Additive effect of AM Fungus and Rhizobium with Molybdenum sources on Growth and Nutrient Content of *Tamarindus indica* L.

Channabasava A.[1,2*] and Lakshman H.C.[2]
1. Department of Botany, SGGVT's Shri Gavisiddeshwar Arts, Science and Commerce College, Koppal-583 231, Karnataka, INDIA
2. P.G. Department of Studies in Botany, Karnatak University, Dharwad-580 003, INDIA
*casahukar85@gmail.com

Abstract
This study investigated the synergistic effects of Arbuscular mycorrhizal (AM) fungi, Rhizobium bacteria and molybdenum on Tamarindus indica L. seedling growth and development. Dual inoculation with AM fungus Rhizophagus fasciculatus and Rhizobium combined with molybdenum treatment significantly enhanced plant growth parameters, nutrient uptake and overall plant health. Key findings include increased shoot and root length, leaf length and leaf number, enhanced mycorrhizal colonization and root nodulation and improved uptake of essential nutrients (N, P, K, Mg and Mo) in plants receiving combined treatment. The results suggest that this approach could reduce the need for inorganic fertilizers, promoting more sustainable agricultural practices. The study highlights the importance of understanding complex interactions between beneficial microorganisms and micronutrients in plant growth.

Combined application of AM fungi, Rhizobium and molybdenum could be an effective strategy for improving Tamarindus indica L. cultivation and potentially other leguminous plants. Future research should focus on optimizing application rates and methods in field conditions, investigating long-term effects on plant productivity and soil health and exploring this approach in different soil types and environmental conditions.

Keywords: *Tamarindus indica*, *Rhizophagus fasciculatus*, *Rhizobium*, molybdenum, nodulation.

Introduction
Arbuscular mycorrhizal (AM) fungi are ubiquitous in soils worldwide, forming associations with approximately 80% of terrestrial plant roots[8]. These microscopic organisms play a crucial role in ecosystem functioning and plant health. The beneficial effects of AM fungi symbiotic associations on plant growth are well-documented and have been extensively studied over the past few decades[1,4,13,20,26]. These fungi primarily benefit their host plants by enhancing the uptake of relatively immobile phosphate ions through the activity of their extra-radical mycelium which extends beyond the phosphate depletion zone that rapidly develops around plant roots[27]. This extension of the plant's nutrient absorption capacity allows for more efficient utilization of soil resources, particularly in nutrient-poor environments.

Legumes, a family of plants known for their ability to fix atmospheric nitrogen, form intricate associations with both AM fungi and nitrogen-fixing bacteria of the genus Rhizobium. These complex interactions are known as tripartite symbioses. The significance of these tripartite associations is twofold: they provide accrued benefits to the plant while also imposing a carbon drain on the host. This balance between cost and benefit is a critical aspect of the symbiotic relationship and can vary depending on environmental conditions and the specific species involved. Generally, plant benefits derived from these symbiotic relationships include enhanced growth and yield, improved nitrogen and phosphorus nutrition, increased drought resistance, disease control and phosphorus solubilization.

The enhanced nutrient uptake, particularly of nitrogen and phosphorus, can lead to improved plant vigor and productivity. Increased drought resistance is especially valuable in water-limited environments, potentially mitigating the effects of climate change on agricultural systems. The ability of AM fungi to confer some level of disease control is an area of growing interest, as it may provide a sustainable alternative to chemical pesticides in some contexts. Numerous researchers have investigated the influence of various AMF species on the growth of nodulated legumes. These studies have explored the complex interactions between the host plant, AM fungi and rhizobia, seeking to understand how these relationships can be optimized for agricultural and ecological benefits.

The research has spanned various legume species including important crops such as soybeans, peas and alfalfa, as well as wild legumes that play crucial roles in natural ecosystems. *Tamarindus indica* L., commonly known as tamarind, is a long-lived, medium-sized bushy tree that can reach impressive heights of 40 to 60 feet. This versatile tree has been cultivated for centuries across tropical regions due to its numerous beneficial properties. The wood of the tamarind tree is highly valued in the furniture industry for its durability and attractive grain, making it a popular choice for high-quality furniture and flooring applications.

