height, root length, number of leaves and nodules, and collar diameter of seedlings.

AMF Colonization

To estimate AMF colonization roots were used to clear in 10% KOH followed by staining with 0.05% trypan blue. Roots were cut into segments of 1 cm in length. Per replicate five microscopic slides were prepared, containing randomly selected ten root segments (Phillips and Hayman, 1970). Formula used for the calculation of Percent root colonization is mentioned below:

$$\text{Percentage root colonization (\%)} = \frac{\text{(sum of number of colonized roots) X100}}{\text{(sum of number of roots observed)}}$$

The significance of the Effect of As concentration, AMF inoculation and their interaction on parameters (Shoot height, Root length, No. of nodules, No. of leaves, Collar diameter and dry and fresh weight of root and shoot) were analyzed by Two-way ANOVA.

Result and Discussion

A considerable improvement in growth response was seen in *D. sissoo* seedlings inoculated with AMF in the nursery. When compared to non-mycorrhizal seedlings, mycorrhizal seedlings showed a substantial growth increase in the following dimensions: Root length, Shoot height, Collar diameter, Root nodules number, Leaf count, fresh weight and biomass of Shoot and Root.

Root AMF Colonization

The control (non-inoculated) plants showed no signs of root infection, whereas all *D. sissoo* seedlings exposed to both inoculums and all concentrations of arsenic experienced effective AMF infections. All of the inoculated plants had vesicles, arbuscules and hyphae. For *Glomus macrocarpum* and *Glomus fasciculatum*, the percent root colonization was from 54 to 79% and 58 to 75%, respectively. With an increase in As in the soil, the % root colonization in both fungus species decreased (Fig. 1).

Effect of arsenic in plant's physical parameters

This study revealed some intriguing impacts of AMF on *D. sissoo* seedling infection, growth, and biomass. In this greenhouse experiment, AMF infection of all plant species under As-contaminated circumstances was effective. Additionally, the data showed that this impact differed depending on the arsenic concentration and AMF. ANOVA analysis shows that effect of arsenic and AMF on seedlings reveals significant ($p < 0.01$) results, whereas their interactions have some non-significant results (Table 1).

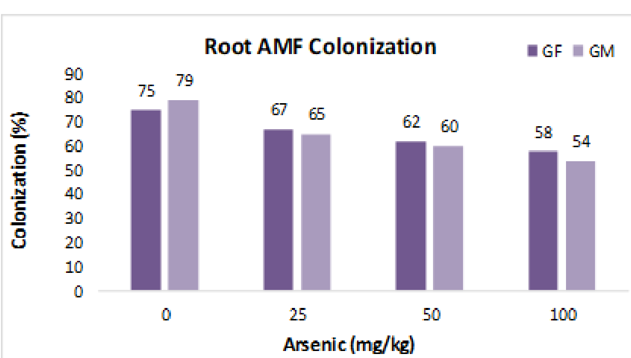

Figure 1: Effect of Arsenic concentrations on *Glomus macrocarpum* (GM) and *Glomus fasciculatum* (GF) colonization of *D. sissoo* roots

Table 1: Arsenic concentration (As), AMF treatments and their interactions (AMF×As) on characteristics of *D. sissoo* seedlings were analyzed using a two-way ANOVA

Parameters	Arsenic	AMF	As x AMF
Root length (cm)	**	**	ns
Shoot height (cm)	**	**	ns
Collar diameter(mm)	**	**	**
No. of nodules	**	**	**
No. of leaves	**	**	**
Root fresh weight (g)	**	**	**
Root dry weight (g)	**	**	**
Shoot fresh weight (g)	**	**	**
Shoot dry weight (g)	**	**	**

Where ns, not significant; ** $p < 0.01$, * $p < 0.05$.

