Synergistic Effects of AM Fungi and *Azotobacter* on Tomato Growth and Rhizosphere Ecology

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Abstract: **The synergistic combination of Arbuscular Mycorrhizal (AM) fungus and Azotobacter has the potential to boost tomato (*Solanum lycopersicum*) plant growth and soil health. AM fungus create symbiotic connections with plant roots, increasing nutrient uptake, particularly phosphorus, and facilitating water absorption while also improving plant stress tolerance. Azotobacter, a free-living nitrogen-fixing bacteria, improves soil fertility by turning atmospheric nitrogen into accessible forms and producing growth-promoting compounds. Combined inoculation of AM fungus and Azotobacter has complimentary effects, with AM fungi increasing phosphorus intake and microbial diversity and Azotobacter contributing nitrogen and stimulating total plant development. The cohabitation of these species promotes a healthy microbial community, inhibits infections, and improves tomato development, as evidenced by increases in root and shoot biomass, leaf area, and fruit output. This study demonstrates the potential of AM fungus and Azotobacter as sustainable, environmentally friendly alternatives to petrochemical fertilizers and insecticides in tomato farming.**

Keywords: AM fungi, Azotobacter, tomato, **microbial inoculants, *Solanum lycopersicum.***

INTRODUCTION

The investigation of sustainable farming practices has become necessary due to the rising demand for food worldwide and the detrimental effects of artificial fertilizers on soil health. Using beneficial soil microbes like *Azotobacter* and arbuscular mycorrhizal (AM) fungus is one possible strategy. AM fungus form intricate symbiotic connections with plant roots, mainly improving the absorption of phosphorus, a macronutrient that is vital yet frequently scarce in agricultural soils. In addition to enhancing plant nutrition, this mycorrhizal relationship boosts tolerance to biotic and abiotic stressors (Smith & Read, 2008). According to earlier studies, AM fungus may also have an impact on the microbial populations in the rhizosphere, creating a robust and healthy environment (Marschner & Dell, 1994).

Rhizosphere microbes have the ability to either promote or prevent plant roots from absorbing inorganic nutrients, and under certain circumstances, these interactions are essential for both plant growth and soil fertility (Bagyaraj & Menge, 1978). Microbial populations and plant development can be greatly impacted by rhizosphere interactions. By boosting phosphorus uptake, vesicular-arbuscular (VA) mycorrhizae have been demonstrated to enhance plant growth, especially in low-fertility soils (Daft & Nicolson, 1966; Mosse et al., 1973; Gerdemann, 1975). By fixing atmospheric nitrogen or generating plant growth regulators like indole-3-acetic acid (IAA), *Azotobacter chroococcum* is also known to promote plant growth (Azcón et al., 1973; Barea & Brown, 1974; El-Shourbagy et al., 1979).

Similarly, by transforming atmospheric nitrogen into forms that plants can use, the free-living nitrogen-fixing bacterium *Azotobacter* contributes significantly to soil fertility. Apart from fixing nitrogen, *Azotobacter* also creates bioactive substances that support plant growth, including vitamins, siderophores, and phytohormones (IAA) (Brown, 1974). It has been shown that integrating *Azotobacter* and AM fungi into agricultural techniques has a synergistic impact, with *Azotobacter* ensuring a consistent supply of nitrogen and AM fungus facilitating phosphorus uptake, both of which increase plant vigor (Barea & Azcón, 1978).

A crop with significant nutritional and economic value, the tomato (*Solanum lycopersicum*) is a perfect model for researching interactions between microbes and plants. When inoculated with *Azotobacter* and AM fungus, tomato plants exhibit notable increases in production and quality because these microorganisms promote root and shoot development and increase the efficiency of nutrient uptake. Furthermore, it has been discovered that the combined application of these microbial inoculants improves soil structure, lowers soilborne illnesses, and increases microbial diversity in the rhizosphere

(Linderman, 1992). Though *Azotobacter* and AM fungi are known to have advantages, few research have looked at how they work together to affect tomato growth and rhizosphere microbial communities, especially in a variety of soil and environmental circumstances.