The fruits of the tamarind tree are not only delicious but also packed with essential nutrients. They are particularly rich in vitamin B and calcium, making them a valuable addition to a healthy diet. The tangy-sweet flavor of tamarind fruit is widely used in culinary traditions around the world, from Southeast Asian cuisines to Latin American dishes. Traditional medicine systems have long recognized the medicinal properties of various parts of the tamarind tree. A decoction prepared from the stem bark and leaves, when mixed with potash, is used as a remedy for a range of ailments. This concoction is believed to be effective in treating stomach disorders, alleviating body pain, combating jaundice and yellow fever and purifying the blood by reducing toxicity.

The fruit juice of the tamarind is particularly prized for its antiseptic properties. It is commonly used to address a variety of health issues, including colic, conjunctivitis and constipation. Additionally, it is believed to have beneficial effects on managing diabetes, soothing coughs, providing relief from asthma symptoms and alleviating chest pain. In recent years, scientific research has focused on the role of micronutrients in plant growth and development. One such micronutrient that has gained significant attention is molybdenum (Mo). This trace element plays a crucial role in biological nitrogen fixation and has become increasingly important in intensive crop production systems[25].

The ability of plants to efficiently utilize atmospheric nitrogen is vital for sustainable agriculture and molybdenum is an essential component in this process. The effects of Arbuscular mycorrhizal (AM) fungi and Rhizobium bacteria on various plant species have been extensively studied and well-documented in scientific literature[15,16,21]. These beneficial microorganisms form symbiotic relationships with plant roots, enhancing nutrient uptake, improving soil structure and increasing plant resistance to various environmental stresses.

The positive impacts of these associations on plant growth, yield and overall health have been observed across a wide range of agricultural and horticultural crops. However, despite the wealth of knowledge on the individual effects of AM fungi and Rhizobium, there is a notable gap in our understanding of their combined effects, particularly when considered alongside the influence of molybdenum sources on *Tamarindus indica* L. This lack of comprehensive research presents an exciting opportunity for further investigation.

The present study aims to address this knowledge gap by assessing the differential effects of AM fungi and rhizobium in the presence of various molybdenum sources on the growth and development of *Tamarindus indica* L. This research seeks to provide valuable insights into the potential synergistic relationships between these beneficial microorganisms and the essential micronutrient molybdenum in enhancing the growth, productivity and overall health of tamarind trees. By exploring these interactions, the study aims to contribute to our understanding of sustainable agricultural practices for tamarind cultivation. The findings could potentially lead to the development of more effective and environmentally friendly cultivation techniques, ultimately benefiting farmers and the ecosystem alike.

Material and Methods

Experimental Design: The seeds of *Tamarindus indica* L. were collected from an 8-year-old plant. These seeds underwent surface sterilization with 2% sodium hypochlorite and were subsequently washed twice with distilled water to remove any traces of the sodium hypochlorite solution. The surface-sterilized seeds were then sown in earthen pots containing 8 kg of sterilized potting mixture (loam soil and pure sand, 1:1 v/v). This potting mixture was subjected to chemical analysis (Table 1). The experimental pots were arranged in a completely randomized block design (CRD) in triplicate per treatment. The following treatments were maintained under polyhouse conditions:

T1: AM fungus (*Rhizophagus fasciculatus*) inoculation
T2: Rhizobium inoculation

Table 1
Physico-chemical properties of soil used for pot experiments.

Soil	Values
Soil type	Sandy loamy
Soil moisture	26.11
pH	6.89
Electric conductivity (mmhos/cm at 25^{0}C organic carbon	0.54
(%)	0.26
Available Phosphorus (%)	0.17
Available Potassium (%)	22.73
Available Nitrogen (%)	18.16
Iron (%)	13.17
Zinc (%)	5.91
Copper (%)	3.59
Magnesiumm (%)	2.58
Molybdenum (%)	0.03

Each value is mean of 3 samples

T3: Molybdenum treatment
T4: Dual inoculation of AMF + Rhizobium
T5: AMF inoculation with Molybdenum
T6: Dual inoculation of AMF and Rhizobium with Molybdenum
T7: Control

Rhizobium Inoculation of *Tamarindus indica* Seedlings: Rhizobium culture was obtained from the University of Agricultural Sciences Dharwad (Karnataka, India) and multiplied in the laboratory of the Department of Studies in Botany, Karnatak University, Dharwad (India) using yeast extract agar (YEA) media. A thin film of rhizobium culture was applied to surface-sterilized seeds following the method described by Varma and Subba Rao[30].

