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Nutrient Uptake and Yield Potential of Bhendi as Influenced by Humic Acid, Micronutrients Mixture and Plant Growth Regulators

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Abstract

Sustainable intensification of agriculture increasingly depends on biological processes that enhance soil health and nutrient-use efficiency. Arbuscular mycorrhizal fungi (AMF) constitute one of the most important functional groups of soil microorganisms, playing a vital role in nutrient acquisition, soil aggregation, and plant tolerance to environmental stresses. The present study evaluates the contribution of AMF to soil fertility and crop productivity in representative agricultural fields of Nashik District, Maharashtra, India. Ten fields under major crops onion, maize, wheat, soybean, and grape were assessed for AMF root colonization, spore density, soil physicochemical properties, and crop performance. Fields exhibiting higher AMF colonization showed significantly greater soil organic carbon, available nitrogen, phosphorus, and potassium, along with enhanced crop growth and yield. Yield improvement ranged from 12-20% across crops, with onion and soybean showing the highest responses. Strong positive correlations between AMF abundance and soil fertility indices confirm the central role of mycorrhizal symbiosis in sustainable nutrient management. The findings establish AMF as effective biological inputs capable of reducing dependency on chemical fertilizers while improving soil resilience. Integration of AMF into conventional and integrated farming systems is recommended to support climate-resilient and sustainable agriculture in semi-arid regions of India.

Key words: Arbuscular mycorrhizal fungi, Soil fertility, Crop yield, Sustainable agriculture, Biofertilizers

Agricultural intensification over the past five decades has significantly enhanced global food production; however, it has also accelerated soil degradation, depletion of organic matter, nutrient imbalance, and loss of biological diversity in agroecosystems [1-2]. In India, especially in semi-arid regions, indiscriminate and prolonged use of chemical fertilizers and pesticides has disrupted natural nutrient cycling processes and weakened soil resilience. These challenges have encouraged a gradual shift toward ecologically based agricultural systems that emphasize biological inputs for sustaining productivity while minimizing environmental impact. Among beneficial soil microorganisms, arbuscular mycorrhizal fungi (AMF) are widely recognized as keystone components of soil plant systems. These obligate symbionts associate with nearly 80-90% of terrestrial plant species and play a pivotal role in plant nutrition, particularly by enhancing phosphorus uptake in nutrient-poor soils [3-4]. AMF form extensive extraradical hyphal networks that increase the effective absorptive area of plant roots, facilitating the uptake of nitrogen, potassium, and essential micronutrients such as zinc and copper. In addition to nutrient acquisition, AMF significantly improve soil physical structure by promoting aggregation through the production of glomalin, a glycoprotein that enhances soil stability, water infiltration, and resistance to erosion [5].

Recent research has demonstrated that AMF enhance plant tolerance to abiotic stresses such as drought, salinity, and temperature extremes by improving water relations, osmotic adjustment, and antioxidant defense mechanisms [6-7]. These attributes make arbuscular mycorrhizal fungi (AMF) indispensable partners in climate-resilient agriculture. Nashik District, Maharashtra, is a major agricultural hub characterized by intensive cultivation of onion, grape, maize, wheat, soybean, and vegetables. The region experiences marked rainfall variability and increasing dependence on chemical inputs, raising concerns about long-term soil sustainability. Although AMF are ubiquitous in agricultural soils, systematic field-based assessments of their contribution to soil health and crop productivity in Nashik remain limited. Earlier investigations by [8-11] demonstrated the relevance of arbuscular mycorrhizal fungi (AMF) in disease suppression, nutrient uptake, and crop growth in this region, yet comprehensive field validation under diverse cropping systems has been scarce. The present investigation aims to bridge this gap by evaluating the ecological and agronomic significance of AMF under real farming conditions in Nashik District. By linking AMF colonization patterns with soil fertility parameters and crop performance, this study provides a scientific basis for integrating mycorrhizal technology into sustainable agricultural systems.

Ecology and evolution of arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi represent one of the most ancient and widespread plant–microbe symbioses, with origins dating back more than 400 million years. Fossil and molecular

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evidence suggests that AMF facilitated the early colonization of terrestrial environments by plants, enabling efficient nutrient acquisition from primitive soils [12]. Belonging to the phylum *Glomeromycota*, AMF occur across almost all terrestrial ecosystems and associate with most agricultural crops, underscoring their fundamental ecological importance [13].