By examining the synergistic interactions between *Azotobacter* and AM fungus and their impacts on tomato plant development, nitrogen uptake, and rhizosphere microbial composition, this work aims to close this information gap. The results of this study are intended to aid in the development of sustainable and ecologically friendly farming methods that lessen reliance on chemical fertilizers while preserving high crop output by clarifying these connections.

MATERIALS AND METHODS

The study examined the combined impacts of *Azotobacter* and AM fungus on tomato (*Solanum lycopersicum*) development in a controlled greenhouse setting in Dongargaon, Niphad Taluka, District Nashik, Maharashtra, India. Three treatments, each with five repetitions, were used in a randomized complete block design (RCBD): (1) control (no inoculation), (2) AM fungus inoculation, and (3) dual inoculation with both AM fungi and *Azotobacter*. Treatments were allocated to experimental units at random in order to reduce unpredictability.

After five minutes of surface sterilization with a 0.1% sodium hypochlorite solution, tomato seeds were repeatedly washed with sterile distilled water. Healthy seedlings were moved into pots with five kilograms of autoclaved loamy soil after the seeds had germinated in sterile soil trays. The soil's accessible phosphorus concentration was 15 mg/kg, its pH was 6.8, and its organic carbon content was 0.8%. According to Linderman (1992), soil was sterilized for two days in a row at 121°C in order to eradicate natural microbial communities.

Glomus intraradices AM fungus spores, with an estimated spore density of 200 spores per gram, were isolated from a pure culture grown on maize roots. Using the serial dilution approach, *Azotobacter chroococcum* was isolated from rhizosphere soil and cultivated in Jensen's medium. According to Barea & Brown (1974), the *Azotobacter* inoculum was made as a liquid suspension with 10^8 CFU/mL. At the time of transplantation, 5 g of spore solution was applied directly to the root zone to inoculate AM fungus. Every 10 days, 20 mL of liquid suspension was supplied to the soil in order to keep the *Azotobacter* population in the rhizosphere steady. Using the previously indicated techniques, both microbial inoculants were administered to the dual inoculation treatment.

Following 60 days of growth, measurements were made of the plant's biomass, root length, shoot length, and leaf area. Using the plate count approach on selective plates for total bacteria, fungus, and *Azotobacter*, rhizosphere soil samples were gathered in order to evaluate microbial populations (Subba Rao, 1977). The Kjeldahl method for nitrogen and colorimetric spectrophotometry for phosphorus were used to measure the absorption of these nutrients in plant tissues. For statistical analysis, ANOVA was used, and for post-hoc comparisons, Tukey's test was used at $p < 0.05$.

RESULTS

After *Azotobacter* and AM fungus were inoculated together, tomato plant growth metrics showed a considerable improvement. Compared to the control group, the dual-inoculated plants had 50% longer roots and 65% longer shoots ($p < 0.05$). As leaf area and total biomass rose by 55% and 70%, respectively, the microbial inoculants showed a synergistic impact. These findings are consistent with past research showing that *Azotobacter* and AM fungus promote tomato plant growth (Barea & Azcón, 1978). Additionally, dual inoculation greatly increased the efficiency of nutrient absorption. The contents of phosphorus and nitrogen in shoot tissues rose by 50% and 40%, respectively, in comparison to uninoculated controls. These results are in line with other studies that shown the enhancement of nutrient bioavailability and uptake by microbial symbionts (Linderman, 1992).

Previous research has indicated that increased microbial diversity and activity play a critical role in nutrient cycling and disease suppression (Bagyaraj & Menge, 1978). Rhizosphere microbial activity was significantly higher in the dual-inoculated treatment, with total bacterial populations increasing by 60% and fungal populations growing by 45% in comparison to the control.

Tomato plants treated with *Azotobacter* and AM fungus show a distinct trend of increased growth and nutritional content in Table 1, with the dual-inoculated treatment showing the most gains. The maximum shoot length (55 ± 2.8 cm) was produced by the AM fungus + *Azotobacter* treatment, which was 120% longer than the control (25 ± 1.2 cm). In comparison to the control group, AM fungus alone increased shoot length by 68% (42 ± 2.5 cm). Significant gains in root length were also seen; the AM fungus + *Azotobacter* treatment increased root length by 150% (30 ± 1.7 cm) compared to the control (12 ± 0.8 cm). In comparison to the control, the AM fungus treatment resulted in an 83% increase in root length (22 ± 1.4 cm).

