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Rhizobium and Mycorrhizal Interactions: Dual Inoculation for Enhanced Nitrogen and Phosphorus Uptake

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Abstract

The integration of *Rhizobium* and arbuscular mycorrhizal fungi (AMF) as dual inoculants offers a sustainable strategy to improve nutrient uptake, particularly nitrogen (N) and phosphorus (P), in leguminous crops. This study investigates the synergistic interactions between *Rhizobium* and AMF in promoting plant growth, nodulation efficiency, soil fertility, and nutrient uptake. The results indicate that co-inoculation significantly enhances plant biomass, nitrogen fixation, and phosphorus assimilation compared to single inoculation or control. These findings emphasize the potential of microbial consortia as biofertilizers for improving soil health and crop productivity under sustainable agricultural practices.

Introduction



Modern agriculture relies heavily on chemical fertilizers, leading to soil degradation, environmental pollution, and reduced microbial diversity. Sustainable alternatives such as biofertilizers harness beneficial microbes to improve soil fertility naturally.

Rhizobium bacteria form symbiotic relationships with legumes, fixing atmospheric nitrogen through root nodules. Similarly, arbuscular mycorrhizal fungi (AMF) colonize root cortical cells, extending hyphal networks that improve phosphorus and micronutrient uptake, Smith et al., 2010, Gopalakrishnan et al., 2018.

Dual inoculation of *Rhizobium* and AMF can create a synergistic system that enhances nutrient availability, improves plant vigor, and reduces dependence on synthetic fertilizers, Adesemoye et al., 2018.

Rhizobium and mycorrhizal fungi engage in a symbiotic relationship that significantly enhances plant nutrient uptake, specifically nitrogen (N) and phosphorus (P). *Rhizobium* bacteria fix atmospheric nitrogen into bioavailable forms for plants, primarily legumes, while arbuscular mycorrhizal fungi (AMF) enhance phosphorus absorption and other nutrients by extending the root system through their hyphal networks. Dual inoculation with both *Rhizobium* and AMF has demonstrated synergistic effects on plant growth, nutrient content, and yield, Rajendra et al, 2022 .

The most notable benefits of this dual inoculation include:

1. Enhanced Nitrogen Uptake: *Rhizobium* safely fixes nitrogen from the atmosphere, increasing the nitrogen available to the plant. Studies



found that soybeans inoculated with both Rhizobium and AMF showed significant increases in nitrogen uptake compared to single inoculations or non-inoculated controls. The transfer of nitrogen also benefits intercrops, such as maize adjacent to soybean, improving overall system nitrogen availability.

2. Improved Phosphorus Absorption: AMF improves phosphorus uptake by increasing the root's absorptive surface area and mobilizing phosphorus in soil, which is otherwise poorly available to plants. This in turn enhances energy metabolism that supports nodulation and nitrogen fixation by Rhizobium.

3. Increased Plant Growth and Biomass: Dual inoculated plants generally show enhanced root colonization by AMF and increased nodulation by Rhizobium, resulting in improved nutrient uptake and growth parameters such as shoot length, biomass, and grain yield compared to single inoculated or uninoculated plants.

4. Synergistic Interactions: The presence of AMF elevates the efficiency of Rhizobium nodulation and nitrogen fixation, while Rhizobium-fixed nitrogen is critical for the energy-intensive processes of mycorrhizal development and function. This mutual enhancement results in better establishment and functionality of both symbionts.

5. Context-Specific Effects: While dual inoculation often improves



nutrient uptake, the magnitude of benefits can be influenced by soil nutrient status, plant species, microbial strains, and environmental conditions.

Some studies note that the benefits are more pronounced in nutrient-poor soils, while in nutrient-rich soils, inoculation effects may be less evident.

Materials and Methods

Experimental Design

A greenhouse experiment was conducted on chickpea (*Cicer arietinum L.*) under controlled conditions. Four treatments were established:

Treatment Code	Description
T ₁	Control (no inoculation)
T ₂	<i>Rhizobium</i> inoculation only
T ₃	Mycorrhizal inoculation only
T ₄	Dual inoculation (<i>Rhizobium</i> + Mycorrhiza)

Each treatment was replicated three times in a randomized block design (RBD).

Inoculum Preparation

- *Rhizobium* strain: Isolated from local legume rhizosphere and



cultured in yeast extract mannitol broth.

- Mycorrhizal fungi: *Glomus mosseae* spores procured from ICAR culture collection.
- Seeds were surface sterilized with 0.1% $HgCl_2$, rinsed with sterile water, and coated with inoculum before sowing.

