

Arbuscular mycorrhizal fungal inoculation enhances growth and phosphorus uptake in nutmeg (*Myristica fragrans*) seedlings under varying phosphorus levels

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Abstract

A pot experiment conducted during 2022–23 and 2023–24 evaluated the effects of arbuscular mycorrhizal (AM) fungi and phosphorus (P) levels on growth, nutrient uptake, and soil enzyme activity in nutmeg seedlings. AM fungi, P, and their interaction significantly influenced growth and nutrient accumulation, particularly phosphorus. The highest shoot length (174 cm), root length (55.5 cm), and stem girth (9.5 mm) were recorded with 50 g AM fungi $\times$ 100% P, indicating a strong AM–P interaction. While the highest dry matter (600 g) and phosphorus recovery efficiency (17.17%) occurred with 50 g AM fungi $\times$ 50% P. AM fungal spore density peaked (35 spores 50 g⁻¹ soil) under 50 g AM fungi $\times$ 75% P. Nutrient accumulation varied among plant parts, with stems as the main sink. AM fungi also enhanced soil enzyme activities, demonstrating their synergistic role with moderate P in sustainable nutmeg seedling growth.

Keywords: Arbuscular mycorrhizal fungi, apparent phosphorus recovery efficiency, nutrient uptake, soil enzyme activity

Introduction

Nutmeg (*Myristica fragrans* Houtt.) is a dioecious, evergreen tree of the family Myristicaceae, originally native to the Moluccas of Indonesia. Today, it is grown widely throughout tropical regions such as Indonesia, India, Grenada, China, Sri Lanka, Malaysia, Sumatra, Western Zanzibar and Mauritius. In India, nutmeg cultivation is concentrated in the humid tropical zones of Kerala, Karnataka, Tamil Nadu, Maharashtra and Goa. Nutmeg is

valued for its dried kernel and mace that are rich in bioactive compounds like myristicin, elemicin, and eugenol, known for their antioxidant and antimicrobial properties (Krishnamoorthy and Rema 2006). Commercial plantations of nutmeg are mostly established through vegetative propagation methods like grafting and budding, which ensures true-to-type progenies with early bearing and uniform yield and quality. However, inadequate root development, slow seedling growth, and high

incidence of nursery diseases remain major constraints to producing healthy, high-quality planting material (Biju *et al.*, 2021). Hence, the faster growth and early seedling vigour are crucial for the success of effective planting material production in nutmeg (Nissar *et al.*, 2019).

To address the challenges in raising healthy nutmeg seedlings, traditional propagation practices need improvement through better input management. Adding beneficial bio-inoculants to the potting mix provides an eco-friendly way to boost seedling growth and vigour. Among these, arbuscular mycorrhizal (AM) fungi have gained attention for improving plant growth, ensuring more uniform seedlings, lowering transplant shock, and supporting sustainable nursery production (Sarathambal *et al.*, 2022).

AM fungi support plant growth by improving the uptake of nutrients and water through their extensive hyphal networks, which greatly expand the effective root surface area and connect with the cortical cells inside the roots. In return, the host plant supplies the fungi with carbon produced through photosynthesis (Wilkes *et al.*, 2020). This mutually beneficial relationship is well known for enhancing plant vigour and overall growth. As a practical and efficient biostimulant, AM fungi can give nutmeg seedlings an early advantage, helping them establish better in the field. Previous studies have also shown the positive effects of AM fungal colonisation on the growth and development of many crops, including perennial species such as *Piper nigrum*, *Poincianella pyramidalis*, *Cnidioscolus quercifolius*, *Araucaria araucana* and *Tamarindus indica* (Sarathambal *et al.*, 2023a; Frosi *et al.*, 2016; Chavez *et al.*, 2023; Kareem *et al.*, 2023).

Phosphorus (P) is an essential macronutrient that plays a major role in early seedling growth by supporting cell division, root development and the formation of a strong photosynthetic system (Hussain *et al.*, 2022). It is required

for building nucleic acids and phospholipids and is central to energy transfer through ATP, which drives many key metabolic processes during early plant establishment (Nadeem *et al.*, 2021). Despite its importance, phosphorus is often one of the least available nutrients in agricultural soils because it easily becomes fixed into insoluble forms.

To address the problem of low phosphorus availability, AM fungi develop a symbiotic association with plant roots, helping seedlings absorb more phosphorus during their early growth stages when nutrient demand is highest (Begum *et al.*, 2019). Through their widespread hyphal network, AM fungi reach soil zones that roots alone cannot access, improving phosphorus uptake and supporting stronger early growth, better root development, and greater stress tolerance (Gonzalez-Guerrero *et al.*, 2022). This eco-friendly strategy increases phosphorus-use efficiency and helps plants establish well, even under low-phosphorus conditions.

Considering the critical role of phosphorus in early seedling development and the potential of AM fungi to enhance nutrient acquisition, it is important to explore their synergistic effects in nutmeg, a high-value spice crop. Despite the well-established benefits of arbuscular mycorrhizal (AM) fungi in enhancing phosphorus acquisition, information on the optimum combination of AM inoculum dose and phosphorus fertilization for nutmeg seedlings is limited. Therefore, the objective of the present study was to assess the effects of AM fungi inoculation on the growth and nutrient uptake pattern in nutmeg seedlings under varying phosphorus levels.

