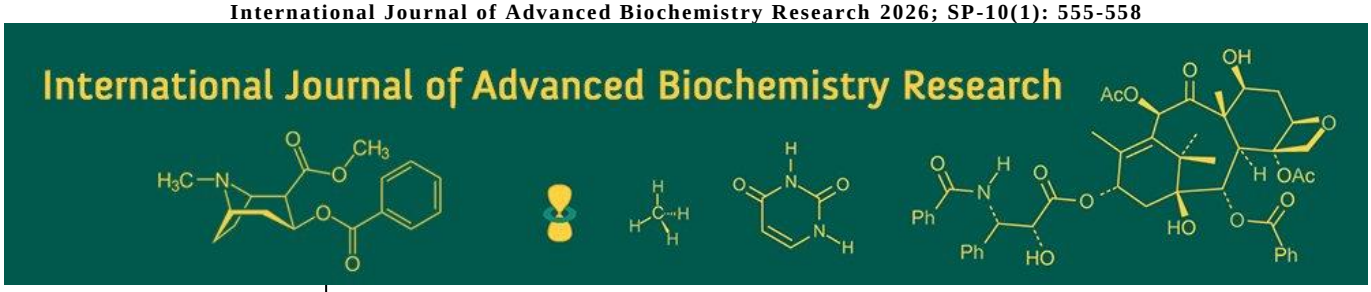

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Diversity and regenerative efficiency of arbuscular mycorrhizal fungi isolated from natural forest soils

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Abstract
Arbuscular mycorrhizal fungi (AMF) are central to soil biochemical processes involving carbon stabilization and the formation of aggregates. This study isolated native AM fungi from evergreen, deciduous, and degraded forest soils in Uttara Kannada district, Karnataka. The regenerative efficiency of twenty-two AM fungal isolates, primarily from *Glomus* and *Acaulospora* genera, was assessed under controlled conditions using wheat as a test crop. Various biochemical indicators such as organic carbon, glomalin-related soil proteins, microbial biomass carbon, and water-stable aggregates were measured. Notably, the isolate JAMFI-8 exhibited the highest organic carbon content (6.62 g kg^{-1}), microbial biomass carbon (632.87 µg g^{-1}), total glomalin (0.84 mg g^{-1}), and water-stable aggregates (73.89%). These findings underscore the significant biochemical diversity among forest-derived AM fungi and suggest their potential for soil regeneration and carbon stabilization.

Keywords: Arbuscular mycorrhizal fungi, glomalin, soil biochemistry, carbon sequestration, humic substances

Introduction
Soil chemical degradation, manifested by changes in soil organic carbon pools, microbial activity, and soil aggregate stability, has emerged as a significant global issue impacting sustainability in both ecosystems and agriculture. Soil organic carbon (SOC) plays a crucial role in regulating soil biochemical processes, such as nutrient availability, microbial functions, and the long-term storage of carbon. Intensified land use practices and disturbances disrupt these biogeochemical balances, leading to increased carbon mineralization and degradation of soil structure (Lal, 2004; Six *et al.*, 2002) [6, 15].

Within the soil environment, arbuscular mycorrhizal fungi (AMF) are recognized as key biological factors influencing soil biochemical cycles. These symbiotic fungi, forming associations with over 80% of terrestrial plant species, significantly contribute to carbon transfer from plants to soils (Smith and Read, 2008) [16]. AM fungi enhance nutrient uptake, particularly phosphorus, through their extensive hyphal networks, while also depositing substantial amounts of biologically stable carbon into soil systems (Rillig *et al.*, 2001) [13].

