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The Utilization of Arbuscular Mycorrhizal Fungi and Accessible Phosphorus in Enhancing Garlic (Allium Sativum L.) Yield.

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Abstract

Enhancing the economic productivity of widely cultivated crops is a critical challenge facing both society and science in contemporary agriculture. This study aimed to explore the effectiveness of arbuscular mycorrhizal fungi (AMF) in optimizing phosphorus (P) utilization and boosting the yield of garlic plants cultivated in sandy soil with an open irrigation system. The results demonstrated that the inoculation of garlic with AMF, combined with the application of 120 kg of P fertilizer per hectare, significantly improved both the fresh and dry weights, as well as the chlorophyll content in the roots, shoots, and bulbs over two growing seasons. Furthermore, AMF enhanced the bioavailability of P in the rhizosphere and markedly improved nitrogen utilization in the inoculated plants. These results suggest that field inoculation of garlic with AMF can be highly effective in increasing garlic yields. Additionally, this research positions AMF as a cost-effective and promising option for the sustainable cultivation of garlic in reclaimed sandy soils using a drip irrigation system.

Keywords- Arbuscular Mycorrhizal Fungi (AMF),Yield , chemical and physical Properties etc.

Introduction

One of the most significant commercial crops, garlic is ranked as the second most economical value after some cultivated vegetables (FAO, 2020). The plant has a unique flavor and is used in dishes, soups, sandwiches, and salads, as well as cooked on its own as a vegetable. Garlic is consumed when it matures as a dry bulb or when it is still young and contains proteins, starch, sugars, and some vitamins (Jilani et al., 2010). In addition to its nutritional value, garlic has shown some medical applications due to the presence of several anticancer agents (Bagali et al., 2012) that have been shown to prevent cancer in animals. Typically, the edible parts of Allium spp. plants contain volatile sulfur-containing compounds that have a distinct flavor and are responsible pungent odor. Garlic flavor is dominated by physiologically active organo-sulfuric chemicals called S-alk(en)yl-L-cysteine sulfoxides, which include g-glutamylcysteines and alliin. Furthermore, the cytoplasm and the vacuole contain sulfoxides. These chemicals are responsible for the unique smell and taste of onion and most of its biological qualities Lanzotti (2006).

Chemical fertilizers are currently used to boost the production of most crops and meet the growing demands for food due to the growing human population, but they have high production costs and have a negative impact on the environment. As a result, there is a growing and will continue to be interest in finding alternative green sources, such as biofertilizers, as eco-friendly systems that can improve crop yield and lower production costs. Recently, the use of microbial populations as bio-fertilizers has emerged as a promising alternative to these chemicals for lower production costs, improved environmental sustainability, and increased yield (Egamberdiyera, 2007).

Arbuscular mycorrhizal fungi (AMF) have been shown in several studies to enhance plant development by improving the bioavailability of phosphorus (P) to host and enhancing both water and nutrient absorption (Golubkina et al., 2020) . Arbuscular mycorrhizal fungi (AMF) exhibit synergistic interactions with various microorganisms, leading to increased crop yields (Lukiwatid and Simanungkalit, 2002; Heggo and Barakah, 2003; Marulanda et al., 2003). The relationship between plants and AMF is a crucial one within the rhizosphere, contributing to enhancements in the physical, chemical, and biological characteristics of the soil (Smith and Smith, 2011). AMF function in the rhizosphere through a variety of mechanisms, including the solubilization of phosphorus via organic acid production (Eldhuset et al., 2007). Utilizing AMF as phosphate-solubilizing microorganisms has been suggested as an energy-efficient and cost-effective strategy to improve the efficacy of phosphate fertilizers (Richardson, 2001; Gyaneshwar et al., 2002). Additionally, nitrogen fertilizers play a significant role in agricultural pollution, contributing to issues such as nitrate leaching, ammonia volatilization, and nitrous oxide emissions; therefore, optimizing fertilizer use efficiency is essential (Lobos Ortega et al., 2016).

