Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 23(3) September-December 2025]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology
Pantnagar, U.S. Nagar; Uttarakhand, Website : gbpuat.res.in/PJR

PANTNAGAR JOURNAL OF RESEARCH

Vol. 23(3) September-December, 2025

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Arbuscular Mycorrhizal Fungi (AMF) root colonisation and glomalin variability across bamboo species integrating UV–vis spectral characterisation

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ABSTRACT: Glomalin-related soil proteins (GRSPs), produced exclusively by arbuscular mycorrhizal fungi (AMF), are key contributors to soil aggregation, carbon sequestration, and rhizosphere stability. The present study investigated species-specific variation in Total Glomalin-related soil protein (TGRSP) and easily extractable glomalin-related soil protein (EE-GRSP) in the rhizosphere soils of ten bamboo species and examined their association with fungal abundance, AMF colonisation, biochemical characteristics, and plant growth responses. Both TGRSP and EE-GRSP contents were significantly higher in the rhizosphere of all bamboo species compared to bulk soil, with pronounced interspecific differences; *Dendrocalamus asper* exhibited the highest EE-GRSP, while *Bambusa nutans* showed the lowest EE-GRSP content. Fungal population analysis revealed CFU values within ecologically functional ranges, and microscopic examination confirmed the presence of AMF hyphae, vesicles, and spores, indicating active mycorrhizal colonisation. UV–visible spectrophotometric analysis of glomalin extracts showed broad absorption features with peaks around 230 and 320 nm, suggesting a structurally complex, proteinaceous macromolecule containing aromatic and amine functionalities rather than simple phenolic compounds. Seed germination bioassays demonstrated a clear dose-dependent response, with half-strength glomalin showing minimal inhibition, whereas full-strength glomalin and aromatic amines markedly suppressed germination. Overall, the results highlight the strong influence of bamboo species on AMF-mediated glomalin production and demonstrate the ecological significance of GRSPs in rhizosphere functioning, soil stability, and plant–soil interactions, with implications for sustainable soil management and bamboo-based agroecosystems.

Keywords: AMF, bamboo, Glomalin rhizosphere, TGRSP

The global need for environmentally friendly alternatives to synthetic fertilisers has sparked interest in the discovery of microbial bio-stimulants as the primary focus of research (Sible *et al.,* 2021). Bio-stimulants are useful products composed of beneficial microorganisms and their metabolites that help plants access more nutrients, promote growth and mitigate environmental stress (Shao *et al.,* 2023). The arbuscular mycorrhizal fungi (AMF) are one of the most valuable species as they establish root symbioses with land plants and play an important role in maintaining soil health and ecosystem stability (Sen, 2003).

One of the key products of AMF is glomalin, or glomalin-related soil proteins (GRSP), which is released mainly by their spores and hyphae into the surrounding soil. Glomalin is a hydrophobic, resilient, and thermostable glycoprotein, which typically contains 3–5% N, 36–59% C, 4–6% H, 33–49% O, 0.03–0.1% P, and 2–5% Fe (Wu *et al.*, 2014). GRSP can be divided into two fractions based on how they were extracted from the soil matrix: difficult-to-extract (DEG) and easy-to-extract (EEG). The DEG is a more resistant fraction where glomalin binds to soil minerals, whereas the EEG is assumed to be the more recent and active fraction (Wu *et al.,* 2015). The EEG portion is primarily unbound and has been demonstrated to improve soil aggregate stability, which is crucial for soil carbon sequestration (Liu *et al.,* 2024).

Studies have strongly confirmed the importance of glomalin in soil science as an effective force of soil aggregation, a factor that enhances the soil structure, water retention and aeration (Rosier *et al.,* 2006). GRSP has several crucial properties that support its ecological significance: (I) GRSP is insoluble in water, which makes it inert to microbial decomposition and, consequently, prolonged persistence in soil systems (Rillig *et al.,* 2001); (II) it regulates plant stress resistance, as it has been found that plant responses to stress are enhanced by

increasing the levels of GRSP in soil (Hammer and Rillig, 2011); (III) it functions as a chelator via diverse chemical functional groups that reduce the bioavailability of heavy metals and, therefore, contribute to the alleviation of soil contamination (Yuan *et al.,* 2022). Moreover, glomalin is an important element of the global carbon cycle due to its stability and high carbon levels, as well as it plays an important part in long-term carbon and nitrogen sequestration of soil(Walley *et al*., 2013).

