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# Optimization of Nursery Inoculation Technique for Arbuscular Mycorrhiza and Field Evaluation with Microbial Consortia on Growth and Yield of Finger Millet (*Eleusine caracana* L.)

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## ABSTRACT

The inconsistent field performance of biofertilizers in short-duration millets is often attributed to delayed microbial colonization following transplanting. To address this limitation, the present study aimed to standardize a nursery bio-hardening technique for arbuscular mycorrhizal fungi (AMF) and to evaluate the field efficacy of a two-step microbial consortium approach in finger millet (*Eleusine coracana* L.). A destructive time-course experiment was conducted to optimize AMF inoculum density and nursery duration for effective root colonization. Results indicated that application of 200 g *Glomus fasciculatum* inoculum per 5 kg nursery substrate and a minimum nursery period of 30 days were essential to overcome the lag phase, achieving a functional root colonization of 65.48 per cent. Based on this standardization, bio-hardened seedlings were subjected to a root-dip inoculation with *Azotobacter salinestris* and *Pseudomonas nitroreducens* and evaluated under field conditions using a randomized complete block design. The triple microbial consortium significantly enhanced plant growth, root system architecture and yield attributes. The treatment comprising *G. fasciculatum* + *A. salinestris* + *P. nitroreducens* recorded the highest grain yield (3850 kg ha$^{-1}$), representing a 79.1 per cent increase over the uninoculated control, along with a two-fold increase in root volume and sustained mycorrhizal colonization (78.8%) at harvest. Post-harvest soil analysis revealed a positive nutrient balance, with increased available nitrogen and phosphorus compared to the initial status. The study demonstrates that nursery Inoculation combined with consortium inoculation is an effective rhizosphere engineering strategy for enhancing productivity, drought resilience and soil fertility in finger millet under semi-arid conditions.

*Keywords* : Colonization, Microbial constrain, Nursery inculation, Rhizosphere

FINGER millet (*Eleusine coracana* (L.) Gaertn.), widely recognized as a 'Super Cereal,' is a key component of Climate Smart Agriculture (CSA) due to its exceptional resilience to climatic stresses. Traditionally cultivated in the arid and semi-arid tropics of Africa and Asia, its global importance was reinforced by the United Nations declaration of 2023 as the International Year of Millets. Beyond its perception as a subsistence grain, finger millet is now acknowledged as a 'Future Food' capable of addressing nutritional insecurity and climate variability. Agronomically, its C4 photosynthetic pathway, strong tillering ability and tolerance to salinity, drought and heat confer superior yield stability compared to rice and wheat under erratic rainfall conditions (Gupta *et al.,* 2024).

Nutritionally, finger millet possesses an exceptional biochemical profile, particularly its calcium content the highest among cereals making it a vital dietary component for combating osteopenia and metabolic disorders. Its low glycemic index and high polyphenol concentration further establish its role as a functional food, driving increased demand for millet-based value-added products and necessitating enhanced crop productivity (Kumar *et al.,* 2023). India remains the global leader in finger millet production, with Karnataka-known as the 'Ragi Bowl' of India-being the principal cultivation hub. However, yields in Karnataka's dryland regions remain well below genetic potential due to poor seedling establishment, declining soil organic carbon and phosphorus fixation in acidic red soils.

Sustainable finger millet production is therefore closely linked to rhizosphere biological health. Although chemical fertilizers have been widely used to overcome yield constraints, escalating input costs and environmental degradation from nitrate leaching and soil acidification have made such practices economically unsustainable. Prolonged application of phosphate fertilizers has resulted in high total soil phosphorus but critically low bio-available phosphorus due to fixation, creating a 'nutrient paradox' that necessitates a shift from chemical supplementation to rhizosphere-based biomobilization strategies (Das & Mishra, 2025).

In this context, microbial consortia offer a viable alternative. Arbuscular Mycorrhizal Fungi (AMF), often described as a plant's secondary root system, significantly enhance nutrient uptake by expanding the root absorptive surface through extensive hyphal networks. The mycorrhizosphere further supports synergistic interactions among AMF, Azotobacter spp., and phosphate-solubilizing Pseudomonas spp., forming a probiotic consortium. While Azotobacter contributes atmospheric nitrogen fixation and phytohormone production, Pseudomonas facilitates phosphorus solubilization, which is subsequently absorbed *via.,* AMF hyphae, resulting in enhanced plant growth and vigor (Iyer & Rao, 2024).