AMF and soil fertility

Phosphorus deficiency remains a major constraint to crop productivity in tropical and subtropical soils. AMF enhance phosphorus uptake by mobilizing sparingly soluble phosphate pools through enzymatic activity and extensive hyphal exploration beyond the root depletion zone [4]. In addition to phosphorus, AMF improve the acquisition of nitrogen, potassium, and essential micronutrients, while stimulating beneficial rhizosphere microorganisms that contribute to nutrient cycling and disease suppression [14-15].

AMF and soil structure

Beyond nutrient dynamics, AMF play a crucial role in improving soil physical properties. The glycoprotein glomalin produced by AMF contributes substantially to soil carbon sequestration and aggregate stability [5]. Enhanced aggregation improves soil porosity, water-holding capacity, and resistance to erosion attributes that are especially important in drought-prone agricultural regions.

Relevance in Indian agriculture

Several studies in India consistently report positive responses of cereals, pulses, vegetables, and horticultural crops to AMF inoculation, including improved growth, yield, and stress tolerance [16-17]. Region-specific investigations by Patale and Shinde [8] demonstrated the influence of *Glomus* species on disease dynamics in Bt cotton, while Patale (2016) documented variability in AMF abundance in brinjal rhizospheres of Yeola taluka. More recently, Patale [9-10] established the role of AMF in enhancing onion and eggplant productivity under semi-arid conditions, highlighting their practical value in local farming systems. Specific objectives of this investigation are as:

1. To quantify AMF root colonization and spore density in agricultural soils of Nashik District.
2. To evaluate the influence of AMF on soil physicochemical and biological properties.
3. To assess the impact of AMF association on crop growth and yield.
4. To promote AMF-based bioinputs for sustainable agricultural development in Maharashtra.

MATERIALS AND METHODS

Study area

The investigation was conducted in Nashik District, Maharashtra, India (19°33′–20°53′ N; 73°16′–74°56′ E). The region experiences a semi-arid to sub-humid climate with average annual rainfall of 700-900 mm. Dominant crops include onion, grape, maize, wheat, soybean, and seasonal vegetables.

Field selection and sampling

Ten representative agricultural fields were selected across different talukas to capture variability in cropping systems and management practices. Composite soil samples were collected from the rhizosphere (0-20 cm depth) using a randomized sampling approach. Fine root samples were simultaneously collected from standing crops during the active growth stage.

Estimation of AMF colonization

Roots were cleared in potassium hydroxide and stained with trypan blue following Phillips and Hayman [18]. Percentage root colonization was determined using the gridline intersect method of Giovannetti and Mosse [19].

Spore density

AMF spores were isolated from soil using the wet-sieving and decanting technique [20] and counted under a stereomicroscope. Spore density was expressed as the number of spores per 100 g of dry soil.

Soil and crop analysis

Soil pH, electrical conductivity, organic carbon, and available nitrogen, phosphorus, and potassium were analyzed using standard methods [21]. Crop growth parameters (plant height and biomass) and yield were recorded from representative plants in each field.

Statistical analysis

Descriptive statistics and Pearson correlation analyses were used to evaluate relationships between AMF colonization, soil fertility parameters, and crop productivity.

RESULTS AND DISCUSSION

AMF colonization and spore density

Significant variation in AMF colonization and spore density was observed among the surveyed fields, reflecting differences in crop species, soil conditions, and management practices. Root colonization ranged from 45% to 82%, while spore density varied from 150 to 450 spores per 100 g soil. Grape and soybean fields recorded the highest AMF abundance, indicating that crop type and rhizosphere environment strongly influence AMF propagation.

Table 1 AMF colonization and spore density in selected fields

Field	F_1	F_2	F_3	F_4	F_5	F_6
Crop	Onion	Maize	Wheat	Soybean	Onion	Grape
AMF Colonization (%)	45	52	60	68	75	82
Spore Density (per 100 g soil)	150	180	210	260	320	450

The data presented in (Table 1) reveal substantial variability in arbuscular mycorrhizal fungi (AMF) colonization and spore density across the six surveyed fields (F_1-F_6), clearly indicating the influence of crop type and rhizospheric conditions on AMF dynamics. AMF root colonization ranged from 45% in F_1 (onion) to 82% in F_6 (grape), while spore density increased correspondingly from 150 to 450 spores per 100 g of soil. This progressive increase suggests a strong positive relationship between root colonization and spore population, implying that fields supporting higher fungal establishment within roots also tend to harbor greater propagule reserves in the soil.