Bamboo, being one of the fast-growing species of the grass family, forms an extensive and dense fibrous root system where beneficial microorganisms such as AMF find a vast surface area for colonization. The exclusive biological and ecological features of bamboo provide a very favourable condition for the manufacture and storage of GRSP. The bamboo rhizosphere and AMF develop an extremely productive mutualistic relationship in which the plant provides the fungi with photosynthetically derived carbon needed to grow, and the fungi improve nutrient uptake through their extensive hyphal structure. This close symbiosis maintains a high fungal biomass, which ultimately releases significant levels of glomalin in the immediate soil. The glomalin so released acts as a potent soil adhesive that binds organic matter and mineral particles into stabilized aggregates (Mishra *et al.,* 2024), thereby enhancing the soil structure and further benefiting the bamboo as well as its associated microbial community. Hence, the bamboo rhizosphere signifies a natural model system where glomalin production is not merely incidental but also an important part of the stability and functioning of the ecosystem, and would be a perfect place to study the dynamics of this important soil protein.

Regardless of its importance, there are still some serious gaps in our learning of glomalin. The former of these is the quantity of glomalin secreted by the rhizosphere, depending on what host-plant species is being used, and what sort of glomalin is being produced. It is quite likely that various plant species (even within the same genus) might support different microbial communities in the rhizosphere, thereby generating different amounts and types of glomalin (Pantigoso *et al*., 2022). Second, scientists are still investigating the molecular structure of glomalin and the relation between its molecular structure and glomalin activity. It remains unclear whether its potential to enhance plant growth is because of its complex glycoprotein nature or may be linked to simpler bioactive compounds associated with glomalin. Very few studies have been conducted to give comparisons between the spectroscopically measured properties of glomalin and known bioactive compounds. Moreover, its effects on the physical characteristics of soil are well-established; nevertheless, the direct biochemical effect of glomalin on promoting plant growth is a new field of study.

Therefore, the present study was carried out to examine the effect of different bamboo species on glomalin production and to evaluate the role of the produced glomalin in promoting plant growth. To test this, the following objectives were set: (1) to measure total and easily extractable glomalin in the rhizosphere soil of ten bamboo species; (2) to analyse glomalin extracts, through absorption spectroscopy and compare its spectral pattern with known phenolic compounds; (3) to study the dominant fungal species and AMF root colonisation; (4) to perform a direct seed germination test to confirm its plant growth promoting ability. This study offers new knowledge to investigate the plant-fungus-glomalin interactions, which brings new facts to the research system and proves its economic significance.

MATERIALS AND METHODS

Sample site description

Soil samples were collected from the Agroforestry Research Centre (AFRC), Haldi, Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantnagar-263145, Uttarakhand, India (Latitude: 29.0°N, Longitude: 79.3°E, 243.84 m in altitude). The climate at this location is subhumid subtropical, with average annual rainfall of about 1300 mm and summer temperatures between 31.0-41.0°C, while winter temperatures between 2-10 °C. The area's soils have a pH of roughly 7.5 and are

categorised as clay loamy.

Soil sampling and processing

Ten soil samples were collected from the rhizosphere of following bamboo species: *Dendrocalamus hamiltonian, Dendrocalamus asper, Dendrocalamus strictus, Bambusa nutans, Bambusa balcooa, Bambusa bamboos, Bambusa tulda, Bambusa multiplex, Bambusa vulgaris waimin,* and *Bambusa vulgaris striata*. Samples were collected by digging 10 cm deep soil from each species, while a soil sample collected from barren land was taken as a control. Loosely adhered soil particles were detached from the roots by gently shaking, and firmly adhered soil was considered the rhizosphere, which was then separated from the roots using a paintbrush. Plant roots and litter were manually removed and sieved through a 4-mm sieve. Each sample was divided into two portions. One was air-dried and used for chemical analysis, whereas the fresh sample was used for fungal isolation.