2. Material and methods

2.1. Garlic crop and soil-

Garlic (Allium sativum L.) clove were obtained from the National Horticulture and Research Development Foundation (NHRDF) located in Lasalgaon, Tal. Niphad, Dist. Nashik. The field soil utilized in this research was reclaimed from sandy soil and underwent a series of chemical, physical, and mechanical analyses as outlined by Carter and Gregorich , 2007. A comprehensive list of all examined parameters can be found in Table 1.

2.2. Isolation arbuscular mycorrhizal fungi (AMF) and inoculum preparation

Spores of arbuscular mycorrhizal fungi (AMF) were isolated from the rhizosphere of fertile soil cultivated with garlic plants in Panchakeshwar, Tal-Niphad, Dist-Nashik (MH). The extraction of mycorrhizal spores was performed using the wet sieving and decanting method as described by Gerdemann and Nicolson (1963). The isolated AMF spores were preserved at 4°C until further use. A comprehensive examination of the morphological features of attached hyphae, azygospores, chlamydospores, and sporocarps from the collected AMF was conducted. Identification and characterization were based on the key provided by Schenck and Perez (1990). The distribution of the extracted AMF genera in a 1 kg soil sample was as follows: Glomus sp. 80%, Gigaspora sp. 15%, Acaulospora sp. 3%, and Sctellospora sp. 2%. AMF inoculation was performed after each growing season, with 100 mL of spore suspension (containing 30,000 spores) added to 1 kg of sterilized soil, evenly distributed across the plot, and incorporated into the soil through tillage at the time of planting.

Table 1 *chemical and physical properties of soil.*

Chemical properties		Physical properties	
0.91%	Organic matter	17.21%	Field capacity
35 (mg kg-1)	Available N	6.07%	Wilting point
18 (mg kg-1)	Available P	11.22%	Available water
141 (mg kg-1)	Available K	1.65 (g cm 3)	Bulk density
0.68	EC (ds m-1)	7.69	pH (1: 2.5)

2.3. Field experiment

Two field experiments were conducted at the experimental farm in Panchakeshwar village, located in the Niphad tahasil of Maharashtra, during the growing seasons of 2023/2024 and 2024/2025. The objective was to investigate the impact of arbuscular mycorrhizal fungi (AMF) inoculation in conjunction with mineral phosphorus fertilizer on the growth and yield of garlic plants. Garlic cloves were initially planted in pots for a duration of 25 days before being transplanted into the field. The transplanting was arranged along both sides of an irrigation line equipped with a dripper spaced 25 cm apart. The experimental area was divided into equalsized plots, each measuring 1.5 by 2 meters (3 m²). The experiments incorporated three levels of monocalcium phosphate fertilizer (15.5% P2O5) at rates of 40, 80, and 120 kg per hectare, with or without AMF inoculation serving as the control. A single level of nitrogen fertilizer was applied at a rate of 140 kg N per hectare in the form of ammonium sulfate (21.2% N), administered in two equal doses: the first application occurred 21 days after transplanting, followed by the second application 21 days later. To assess nitrogen efficiency as N-utilized, (15NH4)SO4 enriched with 5% N atom excess was mixed with standard fertilizer in a microplot measuring 0.60 m long and 0.50 m wide, totaling 0.30 m². Additionally, potassium sulfate (48% K2SO4) was applied at a rate of 200 kg per hectare prior to transplanting.

2.4. Estimation of root colonization

Root samples were obtained during both seasons of the experiment at intervals of 30, 60, and 90 days post-transplanting. The samples were stained following the procedure outlined by Phillips and Hayman (1970). In summary, the garlic roots were rinsed with sterile distilled water, treated with 10% KOH for 24 hours to soften, and then washed again with sterile distilled water. Subsequently, the roots were acidified in 5% lactic acid for one hour at room temperature and stained for 24 hours with 0.01% aniline blue in pure lactic acid. Finally, the roots were sectioned into 1 cm pieces, mounted on slides with glycerol, and examined using a binocular light microscope at SNJB's Arts, Commerce & Science College in Chandwad, Dist-Nashik, Maharashtra. The percentage of AMF colonization was determined based on the method established by Trouvelot et al. (1986).