Among the crops studied, grape (F_6) exhibited the highest AMF colonization (82%) and spore density (450), followed by onion (F_5) and soybean (F_4). The elevated AMF association in grape fields can be attributed to the perennial nature of the crop, which provides a stable and undisturbed rhizosphere that favors continuous fungal growth, sporulation, and hyphal network development. In contrast, annual crops such as onion, maize, and wheat showed comparatively lower colonization levels, particularly in F_1 (onion), where minimal colonization (45%) and spore density (150) were recorded. This may be due to frequent soil disturbances, shorter crop duration, and possible agrochemical inputs that negatively affect AMF survival and proliferation.

A gradual increase in AMF colonization from maize (52%) to wheat (60%) to soybean (68%) further indicates crop-specific dependency on mycorrhizal associations. Leguminous crops like soybean typically exhibit higher mycorrhizal responsiveness due to their greater nutrient demand, especially phosphorus, and their symbiotic compatibility with soil microorganisms. This is reflected in the relatively higher colonization and spore density observed in soybean fields. Interestingly, the difference between the two onion fields (F_1 and F_5) is noteworthy. While F_1 recorded the lowest AMF parameters, F_5 showed substantially higher colonization (75%) and spore density (320). This variation within the same crop suggests that soil management practices, organic matter content, irrigation regimes, and previous cropping history play a crucial role in determining AMF abundance, sometimes even more than crop type alone [22].

The strong correspondence between spore density and colonization percentage also indicates that spore count can serve as a reliable indicator of AMF activity and soil biological health. Higher spore density reflects a robust fungal community capable of rapid colonization and nutrient exchange, thereby contributing to improved soil fertility and plant performance. Overall, the findings emphasize that AMF distribution is not uniform but is shaped by a combination of biological, environmental, and agronomic factors [23]. Fields with perennial crops and favorable soil conditions tend to support higher AMF populations, while intensively managed annual cropping systems may limit their development. These insights highlight the need for adopting AMF-friendly agricultural practices, such as reduced tillage, organic amendments, and crop diversification, to enhance soil microbial health and sustain long-term productivity [24].

Soil fertility parameters

Fields were categorized into low-AMF and high-AMF groups based on colonization levels. Soils with higher AMF colonization exhibited significantly improved fertility status. Organic carbon increased by nearly 71%, while available phosphorus showed more than a twofold increase, demonstrating the efficiency of AMF-mediated nutrient mobilization.

Table 2 Soil fertility parameters (Mean ± SD)

Parameter	Organic carbon (%)	Available N (kg ha⁻¹)	Available P (kg ha⁻¹)	Available K (kg ha⁻¹)
Low-AMF fields	0.48 ± 0.05	210 ± 12	14 ± 2	280 ± 18
High-AMF fields	0.82 ± 0.06	265 ± 15	31 ± 3	345 ± 20

The comparative assessment of soil fertility parameters between low-AMF and high-AMF fields provides compelling evidence of the positive role of arbuscular mycorrhizal fungi (AMF) in enhancing soil quality and nutrient dynamics. The clear differences observed across all measured parameters organic carbon, available nitrogen (N), phosphorus (P), and potassium (K) indicate that AMF colonization is closely associated with improved soil fertility status. One of the most notable findings is the substantial increase in soil organic carbon (SOC), which rose from 0.48% in low-AMF fields to 0.82% in high-AMF fields, representing an increase of nearly 71%. This improvement can be attributed to the role of AMF in promoting carbon sequestration through the production of glomalin, a glycoprotein that contributes to soil aggregation and stabilization of organic matter. Enhanced root biomass and microbial activity in AMF-rich soils further contribute to the buildup of organic carbon, which is a key indicator of soil health and long-term fertility [25].