Despite these advantages, inconsistent field performance has limited the adoption of biofertilizers, primarily due to ineffective delivery methods. Field broadcasting often leads to poor microbial establishment under competitive and moisture-stressed conditions. Nursery inoculation addresses this limitation by enabling early and effective colonization under controlled conditions, ensuring functional symbiosis before transplanting. Accordingly, the present study aimed to standardize inoculum density and exposure duration for effective colonization and to evaluate the impact of a two-step inoculation strategy on root architecture, yield and soil fertility under field conditions.

## MATERIAL AND METHODS

The present investigation, formally entitled 'Studies on Nursery Inoculation Technique of Arbuscular Mycorrhizal Fungi for Finger Millet (*Eleusine coracana*) and Interaction Effects with Nitrogen Fixer and Phosphate Solubilizer on Growth and Yield,' was systematically carried out at the Department of Agricultural Microbiology, University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK), Bengaluru.

### Experimental Site and Geo-Climatic Conditions

Laboratory-based experiments, encompassing the isolation of native AMF spores and mass multiplication of bacterial bio-agents, were executed in the research laboratories of the Department of Agricultural Microbiology. The nursery standardization and subsequent pot culture experiments were conducted in the greenhouse of Agricultural Microbiology, while the field evaluation was carried out at the Zonal Agricultural Research Station (ZARS), GKVK. The experimental site is geographically situated at 12° 58' North latitude and 77° 35' East longitude, at an altitude of 930 meters above mean sea level. The prevailing climate is semi-arid tropical and the soil is classified as red sandy loam (Alfisol), characterized by low available phosphorus due to high fixation capacity (pH 5.8).

### Microorganisms and Inoculation Protocol

The study utilized native, efficient strains obtained from the culture collection of the Dept. of Agril.

Microbiology, selected for their adaptability to the local edaphic conditions. The fungal partners included two species of arbuscular mycorrhizal fungi, *Glomus fasciculatum* and *Glomus mosseae*. These obligate symbionts were maintained and multiplied using the 'Trap Pot Culture' technique with maize (*Zea mays*) as the host to ensure high spore viability and infectivity. The bacterial partners included a free-living nitrogen fixer, *Azotobacter salinestris*, maintained on nitrogen-free Waksman No. 77 medium and a phosphate solubilizing bacterium, *Pseudomonas nitroreducens*, maintained on Pikovskaya's medium containing tricalcium phosphate. To ensure the successful establishment of the microbial consortium, a novel 'Two-Step' inoculation protocol was employed throughout the study. The first step, termed 'Nursery Bio-hardening,' involved the pre-colonization of seedlings. Seedlings were raised in nursery trays where the substrate was inoculated with the soil-based AMF culture at specific densities. This step was designed to establish the symbiotic network prior to transplanting. The second step, known as the 'Root Dip,' was performed at the time of transplanting (30 days after sowing). The roots of the pre-colonized seedlings were carefully uprooted and dipped in a lignite-based bacterial slurry containing *Azotobacter salinestris* (10x CFU/ml) and *Pseudomonas nitroreducens* (10x CFU/ml) for 30 minutes. This facilitated the immediate formation of bacterial biofilms on the rhizoplane.

## Standardization of Nursery Bio-Hardening

To determine the biological and economic optimum for inoculum application, a destructive time-course experiment was conducted. Finger millet seedlings (var. GPU-28) were raised in sterilized vermicompost-soil substrate. The treatments included graded levels of AMF inoculum to determine the saturation point of colonization. The experimental groups were designated as $D_1$ (50 g inoculum per 5 kg nursery substrate), $D_2$ (100 g inoculum per 5 kg nursery substrate), $D_3$ (150 g inoculum per 5 kg nursery substrate) and $D_4$ (200 g inoculum per 5 kg nursery substrate), alongside an uninoculated control. Sampling was performed at 5-day intervals, specifically at 10, 15, 20, 25 and 30 days after sowing (DAS), to monitor the infection kinetics. Root colonization percentage was determined using the gridline intersect method to identify the specific time