2.5. Measurement of plant parameters

Plant samples were collected 110 days post-transplantation to assess both fresh and dry weight (g per plant) as well as total chlorophyll content (mg per g of dry weight) in the leaves, following the procedure outlined by Gross (1991). In summary, the leaves were ground in cool water under dark conditions and diluted to a final volume of 25 mL with distilled water. Subsequently, 0.5 mL of this solution was combined with 4.5 mL of 80% acetone and centrifuged at 3000 rpm for 10 minutes. The supernatant was then used for spectrophotometric analysis (Gross, 1991). At the time of harvest, the economic yield was quantified as bulb weight (kg per hectare). Additionally, the shoots, roots, and bulbs were dried and ground to analyze phosphorus content, following the method described by Cottenie et al. (1982). A plant sample was placed in a 100 mL flask, to which Darco-G-60 was added, followed by the addition of 50 mL of a 0.5 M NaHCO3 solution. The flask was then sealed with a cork and shaken for 30 minutes. After this period, the mixture was filtered. A 5 mL aliquot of the filtrate was transferred to a 25 mL volumetric flask, and two drops of 2,4-paranitrophenol were introduced. Gradually, 5 N H2SO4 was added dropwise with continuous shaking until the yellow color disappeared. The solution was then diluted to approximately 20 mL with distilled water, and 4 mL of ascorbic acid was incorporated. The mixture was shaken thoroughly, and the intensity of the blue color was measured at 660 nm using a Spectrophotometer.

The absorbance values were compared, and the concentrations of phosphorus were calculated using standard values.