Extraction and quantification of EE-GRSP and T-GRSP

Extraction of glomalin was performed by autoclaving soil in a citrate buffer solution (Wright and Upadhyaya, 1998), where Easily Extractable Glomalin Related Soil Protein (EE-GRSP) was isolated by taking1.0 g of soil sample in 8 mL of 20mM sodium citrate buffer (pH 7.4) and autoclaved for 60 min. at 121°C. After extraction, samples were immediately centrifuged at 5000 xg for 15 min to pellet the soil particles. The supernatant containing the protein was stored at 4 °C for further analysis. While the Total Glomalin Related Soil Protein (TGRSP)was extracted by taking 1.0 g soil sample in 8 mL of 50mM sodium citrate buffer (pH 8.0) by autoclaving at 121°C for 90 min. Then, centrifugation of samples was immediately done at 5000 xg for 15 min to pellet out the soil particles. The protein suspended in the supernatant was stored at 4°C for further quantification. The total soluble protein was estimated using the Lowry method (Lowry *et al.,* 1951).

Four different concentrations, *i.e.,* 100 µL, 200 µL, 400 µL, and 500 µL of extracted protein (EE-GRSP and TGRSP) were taken, and the total volume was made up to 1 mL with distilled water. Then, 5 mL of freshly prepared solution C (50 parts of 2% Na_2CO_3 in 0.1N NaOH and 1 part of 0.5% Cu_2SO_4 in 1% sodium potassium tartrate) was added, followed by 0.5 mL of FCR. Incubated in the dark for 30 min, and the absorbance of colour developed was measured at 660 nm.

Isolation and staining of fungal communities

One gram of soil sample from the rhizosphere of *Dendrocalamus asper* (highest EE-GRSP) and *Bambusa nutans* (lowest EE-GRSP) was weighed, and serial dilution was done using Potato Dextrose Agar (PDA), inoculated with antibiotics for 5 days at 25°C. The colonies so obtained were morphologically characterised (colony colour, texture, margin, elevation, growth rate), and each distinct colony was sub-cultured on PDA to obtain a pure isolate. Identification was done by staining with lacto-phenol cotton blue (20% phenol, 20% lactic acid, 40% glycerol and 0.05% cotton blue) by teasing a portion of fungal isolate on a glass slide using a sterile needle and observed under a microscope. The following observations, such as, type of hyphae, mycelium colour, type of spores, characteristics of hyphae and sporangia, features of conidia, and arrangement of sporangiophore and conidiophores were recorded and captured.

Root processing for AMF spore analysis

Root samples were thoroughly washed, cut into 1 cm segments, and incubated at 90°C with 10% KOH until cleared. The roots were then rinsed multiple times with tap water and acidified in 1% HCl for 5 min to neutralise residual alkali and enhance dye absorption. Acidification improves tissue permeability and prevents dye precipitation, allowing clearer visualisation of fungal structures. The roots were then stained in 0.05% (w/v) Trypan Blue prepared in lactoglycerol and gently heated for 15 min. Excess stain was removed by transferring the roots to fresh lactoglycerol, after which the stained segments were mounted on slides and examined under a light microscope for the presence of AMF structures such as hyphae, vesicles, and arbuscules following Phillips and Hayman (1970).

Absorption Spectroscopy

Glomalin protein extracted from soil was reconstituted with distilled water to a final concentration of 0.5 mg/mL and scanned over a wavelength range of 200-500 nm using a UV-visible spectrophotometer in 1 cm quartz cuvettes at ambient temperature. Citrate buffer was used as the blank. A comparative analysis was done with specific plant growth regulators, such as ferulic acid, benzoic acid, para-aminobenzoic and n-methylaniline acid. 1mM stock solution of these compounds was diluted to a 100 µM working solution and scanned in a range of 200-500 nm. The spectra so obtained were then compared to spectra of glomalin extract.

Seed Germination Bioassay

For the germination bioassay, one negative control with distilled water, one treatment for each compound (ferulic acid, BA, NMA, PABA), and two treatments, i.e., half-strength (0.25 mg/mL) and full-strength (0.5 mg/mL), for glomalin extract were performed, each in triplicate. Each Petri dish contained an equal number of surface-sterilised soyabean seeds, placed on a well-moistened sterile filter paper with its respective solution. The plates were incubated in the dark at ambient temperature, and the germination rate was observed for 7 days and calculated as follows:

$$\text{Germination percentage (G\%)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Statistical analysis

Each treatment consisted of three replications and results were interpreted as mean ± standard deviation. The statistical analysis of the experimental data used ANOVA and Tukey's post hoc test at $p<0.05$. Each experimental value was compared with the control.