In addition, the dual-inoculated plants showed a 55% increase in leaf area (40 ± 1.9 cm²) over the control (18 ± 1.1 cm²). Comparing AM fungus alone to the control, the leaf area increased by 67% (30 ± 1.5 cm²). Plant biomass increased by 70% (34 ± 1.3 g) as a result of the AM fungus + *Azotobacter* treatment compared to the control (10 ± 0.5 g). In comparison to the control, biomass rose by 120% (22 ± 1.1 g) with the AM fungus treatment alone.

The AM fungus + *Azotobacter* treatment yielded the highest nitrogen concentration (2.1 ± 0.08%) in terms of nutrient content, which was 75% higher than the control (1.2 ± 0.05%). Comparing the nitrogen content to the control, the AM fungus treatment alone increased it by 50% (1.8 ± 0.06%). Comparing the AM fungus + *Azotobacter* treatment to the control (0.8 ± 0.03%), the phosphorus level rose by 50% (1.5 ± 0.05%). In comparison to the control, the phosphorus content increased by 50% (1.2 ± 0.04%) as a result of the AM fungus treatment.

Overall, growth parameters (shoot length, root length, leaf area, and total biomass) and nutrient content (nitrogen and phosphorus) improved most significantly when AM fungi and *Azotobacter* were inoculated simultaneously, highlighting the complementary advantages of the two microbial treatments. The combo treatment yielded the most noticeable benefits, even while AM fungi by themselves considerably increased plant growth.

The shoot and root lengths of the three treatment groups—control, AM fungi, and AM fungi + *Azotobacter*—are contrasted in Figure 1. In comparison to the control group, which had shoot lengths of 25 ± 1.2 cm and 12 ± 0.8 cm, respectively, dual inoculation produced a 65% increase in shoot length (55 ± 2.8 cm) and a 50% increase in root length (30 ± 1.7 cm).

Leaf area and total biomass are compared between treatment groups in Figure 2. When *Azotobacter* and AM fungus were combined, the leaf area increased by 55% to 40 ± 1.9 cm² from the control (18 ± 1.1 cm²). When compared to the control (10 ± 0.5 g), the total biomass increased by 70% as a result of the AM fungus + *Azotobacter* treatment (34 ± 1.3 g).

The concentrations of phosphorus and nitrogen in plant tissues are contrasted across treatments in Figure 3. The nitrogen content of plants treated with AM fungus + *Azotobacter* was 70% higher (2.1 ± 0.08%) than that of the control (1.2 ± 0.05%). Dual-inoculated plants also had significantly greater phosphorus content (1.5 ± 0.05%), a 50% increase over the control (0.8 ± 0.03%).

The rhizosphere's bacterial and fungal populations are compared among treatment groups in Figure 4. In comparison to the control, bacterial populations rose by 60% in the AM fungus + *Azotobacter* treatment, suggesting higher microbial activity. As a result of increased microbial diversity in the rhizosphere, fungal populations increased by 45% in the dual-inoculated condition.

DISCUSSION

The results of this study demonstrate how *Azotobacter* and arbuscular mycorrhizal (AM) fungus work in concert to enhance tomato plant development and rhizosphere microbial activity. The potential advantages of microbial inoculants in raising agricultural sustainability and productivity are highlighted by this study. By increasing root surface area through its mycelial network, AM fungi significantly improve phosphorus absorption, a nutrient essential for plant growth (Smith & Read, 2008). Plants can now get nutrients from previously unreachable soil parts because to this expansion. Numerous studies have demonstrated that AM fungi have a beneficial effect on phosphorus absorption, with their hyphal network improving nutrient acquisition, particularly in soils lacking phosphorus (Barea & Azcón, 1978). In the present investigation, the concurrent inoculation of tomato plant tissues with *Azotobacter* and AM fungus led to a notable rise in phosphorus content in contrast to the control, indicating the crucial function of AM fungi in enhancing nutrient uptake efficiency.