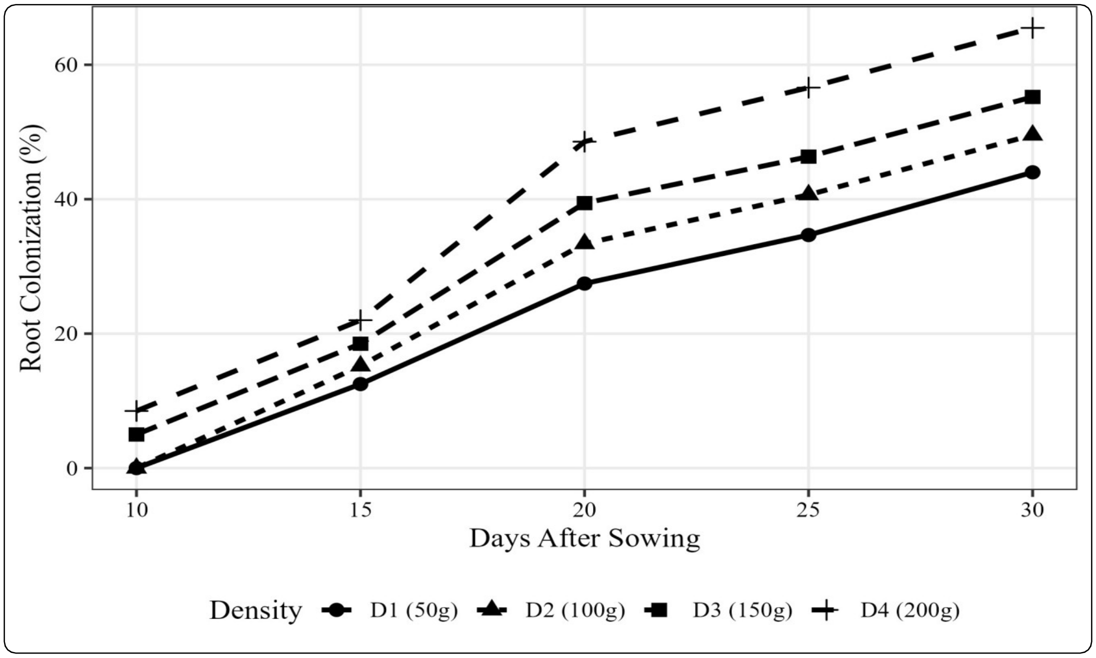

Fig. 1 : Kinetics of root colonization at different intervals

point where colonization plateaued (Giovanetti and Mosse, 1980).

### Field Evaluation

Based on the results of the standardization phase, the optimal protocol utilizing 200 g inoculum and a 30-day duration was used to raise bio-hardened seedlings for the field trial. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The plot size was maintained at 3.0 m × 2.4 m. The treatment structure was designed to dissect the individual and combined effects of the microbial partners, as detailed in Table 1.

### Data Collection and Statistical Analysis

Comprehensive biometric observations were recorded at physiological maturity. Vegetative parameters such as plant height and the number of productive tillers were measured to assess vigor. A detailed analysis of root architecture was conducted by retrieving the root system using a core sampler. The roots were washed, and root volume was measured *via.,* the water displacement technique. Yield attributes, including grain and straw yield, were recorded from the net plot area and converted to kg/ha. To assess the environmental impact, post-harvest soil analysis was conducted. Soil samples were analyzed for available nitrogen using the alkaline permanganate method (Subbih and Asija, 1956), available phosphorus using Olsen's extraction method (Olsen *et al.,* 1954) and available potassium using the ammonium acetate extraction method (Hanway and Heidel, 1952). The experimental data were subjected to rigorous statistical scrutiny using Analysis of Variance (ANOVA). Fisher's Least Significant Difference (LSD) was used to separate treatment means at a probability level of $p = 0.05$.

## RESULTS AND DISCUSSION

The results obtained from findings of the present investigation, which was meticulously designed to standardize the bio-hardening technique and evaluate the 'Triple Synergy' under field conditions, are presented below. The data provide compelling evidence that manipulating the rhizosphere microbiome through a structured inoculation protocol can fundamentally alter crop physiology and productivity.