AMF Inoculation: Soil-based mixed inoculum of AM fungus *Rhizophagus fasciculatus* (15g/pot) containing 200-250 chlamydospores/50g soil was applied 5cm below the surface of the potting mixture in each pot. This AMF inoculum was prepared using Sorghum vulgare as a host plant.

Molybdenum Treatment: Different doses of sodium molybdate (3, 6 and 8g molybdenum/L water) were applied as foliar spray once a month when plants reached 5 cm in height after inoculation. Plants were watered every alternate day and 15 ml of Hoagland solution without phosphorus was applied per pot fortnightly.

Analysis of Plant Growth Parameters and Nutrient Uptake: Plants were harvested 90 days after sowing (DAS) and analyzed for growth parameters including plant height, root length; shoot dry weight, number of leaves and leaf length. Percent mycorrhizal colonization was determined according to the method described by Phillips and Hayman[19]. Chemical analysis of the plants was carried out following Jackson's[11] procedure. The results were subjected to Analysis of Variance (ANOVA) and tested for significant differences (P<0.05) using statistical analysis.

Results and Discussion

The plants inoculated with either Rhizobium or AM fungi alone showed significantly increased shoot and root length compared to the control. Dual inoculation of AM fungus and rhizobium resulted in maximum values for percent mycorrhizal colonization, root length and shoot length compared to single inoculation treatments (Table 2). Rhizobium-inoculated plants and control plants did not exhibit mycorrhizal colonization. The highest frequency of mycorrhizal infection was observed in roots of dual-inoculated plants and AM fungi-inoculated plants (Table 2).

The soil used for pot experiments was phosphorus-deficient (Table 1). Significant plant height was achieved when seedlings were dual-inoculated with AM fungus *Rhizophagus fasciculatus* and rhizobium, along with molybdenum treatment (Table 2).

However, no favorable increase in plant height or percent root colonization was observed in plants treated with rhizobium or molybdenum alone. Experimental results also revealed a significant increase in leaf length and number of leaves in plants inoculated with AM fungus and Rhizobium, combined with molybdenum treatment (Table 3).

Nutrient contents (N, P, K, Mg and Mo) in the shoot of *Tamarindus indica* L. were significantly increased in plants with dual inoculation of AM fungus and rhizobium in the presence of molybdenum (8g/liter distilled water) (Table 4). This trend aligns with findings from earlier studies[7,24]. Several researchers have reported significant enhancement of nutrient uptake by experimental plants after inoculation with biofertilizers, with and without heavy metal treatments[6, 22].

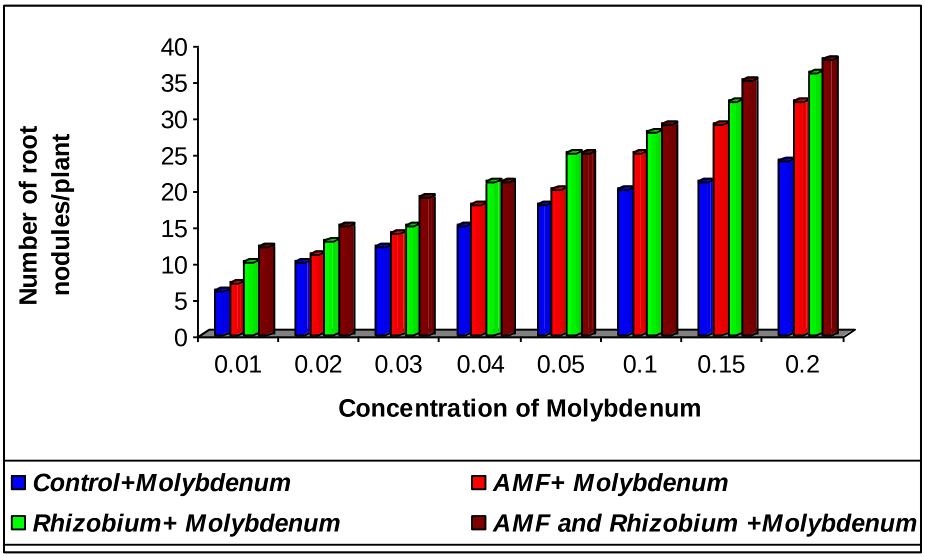

Fig. 1: Effect of AM fungus and Rhizobium with different levels of molybdenum spray on the number of nodules formed in the roots of *Tamarindus indica* L., at 120 days