AM fungi play a unique role in producing glomalin-related soil proteins (GRSP), consisting of organic glycoproteins that persist in soil for extended periods. Glomalin acts as a biochemical adhesive, enhancing soil aggregate stability and shielding organic carbon from rapid microbial degradation (Wright and Upadhyaya, 1998; Rillig, 2004) [21, 12]. Numerous studies have demonstrated a positive correlation between GRSP levels and soil organic carbon, microbial biomass, and aggregate stability (Wilson *et al.*, 2009; Peng *et al.*, 2013) [19, 11], underscoring the significant contribution of AM fungi to long-term carbon sequestration.Forest ecosystems represent undisturbed environments characterized by continuous organic inputs to the soil and stable plant-microbe interactions. These interactions serve as reservoirs for highly efficient arbuscular mycorrhizal (AM) fungal communities. AM fungi naturally present in forest soils are well-adapted to nutrient-poor and stressful conditions, exhibiting greater biochemical efficiency compared to introduced or commercial inoculants. For instance, AM fungal taxa in both evergreen and deciduous forest soils can boost glomalin levels, humification, and stimulate microbial biomass, all contributing to crucial soil structural stability.

While the essential role of AM fungi in soil biochemistry is widely acknowledged, a detailed quantitative evaluation of native forest-derived AM fungal communities with regard to regenerative biochemical traits remains largely unexplored, especially in controlled experiments. Rather than focusing solely on diversity assessments or crop responses at the field level, only a few studies have conducted comprehensive screening of AM fungal isolates using laboratory and pot-culture methods. Understanding isolate-level variations in glomalin production, carbon fixation, humified substance formation, and microbial biomass increase is crucial for selecting effective AM fungal strains to support soil restoration and sustainable land management practices.

This study aims to isolate and characterize AM fungi from natural forest soils, assess their diversity based on morphological types, and measure their regenerative potential in terms of key soil biochemical parameters, such as organic carbon, glomalin-related soil proteins, microbial biomass carbon and water-stable aggregates within a controlled pot-culture system. By providing biochemical evidence, this research intends to underscore the beneficial role of non-crop (wild) AM fungi as valuable assets for soil reclamation and carbon sequestration.

Materials and methods

Study Area and Soil Sampling

The Rhizosphere soil was collected from natural forest ecosystems of Uttara Kannada district, Karnataka State, India which includes evergreen, deciduous and degraded forest patches. Sample areas were Haliyal, Dandeli, Joida, Gutti and Mirjan-Ramnagar forest ranges. Soil samples were taken at a depth of 0-10 cm after removal of surface litter, placed in sterile polyethylene bags, and stored at 4 °C until analyzed. Are dried and passed through a 2-mm sieve before AMF isolation.

Trap Pot Culturing of AM Fungi

The pot culturing was performed in trap pots.Trap pot cultures were used to improve spore recovery and viability since some species of AM fungi do not produce spores by the time of soil sampling (Patricia *et al.*, 2009) [10]. Forest soil was mixed with sterilized vermiculite in a 1:1 proportion and finger millet (*Eleusine coracana*) was used as the host plant. Plants were grown in pots under greenhouse conditions and watered regularly. Hoagland's nutrient solution (0.1 mL/pot) was added weekly. The AM fungal spores were extracted from the trap culture after three months for identification and multiplication.

Collection of AM Fungal Spores

AMF spores were extracted by the wet sieving and decanting technique (Gerdemann and Nicholson, 1963) [4]. Soil suspensions were sieved through 500, 250, 106, 75, 45 and 37 µm. Sonicates from sediment trapped on the fine meshes were purified using sucrose density gradient centrifugation (Ohms, 1957) [9]. Subsequent to the above mentioned treatments, spores were disinfected with 200ppm streptomycin sulphate and 2% chloramine-T and stored for further tests.

Morphological Identification of AMFs

The spores were isolated and mounted in lactophenol for observation under a compound microscope. They were identified according to standard taxonomic keys (Rodrigues and Muthukumar, 2009) [14] and reference descriptions present in the INVAM database using spore size, color, wall structure, surface ornamentation, and hyphal attachment. With unique morphotypes were incoded the site of origin.

Multiplication and Scaling-Up of AM Fungi Inoculum

Single representative spores were propagated using funnel and pot culture with a sterile sand:soil mix (1:1) and finger millet as the host plant. Hoagland nutrient solution was given weekly. Upon successful colonization, cultures were transferred to vermiculite pot cultures and maintained there during 45 days in order to obtain enough inoculum of spores-colonized root fragments and extraradical hyphae.