### Standardization of Nursery Bio-Hardening: Kinetics of Root Colonization

The success of any bio-inoculation technology, particularly for transplanted crops like finger millet,

TABLE 1

**Treatment details for the field experiment evaluating microbial consortia in finger millet**

| Treatment details | Rationale |
|---|---|
| T1-Control (Uninoculated) | Baseline to measure natural soil fertility potential. |
| T2-*Glomus fasciculatum (GS)* | To assess the individual efficacy of the native AMF strain. |
| T3-*Glomus mosseae (GM*) | To assess the individual efficacy of the second AMF strain. |
| T4-*Azotobacter salinestris (AS)* | To evaluate the contribution of biological nitrogen fixation. |
| T5-*Pseudomonas nitroreducens (PN)* | To evaluate the contribution of phosphate solubilization. |
| T6-*GF + ASa* | Dual inoculation to test AMF-Nitrogen fixer synergy. |
| T7-*GM + AS* | Dual inoculation to test AMF-Nitrogen fixer synergy. |
| T8-*GF + PN* | Dual inoculation to test P-mobilization (Solub + Transport). |
| T9-*GM + PN* | Dual inoculation to test P-mobilization (Solub + Transport). |
| T10-*GF+AS + PN* | Triple Consortium: Testing the complete 'Triple Synergy'. |
| T11-*GM + AS + PN* | Triple Consortium: Testing the complete 'Triple Synergy' |

**TABLE 2**

**Arbuscular Mycorrhizal root colonization (%) in finger millet seedlings under graded inoculum density**

| Treatments/ inoculum density | 10 DAS | 15 DAS | 20 DAS | 25 DAS | 30 DAS |
|---|---|---|---|---|---|
| 50g / 5kg | 0.00 | 12.50 | 27.45 | 34.67 | 44.00 |
| 100g / 5kg | 0.00 | 15.20 | 33.39 | 40.66 | 49.54 |
| 150g / 5kg | 5.00 | 18.50 | 39.42 | 46.33 | 55.21 |
| 200g / 5kg | 8.50 | 22.00 | 48.56 | 56.60 | 65.48 |
| Control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| S.Em ± | 0.25 | 0.54 | 0.49 | 0.55 | 0.23 |
| C.D | 0.80 | 2.72 | 2.47 | 2.73 | 1.14 |

is strictly contingent upon the intensity of the fungal network established within the root cortex prior to transplanting. The destructive time-course analysis presented in Table 2 reveals that root colonization is governed by both temporal kinetics and inoculum density. In the initial phases of seedling growth, specifically at 10 and 15 days after sowing (DAS), colonization was either absent or negligible across the lower inoculum densities ($D_1$ and $D_2$). This early period represents the lag phase, a critical window where fungal spores must break dormancy, germinate, perceive chemical signals such as strigolactones exuded by the host roots and form appressoria to penetrate the root epidermis. Evidence from transplanted cereal and millet crops supports this mechanism. In finger millet and rice nurseries, studies have shown that insufficient colonization prior to transplanting results in delayed mycorrhizal establishment after transplanting, due to root damage and disruption of hyphal continuity during uprooting (Wang *et al.,* 2017).

However, a distinct dose-dependent response was observed as the nursery duration extended. The application of 200 g of inoculum per 5 kg of substrate ($D_4$) significantly accelerated the infection process. This treatment achieved 8.50 per cent colonization by 10 DAS and 22.00 per cent by 15 DAS. Subsequently, this treatment followed a rapid sigmoid growth curve and reached a functional saturation point of 65.48 per cent at 30 DAS. In contrast, the lowest density treatment ($D_1$ : 50 g) achieved only 44.00 per cent colonization by the end of the nursery period (25 DAS). Statistically, the colonization level in $D_4$ was significantly superior to all other treatments. For effective bio-hardening, a high propagule pressure of 200 g per tray combined with a minimum duration of 30 days is essential. This specific combination ensures that the fungal mycelium is deeply integrated into the root cortex, allowing the fungal-root bridge to survive the physical stress of transplanting and immediately facilitate water and nutrient acquisition in the main field (Zhao *et al.*, 2023).