RESULTS AND DISCUSSION

Easily Extractable Glomalin Related Soil Protein (EE-GRSP)

The analysis of EE-GRSP showed clear and significant variation among the rhizosphere soils of the ten bamboo species (Fig 1). EE-GRSP content in rhizosphere soils of ten bamboo species varied from 0.388±0.04% to 0.217±0.04% with *Dendrocalamus asper* recorded the maximum (0.388±0.04%) EE-GRSP concentration, followed by *Bambusa bamboos* (0.368±0.03%), which shows statistical similarity to *Dendrocalamus hamiltonian* (0.357±0.01%)and *Bambusa multiplex* (0.354± 0.05%). The lowest value was reported in *Bambusa nutans* (0.217±0.04%), which is lower than the control, whereas the remaining species showed consistently elevated levels relative to the control (0.270±0.01%).

Total Glomalin Related Soil Protein (TGRSP)

The estimated status of TGRSP in the bamboo species was observed Fig 1. The rhizosphere of all bamboo species had higher TGRSP content than the control soil, with a significant variation of 0.740±0.06% to 0.372±0.07%. *Bambusa vulgaris waimin* exhibited the highest TGRSP content, followed by *Bambusa vulgaris striata* (0.707±0.03%), and the lowest in *Bambusa tulda* (0.372±0.07%). *Dendrocalamus asper* (0.602 ± 0.01%) showed statistically the same TGRSP value as both *D. strictus* (0.609 ± 0.02%) and *Bambusa multiplex* (0.578 ± 0.03%). Similarly, the value of *B. balcooa* (0.565±0.04) was statistically the same as *B. nutans* (0.543±0.01), and *B. multiplex* meaning that the same pattern of glomalin accumulation was observed in these species.

Fungal analysis

Fungal population were reported as colony-forming units per gram (CFU g^{-1}) of dry soil (Table 1). After incubation at 25 °C, visible colonies formed (Fig 2), and the plate with the second dilution was used to calculate CFU as it had 53 colonies, which is in the statistically acceptable range of 30-300 colonies. Thus, fungal load of *Dendrocalamus asper* rhizosphere soil has been reported as 5.3×10^4 CFU g^{-1} soil and in *B nutans* was 4.3×10^4 CFU g^{-1} soil. The cultured plates were of varied colony morphotypes such as a fluffy, cottony white colony with loose aerial mycelial growth, an orange and round colony with a smooth surface and a yellow peripheral margin, a densely fluffed white colony

with compact mycelial growth and a greenish colony with velvety texture. Microscopic observation of stained samples of representative PDA colonies showed definite fungal hyphae, conidia, and spores, confirming the fungal character of the colonies used in CFU counts (Fig 3).

AMF spore analysis

Microscopic examination of stained root samples of *Dendrocalamus asper* rhizosphere, revealed the presence of AMF spores (Fig 4) that were identifiable through high staining contrast, allowing reliable assessment of spore abundance and morphology. The characteristic AMF hyphae and vesicles were observed, along with the moderate abundance of spore. The presence of good, healthy and well-developed spores indicates favourable rhizospheric environment that facilitates active AMF colonization in the rhizosphere.

Absorption Spectrum

Two distinct peaks were observed in the UV–vis absorption spectrum of extracted glomalin, with a primary absorption peak at 230 nm (absorbance 0.396), and a secondary peak with an absorbance value of 0.168 at 320 nm, Fig 5. Beyond 330 nm, there was a decline in the spectrum, indicating minor constituents or secondary modifications in the extract. Comparative analysis with related aromatic compounds (Fig 6) revealed characteristic absorption maxima: benzoic acid at ~280 nm (A= 0.75), aniline at ~290 nm (A= 0.9), N-methylaniline at ~300 nm (A= 0.85), p-aminobenzoic acid (PABA) at ~330 nm (A= 0.95), and ferulic acid with two peaks at 220 nm (A= 0.40) and 340 nm (A= 1). Glomalin's secondary peak near 320 nm closely resembled with PABA. Similarly, the primary peak at 230 nm reflected features similar to the minor peak of ferulic acid, suggesting the presence of amine and aromatic functionalities.

Table 1: Microbial load expressed as CFU g^{-1} soil based on serial dilution.