Materials and methods

Pot experiment setup

The pot experiment was conducted in a polyhouse at the Division of Crop Protection, ICAR-Indian Institute of Spices Research

(11.29395°N, 75.82038°E), Kozhikode, India during 2022-23 and 2023-24 to evaluate the interactive effects of AM fungi inoculation and phosphorus (P) levels on nutmeg seedlings. Each pot contained 10 kg of standardized potting mixture of sterilized soil, compost and sand in 2:1:1 ratio. All the nutrients (nitrogen, potassium and other micronutrients) except phosphorus were applied uniformly across the twelve treatments as per the package of practices to avoid confounding effects (Anandaraj *et al.*, 2005). The initial nutrient composition of the potting mixture was analyzed as per the standard procedures and the results were pH 6.23, organic carbon 2.1%, nitrogen 122.5 mg kg⁻¹, phosphorus 46 mg kg⁻¹, potassium 187.5 mg kg⁻¹, calcium 981 mg kg⁻¹, magnesium 375 mg kg⁻¹, Iron 32 mg kg⁻¹, zinc 1.9 mg kg⁻¹, manganese 21.6 mg kg⁻¹ and copper 1.2 mg kg⁻¹.

Phosphorus was applied using single super phosphate (SSP, containing 16% P) at 0%, 50%, 75%, and 100% of the recommended dose for nutmeg. The respective quantities were thoroughly mixed with the potting mixture prior to transplanting. Arbuscular mycorrhizal (AM) fungi specifically, *Rhizophagus irregularis* (PV916399), inoculum containing spores, colonized root fragments, and mycelium, was applied at 0 g (control), 25 g, and 50 g per pot. The inoculum was incorporated directly into the root zone during transplanting.

Assessment of growth parameters of nutmeg seedlings

To assess the initial response of AM fungi inoculation and varying phosphorus (P) levels, nutmeg seedlings were evaluated at annual intervals. Whole plants were carefully uprooted, and biometric measurements were recorded. Growth parameters such as shoot length, root length, stem girth, and dry weight were recorded following the procedure outlined by Newman (1966).

Extraction and quantification of AM Spores

AM fungal spores were isolated following the wet-sieving and decanting procedure of Gerdemann and Nicolson (1963). For this, 50 g of rhizosphere soil was mixed with 500 mL of water in a beaker and left undisturbed for one hour. The mixture was then poured through a set of sieves arranged from coarse to fine mesh sizes (600 µm to 37 µm) to separate the spores. The material retained on the smaller sieves was collected in Petri dishes and examined under a stereo zoom microscope (Zeiss-Discovery V8) to count the AM spores and sporocarps.

Assessment of nutrient uptake and soil enzyme analysis

For nutrient uptake assessment, plant tissues were oven-dried at 60 °C and ground to a fine powder using a mixer grinder. Nitrogen (N) content was quantified using the Kjeldahl procedure as detailed by Nelson and Sommers (1973). Phosphorus (P) was analyzed by digesting one g of the ground material in a nitric acid–hydrochloric acid mixture (9:4, v/v; 60% HCl), and the digest was read at 660 nm using a spectrophotometer (Jackson, 1973). Exchangeable potassium (K), calcium (Ca), magnesium (Mg), and micronutrients including iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were determined with an atomic absorption spectrophotometer (Varian AA 240FS) following the method of Thomas (1982).

For the enzyme activity assays, one g of soil was taken in

a 50 mL Erlenmeyer flask, followed by the addition of 250 µL of toluene and 4 mL of modified universal buffer (adjusted to pH 6.5 and pH 11.0 for acid phosphatase and alkaline phosphatase respectively). Then, 1 mL of p-nitrophenyl phosphate solution was added and the mixture was incubated at 37 °C for one hour in sealed form. After incubation, 1 mL of 0.5 M CaCl₂ and 4 mL of 0.5 M NaOH were added, mixed thoroughly, and it was filtered through

Whatman No. 2 filter paper. The amount of p-nitrophenol released was measured using a spectrophotometer (Shimadzu UV-1800) at 420 nm (Tabatabai and Bremner, 1969).

Soil dehydrogenase (DHA) activity was measured following Casida *et al.* (1964) with minor modifications. In this procedure, 18 g of soil was treated with a 3% solution of 2,3,5-triphenyltetrazolium chloride (TTC) and incubated at 37 °C for 24 hours. During incubation, TTC is reduced to triphenyl formazan (TPF), which was extracted with methanol. The absorbance of the methanolic extract was then read at 485 nm using a spectrophotometer to determine enzyme activity.

Apparent Recovery Efficiency of Phosphorus (AREP)

AREP was estimated to assess the proportion of applied phosphorus assimilated by the plants. It was calculated using the following equation (Ran *et al.*, 2023):

$$\text{AREP}(\%) = \frac{\text{P uptake (treated)} - \text{P uptake (control)}}{\text{P applied}} \times 100$$

Here, P uptake (treated) and P uptake (control) refer to the total phosphorus absorbed by plants (g plant⁻¹) in the phosphorus-treated pots and the control pots, respectively. P applied represents the amount of phosphorus added through the fertilizer (g plant⁻¹).