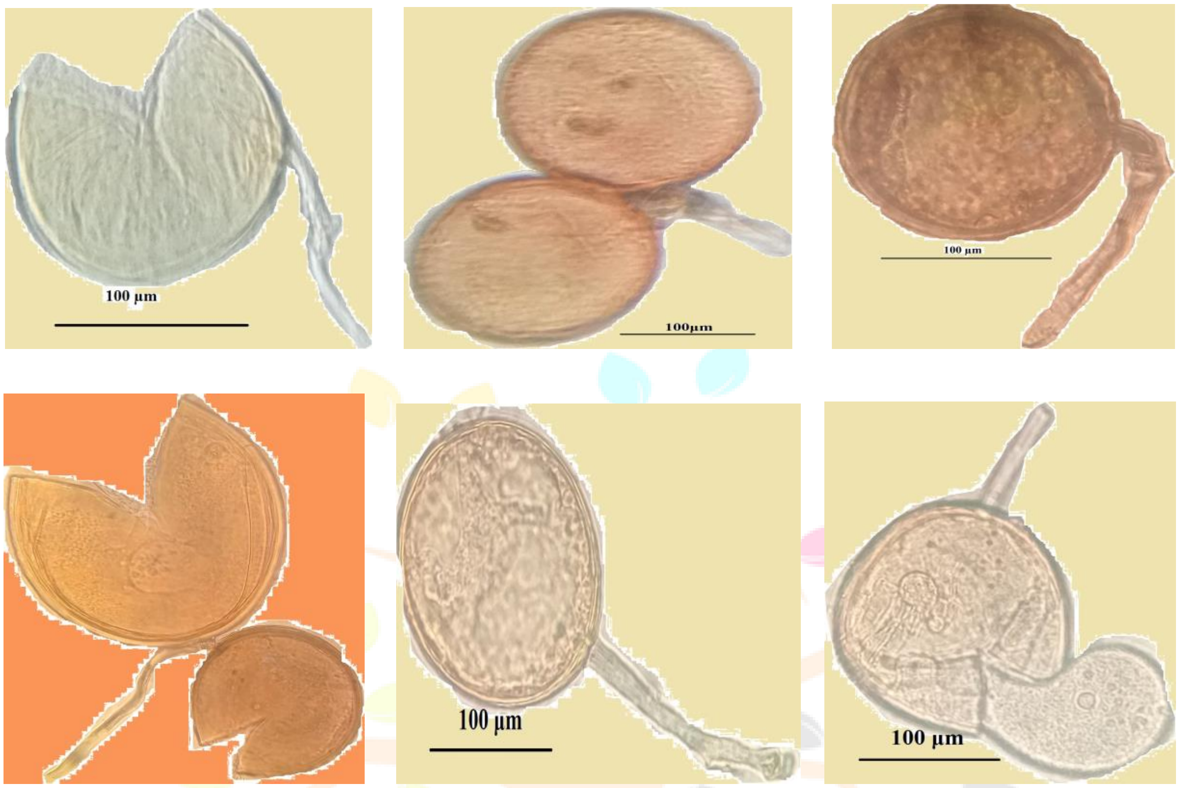

Fig. 1. Microscopic visualization (100x) of isolation of arbuscular mycorrhizal fungi from soil sample showing arbuscules spores and spores and hyphae

Fig. 2. Microscopic visualization (45x) of arbuscular mycorrhizal fungi showing arbuscules and spores and spores and hyphae in the Garlic root

3. Results

3.1. Effect of Arbuscular Mycorrhizal Fungi inoculation on root colonization

The impact of AMF inoculation on root colonization was assessed at 30, 60, and 90 days post-transplanting. Figure 1 illustrates the microscopic examination of AMF, highlighting the presence of arbuscules, spores, and hyphae within garlic roots. The results indicated a complete absence of colonization at the 30-day mark. Data presented in Table 2 confirmed that after 60 days of planting, AMF colonization in garlic roots reached 70% with the application of 80 kg P fertilizer per hectare. Likewise, Table 3 demonstrated a significant increase in AMF colonization, peaking at 81% after 90 days with the same fertilizer application rate across two growing seasons. The combination of AMF inoculation and phosphorus fertilizer application proved to be particularly effective in promoting root colonization. The highest percentages of AMF infection were observed in onion plants treated with 80 kg P fertilizer per hectare throughout the two growing seasons. However, mycorrhizal colonization of roots decreased when the phosphorus application exceeded 80 kg P2O5 per hectare, as shown in Tables no. 2 and 3.

3.2. Effect of Arbuscular mycorrhizal fungi inoculation on availability of P in the rhizosphere

Table 3 illustrates the impact of arbuscular mycorrhizal fungi (AMF) on phosphorus (P) availability in the soil rhizosphere after 90 days post-planting. The data presented indicate that AMF inoculation led to an increase in soil P concentration when compared to the control treatment that did not utilize AMF. Additionally, the results revealed that higher levels of phosphorus fertilization corresponded with elevated P levels in the rhizosphere. The peak concentrations of available P recorded during the two growing seasons were 54.39% for the 2023/2024 season and 76.13% for the 2024/2025 season, both observed at a fertilization rate of 120 kg P2O5 per hectare. In the second season, the maximum available P at this fertilization level reached 76.13%, significantly higher than the control level of 20.20%. Similar, albeit less pronounced, trends were observed during the first season.

3.3. Effect of Arbuscular Mycorrhizal Fungi inoculation on growth parameters of the garlic plant

The data illustrated in Fig. 2 indicate that both the fresh and dry weights of garlic plants exhibited a positive response to varying levels of AMF inoculation fertilizer when compared to the control group (which did not receive AMF). The maximum recorded values for fresh and dry weights were observed with mycorrhizal inoculation at a phosphorus application rate of 120 kg P2O5 per hectare, which is the recommended dosage. Notably, there was only a slight difference in the values of dry and fresh weights when comparing the second level of phosphorus (80 kg P2O5 per hectare) to the third level (120 kg P2O5 per hectare). Mycorrhizal inoculation resulted in increases in fresh weight of 34.8%, 22.7%, and 11.7% for the first season, and 23.1%, 22.4%, and 12.6% for the second season, relative to the control. The findings also demonstrated that the dry weight per plant rose with the increase in phosphate fertilizer application from 40 to 120 kg P2O5 per hectare during the 2023/2024 and 2024/2025 growing seasons, as depicted in Fig. 2. The highest dry weights recorded were 33.7 and 32.6 in the first season, and 41.0 and 35.3 in the second season, with and without mycorrhizal inoculation, respectively.