Dilution	*D. hamiltonian* CFU/g soil	*B. nutans* CFU/g soil
10^{-1}	-	-
10^{-2}	5.3×10^4	4.3×10^4
10^{-3}	1.1×10^5	0.9×10^5
10^{-4}	6×10^5	4×10^5

Table 2: The effect of glomalin and aromatic compounds on seed (soybean seeds) germination. FA-Ferulic acid; BA-Benzoic acid; A-Aniline; NMA-n-methylaniline; PABA-p-aminobenzoic acid.

Germination % in Soyabean seeds	
Control (d. water)	93.33±2.5
Glomalin half	83.33±3.2
Glomalin Full	20.67±1.5
PABA	16.67±3.5
NMA	15.00±2.2
BA	23.33±2.5
FA	25.00±3.5

Seed Germination Bioassay

The germination assay test showed variations across the treatments (Table 2). The control group (distilled water) exhibited the highest germination percentage (93.33%), reflecting normal and unhindered seed emergence. Seeds exposed to half-strength glomalin extract displayed a lowered germination rate (83.33%) while those treated with full-strength glomalin extract experienced a significant drop, to 20.67%, highlighting a pronounced dose-dependent suppression. Within the group of compounds, ferulic acid (FA) exhibited a somewhat greater germination rate (25.00%) relative, to benzoic acid (BA; 23.33%) p-aminobenzoic acid (PABA; 16.67%) and N-methylaniline (NMA; 15.00%) with NMA resulting in the smallest germination percentage. In general, the order of treatments was: Control > Glomalin (half) > Ferulic acid > Benzoic acid > Glomalin (full) > PABA > NMA, showing that high glomalin and aromatic amine compounds strongly suppressed seed germination.

This research quantified TGRSP and EE-GRSP in bamboo species and subsequently compared them with the bulk soil. The rhizosphere of all bamboo species showed higher TGRSP and EE-GRSP content relative to the control soil, reflecting higher glomalin accumulation in the bamboo rhizospheres, except for *Bambusa nutans*, which showed a lower EE-GRSP content than the control. The reduced EE-GRSP level in *Bambusa nutans* might result from due to weaker AMF colonization, species-specific root traits, or less favourable soil–microbe interactions. These results are consistent with the

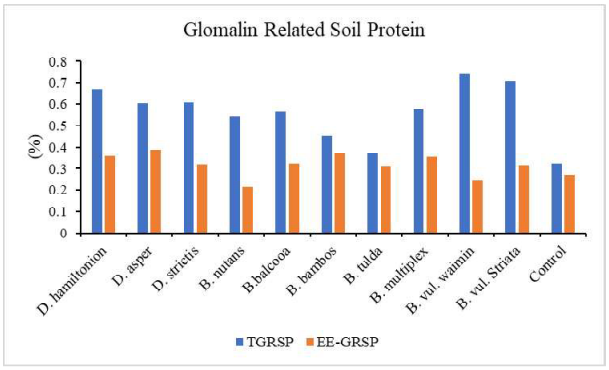

Fig 1: Comparison of Glomalin-Related Soil Protein (GRSP) among 10 bamboo species and control soil-TGRSP-Total Glomalin-Related Soil Protein; EE-GRSP; Easily extractable Glomalin-Related Soil Protein.

study of Quin *et al.* (2017), which states that the bamboo rhizosphere often displays elevated EE-GRSP (0.190 ± 0.29%) and TGRSP (0.442 ± 0.57%) compared to neighbouring vegetation types. Galazka *et al.* (2020) examined changes in glomalin-related soil proteins (GRSP) and microbial diversity based on soil type and found that the highest T-GRSP content was in soil with high biological activity, with TGRSP % and EE-GRSP ranging from 0.258% to 0.643% and 0.172% to 0.267%, respectively.