Statistical analysis

The study followed a two-factor completely randomized design (CRD), incorporating three AM inoculation rates (0, 25, and 50 g pot⁻¹) and four phosphorus levels (0%, 50%, 75%, and 100% of the recommended dose). Data were analyzed using ANOVA to evaluate the effects of individual treatments and their interactions. When significant differences were detected, means were compared using the least significant difference (LSD) test at the 5% level. All statistical analyses were carried out in R (version 4.3.2; R Core Team, 2024), employing

the base stats functions, with post-hoc tests conducted using the agricolae package (de Mendiburu, 2021).

Results and discussion

Influence of AM fungi and phosphorus levels on nutmeg seedling growth and AM fungi sporulation

The effects of AM fungi, phosphorus (P) supply, and their interaction on plant growth parameters and AM fungi spore production are presented in Table 1 and Supplementary Table 1. Shoot growth responded significantly to P supply ($p = 0.0017$), wherein the highest shoot length (174 cm) was observed with 50 g AM fungi at 100% P, followed by 172 cm in the same AM fungi level with 75% P. Root length, however, was significantly affected by both P ($p < 0.001$) and the AM fungi $\times$ P interaction ($p = 0.012$). The maximum root length (55.5 cm) was noticed with 50 g AM fungi combined with 100% P.

AM fungi spore production was strongly affected by AM fungi ($p < 0.001$), P supply ($p < 0.001$), and their interaction ($p < 0.001$). The maximum spore density (35 spores 50 g⁻¹ soil) was observed in 50 g AM fungi at 75% P, followed by 30 spores 50 g⁻¹ soil with 100% P. In uninoculated plants, spore counts were negligible across all P levels. A consistent trend indicated that moderate P (50–75%) favoured sporulation more than full P supply, suggesting possible suppression of AM fungi activity under high P availability.

Phosphorus application had a clear positive effect on all growth traits, while AM fungi inoculation specifically improved dry weight, stem girth and spore production. The significant AM fungi $\times$ P interactions seen for root length, girth and spore count highlight the importance of combining nutrient management with microbial inputs in tree-spice nurseries.

Treatments that received 50 g of AM fungi along with 50–75% of the recommended P

Table 1. Analysis of variance (ANOVA) for shoot length, root length, stem girth, dry weight, and AM fungal spore count of nutmeg seedlings

Parameter	Source	df	F value	P value
Shoot length (cm)	AMF	2	0.46	NS
	P	3	6.83	<0.001
	AMF×P	6	1.63	NS
Root length (cm)	AMF	2	1.69	NS
	P	3	28.9	<0.001
	AMF×P	6	3.47	NS
Girth (mm)	AMF	2	7.26	0.003
	P	3	21.52	<0.001
	AMF×P	6	7.47	<0.001
Dry weight (g)	AMF	2	18.11	<0.001
	P	3	26.57	<0.001
	AMF×P	6	2.23	NS
Spore count (50 g ⁻¹ soil)	AMF	2	170.29	<0.001
	P	3	14.92	<0.001
	AMF×P	6	15.97	<0.001

consistently performed better, indicating that P fertilizer can be reduced without affecting plant growth. The results emanated from the present study is in corroboration with the findings in other spice crops like ginger, turmeric cardamom, and black pepper where AM fungi combined with moderate nutrient inputs enhanced growth and nutrient uptake more effectively than sole nutrient application (Sarathambal *et al* 2022, Sarathambal *et al* 2023b, Sarathambal *et al.*, 2024a; Sarathambal *et al.*, 2025). Similarly, in woody forest plants, *viz.*, *Poincianella pyramidalis* *Cnidoscolus quercifolius* and *Araucaria araucana* an increase in biomass under different availability levels of phosphorus (P) in the soil was reported (Forsi *et al* 2016, Chávez *et al.*, 2023). Similar trends were also observed in *Triticum aestivum* and *Zea mays*, in which AM fungi inoculation combined

with phosphorus application enhanced growth parameters, yield attributes, P uptake, and chlorophyll content (Rashwan & El-Gohary, 2019; Pereira *et al.*, 2020).

Effects of AM fungi and phosphorus on nutrient accumulation in different plant parts