The morphological characteristics of plants alter, proving that As has an impact on plant development (Fig. 2). In comparison to controls, the plants inoculated with mycorrhiza exhibited increased shoot and root development. While growth declines as soil arsenic concentrations increases. The root length of *Glomus macrocarpum* plants is more than that of *Glomus fasciculatum* and control plants at 0 mg/kg arsenic concentration, and *Glomus fasciculatum* plants have longer roots than *Glomus macrocarpum* plants at all other arsenic concentrations (25, 50, and 100 mg/kg soil), despite that when the quantity of arsenic rises, all the plants in both mycorrhizal inoculations show a notable decrease

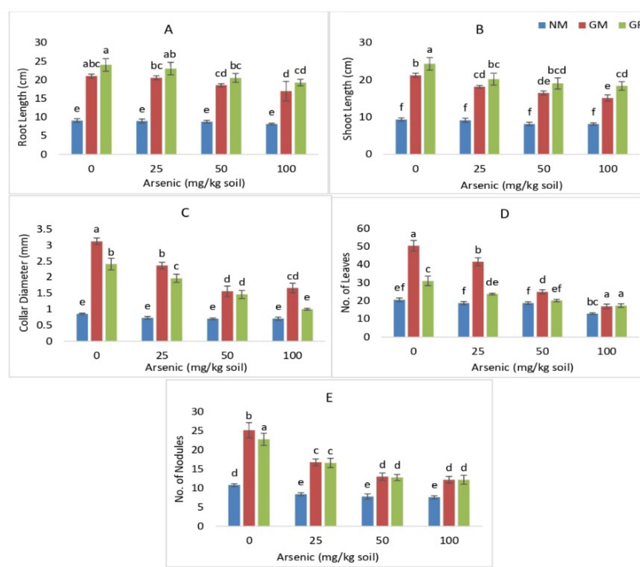

Figure 2: Effect of As concentrations and AMF treatments on *D. sissoo* seedlings (A) Root length (B) Shoot height; (C) Collar diameter; (D) Number of leaves ; and (E) No. of Nodules.. The data is shown as mean±SE (n = 5). According to the Duncan's multiple comparison test, bars with different lettering indicate significant difference among treatments (p 0.05); where (GM) stands for *Glomus macrocarpum* and (GF) for *Glomus fasciculatum*

in root development (Fig. 2A). The height of shoot, no. of nodules in roots and collar diameter are also decreasing in the plats with inclining level of arsenic concentration in soil, while plants inoculated with *Glomus fasciculatum* has higher responses in all these parameters as compared to *Glomus macrocarpum* and control plants (Fig. 2B, 2E and 2C). *D. sissoo* plants inoculated with *Glomus macrocarpum* had more leaves than plants inoculated with *Glomus fasciculatum* and control at all three arsenic concentrations (0, 25, and 50 mg/kg soil), but at 100 mg/kg soil As, Compared to *Glomus fasciculatum, Glomus macrocarpum* had more leaves as concentration increased (Fig. 2D).

According to earlier studies, *D. sissoo* seedlings can tolerate environmental stress from heavy metals and other severe elements such as Pb, Cd, Co, Cr, marginal, degraded land and salinity etc. (Edrisi *et al*., 2019; Shah *et al*., 2008; Yousaf *et al*., 2022 and Verma and Bhushan, 2016).

Recent studies have confirmed the importance of various AMF in promoting growth and arsenic uptake from the arsenic-contaminated soil (Jankong and Visoottiviseth, 2008; Orlowska *et al*., 2012a; Zhang *et al*., 2020). Bisht *et al*., (2009) examined the effects of a strain of *Rhizobium leguminosarum* DSP2 (N2 fixer), strain of *Pseudomonas fluorescens*, a plant growth-promoting bacterium, and AMF, which promote plant growth by increasing nutrient uptake, on the growth and nutrient acquisition of *D. sissoo* Found that AMF, strain of *P. fluorescens* GRPr, strain of *R. leguminosarum* DSP2, and *D. sissoo* tetrapartite interaction boosted response of plant growth in the Entisol opposed to uninfested plants, suggesting that the bacteria-AMF combination was responsible for the higher plant growth. On the other hand, Bradyrhizobium and *G. fasciculatum* inoculation of *D. sissoo* seedlings resulted in a synergistic increase in growth, nodulation, and nitrogen fixation (Niranjan *et al*., 2007).