The rhizosphere soil of *Dendrocalamus asper* and *Bambusa nutans* was selected to analyse AMF and fungi, due to its high and low EE-GRSP content, respectively. EE-GRSP has been broadly considered as the more labile, newly-formed fraction of glomalin that is highly sensitive to alterations in AMF hyphal production and reflects an active AMF input to the rhizosphere protein pool (Riling, 2004). The rhizosphere soil presented a high population of fungi (5.3 × 10t CFU g^{-1} of soil) and presence of AMF hyphal structures, vesicles, and characteristics spores indicate that active fungal community able to establish successful symbiotic association between the host plant and the fungal species (Smith and Read, 2008). The current investigation found a positive correlation between fungus population density and glomalin concentration. Fungal CFU counts were consistently higher in soils with higher glomalin concentrations, while soils with lower glomalin concentrations showed relatively lower fungal populations. This symbiotic interaction is known to stimulate glomalin secretion, as AMF are the exclusive producers of GRSP (Wright and Upadhyaya, 1996). Earlier studies reported that rhizosphere fungal populations typically range between 10^3–10u CFU g^{-1}, depending on soil type, moisture, and root exudation patterns (Garbeva *et al.*, 2004; Rousk and Baath, 2011). Therefore, the CFU value obtained in our study falls within the ecological functional range and presents a healthy colonization pattern of rhizosphere fungi. Subsequent studies by Driver *et al.* (2005) and Rosier *et al.* (2006) reported a positive relationship between AMF hyphal density and GRSP, providing evidence that glomalin reflects AMF density. Field evidence from Wu *et al.* (2014) also reports that higher AMF colonization stimulates both easily extractable (EE-GRSP) and total GRSP fractions.

The chemical structure of GRSP is still unknown but GRSP extracts are reported be heterogenous mixtures of proteins, lipids, humic-like material and soil organic matter but spectroscopic and compositional studies have identified aromatic

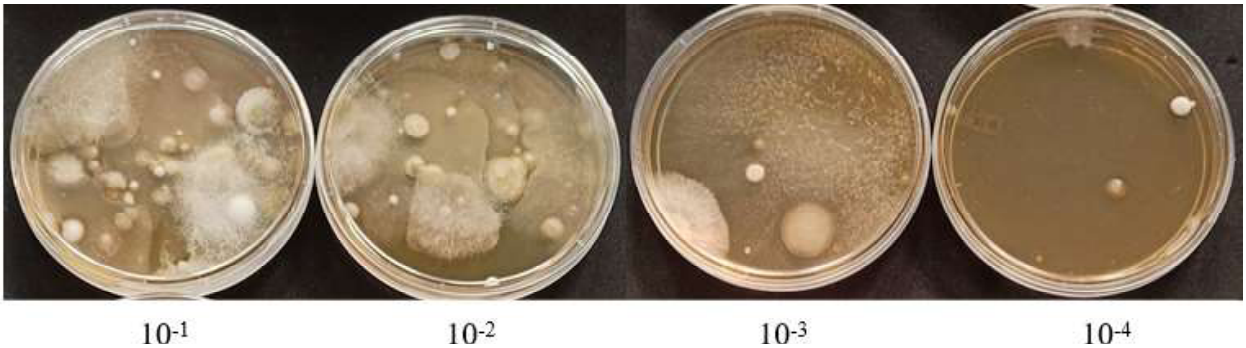

Fig 2: Serial dilution of fungal colonies isolated from the rhizosphere of *Dendrocalamus asper.*

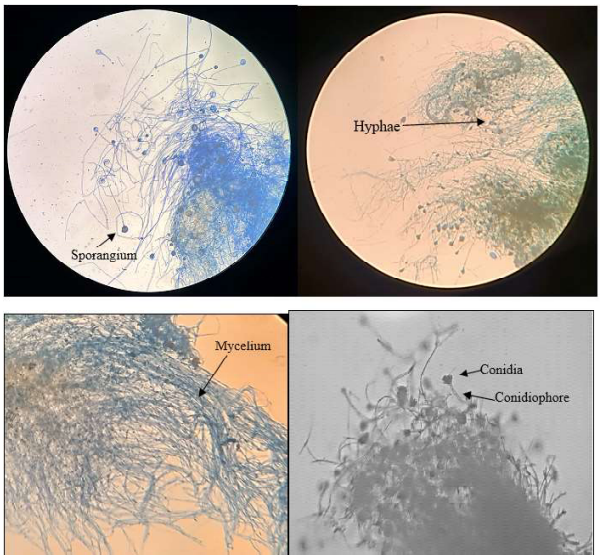

Fig 3: Staining of fungal colonies isolated from the rhizosphere of *Dendrocalamus asper.*

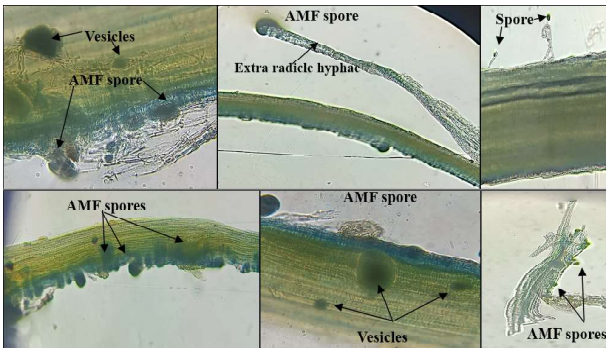

Fig 4: Staining the rhizosphere roots of *Dendrocalamus asper* showing AMF Spores, vesicles, and hyphae.