The uptake of macronutrients (N, P, K, Ca, and Mg) varied significantly among plant parts and was strongly influenced by AM fungi inoculation, phosphorus (P) levels, and their interaction (Fig 1). Nitrogen accumulation was highest in stems and roots compared to leaves, with a maximum of 3.61 g plant⁻¹ in stems and 2.37 g plant⁻¹ in roots under 50 g AM fungi with 100% P, while leaves consistently recorded lower uptake. Uptake of P was significantly influenced by AM fungi, P levels, and their interaction. Phosphorus content was greater in

stems ($1.29 \text{ g plant}^{-1}$) obtained in the 50g AM fungi inoculated seedlings supplied with 50% P. Potassium was predominantly concentrated in the stem, reaching $5.65 \text{ g plant}^{-1}$ under 50 g AM fungi with 100% P, followed by the root fraction, whereas leaves accumulated relatively little. Uptake of K was highly significant across all factors and their interaction. Calcium uptake was also higher in the stem, with a maximum of $4.79 \text{ g plant}^{-1}$ under 25 g AM fungi with 75% P, while roots and leaves showed comparatively lower accumulation. AM fungi, P, and their interaction had significant effects on stem and leaf Ca, while root Ca showed only a modest response. Similarly, magnesium was largely concentrated in the stem, recording $3.34 \text{ g plant}^{-1}$ under 50 g AM fungi with 50% P, whereas the root contained the least. Overall, stems acted as the major sink for N, P, K, Ca, and Mg, roots contributed a secondary reservoir role, and leaves accumulated only minor quantities.

The uptake of micronutrients (Fe, Mn, Zn, and Cu) varied significantly among plant parts and was strongly influenced by AM fungi inoculation, phosphorus (P) application, and their interaction (Fig 2). Iron (Fe) was predominantly accumulated in stems, with the maximum value of $75.90 \text{ mg plant}^{-1}$ at 50 g AM fungi with 75% P while roots also contributed considerably $57.22 \text{ mg plant}^{-1}$ under 25 g AM fungi with 50% P, followed by $56.61 \text{ mg plant}^{-1}$ under 50 g AM fungi with 50% P whereas, the leaves accumulated comparatively less. Root and leaf Fe uptake were highly significant for AM fungi, P, and their interaction, whereas stem Fe was primarily influenced by P and AM fungi $\times$ P. Manganese (Mn) accumulation was highest in stems, reaching $78.38 \text{ mg plant}^{-1}$ under 25 g AM fungi with 100% P, whereas roots recorded moderate amounts (up to $19.09 \text{ mg plant}^{-1}$) and leaves showed only minor uptake. Uptake of Mn in all plant parts was highly significant under the main and

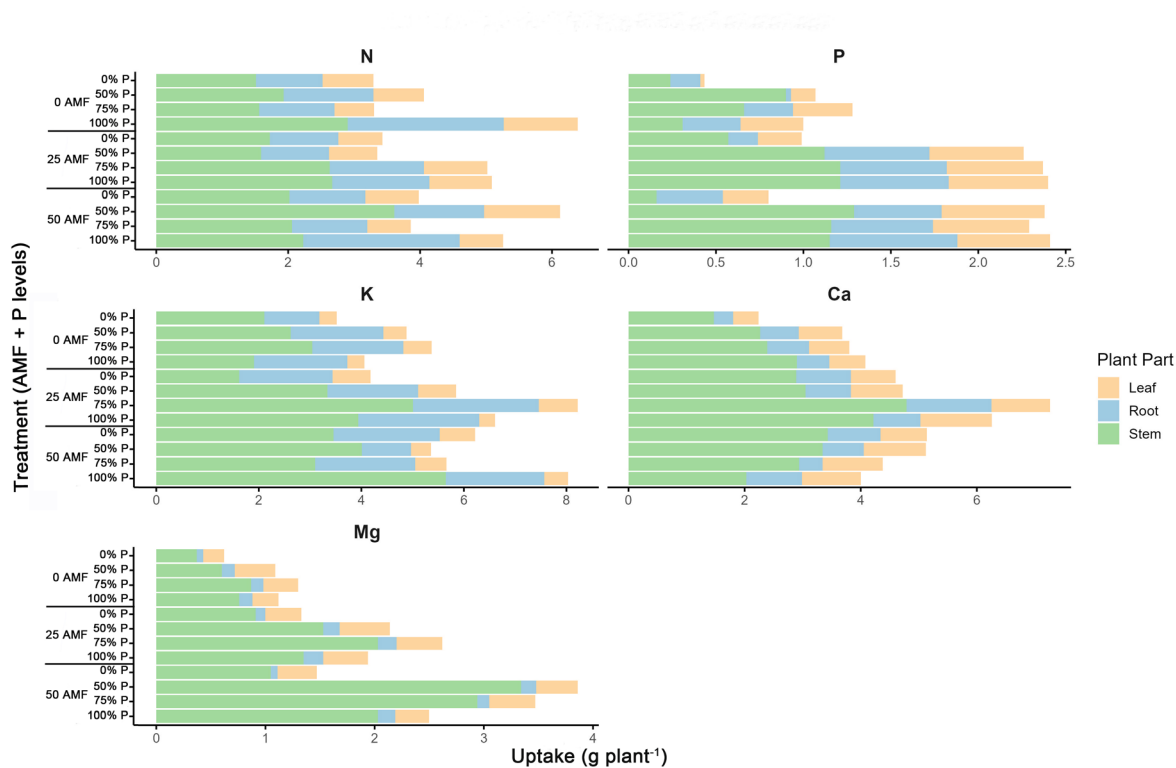


Fig. 1. Effect of AM fungi inoculation and different levels of phosphorus on uptake of macro nutrients in nutmeg seedlings

interaction effects of AM fungi, and P. Zinc (Zn) uptake was distributed between stems and roots, with maximum values of 15.95 and 12.45 mg plant⁻¹, respectively, under 25 g AM fungi with 75% P, while leaves accumulated much less. Zn uptake was significantly affected by AM fungi, P, and their interaction across plant parts. Copper (Cu) uptake was comparatively low across plant parts but showed distinct distribution patterns. Stems accumulated the highest amounts, with a maximum of 14.87 mg plant⁻¹ under 50 g AM fungi with 75% P, followed by moderate uptake in roots (2.53 mg plant⁻¹ in non-AM seedlings with 100% P). Leaves recorded only trace amounts (<0.4 mg plant⁻¹ across all treatments).