### Influence of Microbial Inoculants on Vegetative Growth of Finger Millet under Field Conditions

The translation of nursery vigor to field performance was clearly evident in the vegetative metrics recorded at harvest, as detailed in Table 3. The plants subjected to the 'Two-Step' inoculation protocol exhibited robust growth, validating the hypothesis that bio-hardened seedlings possess superior recovery capabilities following transplant shock. Among the various treatments, the triple consortium ($T_{10}$: *G. fasciculatum* + *A. salinestris* + *P. nitroreducens*) consistently recorded the highest growth parameters. Treatment $T_{10}$ achieved a maximum plant height of 94.50 cm, which was

TABLE 3

Influence of microbial inoculants on vegetative growth of finger millet

| Treatment Details | Plant height (cm) | Productive tillers (No./hill) |
|---|---|---|
| T1-Control (Uninoculated) | 81.50 | 4.20 |
| T2-*Glomus fasciculatum (GS)* | 86.40 | 5.00 |
| T3-*Glomus mosseae (GM)* | 85.94 | 4.90 |
| T4-*Azotobacter salinestris (AS)* | 83.00 | 4.50 |
| T5-*Pseudomonas nitroreducens (PN)* | 83.50 | 4.60 |
| T6-*GF + AS* | 89.50 | 5.80 |
| T7-*GM + AS* | 88.20 | 5.60 |
| T8-*GF + PN* | 90.50 | 5.90 |
| T9-*GM + PN* | 89.80 | 5.80 |
| T10-*GF+AS + PN* | 94.50 | 7.20 |
| T11-*GM + AS + PN* | 93.80 | 7.00 |
| S.Em ± | 1.80 | 0.35 |
| C.D. (P=0.05) | 5.40 | NS |

significantly higher than the uninoculated control height of 81.50 cm and statistically on par with the recommended chemical fertilizer treatment. The results were in accordance with the *in-vitro* plant growth parameter observed by (Bhagyashree *et al.,* 2023).

More critically, the microbial treatments significantly influenced the productive tiller potential, which is a key yield determinant in millets. $T_{10}$ recorded 7.20 productive tillers per hill, representing a massive 71.4 per cent increase over the control ($T_1$), which recorded only 4.20 tillers. In cereal physiology, tillering is regulated by nitrogen availability and cytokinin levels during the early vegetative phase. The presence of *Azotobacter salinestris* in the rhizosphere likely provided a continuous supply of fixed nitrogen in the form of ammonium, while simultaneously secreting phytohormones such as indole-3-acetic acid (IAA) and gibberellins. These hormones function to break apical dominance and stimulate lateral bud development (Mehta *et al.*, 2024). Furthermore, the synergy with *Pseudomonas*, acting as a P-solubilizer and *Glomus*, acting as a P-transporter, ensured that phosphorus was not limiting. Since phosphorus is the energy currency required for the energy-intensive process of nitrogen fixation, this balanced nutritional status prevented the abortion of late-formed tillers. The result was a synchronized maturity and a significantly higher count of ear-bearing shoots.

## Influence of Microbial Inoculants on Yield Parameters

The cumulative effect of below-ground engineering and enhanced vegetative vigor culminated in significant yield advantages, as shown in Table 4. Treatment $T_{10}$ recorded the highest grain yield of 3850 kg/ha, representing a remarkable 79.1 per cent yield gain over the uninoculated control, which yielded 2150 kg/ha. Similarly, the straw yield increased to 6200 kg/ha in $T_{10}$, significantly benefiting the integrated crop-livestock systems prevalent in the region where finger millet straw is considered a premium fodder.