Pot Culture Experiment

Single AM fungal isolates were tested under greenhouse conditions in a pot culture experiment. Sterilized degraded red soil was filled in the pots and they were inoculated in the vicinity of drip zone. The test crop was wheat (*Triticum aestivum*). Uninoculated soil was used as control. The plants were cultivated for 45 days and soil samples were collected, air-dried, sieved and processed according to biochemical analysis.

Biochemical Analysis of Soil

Soil Organic Carbon

Soil organic carbon was determined by the Walkley and Black (1934) [18] wet oxidation method. Values were given g kg^{-1} soil.

Glomalin-Related Soil Proteins

Glomalin was isolated as per Wright and Upadhyaya (1996) [20] using 50 mM citrate buffer (pH 8.0). Total protein concentration was determined by the Bradford dye-binding assay (Bradford, 1976) [2] and expressed as mg g^{-1} soil.

Microbial Biomass Carbon

The soil microbial biomass carbon was determined by the chloroform fumigation-extraction method (Brookes *et al.*, 1987) [3] and expressed as µg g^{-1} soil.

Water-Stable Aggregates

Soil aggregate stability was based on the wet sieving technique of Yoder (1936) [22]. Percent of soil weight consisted water-stable aggregates.

Statistical Analysis

Statistical analysis of the data from pot culture experiment was performed by suitable analysis of variance. Mean values were compared statistically using the Student t-test at 1 and 5% level of probability according to standard procedures (Gomez and Gomez, 1984) [5].

Results

Variety of Arbuscular Mycorrhizal Fungi

The isolation and trap culturing of forest soils yielded 22 morphologically identifiable AMF isolates. The fungal isolates predominantly were that of the genus *Glomus* (12), *Acaulospora* (9) and one *Funneliformis*. There was quite a variation in morphology among the isolates. The spores were 32.0-74.6 µm in diameter and the thickness of the spore wall was 2.1-8.99 µm; the pale-yellow to dark-brown colour and smooth to rough surface ornamentation of tubes were recorded. This morphological variety reflects

functional diversity of AG(Db) AM fungi obtained from forest soil.

Influence of AM Fungal Isolates on Soil Organic Carbon concentration

Soil organic carbon (SOC) content was significantly different across the AM fungal treatments. SOC content of AMF inoculated soils varied from 4.62 to 6.62 g kg^{-1}, whereas that in the uninoculated control was 3.75 g kg^{-1}. JAMFI-8 (6.62 g kg^{-1}) had the greatest SOC, followed by JAMFI-7 (5.39 g kg^{-1}) and MAMFI-15 (5.38 g kg^{-1}) (Table. 1).

Glomalin-Related Soil Protein Content

AM fungal inoculation strongly increased GRSP levels. The concentrations of EZ-glomalin (EE-GRSP) varied from 0.011 to 0.054 mg g^{-1} of soil, and that for T-GRSP, ranged between 0.19 and 0.84 mg g^{-1} of soil. The superior values for EE-GRSP (0.054 mg g^{-1}) and T-GRSP (0.84 mg g^{-1}) were found in isolate JAMFI-8, whereas control had only 0.005 mg g^{-1} EE-GRSP and 0.10 mg g^{-1} T-GRSP (Table. 1).

Microbial Biomass Carbon

The content of soil microbial biomass carbon (MBC) was remarkably increased due to inoculation with AM fungi. MBC was ranging from 282.99 to 632.87 µg g^{-1} soil in AMF-soil, compared with the value of MBC = 106.77 µg g^{-1} soil for the control. JAMFI-8 recorded the highest MBC (632.87 µg g^{-1}), while JAMFI-7 recorded the least (534.78 µg g^{-1}) (Table. 1).

Water-Stable Aggregates

Treatments with AMF had higher WSA (40.71-73.89%) contents than the control (38.00%). JAMFI-8 registered the highest WSA (73.89%) followed by JAMFI-7 and MAMFI-15 with 57.91% and 55.21%, respectively) (Table. 1).