In perennial crops, the way nutrients are distributed among plant organs is important not only for immediate growth but also for maintaining reserves that support future

growth cycles and help plants cope with changing nutrient availability. In this study, the stem acted as the major storage site for macronutrients, especially nitrogen and potassium, with the highest N uptake (3.61 g plant⁻¹) recorded under the 50 g AM + 100% P treatment. This nutrient enrichment in structural tissues reflects the contribution of AM fungi in improving nutrient uptake, supporting biomass formation and long-term productivity. Although leaves stored comparatively smaller amounts of nutrients, they responded strongly to AM fungi under moderate phosphorus supply. The highest N and P uptake in leaves occurred at 50 g AM + 50% P, indicating that mycorrhizal association improves nutrient-use efficiency in photosynthetic tissues. This is particularly important in perennial species, where maintaining a healthy canopy is essential for sustained carbon assimilation and overall plant performance.

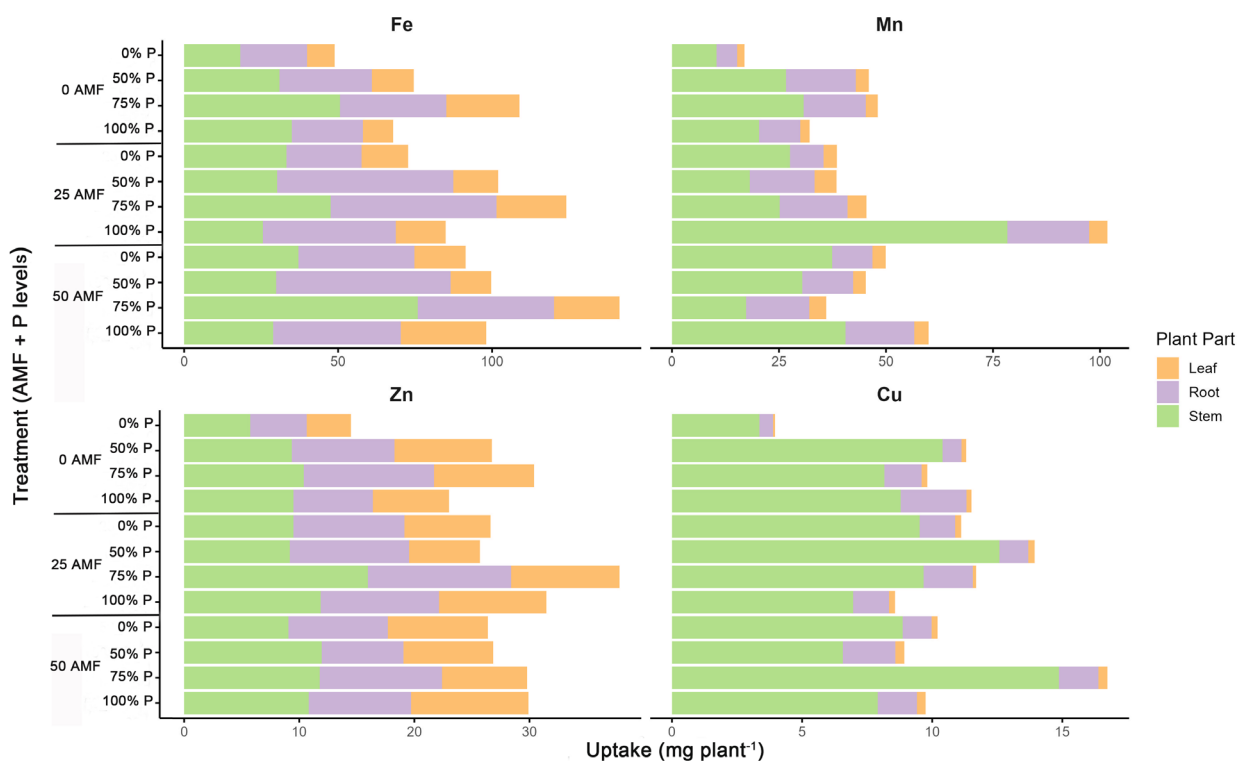


Fig. 2. Effect of AM fungi inoculation and different levels of phosphorus on uptake of micro nutrients in nutmeg seedlings

The stem served as an important storage site for most of the nutrients including Ca, Mg, and micronutrients in treatments receiving 50 g of AM fungi. This accumulation highlights the stem's buffering function in perennial crops, where stored minerals can be remobilized to support new growth flushes and reproductive development. Notably, both the 50% P + AM fungi treatment and the non-mycorrhizal control with 100% P showed the highest root N and P uptake. This indicates that while high fertilizer inputs can partially compensate for the absence of AM fungi, the symbiosis offers clear benefits under lower P levels by improving nutrient capture and internal nutrient storage.