Table 2 *Effect of AMF inoculation on mycorrhizal colonization (%) after 60 days of Garlic plant growth*

Mycorrhizal colonization (%)			
Season 2023/2024		Season 2024/2025	
Control	AMF	Control	AMF
0.001	20.0	9.67	34.33
0.001	65.0	9.33	77.33
0.001	40.0	10.03	48.66
P fertilizer: 1.70		P fertilizer: 1.42	
AMF: 1.35		AMF: 1.11	

Table 3 *Effect of AMF inoculation on mycorrhizal colonization (%) after 90 days of Garlic plant growth*

Mycorrhizal colonization (%)			
Season 2023/2024		Season 2024/2025	
Control	AMF	Control	AMF
0.001	25.33	10.56	40.35
0.001	70.00	10.33	80.33
0.001	42.66	11.03	82.66
P fertilizer: 0.97		P fertilizer: 2.31	
AMF: 0.99		AMF: 1.82	

4. Discussion

The highest percentage of AMF colonization observed after 90 days of AMF inoculation was 81.33%, achieved with the application of 80 kg ha^{-1} of phosphorus fertilizer. According to Alloush and Clark (2001), AMF enhances plant growth and nutrient absorption in conditions of low soil fertility, and it can improve the efficacy of phosphorus fertilizers when compared to the application of phosphate fertilizers alone. Both very low and very high levels of phosphate fertilizer can hinder AMF colonization (Koide, 1991). Soil phosphorus levels exceeding the requirements for plant growth can inhibit the development of arbuscular structures formed by AMF. These arbuscules, which are produced within the cells of host plants, resemble small shrub-like trees

(Dickson et al., 2007). The presence of excessive phosphate prevents the formation of arbuscules, which are crucial for the transfer of nutrients from the fungus to the host plant (Hughes et al., 2008; Golubkina et al., 2018). The findings indicated that the inoculation with arbuscular mycorrhizal fungi (AMF) led to a substantial increase in the availability of phosphorus (P) in the rhizosphere when compared to the control group. Additionally, the results demonstrated a negative correlation between the levels of P in the rhizosphere and the extent of mycorrhizal colonization. Our findings indicate that both extremely high and low levels of available phosphorus (P) in soil can hinder mycorrhizal colonization. This aligns with the conclusions drawn by Koide and Li (1990) and Koide (1991), who noted that phosphorus levels in the rhizosphere significantly affect mycorrhizal establishment, often leading to a marked reduction in colonization. Additionally, an increase in soil phosphorus is associated with decreased fungal spore production, which is crucial for root infection, as noted by Menge et al. (1978). Smith and Read (2008) emphasized that phosphorus is the most critical soil factor influencing mycorrhizal symbiosis. Furthermore, Garcia and Mendoza (2008) found that optimal arbuscular colonization correlates with higher nitrogen and phosphorus concentrations in plant tissues, indicating a relationship with enhanced nutrient transfer between the symbiotic partners.

5. Conclusion

This research demonstrated that arbuscular mycorrhizal fungi (AMF) can enhance allium sp. yield when applied with second and third levels of phosphorus fertilizer under drip irrigation across two consecutive growing seasons. Field inoculation of garlic plants with AMF proved to be beneficial for production, as it improves the fertility of the root zone, thereby facilitating nutrient absorption and ultimately leading to increased yields. It is advisable to inoculate the soil with AMF prior to planting. The economic yield of onions, measured by dry bulb weight, showed a significant increase with the application of 120 kg ha^{-1} of phosphorus fertilizer in conjunction with AMF inoculation. These results indicate that AMF is likely to become a valuable and promising option in sustainable agriculture, serving as an alternative green technology to reduce reliance on synthetic fertilizers.

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