Discussion

The study's findings reveal the high diversity of arbuscular mycorrhizal (AM) fungi in natural forest soils concerning their role in biochemical processes. The predominance of *Glomus* and *Acaulospora* species aligns with prior research indicating their prevalence and functional dominance in intact ecosystems (Smith and Read, 2008; Oehl *et al.*, 2010) [16, 8].

The increased soil organic carbon (SOC) in AMF-inoculated soils suggests the participation of mycorrhizal fungi in organic matter stability through hyphal contributions and enhanced aggregate formation. The notably higher SOC levels with JAMFI-8 isolate suggest isolate-specific efficacy, emphasizing the importance of screening indigenous AM fungi pre-application (Six *et al.*2002) [15].

Glomalin-related soil proteins emerged as key biochemical markers of soil restoration. The elevated total glomalin-related soil protein (T-GRSP) content (0.84 mg g^{-1}) associated with JAMFI-8 supports previous findings on glomalin's role in soil aggregation and carbon protection (Wright and Upadhyaya, 1998; Rillig, 2004) [21, 12]. The significant correlation between GRSP and aggregate stability underscores the contribution of AM fungi as potential binding agents following long-term native AM fungi addition to soil (Wilson *et al.*, 2009) [19].

The increase in microbial biomass carbon (MBC) due to AM colonization signifies the activation of soil microorganisms triggered by additional carbon availability and improved micro-habitat conditions facilitated by extraradical hyphae. Similar enhancements in microbial biomass have been documented previously as a consequence of AM fungal activity (Brookes *et al.*, 1987, Barea *et al.*, 2005) [3, 1].

In conclusion, JAMFI-8 isolate consistently exhibited superior performance across various biochemical parameters compared to other AM fungal isolates, including SOC, GRSP, microbial biomass, and aggregate stability, showcasing its strong regenerative ecophysiological potential. These findings underscore the significance of indigenous forest AM fungi as effective enhancers of soil structure and biochemical properties.

Table 1: Effect of selected arbuscular mycorrhizal fungal isolates on soil biochemical properties

Treatment	Soil organic carbon (g kg^{-1})	EE-GRSP (mg g^{-1} soil)	T-GRSP (mg g^{-1} soil)	Microbial biomass carbon (µg g^{-1} soil)	Water-stable aggregates (%)
Control	3.75	0.005	0.10	106.77	38.00
JAMFI-7	5.39	0.038	0.62	534.78	57.91
JAMFI-8	6.62	0.054	0.84	632.87	73.89
MAMFI-15	5.38	0.031	0.57	498.24	55.21
HAMFI-22	4.62	0.011	0.19	282.99	40.71
SEm ±	0.11	0.002	0.03	12.41	1.82
CD (P = 0.05)	0.32	0.006	0.09	36.18	5.31

Conclusion

The current study presented a significant variation between AMF isolates from natural forest soils and also showed prominent isolate-specific variation in their regenerative biochemical effectiveness. Forest-based AM fungal isolates, in particular those belonging to the genera *Glomus* and *Acaulospora*, showed a high potential to enhance selected soil biochemical parameters under controlled conditions.

Out of the isolates tested, JAMFI-8 consistently performed better than other in improving soil organic carbon, glomalin related soil proteins, microbial biomass carbon and formation of water stable aggregate. Increased glomalin production and aggregate stability-in particular-attest to the central biochemical role of AM fungi during soil structure formation, as well as in organic matter stabilization. An increased microbial biomass is also a sign of the stimulation by mycorrhizal symbiosis of biological activity in soil.

The results also stressed the necessity of the fine biochemical screening of native AM fungal isolates because the functional performance between isolates from a single ecosystem was very variable. AM fungi derived from forest may be major bioresources for promoting structural and chemical functioning of the soil. Of these, the JAMFI-8 isolate particularly emerges with prospects for further testing and application in sustainable management and rejuvenation of soils.

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