AREP

The apparent recovery efficiency of phosphorus (AREP) was influenced by the interaction of AM inoculation and P levels (Fig 3). Under a 50 g AM application, AREP values increased as the level of applied P decreased. The highest AREP (17.17%) was obtained with 50% P, followed by 75% P (10.80%), whereas the lowest efficiency was recorded at 100% P (8.75%). This trend indicates that AM inoculation enhanced P utilization efficiency, particularly under reduced P inputs, suggesting a synergistic role of AM fungi in improving P recovery when fertilizer application was curtailed.

For perennial crop management, promoting AM fungal colonisation along with balanced phosphorus fertilization can lead to better nutrient distribution, higher long-term nutrient-use efficiency, and reduced dependence on heavy fertilizer inputs. These outcomes are consistent with recent evidence showing that AM fungi improve nutrient allocation in perennial species, supporting both current productivity and long-term system sustainability (Zhang *et al.*, 2023; Basiru *et al.*, 2024).

Soil enzyme activities

Phosphorus levels significantly influenced acid phosphatase activity ($p < 0.001$), whereas AM fungi and the AM fungi $\times$ P interaction were not significant. Acid phosphatase activity was highest in the treatment receiving 50 g AM fungi + 100% P ($19.57 \mu\text{g pNP g}^{-1} \text{h}^{-1}$), followed by 50 g AM fungi + 75% P. A similar trend was observed for alkaline phosphatase activity, with the highest value ($16.60 \mu\text{g pNP g}^{-1} \text{h}^{-1}$) recorded in 50 g AM fungi + 100% P, which was higher than most other treatments (Fig. 4a). Dehydrogenase activity also varied with phosphorus levels, and the maximum activity ($0.81 \text{ mg TPF g}^{-1} \text{h}^{-1}$) was observed in 50 g AM fungi + 100% P, while lower phosphorus levels resulted in comparatively reduced enzyme activity across treatments (Fig. 4b).

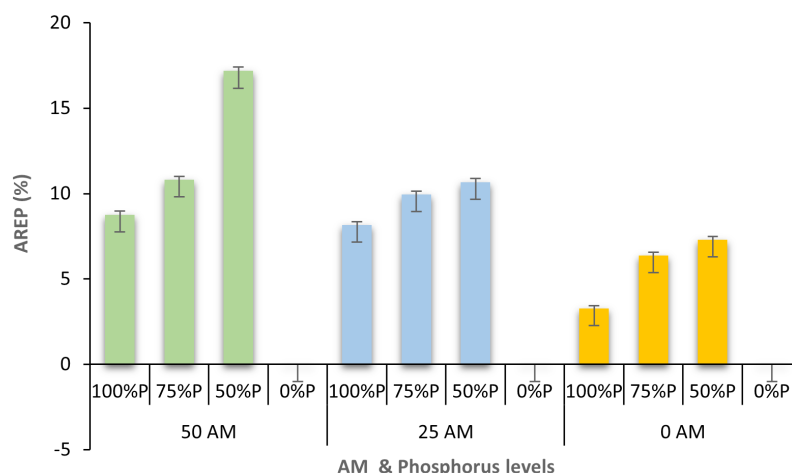


Fig. 3. Effect of AM fungi inoculation and different levels of phosphorus on apparent recovery efficiency of phosphorus (AREP) of nutmeg seedlings

Soil enzymatic activity, an indicator of microbial functioning and nutrient turnover, was also notably influenced by P levels. AM fungi application significantly enhanced dehydrogenase and alkaline phosphatase activities, though acid phosphatase was unaffected. These results corroborated with previous findings in black pepper and cardamom, where AM fungi significantly elevated soil enzymatic activities associated with phosphorus mobilization and microbial respiration (Sarathambal *et al.*, 2023a; Sarathambal *et al.*, 2024b). Such synergism supports the concept of microbial–nutrient interaction enhancing biological soil fertility. For example, in ginger and black pepper systems, AM fungi colonization under moderate P regimes resulted in greater soil biological activity and disease suppression than high P input alone (Sarathambal *et al.*, 2025). This is in line with the findings of Prasad *et al.* (2012), who demonstrated that AM fungi combined with *Pseudomonas fluorescens* significantly enhanced phosphatase activity and phosphorus uptake in *Chrysanthemum indicum*, a perennial ornamental. Enhanced enzymatic activity, especially alkaline phosphatase, plays a pivotal role in the mineralization of organic P and the mobilization of inorganic P bound in soil matrices.

The present findings demonstrate integrated nutrient–bioinoculant approaches can improve phosphorus use efficiency while maintaining seedling growth, thereby reducing phosphorus fertilizer requirements. This strategy offers a practical and sustainable approach for nutrient management in nutmeg nurseries by producing healthy seedlings with lower fertilizer inputs.