Ahlawat *et al*., (2022) examined the effects of three distinct species of AMF—*G. Mosese*, *G. intraradices* and *G. fasciculatum*—of the AMF-infested soil with the seeds of *Acacia nilotica* in the nursery and observed that seedlings put in soil infected with *G. intraradices* followed by *G. fasciculatum* produced considerably longer shoots and roots length.

Alam *et al*., (2019a) investigated the function of arbuscular mycorrhizal fungi (AMF) in reducing As phyto-toxicity in *Lens Culinaris*. Arsenic phytotoxicity was dose-dependent, as seen by the relatively longer shoots and heavier fresh and dried shoot and root weights of lentil plants treated with 5 and 15 mg/kg As compared to the 100 mg/kg As.

Zhang *et al*., (2020) in a pot experiment *Robinia pseudoacacia* seedlings were tested with AMF Rhizoglomus intraradices and controlled for different As stress levels till 4 months. Resultantly As toxicity was reduced and enhanced in the growth of *R. pseudoacacia* seedlings by change in the shape of root, phytohormone concentrations and ratios, and boosting soil glomalin levels.

Yang *et al*., (2015) experimented in greenhouse with AMF (*Funneliformis mosseae* and *Rhizophagus intraradices*) and high Pb stress levels to assess growth, photosynthesis, antioxidant enzyme activities and Pb accumulation in *Robinia pseudoacacia*. AMF symbiosis decreased the levels of lead in the leaves while promoting the synthesis of biomass and photosynthetic pigments. AMF inoculation likely had a more significant role in

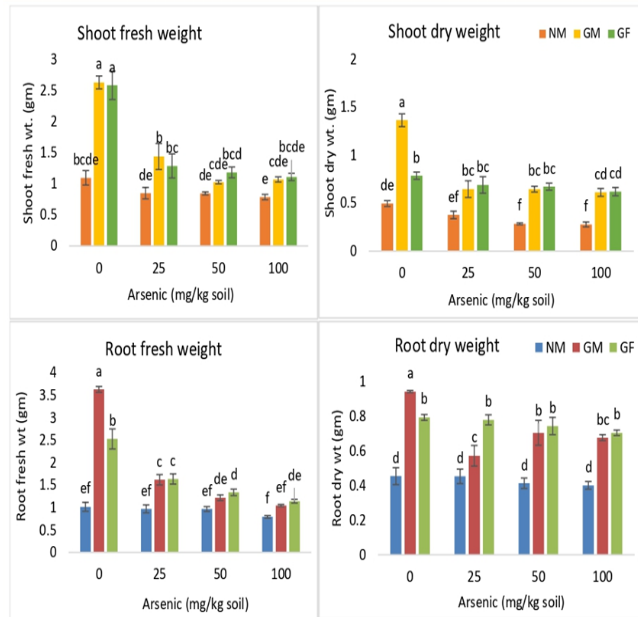

Figure 3: Effect of As concentrations and AMF treatments on *D. sissoo* seedlings fresh and dry weight of shoot and root. The data is shown as mean±SE (n = 5). According to the Duncan's multiple comparison test, bars with different lettering indicate significant difference among treatments (p 0.05); where (GM) stands for *Glomus macrocarpum* and (GF) for *Glomus fasciculatum.*