The availability of nitrogen also showed a marked increase, from 210 kg ha^{-1} to 265 kg ha^{-1} in high-AMF soils. Although AMF are not direct nitrogen fixers, their symbiotic association improves root surface area and nutrient absorption efficiency, thereby facilitating better nitrogen uptake. Additionally, improved soil structure and microbial interactions in AMF-rich environments may enhance nitrogen mineralization and retention, reducing losses through leaching. A particularly significant enhancement was observed in available phosphorus, which increased from 14 kg ha^{-1} in low-AMF fields to 31 kg ha^{-1} in high-AMF fields, more than doubling in concentration [26]. This finding strongly supports the well-established role of AMF in phosphorus mobilization. Through extensive hyphal networks, AMF can access phosphorus beyond the depletion zone surrounding plant roots and convert it into plant-available forms via enzymatic activity. This is especially important in soils where phosphorus is present in insoluble or fixed forms, making AMF a critical component in improving phosphorus use efficiency [27].

Similarly, available potassium increased from 280 kg ha^{-1} to 345 kg ha^{-1} in high-AMF fields. While potassium uptake is less directly associated with AMF than phosphorus, the improved soil structure, enhanced cation exchange capacity, and increased microbial activity in AMF-rich soils likely contribute to better potassium availability and retention [28]. The relatively low standard deviation values across parameters indicate consistency within each group, suggesting that the observed differences are systematic rather than incidental. This strengthens the reliability of the findings and underscores the significant influence of AMF colonization on soil fertility. Overall, the results clearly demonstrate that soils with higher AMF colonization are biologically more active and nutritionally richer. The synergistic effects of improved organic matter content, enhanced nutrient mobilization, and better soil physical properties contribute to a more fertile and sustainable soil system. These findings reinforce the importance of integrating AMF-friendly practices in agricultural management to maintain soil health, optimize nutrient use, and support sustainable crop production [29].

Crop growth performance

All crops recorded improved vegetative growth under AMF-rich conditions. Onion and soybean exhibited the highest growth response, indicating stronger dependence on mycorrhizal pathways.

The data presented in (Table 3) clearly demonstrate the positive influence of arbuscular mycorrhizal fungi (AMF) on the vegetative growth of all selected crops, as reflected by increased plant height across onion, maize, wheat, and soybean. The comparative assessment between AMF-inoculated and non-inoculated plants reveals a consistent enhancement in growth performance, indicating the functional significance of arbuscular mycorrhizal fungi (AMF) symbiosis in crop development.

Table 3 Effect of arbuscular mycorrhizal fungi (AMF) on plant height

Crop	Onion	Maize	Wheat	Soybean
Height without AMF (cm)	52	145	78	42
Height with AMF (cm)	61	165	88	49
% Increase	17.3	13.8	12.8	16.6

Plant height, being a key indicator of overall vegetative vigor, showed marked improvement in AMF-associated treatments. Onion exhibited an increase from 52 cm to 61 cm (17.3%), soybean from 42 cm to 49 cm (16.6%), maize from 145 cm to 165 cm (13.8%), and wheat from 78 cm to 88 cm (12.8%). This enhancement can be attributed to the improved nutrient acquisition facilitated by AMF, particularly phosphorus uptake, along with better absorption of micronutrients such as zinc and copper. Additionally, AMF enhances water uptake efficiency and root surface area through the development of extensive hyphal networks, which collectively contribute to improved plant growth [30-31].

Among the crops studied, onion and soybean recorded the highest percentage increases in plant height. This suggests a relatively higher dependency of these crops on mycorrhizal associations, possibly due to their comparatively less efficient root systems or higher nutrient demand during early growth stages. The stronger response in these crops may also be linked to their physiological compatibility with arbuscular mycorrhizal fungi (AMF), leading to more effective symbiotic interactions and nutrient exchange mechanisms.

In contrast, maize and wheat showed comparatively lower but still significant increases in height. These cereals generally possess more extensive and fibrous root systems, which may reduce their relative dependence on arbuscular mycorrhizal fungi (AMF) for nutrient acquisition. However, the observed growth improvement still highlights the supplementary role of AMF in enhancing nutrient use efficiency and promoting overall plant health. The uniform increase in plant height across all crops indicates that arbuscular mycorrhizal fungi (AMF) contribute not only to nutrient uptake but also to improved hormonal balance, particularly through the production of growth-promoting substances such as auxins and cytokinins. These biochemical influences may further stimulate cell division and elongation, thereby enhancing plant stature. Overall, the findings underscore the agronomic importance of AMF in promoting crop growth, with variable responses depending on crop type. The greater responsiveness of onion and soybean emphasizes the need for targeted arbuscular mycorrhizal fungi (AMF) application in crops that are more reliant on mycorrhizal symbiosis, thereby improving productivity and sustainability in agricultural systems [32].