Table 2
Effect of AM fungus and *Rhizobium* with Molybdenum on shoot length, root length and per cent mycorrhizal colonization (PMC) of *Tamarindus indica* L., seedlings at 90 days under nursery conditions.

Inoculants / Treatment	Shoot length	Root length	PMC
Control	9.6	1.6	-
AM fngus	15.2	1.8	57.5
Rhizobium	14.2	2.1	45.6
Molybdenum	14.9	2.5	50.0
AMF+*Rhizobium*	31.2	2.9	46.2
AMF+Molybdenum	39.5	3.1	53.8
AMF+*Rhizobium*+ Molybdenum	63.2	3.2	55.2
Mean	26.85	2.45	51.36
	CD (P=0.05)	CD(P=0.05)	CD(P=0.05)
Treatment (T)	0.47	NS	0.42
Growth stage (GS)	0.29	0.06	0.51
T x GS	0.85	0.04	0.10

Table 3
Effect of AM fungus and Rhizobium with molybdenum on number of leaves, leaf length and per cent of AM fungal spores /g rhizospheric soil of *Tamarindus indica* L., seedlings at 90 days under nursery conditions.

Inoculants / Treatment	Number of leaves/plant	Leaf length	% of AM spores/g soil
Control	6.3	2.4	4.3
AM Fungus	14.3	3.8	9.8
Rhizobium	13.1	3.9	8.8
Molybdenum	18.5	4.0	10.7
AMF+*Rhizobium*	18.9	4.0	11.6
AMF+Molybdenum	19.8	4.2	12.4
AMF+*Rhizobium*+ Molybdenum	21.0	4.6	15.5
	CD (P=0.05)	CD(P=0.05)	CD(P=0.05)
Treatment (T)	2.94	0.27	1.492
Growth stage (GS)	1.53	0.58	1.024
T x GS	3.86	0.81	2.807

Table 4
Effect of AM fungus and *Rhizobium* with Molybdenum on N, P, K and Mg content in shoots of *Tamarindus indica* L., seedlings at 90 days under nursery conditions.

Inoculants / Treatment	Nutrients N, P, K and Mo (%) in shoots				
	N	P	K	Mg	Mo
Control	1.52	0.06	1.05	0.09	0.01
AMF	2.09	0.14	1.23	0.12	0.03
Rhizobium	2.89	0.12	1.46	0.13	0.02
Molybdenum	3.02	0.16	1.63	0.11	0.04
AMF+*Rhizobium*	3.18	0.21	2.07	0.16	0.05
AMF+Molybdenum	3.35	0.29	2.28	0.17	0.06
AMF+*Rhizobium* + Molybdenum	3.72	0.32	2.81	0.21	0.07
	CD=(P=0.05)	CD=(P=0.05)	CD=(P=0.05)	CD=(P=0.05)	CD=(P=0.05)
Treatment (T)	0.27	0.023	0.09	0.01	0.001
Growth stage (GS)	-	0.032	0.10	0.11	0.002
T x GS	0.59	0.070	0.22	0.02	0.002

Note: AMF - Arbuscular Mycorrhizal Fungus, N-Nitrogen, P-Phosphorous, K- Potassium, Mg-Magnesium and Mo-Molybdenum. Each value is a mean of triplicate.

After 120 days, experimental plants showed a significant number of root nodules when inoculated with AM fungus and rhizobium, combined with molybdenum (Mo) treatment (0.20%). The effect of Mo on increased nodulation can be related to its role as a component of nitrogenous proteins[3]. Paudyal et al[18] reported increased nodulation in two species of tropical legumes with higher levels of Mo treatment (75µM). The influence of Mo may be attributed to its conversion into IAA15 which appears to affect the lengthening, branching and curling of root hairs, favoring the infection process during nodule formation.