Growth and Nutrient Analysis

After 60 days, plant height, shoot and root biomass, nodulation number, and chlorophyll content were measured. Nitrogen and phosphorus contents were analyzed using the Kjeldahl and vanadomolybdate methods, respectively.

Results and Discussion

Growth Parameters

Dual inoculation (T_4) significantly increased all growth parameters compared to control and single inoculations

Table 1. Effect of Rhizobium and Mycorrhiza on Growth and Nutrient Uptake in Chickpea

Parameter	Control (T_1)	Rhizobium (T_2)	Mycorrhiza (T_3)	Dual (T_4)
Plant Height (cm)	32.5 ± 1.2	41.2 ± 1.0	39.8 ± 0.8	51.5 ± 1.1
Root Dry Weight (g)	2.3 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	4.2 ± 0.2
Nodules/Plant	12.5 ± 1.1	22.8 ± 1.4	18.6 ± 1.2	31.2 ± 1.5
N Content (%)	1.22 ± 0.05	1.86 ± 0.07	1.71 ± 0.06	2.41 ± 0.08
P Content (%)	0.32 ± 0.01	0.41 ± 0.01	0.46 ± 0.02	0.61 ± 0.02

**Interpretation:**

The synergistic effect of *Rhizobium* and AMF resulted in the highest nitrogen and phosphorus content, indicating improved root colonization and nutrient exchange efficiency.

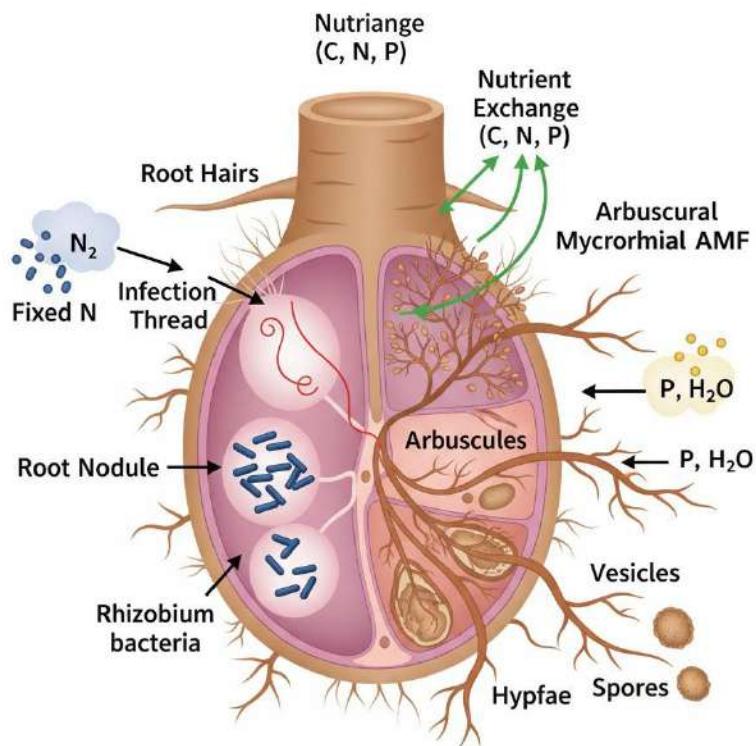


Figure 1. Schematic representation of *Rhizobium*-Mycorrhiza interaction in legume roots.

- The diagram shows a legume root colonized by *Rhizobium* in nodules (responsible for N₂ fixation) and AMF hyphae extending into soil micropores (enhancing P uptake).
- Arrows indicate nutrient flow from soil to root and nodules, and from roots to shoots, highlighting symbiotic synergy.



Mechanistic Insights

The interaction between *Rhizobium* and AMF improves:

- **Root surface area:** Enhancing contact with soil nutrients.
- **Phosphatase activity:** Increasing phosphate solubilization.
- **Nodulation efficiency:** Mycorrhiza enhances root physiology, supporting *Rhizobium* infection and nodulation.
- **Hormonal balance:** Co-inoculation stimulates auxin and cytokinin production.

Conclusion

Dual inoculation of *Rhizobium* and mycorrhizal fungi demonstrates a synergistic relationship that significantly enhances plant growth, nitrogen fixation, and phosphorus uptake. Such biofertilizer

combinations provide a sustainable and eco-friendly solution for improving soil fertility and reducing dependency on chemical fertilizers.

Future Prospects

Future studies should focus on:

- Field-scale validation under diverse agro-climatic zones.
- Development of carrier-based and liquid dual inoculants.
- Integration of molecular tools to understand gene expression in co-inoculated systems.
- Application of AI and remote sensing for nutrient monitoring in biofertilized fields.



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