Table 4 Effect of arbuscular mycorrhizal fungi (AMF) on crop yield

Crop	Onion	Maize	Wheat	Soybean
Yield without AMF (q ha^{-1})	210	48	32	22
Yield with AMF (q ha^{-1})	250	56	36	26

Yield enhancement

Yield enhancement ranged from 12.5% to 19.0%. Onion and soybean recorded the highest yield improvement, confirming the agronomic significance of AMF under field conditions [33]. The present study clearly demonstrates that arbuscular mycorrhizal fungi play a decisive role in improving both soil fertility and crop productivity in Nashik district. Higher AMF colonization was consistently associated with increased soil organic carbon and greater availability of essential macronutrients, particularly phosphorus an element often limiting in the alkaline soils of Maharashtra.

The improvement in soil structure observed in AMF-rich fields can be attributed to glomalin-mediated aggregation, which enhances water infiltration, reduces erosion, and increases resistance to drought stress. Such structural benefits are critical in semi-arid agroecosystems where rainfall variability frequently constrains crop productivity. The observed enhancement in crop growth and yield aligns with global meta-analyses reporting consistent productivity benefits of AMF under field conditions [34-35]. The relatively greater response of onion and soybean corroborates earlier findings by Patale [10-11], emphasizing the potential of AMF-based bioinputs in intensively managed horticultural systems. Beyond nutrition, AMF-mediated improvements in plant water relations and stress tolerance likely contributed to yield stability under fluctuating climatic conditions. These results reinforce the role of AMF as strategic biological tools in climate-smart agriculture.

The present findings strongly corroborate the established role of arbuscular mycorrhizal fungi (AMF) as key drivers of soil health and crop productivity, particularly in resource-constrained agroecosystems. The observed improvements in soil structure through glomalin-mediated aggregation align with widely reported mechanisms whereby AMF enhance soil stability, water infiltration, and moisture retention, thereby mitigating the adverse effects of drought and rainfall variability. This improved soil physical environment directly supports better root growth and nutrient acquisition, which is reflected in the significant yield increases recorded across all crops in (Table 4). The comparatively higher yield responses in onion and soybean further substantiate earlier studies indicating a greater dependency of horticultural and leguminous crops on mycorrhizal associations for efficient phosphorus uptake and stress resilience. Moreover, the consistency of these results with global meta-analytical evidence reinforces the reliability and broader applicability of AMF-based interventions under field conditions. Beyond nutrient dynamics, the contribution of AMF to improved plant water relations and physiological stability under fluctuating climatic conditions highlights their integral role in enhancing yield sustainability [36]. Collectively, these findings validate the strategic importance of AMF as eco-friendly bioinputs that integrate soil restoration, crop productivity enhancement, and climate resilience within sustainable agricultural systems.

CONCLUSION

The present investigation clearly establishes that arbuscular mycorrhizal fungi (AMF) play a pivotal role in enhancing soil fertility, plant growth, and overall crop productivity under field conditions. The observed variation in AMF colonization and spore density across different crops highlights the strong influence of crop type, rhizosphere environment, and management practices on mycorrhizal development. Higher colonization levels, particularly in grape and soybean fields, indicate favorable soil plant microbe interactions that promote AMF proliferation. A significant improvement in soil fertility parameters in high-AMF fields underscores the functional importance of AMF in nutrient cycling. The marked increase in organic carbon, along with enhanced availability of nitrogen, phosphorus, and potassium, demonstrates the efficiency of AMF in mobilizing essential nutrients, especially phosphorus, which is often a limiting factor in many agricultural soils. These improvements also contribute to better soil structure and moisture retention, thereby increasing resilience under stress conditions. The positive impact of AMF on crop growth and yield further reinforces their agronomic significance. All studied crops exhibited enhanced plant height and yield under AMF-rich conditions, with increases ranging from approximately 12% to 19%. Crops like onion and soybean showed comparatively higher responsiveness, indicating their greater dependency on mycorrhizal associations for nutrient uptake and growth. Overall, the study highlights AMF as a sustainable and eco-friendly biological input with immense potential to improve soil health, reduce dependency on chemical fertilizers, and enhance crop productivity. The integration of AMF-based practices into conventional farming systems can contribute significantly to sustainable agriculture, particularly in regions facing soil degradation and climatic uncertainties.

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