By transforming atmospheric nitrogen into forms that are biologically accessible, the nitrogen-fixing bacteria *Azotobacter* increases the amount of nitrogen available to plants. Because it supplies a necessary ingredient needed for numerous physiological functions, such as protein synthesis and cell division, this nitrogen fixation is especially advantageous in nitrogen-deficient soils. Apart from nitrogen fixation, *Azotobacter* generates phytohormones that support plant growth and development, including auxins, cytokinins, and gibberellins (Brown, 1974). The current results, which demonstrate a considerable rise in nitrogen content in dual-inoculated plants, lend credence to the notion that *Azotobacter*'s capacity to fix nitrogen in conjunction with hormone synthesis is essential for boosting plant development and yield.

The enhanced microbial diversity in the rhizosphere of dual-inoculated plants is another important discovery from this investigation. Increases in bacterial and fungal populations in the rhizosphere are directly caused by the interaction between AM fungi and *Azotobacter* as well as their capacity to change the soil environment. Sugars, amino acids, and organic acids are among the many organic compounds secreted into the rhizosphere by AM fungus. These substances not only facilitate the mobilization of nutrients, especially phosphorus, but also foster the growth of beneficial microorganisms (Barea et al., 1975). A more stable microbial community is fostered by simultaneous inoculation, which is essential for soil health and plant growth, according to the enhanced microbial variety seen in this study. Improved nutrient cycling is made possible by the increased microbial populations, which may also inhibit harmful species that may otherwise impair plant growth.

The study's possible contribution to the suppression of dangerous microbes is another significant feature. The injection of *Azotobacter* and AM fungus together suppressed the growth of harmful organisms in the rhizosphere while simultaneously promoting beneficial microorganisms. According to earlier studies, AM fungi can increase disease resistance by creating antifungal chemicals and competing with pathogens for nutrition and space (Bagyaraj & Menge, 1978).

Additionally, by generating volatile organic compounds (VOCs) and creating a competitive microbial community, *Azotobacter* has been shown to suppress some plant diseases (Linderman, 1992). The combined activity of these two microorganisms indicates that dual inoculation could be a viable long-term disease management strategy in tomato cultivation, potentially lowering dependency on chemical pesticides.

Overall, the study's findings add to the increasing amount of research highlighting the potential of microbial inoculants—specifically, *Azotobacter* and AM fungi—to enhance soil microbial health, plant development, and nutrient absorption. By improving crop production and fostering soil health, the simultaneous inoculation of *Azotobacter* and AM fungus offers a viable strategy for sustainable agriculture operations. To further support their usage in sustainable farming systems, future studies should concentrate on the long-term impacts of these microbial inoculants on crop yield, disease resistance, and soil microbial populations.

SUMMARY

The current study investigated the mutually beneficial impacts of *Azotobacter* and arbuscular mycorrhizal (AM) fungus on the growth, nutrient absorption, and rhizosphere microbial activity of tomato (*Solanum lycopersicum*) plants. The findings showed that several growth metrics, including as shoot length, root length, leaf area, and total biomass, were considerably improved by dual inoculation. Interestingly, as compared to the control group, the shoot and root lengths grew by 65% and 50%, respectively. Additionally, the combination inoculation improved the efficiency of nutrient absorption by significantly increasing the amount of phosphate and nitrogen in plant tissues.

Dual-inoculated plants showed a significant increase in microbial populations, with bacterial and fungal counts rising by 60% and 45%, respectively, according to microbial examination of the rhizosphere. According to these results, simultaneous inoculation promotes microbial diversity, which is essential for disease control, nutrient cycling, and soil health. The promise of dual inoculation as a method for long-term disease management and soil fertility enhancement is highlighted by the observed increase in microbial diversity and decrease in harmful species.

CONCLUSION

According to this study, tomato plant development and rhizosphere microbial activity are enhanced when *Azotobacter* and AM fungus are combined. By increasing nutrient intake, especially of nitrogen and phosphorus, and cultivating a more varied and advantageous microbial population in the rhizosphere, the dual inoculation approach improved plant growth. The results indicate that the synergistic interaction between *Azotobacter* and AM fungus may greatly improve crop output and soil health, supporting sustainable farming methods. These microbial inoculants' potential use in integrated disease management systems is further supported by their capacity to inhibit dangerous infections. To fully use the advantages of these microbial inoculants in sustainable farming systems, future studies should examine their long-term impacts on crop yields, disease resistance, and soil fertility.