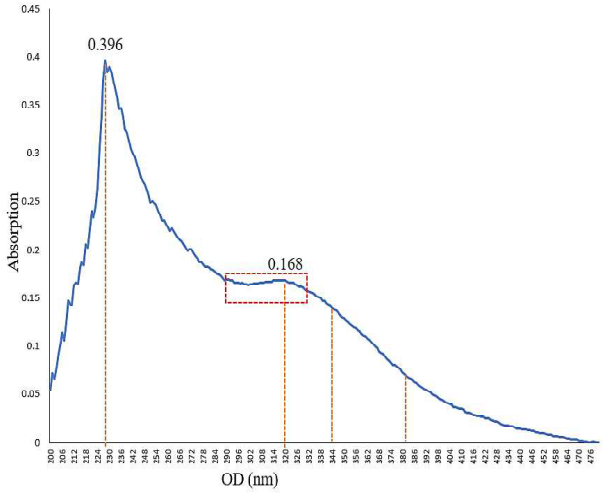

Fig 5: UV-VIS absorption spectra of glomalin.

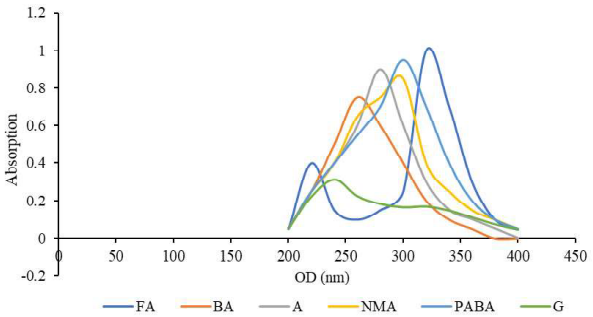

Fig 6: UV-VIS absorption spectra of glomalin. FA-Ferulic acid; BA-Benzoic acid; A-Aniline; NMA-n-methylaniline; PABA-p-aminobenzoic acid; G-glomalin.

carbon and carboxyl functional groups as well as proteinaceous fraction in GRSP with the implication that it could contain conjugated (aromatic) substructures (Gillespie *et al.,* 2010). Thus, the UV-Vis peak observed at 320 and 220 nm in our samples is more likely reflecting compound conjugated/ aromatic or amine containing domains in GRPS rather than simple small aromatics or glycoprotein. This is in accordance with the study by Son *et al.* (2024), stating that GRSP extracted from soils has functional groups like carboxyl, amide/peptide, perhaps lipids (detectable by spectroscopic / XPS / NMR analysis) rather than small aromatic compounds or neat amino acid chromophores. UV–VIS characterisation of our GRSP extracts is consistent with previous reports showing broad absorbance across the UV region ($\simeq$ 220–400 nm) rather than a single, sharp chromophore peak (Zhou *et al.,* 2022). Some field observations showed a mid-UV maximum around 286–295 nm observed by Wang *et al.* (2014), but many characterizations emphasize an overall exponential decline in absorbance with wavelength (Zhou *et al.*, 2022). Comparative analysis with related compounds could point out that glomalin's spectra have broader and less detailed peaks as a consequence of its bigger and more complex structure. Comparison on their spectra indicates a similar structure of glomalin and these tested aromatics. The major peak at 230 nm (similar to minor peak at 220 nm for ferulic acid) is primarily due to $\pi \rightarrow \pi^*$ transitions in conjugated systems, which would be expected if aromatic or peptide-like chromophores exist in glomalin. The shoulder at 320 nm, which overlaps with PABA's