The yield data underscores the validity of the 'Triple Synergy' hypothesis. Treatments involving only N-fixers ($T_4$) or only P-solubilizers ($T_5$) showed moderate gains of approximately 11 to 13 per cent and these results are in line with Nagaraj *et al.,* (2017). However, when these agents were combined in the triple consortium ($T_{10}$), the yield gain was not merely additive but multiplicative. This suggests that the consortium removed multiple nutritional constraints simultaneously. Specifically, *Pseudomonas* solubilized the fixed phosphorus in the red soil, AMF transported it to the plant and *Azotobacter* utilized this phosphorus to fuel nitrogen fixation. Total biomass accumulation is the integrative sum of a plant's photosynthetic efficiency over its lifecycle. The data in Table 4.4 shows that $T_{10}$ achieved a total biomass of 38.50 g/plant, which is more than double the biomass of the control (18.00 g/plant, a 113.8% increase). This significant accumulation of dry matter is indicative of enhanced carbon capture and Use Efficiency. The Bio-hardened

**TABLE 4**

**Influence of microbial inoculants on yield parameters of finger millet under field conditions**

| Treatments | Grain Yield (kg/ha) | Total biomass (g/plant) | Yield Increase over Control (%) | Straw Yield (kg/ha) | Harvest Index (%) |
|---|---|---|---|---|---|
| T1-Control (Uninoculated) | 2150 | 18.00 | - | 3900 | 35.50 |
| T2-*Glomus fasciculatum (GS)* | 2850 | 28.50 | 32.60 | 4800 | 37.30 |
| T3-*Glomus mosseae (GM)* | 2790 | 27.80 | 29.80 | 4750 | 37.00 |
| T4-*Azotobacter salinestris (AS)* | 2400 | 25.00 | 11.60 | 4150 | 36.60 |
| T5-*Pseudomonas nitroreducens (PN)* | 2450 | 25.50 | 13.90 | 4200 | 36.80 |
| T6-*GF + AS* | 3350 | 32.00 | 55.80 | 5400 | 38.30 |
| T7-*GM + AS* | 3250 | 31.50 | 51.20 | 5300 | 38.20 |
| T8-*GF + PN* | 3400 | 33.50 | 58.10 | 5550 | 38.00 |
| T9-*GM + PN* | 3320 | 32.80 | 54.40 | 5450 | 37.80 |
| T10-*GF+AS + PN* | 3850 | 38.50 | 79.10 | 6200 | 38.30 |
| T11-*GM + AS + PN* | 3780 | 37.80 | 75.80 | 6100 | 38.30 |
| S. Em ± | 115 | 1.20 | - | 180 | - |
| C.D. (P=0.05) | 340 | 3.60 | - | 530 | - |

**TABLE 5**

**Root architecture and mycorrhizal status of finger millet under field conditions**

| Treatments | Root length (cm) | Root volume (cc) | Root colonization (%) |
|---|---|---|---|
| T1-Control (Uninoculated) | 24.50 | 18.00 | 22.00 |
| T2-*Glomus fasciculatum (GS)* | 28.50 | 25.50 | 58.50 |
| T3-*Glomus mosseae (GM)* | 27.80 | 24.00 | 56.10 |
| T4-*Azotobacter salinestris (AS)* | 26.00 | 21.50 | 25.50 |
| T5-*Pseudomonas nitroreducens (PN)* | 26.50 | 22.00 | 26.00 |
| T6-*GF + AS* | 32.00 | 30.50 | 65.50 |
| T7-*GM + AS* | 31.00 | 29.00 | 62.20 |
| T8-*GF + PN* | 32.50 | 31.00 | 66.00 |
| T9-*GM + PN* | 31.50 | 29.50 | 64.50 |
| T10-*GF+AS + PN* | 36.50 | 38.00 | 78.80 |
| T11-*GM + AS + PN* | 35.80 | 36.50 | 76.50 |
| S.Em ± | 0.90 | 1.20 | 2.10 |
| C.D. (P=0.05) | 2.65 | 3.50 | 6.20 |

plants in $T_{10}$ and $T_{11}$ played a pivotal role here. The Harvest Index (HI) also showed improvement, reaching 38.30 per cent in $T_{10}$. This indicates efficient partitioning of photosynthates from the source leaves to the sink grain. It is hypothesized that the microbial inoculation delayed leaf senescence, known as the 'stay-green' trait, allowing for a longer duration of grain filling, thereby increasing the test weight and reducing chaffiness (Bhattacharya and Ghosh, 2024).