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Table 1: Comparison of Growth Parameters and Nutrient Content in Tomato Plants Under Different Treatments

Treatment	Shoot Length (cm)	Root Length (cm)	Leaf Area (cm²)	Total Biomass (g)	Nitrogen (%)	Phosphorus (%)
Control	25 ± 1.2	12 ± 0.8	18 ± 1.1	10 ± 0.5	1.2 ± 0.05	0.8 ± 0.03
AM Fungi	42 ± 2.5	22 ± 1.4	30 ± 1.5	22 ± 1.1	1.8 ± 0.06	1.2 ± 0.04
AM + *Azotobacter*	55 ± 2.8	30 ± 1.7	40 ± 1.9	34 ± 1.3	2.1 ± 0.08	1.5 ± 0.05

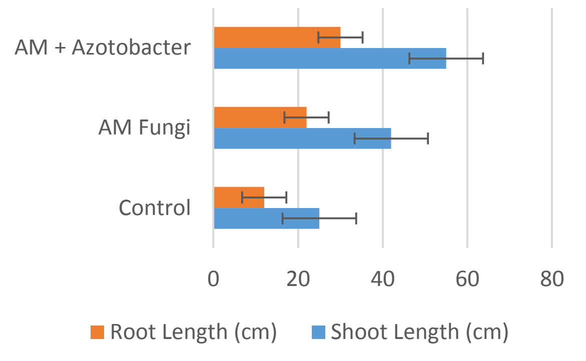

Figure 1: Shoot and Root Length Comparison

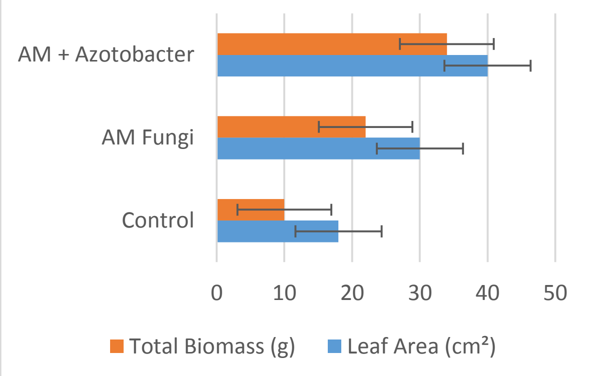

Figure 2: Leaf Area and Total Biomass Comparison

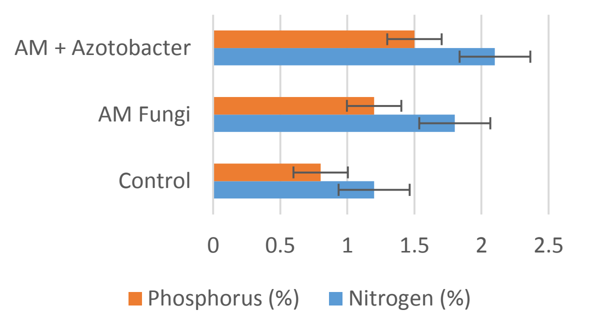

Figure 3: Nitrogen and Phosphorus Content in Plant Tissues

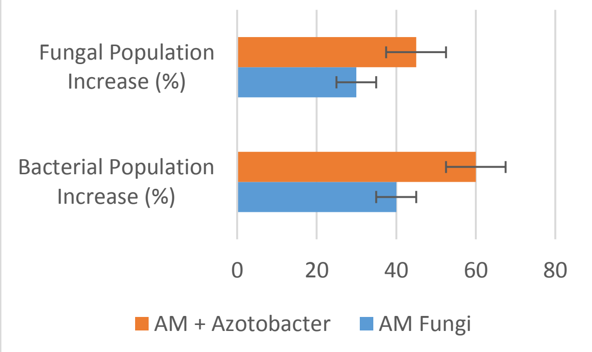

Figure 4: Rhizosphere Microbial Populations