Conclusion

The study demonstrated that AM fungi inoculation, in combination with phosphorus (P) application significantly enhanced the growth of nutmeg seedlings besides promoting nutrient uptake, and soil enzyme activity. The interaction between AM fungi and P improved shoot and root development, nutrient accumulation, and phosphorus use efficiency. Stem served as the major nutrient sink, and AM fungi promoted higher phosphorus recovery under moderate P levels. Enhanced soil enzymatic activity reflected improved soil biological health. Overall, AM fungi inoculation with optimum phosphorus supply proved effective in promoting growth and nutrient efficiency, highlighting its potential for sustainable planting material production in nutmeg. Incorporating AM fungi as a component in nursery practices serves as a sustainable and effective strategy to optimize

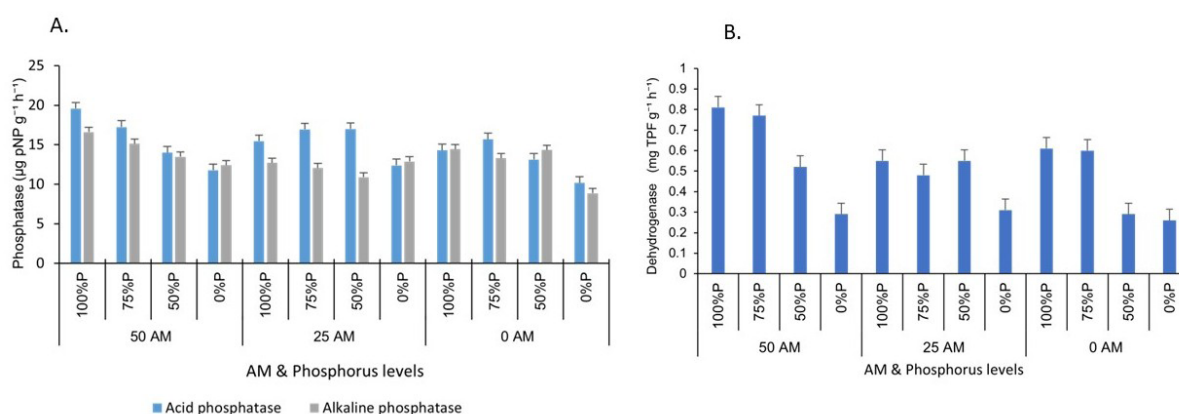


Fig. 4. Effect of AM fungi inoculation and different levels of phosphorus on rhizosphere soil A) alkaline, acid phosphatase and B) dehydrogenase activity of nutmeg seedlings

plant growth and health of nutmeg seedlings, ultimately leading to successful plant establishment under field conditions.

References

- Anandaraj M, Devasahayam S, Zachariah T J, Krishnamoorthy B, Mathew P M & Rema J 2005 Nutmeg (Extension Pamphlet). Indian Institute of Spices Research, Kozhikode.
- Basiru S, Olanrewaju O S & Ayeni F J 2024 Arbuscular mycorrhizal fungi and phosphorus fertilization: synergistic roles in plant nutrient uptake and ecosystem sustainability. *BMC Plant Biol.* 24: 210. <https://doi.org/10.1186/s12870-024-05638-9>.
- Begum N, Qin C, Ahanger M A, Raza S, Khan M I, Ashraf M, Ahmed N & Zhang L 2019 Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Front. Plant Sci.* 10: 1068. <https://doi.org/10.3389/fpls.2019.01068>.
- Biju C N, Jeevalatha A, Peeran M F, Suseela Bhai R, Basima F, Muhammed Nissar V A, Srinivasan V & Thomas L 2021 Association of *Lasiodiplodia theobromae* with die-back and decline of nutmeg as revealed through phenotypic, pathogenicity, and phylogenetic analyses. *3 Biotech* 11: 422. <https://doi.org/10.1007/s13205-021-02961-y>.
- Casida L E Jr, Klein D A & Santoro T 1964 Soil dehydrogenase activity. *Soil Sci.* 98(6): 371–376.
- Chávez D, Rivas G, Machuca Á, Santos C, Deramond C, Aroca R & Cornejo P 2023 Contribution of arbuscular mycorrhizal and endophytic fungi to drought tolerance in *Araucaria araucana* seedlings. *Plants* 12: 2116. <https://doi.org/10.3390/plants12112116>.
- de Mendiburu F 2021 agricolae tutorial (Version 1.3-5). Universidad Nacional Agraria La Molina, Peru.
- Frosi G, Barros V A, Oliveira M T, Cavalcante U M T, Maia L C & Santos M G 2016 Increase in biomass of two woody species from a seasonal dry tropical forest in association with AM fungi with different phosphorus levels. *Appl. Soil Ecol.* 102: 46–52. <https://doi.org/10.1016/j.apsoil.2016.02.009>.
- Gerdemann J W & Nicolson T H 1963 Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* 46: 235–244.
- González-Guerrero M, Escudero V, Saéz Á & Tejada-Jiménez M 2022 Transition metals and phosphorus homeostasis interaction in plants: insights from arbuscular mycorrhizal symbiosis. *Front. Plant Sci.* 13: 860226.
- Hussain M, Tariq A, Ayub M & Asghar W 2022 Arbuscular mycorrhizal fungi improve phosphorus acquisition and early seedling growth of cereals under low-phosphorus soils. *J. Soil Sci. Plant Nutr.* 22(4): 2971–2984.
- Jackson M L 1973 Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi.
- Kareem A A, Ogunwande O A, Olaitan O A & Oyelowo O J 2023 Influence of arbuscular mycorrhiza on the growth potential of *Tamarindus indica* L. seedlings. *J. Cameroon Acad. Sci.* 19(2): 141–148. <https://doi.org/10.4314/jcas.v19i2.3>.
- Krishnamoorthy B & Rema J 2006 Nutmeg and mace. In: Peter K V (Ed.) *Handbook of Herbs and Spices*, Vol. 3, pp. 238–246. Woodhead Publishing, Cambridge.
- Nadeem S M, Li C, Shahzad S M & Wang X 2021 Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in sustainable agriculture. *Front. Plant Sci.* 12: 680906.
- Nelson D W & Sommers L E 1973 Determination of total nitrogen in plant material. *Agron. J.* 65: 109–112. <https://doi.org/10.2134/agronj1973.00021962006500010033x>.
- Newman E I 1966 A method of estimating the total length of root in a sample. *J. Appl. Ecol.* 3: 139–145.
- Nissar V A M, Sasikumar B & Rema S A J 2019 Air layering in nutmeg (*Myristica fragrans* Houtt.). *J. Spices Aromat. Crops* 28(1): 66–69.