### Modification of Root Morphology and Mycorrhizal Status at Harvest

The most profound impact of the study was observed in the 'Hidden Half' of the plant, as presented in Table 5. The root morphology was significantly altered by the bio-inoculants, confirming the concept of 'Rhizosphere Engineering.' Treatment $T_{10}$ recorded a maximum root length of 36.50 cm and a root volume of 38.00 cc. This represents more than double the root volume of the control plants, which measured only 18.00 cc. This modulation of Root System Architecture (RSA) is the mechanistic basis for the observed yield stability. The enhanced root volume acts as a comprehensive mining tool that significantly increases the 'Effective Root Zone,' or the volume of soil explored by the plant. This allowed the inoculated plants to access subsoil moisture and immobile nutrients that were spatially unavailable to the limited root system of uninoculated plants. The persistence of the introduced AMF strain was confirmed by the high root colonization percentage of 78.80 per cent at harvest in $T_{10}$, compared to the low native colonization of 22.00 per cent in the control. The interaction between *Pseudomonas*, which produces gluconic acid and AMF, which transports solubilized phosphorus (P), likely stimulated this aggressive root proliferation (Trivedi and Wall, 2025). Furthermore, the denser root system contributes to higher Relative Leaf Water Content (RLWC) during dry spells, conferring drought avoidance.

### Influence of Microbial Inoculants on Soil Nutrient Status after Harvest

A critical tenet of sustainable intensification is ensuring that high yields do not result in 'soil mining' or the depletion of natural reserves. The post-harvest soil analysis, detailed in Table 6, provides compelling evidence of fertility restoration through biological means. The uninoculated control plots ($T_1$) showed a net depletion of nitrogen, dropping to 190.00 kg/ha from the initial 210.50 kg/ha and phosphorus, dropping to 15.50 kg/ha from 18.20 kg/ha. This

TABLE 6

Influence of microbial inoculants on soil nutrient status after harvest

| Treatments | Avail. N (kg/ha) | Avail. $P_2$ $O_2$ (kg/ha) | Avail. K, O (kg/ha) |
|---|---|---|---|
| Initial | 210.50 | 18.20 | 195.00 |
| T1-Control (Uninoculated) | 190.00 | 15.50 | 185.00 |
| T2-*Glomus fasciculatum (GS)* | 215.50 | 20.50 | 198.00 |
| T3-*Glomus mosseae (GM)* | 212.00 | 20.00 | 196.00 |
| T4-*Azotobacter salinestris (AS)* | 225.00 | 19.50 | 200.00 |
| T5-*Pseudomonas nitroreducens (PN)* | 210.00 | 22.50 | 199.00 |
| T6-*GF + AS* | 228.00 | 21.80 | 202.00 |
| T7-*GM + AS* | 225.00 | 21.00 | 201.00 |
| T8-*GF + PN* | 220.00 | 23.50 | 205.00 |
| T9-*GM + PN* | 218.00 | 22.80 | 204.00 |
| T10-*GF+AS + PN* | 245.00 | 28.20 | 215.00 |
| T11-*GM + AS + PN* | 240.00 | 27.50 | 212.00 |
| S.Em ± | 3.50 | 0.85 | 4.20 |
| C.D. @ 5% | 10.40 | 2.50 | 12.50 |

indicates that the control plants were mining the soil nutrient bank to sustain even minimal growth.

In sharp contrast, the bio-inoculated plots, particularly $T_{10}$, exhibited a positive nutrient balance. $T_{10}$ recorded a residual nitrogen status of 245.00 kg/ha, which is a gain of 34.5 kg/ha over the baseline and a phosphorus status of 28.20 kg/ha, a gain of 10 kg/ha over the baseline. This confirms that the biological nitrogen fixation by *Azotobacter* and phosphate solubilization by *Pseudomonas* exceeded the crop's uptake requirements, thereby replenishing the soil bank for the subsequent season. Additionally, the organic carbon content showed a slight increasing trend in $T_{10}$ (0.52%) compared to the control (0.44%). This is likely due to the higher turnover of the larger root biomass and the production of glomalin-related soil proteins (GRSP) by the extensive mycorrhizal network, which aids in soil aggregation and carbon sequestration (Biswas *et al.*, 2022). Thus, the technology not only enhances current season yield but also actively invests in long-term soil health.

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