plant Pb tolerance in highly polluted soils, as evidenced by the finding that mycorrhizal dependency on plants increased with rising Pb stress levels. Anand *et al*., (2020) found that the field soil + FYM+ and *Glomus intraradices* had stimulatory effects on shoot parameters, colonisation index, mycorrhizal dependence and seedling quality index, while the media for potting comprising field soil + FYM + *Acaulospora scrobiculata* had stimulatory effects on root parameters, including root length, fresh weight and biomass of root of three and six months old *Ailantus exclesa* seedlings. Rangel *et al*., (2014) studied AMF (*Acaulospora morrowiae*, *Gigaspora gigantea*, Acaulospora species and *Glomus etunicatum*) infected four leguminous plants (*Acacia mangium*, *Crotalaria juncea*, *Enterolobium contortisiliquum* and *Stizolobium aterrimum*) with phytoprotective benefits against As-contaminated soil from a gold mining location and found that the greatest measure of the photoprotective impact of all AMF species in all plant species, in general, was the P/As ratio in shoots.

The biomass of *D. sissoo* after three months of growth (12 week) increased maximum when both arbuscular mycorrhizal fungi were inoculated (Fig.3) Arsenic concentrations rose. The plants responded by reducing their biomass. Both Control and Mycorrhizal plants displayed the reduction, which was influenced by the amount of As in the soil. However, mycorrhizal plants had more biomass than control plants at all As concentrations when compared to them. At 0 mg/kg soil, *Glomus macrocarpum* has more root and shoot biomass than *Glomus fasciculatum*. However, in the remaining arsenic treatments (25, 50, and 100 mg/kg soil), *Glomus fasciculatum* displayed more biomass compare to *Glomus macrocarpum*.

Effect of As addition levels and AMF treatments on *D. sissoo* seedlings fresh and dry weight of shoot and root. The data (n = 5) is displayed as mean±SE. Bars with distinct letters, where (GM) stands for *Glomus macrocarpum* and (GF) for *Glomus fasciculatum*, show a significant difference across treatments (p 0.05), according to the Duncan's multiple comparison test. A favorable impact on biomass production and arsenic buildup has also been demonstrated by three Glomus species (*G. mosseae*, *G. intraradices*, and *G. etunicatum*) in *Pityrogramma calomelanos*, *Tagetes erecta*, and *Melastoma malabathricum* (Jankong and Visoottiviseth, *2008)*.

Kaur *et al*., (2018) studied the resistance of *D. sissoo* to Pb toxicity. The tree might be a viable choice for Pb phytoremediation because it showed normal growth, and accumulation of Pb which was boosted by amalgamation of citric acid in soil under pot experiment and one year of period.

Tu *et al*., (2002) found that the development and absorption of arsenic by ladder brakes were affected by varied arsenic concentrations (50 to 1000 mg/ kg) or types (organic/inorganic and Arsenite/arsenate) applied to soils. Ladder brake fern has a higher tolerance for arsenic up to 500 mg/kg As in soil. And noted that up to 100 mg/kg arsenic may be beneficial for its biomass enhancement by 64 to 107% per kg and at low arsenic concentrations, younger fronds have higher concentration and vice versa for older fronds. Ajeesh *et al*., (2017) observed that inoculated seedlings of *Tectona grandis* with local AMF species (*Funelliformis mosseae*, *Glomus intradises* and *Glomus proliferum*), demonstrated a substantial difference in plant growth, when compared to uninoculated seedlings.

Conclusion

As previously stated, under arsenic stress circumstances, a leguminous tree (*D. sissoo*) and two AMF (*Glomus macrocarpum* and *Glomus fasciculatum*) had a strong symbiotic relationship. Inoculations of *G. macrocarpum* and *G. fasciculatum* may lessen arsenic's negative effects on plant development and biomass. The growth of *D. sissoo* seedlings was also markedly improved by both mycorrhizal inoculations. *D. sissoo* seedlings by themselves had the lowest growth observed, whereas *D. sissoo* + *G. macrocarpum* had the highest response in the majority of the parameters under study. However, more research is needed to determine the *D. sissoo* and AMF interaction's capacity to survive arsenic stress in soil over the long term.

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Author contribution

Dr. B P Chamola, guided me during the research and in the framing of this manuscript.

Conflict of Interest

None

Reference

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