absorption region reflects the presence of aromatic amine functionalities and suggests that glomalin possesses proteinaceous moieties in its structure appended with amino acids such as tyrosine, tryptophan or phenylalanine. Ferulic acid is known to be a phenolic that exhibits both longer and shorter peaks, similar to glomalin, which suggests glomalin may also have a low concentration of phenolic like moieties or conjugated groups that contribute to its UV tail beyond 330 nm. All these comparisons suggest that the molecular structure of glomalin may be a proteinaceous structure with incorporated aromatic and amine functionalities, along with minor phenolic components. Despite the numerous studies on glomalin properties, there is no strong published evidence to support the structural and spectral inferences that claims about a sharp UV-Vis peak at ~320 nm, defined $\pi \rightarrow \pi^*$ and aromatic-amine assignments, as well as that glomalin (or Glomalin-related soil protein, GRSP) acts like a small aromatic-rich molecule like simple aromatics. The exact chemical composition of GRSP is still not fully known, and recent literature suggests that GRSP is structurally complex, heterogeneous and likely a mixture, not a prototypical protein or small aromatic polymer.

It is consistent with the observation that half-strength glomalin extract had a much higher germination percentage (83.33%), which is closer to the water control (93.33%) than any of the phenolic compounds. This suggested that glomalin in low concentrations may have a facilitative but not an inhibitory effect on early plant development. This is in line with the earlier evidence that glomalin-related soil protein (GRSP) in moderate levels enhances soil aggregation, moisture retention as well as nutrient availability (Wright and Upadhyaya, 1998; Rillig, 2004). The observed large scale decrease in germination at complete glomalin concentration agrees with reports that high-molecular-weight glomalin fractions have the ability to create hydrophobic coatings that slow down the process of imbibition and radicle emergence (Rillig *et al.,* 2001, Schindler *et al.*, 2007).The complex polymeric structure of glomalin and aromatic amino acid residues could slow enzyme activation during early germination which could be related to dose-dependent inhibition between half and full concentration (Wright and Upadhyaya, 1998). Phenolic compounds like benzoic acid and ferulic acid also contain inhibitory effects, which support classical allelopathy reports, that phenolic acids disrupt cell division, mitochondrial respiration, auxin metabolism, and inhibit germination and early seedling development (Rice, 1984). The unusually high inhibition by PABA and N-methylaniline is in line with the observation that aromatic amines can cause membrane remodelling, leading to oxidative injury in germinating seeds (Huang *et al.*, 2013). In sum, it can be concluded that the glomalin at high level and aromatic amines have the most profound negative impact on germination, distilled water is the best as it has the least amount of inhibition, and phenolic acids have a rather less negative effect as well.

CONCLUSION

The present study clearly demonstrates that bamboo rhizospheres support enhanced accumulation of glomalin-related soil proteins (TGRSP and EE-GRSP) compared to bulk soil, highlighting the strong influence of bamboo–AMF associations on soil biological quality. Significant interspecific variation among bamboo species indicates that host plant identity plays a key role in regulating AMF activity, fungal abundance, and glomalin production, with *Dendrocalamus asper* exhibiting particularly favourable rhizospheric conditions. The positive relationship observed between fungal population density, AMF structures, and glomalin concentration confirms that active mycorrhizal colonization is a major driver of GRSP accumulation. Spectroscopic analyses suggest that glomalin is a structurally complex, heterogeneous macromolecule composed of proteinaceous frameworks incorporating aromatic, amine, and minor phenolic functionalities rather than simple aromatic compounds. Seed germination assays further revealed a clear dose-dependent biological effect of glomalin, where low concentrations were largely non-inhibitory, while high concentrations exerted strong suppression comparable to aromatic amines, emphasizing its dual

functional role. Overall, the findings underscore the ecological significance of bamboo–AMF–glomalin interactions in improving soil structure and stability, while also highlighting the need for refined molecular characterization of GRSP to better understand its multifunctional role in soil processes and plant–soil interactions.

ACKNOWLEDGMENTS

The Authors are grateful to Directorate of Experiment station and Agroforestry Research Center GBPUA&T Pantnagar, All India Coordinate research project on Agroforestry for all the necessary assistance to coordinate this study. The authors are highly thankful to Department of Biochemistry and Department of Biological Sciences, College of Basic Sciences and humanities, Govind Ballabh Pant University of Agriculture and Sciences, Pantnagar, Uttarakhand for providing necessary field, materials used in the study and laboratory facilities.

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Received: December 16, 2025
Accepted: December 31, 2025