The results of this study strongly support combined inoculation of *Tamarindus indica* L. seedlings with AM fungus and rhizobium, along with molybdenum spray. This approach can help to minimize dependence on inorganic fertilizers and can ensure a pollution-free environment[15]. These findings highlight the need for a proper survey of forest soils to understand their nutrient status. Al-Garni[2] reported tolerance of heavy metals in plants inoculated with a consortium of AM fungus and rhizobium under experimental conditions. The present results are consistent with Al-Garni[2], showing an increased number of root nodules with higher concentrations of molybdenum foliar application to *Tamarindus indica* L. seedlings at 120 days.

The observed results with *Rhizophagus fasciculatus* and rhizobium for total N uptake could be due to the increased di-nitrogen fixing character. Phosphorus favors better root proliferation, nodule formation and nitrogen fixation, leading to improved uptake. *R. fasciculatus* and rhizobium most efficiently increased phosphorus uptake due to their synergistic effect, resulting in more dry matter accumulation[12,14]. The enhanced uptake of essential nutrients (N, P, K, Mg and Mo) observed with increasing levels of molybdenum spray and dual inoculation of Arbuscular mycorrhizal (AM) fungus and Rhizobium may be attributed to several interconnected factors. This synergistic effect likely results from a well-balanced shoot-to-root ratio, an agronomically optimal N/P ratio in the roots, improved nitrogen fixation and availability, increased carbon exchange rate, enhanced photosynthesis, efficient partitioning and translocation of photosynthates and the development of larger sink organs[5,29].

The application of molybdenum spray, combined with the dual inoculation of AM fungus and Rhizobium, creates a favorable environment for nutrient uptake and utilization. Molybdenum, an essential micronutrient, plays a crucial role in nitrogen metabolism and enzyme activities. The AM fungus enhances the plant's ability to absorb nutrients, particularly phosphorus, from the soil, while rhizobium facilitates nitrogen fixation in leguminous plants. This improved nutrient uptake leads to a cascade of positive effects on plant growth and development.

The balanced shoot-to-root ratio ensures optimal resource allocation between above and below ground plant parts. The agronomically balanced N/P ratio in the roots promotes efficient nutrient utilization and supports overall plant health. Enhanced nitrogen fixation and availability provide the plant with an ample supply of this critical macronutrient, supporting various physiological processes. Furthermore, the increased carbon exchange rate and enhanced photosynthesis result in greater production of carbohydrates and other essential compounds. The efficient partitioning and translocation of these photosynthates ensure that nutrients and energy are distributed effectively throughout the plant. Finally, the development of larger sink organs such as fruits or grains, allows for increased storage and utilization of these resources, ultimately contributing to improved crop yield and quality.

Conclusion

This study has provided valuable insights into the synergistic effects of arbuscular mycorrhizal (AM) fungi, rhizobium bacteria and molybdenum on the growth and development of *Tamarindus indica* L. seedlings. The findings demonstrate that dual inoculation with AM fungus *Rhizophagus fasciculatus* and Rhizobium, combined with molybdenum treatment, significantly enhances plant growth parameters, nutrient uptake and overall plant health.

Key outcomes of this research include: 1. Increased shoot and root length, leaf length and number of leaves in dual-inoculated plants with molybdenum treatment. 2. Enhanced mycorrhizal colonization and root nodulation in dual-inoculated plants. 3. Improved uptake of essential nutrients (N, P, K, Mg and Mo) in plants receiving combined treatment and 4. Potential reduction in the need for inorganic fertilizers, promoting more sustainable agricultural practices.

These results highlight the importance of understanding the complex interactions between beneficial microorganisms and micronutrients in plant growth. The study suggests that the combined application of AM fungi, rhizobium and molybdenum could be an effective strategy for improving the cultivation of *Tamarindus indica* L. and potentially other leguminous plants. Future research should focus on optimizing the application rates and methods for these treatments in field conditions, as well as investigating their long-term effects on plant productivity and soil health. Additionally, exploring the potential of this approach in different soil types and environmental conditions could further enhance our understanding of sustainable agricultural practices for tamarind cultivation.

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