- Pereira N C M, Galindo F S, Gazola R P D, Dupas E, Rosa P A L, Mortinho E S & Teixeira Filho M C M 2020 Corn yield and phosphorus use efficiency response to phosphorus rates associated with plant growth promoting bacteria. *Front. Environ. Sci.* 8: 40. <https://doi.org/10.3389/fenvs.2020.00040>.
- Prasad K, Aggarwal A, Yadav K & Tanwar A 2012 Impact of different levels of superphosphate using arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* on *Chrysanthemum indicum* L. *J. Soil Sci. Plant Nutr.* 12(3): 451–462.
- R Core Team 2024 R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ran An, Yu Rui-Peng, Xing Yi, Zhang Jiu-Dong, Bao Xing-Guo *et al.* 2023 Enhanced phosphorus fertilizer-use efficiency and sustainable phosphorus management with intercropping. *Agron. Sustain. Dev.* 43(5): 57. <https://doi.org/10.1007/s13593-023-00916-6>.
- Rashwan E & El-Gohary Y 2019 Impact of different levels of phosphorus and seed inoculation with arbuscular mycorrhiza (AM) on growth, yield traits, and productivity of wheat. *Egypt. J. Agron.* 41(1): 11–20. <https://doi.org/10.21608/agro.2019.6329.1135>.
- Sarathambal C, Dinesh R, Srinivasan V, Sheeja T E, Jeeva V & Manzoor M 2022 Changes in bacterial diversity and composition in response to co-inoculation of arbuscular mycorrhizae and zinc-solubilizing bacteria in turmeric rhizosphere. *Curr. Microbiol.* 79(1): 4.
- Sarathambal C *et al.* 2023a Effect of arbuscular mycorrhizal inoculation on growth, mineral nutrient uptake, photosynthesis, and antioxidant activities of black pepper cuttings. *J. Plant Nutr.* 46(10): 2508–2524.
- Sarathambal C *et al.* 2023b Arbuscular mycorrhizal colonization alters biochemical, molecular defense responses, and root exudate composition against *Phytophthora capsici* infection in black pepper. *Rhizosphere* 25: 100651.
- Sarathambal C *et al.* 2024a Optimizing mycorrhizal fungi application for improved nutrient uptake, growth, and disease resistance in cardamom seedlings. *Heliyon* 10: e39227.
- Sarathambal C *et al.* 2024b Unravelling the synergistic effects of arbuscular mycorrhizal fungi and vermicompost on improving plant growth, nutrient absorption, and secondary metabolite production in ginger. *Front. Sustain. Food Syst.* 8: 1412610.
- Sarathambal C *et al.* 2025 Arbuscular mycorrhizal colonization promotes plant growth and regulates biochemical and molecular defense responses against *Pythium myriotylum* and *Meloidogyne incognita* in ginger. *Rhizosphere* 34: 101071.
- Tabatabai M A & Bremner J M 1969 Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1: 301–307.
- Thomas G W 1982 Exchangeable cations. In: Page A L (Ed.) *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*, pp. 159–165. American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin.
- Wilkes T I, Warner D J, Edmonds-Brown V & Davies K G 2020 Species-specific interactions of *Bacillus innocula* and arbuscular mycorrhizal fungi symbiosis with winter wheat. *Microorganisms* 8(11): 1795.
- Zhang Q, Chen X & Zhao J 2023 Arbuscular mycorrhizal associations alter nutrient partitioning in roots and shoots of woody perennials. *Appl. Soil Ecol.* 187: 104888. <https://doi.org/10.1016/j.apsoil.2023.104888>.