



Effectiveness of HCM Mycorrhizal (AMF) Inoculation on Growth and Yield Performance of Paddy (*Oryza sativa L.*)

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Abstract

Rice (*Oryza sativa L.*) is a staple food crop for a large part of the global population, and increasing its productivity and growth is essential for ensuring food security. A field study was conducted at Kunjpura, Karnal, Haryana to evaluate the effect of HCM Mycorrhizal, a bioinoculant containing *Glomus intraradices*, *Glomus mosseae*, *Glomus aggregatum*, and *Rhizophagus clarus*, on paddy growth and productivity. The experiment included two treatments: T1-HCM Mycorrhiza and T2-control with three replications. The study measured AMF colonization, spore count, dry weight, and grain yield at transplanting, tillering, and flowering stages. Results showed that AMF colonization in T1 is 15.2% at transplanting, 29.7% at tillering and 33.4% at flowering, while the control remained very low (1.1–2.4%). Similarly, spore count in T1 noted 10.7 spores/g soil at transplanting, 17.9 at tillering, and slightly decreased to 15.2 at flowering, whereas the control had negligible spore numbers (0–1.0 spores/g soil). Dry weight of treated plants increased from 10.54 g/plant at transplanting to 19.9 g/plant at tillering and 76.8 g/plant at flowering, compared to 6.90–43.12 g/plant in the control. Grain yield was also higher in T1 (2.29 t/acre) than T2 (1.88 t/acre). This indicates the potential of HCM Mycorrhizal as a sustainable and eco-friendly biofertilizer for maintaining long-term productivity and soil health in paddy cultivation.

Keywords: Paddy; Arbuscular Mycorrhizal Fungi (AMF); HCM Mycorrhizal; Biofertilizer; Sustainable Agriculture

Abbreviation

AMF: Arbuscular Mycorrhizal Fungi; RCBD: Randomized Complete Block Design; ITS: Internal Transcribed Spacer; FAA: Formalin Aceto Alcohol; DMRT: Duncan's Multiple Range Test.

Introduction

Rice (*Oryza sativa L.*) is a major cereal crop and a staple food for more than half of the global population [1]. Rice is not only an important food crop but also plays a vital role in the agricultural economy of many Asian countries by providing jobs and supporting rural livelihoods [2]. In addition, rice cultivation

helps in managing water resources and storing carbon in paddy soils, offering environmental benefits when practiced sustainably [3]. The demand for rice has increased due to the growing global population, necessitating methods to enhance and sustain paddy production [4].

However, conventional intensive rice farming has led to several agronomic and environmental challenges, including soil nutrient depletion, reduced soil fertility, and over-reliance on chemical fertilizers [5]. Such practices not only degrade soil health over time but also cause environmental issues, including soil degradation, water contamination, and a reduction in beneficial soil microbial

populations [6]. In addition, rice plants are constantly facing abiotic challenges such as drought, salt, and nutritional deficits, which can severely limit growth and production potential [7]. These challenges emphasize the critical necessity for sustainable and biologically based approaches to increase rice production while preserving soil ecosystem balance [8].

Among the biological strategies, Arbuscular Mycorrhizal Fungi (AMF) have emerged as an effective and eco-friendly tool to enhance crop performance [9]. AMF form a symbiotic association with plant roots, establishing an extensive hyphal network that increases root surface area and facilitates nutrient absorption [10]. Phosphorus, an essential but often immobile nutrient in paddy soils, is efficiently mobilized and translocated to the host plant via the fungal hyphae [11]. Additionally, AMF assist in the uptake of other macro- and micronutrients, including nitrogen, zinc, and iron, supporting overall plant growth and physiological functions [12]. AMF improve nutrient efficiency in plants, thereby reducing the need for chemical fertilizers and supporting sustainable agriculture while lowering environmental pollution [13].

Rice with higher arbuscular mycorrhizal fungal (AMF) colonization shows greater N and P uptake [14], higher shoot, root, and grain biomass [15], increased stomatal conductance and PS II quantum yield (Fv/Fm) [16], and altered hormonal profiles (lower ABA, higher IAA) than plants with lower colonization [17], with these benefits being stronger under drought than under well-watered conditions, indicating that AMF symbiosis enhances nutrient status, physiology, and drought tolerance in rice [18].

In addition to enhancing nutrient uptake, AMF improve plant stress tolerance and soil health [19]. The hyphal arrangement improves root structure and promotes better water absorption, enhancing the ability of rice plants to resist drought and salinity stresses [20]. AMF also improve soil physical properties such as aggregation, porosity, and aeration, which facilitate microbial activity and efficient nutrient cycling in the rhizosphere [21]. Overall, these benefits lead to improved vegetative growth, increased biomass accumulation, more efficient nutrient use, and higher grain yield [22]. Therefore, AMF offer a sustainable and environmentally friendly strategy to boost rice productivity while preserving soil quality and ecosystem health [23].

Therefore, this study aims to evaluate the effectiveness of the AMF-based product HCM Mycorrhizal on the growth and yield of rice, with a focus on its role in improving nutrient uptake, root development, and overall productivity.

Materials and Methods

Experimental setup

The field experiment was conducted at Kunjpura, Karnal, Haryana, using paddy as the test crop. The study consisted of two treatments—T1: Treated with the AMF product HCM Mycorrhizal and T2: Control—and was laid out in a Randomized Complete Block Design (RCBD) with three replications. The arbuscular mycorrhizal fungi (AMF) product HCM Mycorrhizal consisted of a mixed consortium of AMF species, having a minimum spore count of 10 spores/g and formulated on a bentonite base. For the treated plots (T1), HCM Mycorrhizal was applied at the recommended dose of 4 kg/acre. All plots received uniform agronomic practices including irrigation and plant protection measures.

Molecular characterization of AMF Isolates

Molecular identification of the mycorrhizal isolates present in a formulation was performed through DNA sequencing. Genomic DNA was extracted from purified fungal biomass, and the internal transcribed spacer (ITS) region was amplified using universal primers ITS1 and ITS4. The PCR products were confirmed by agarose gel electrophoresis, purified, and sequenced. Species identification was confirmed by comparison with reference sequences available in the NCBI GenBank database through BLASTn, and phylogenetic analysis was carried out to confirm taxonomic identity. The validated sequence was submitted to GenBank and assigned an accession number.

AMF application and inoculum preparation

HCM Mycorrhizal was applied directly to the nursery bed before sowing by incorporating the inoculum into the top 2–3 cm soil layer. The bentonite-based AMF formulation was evenly distributed to ensure uniform spore availability for root colonization. At transplanting, 8–10 day-old rice seedlings in T1 were subjected to root dipping in a suspension prepared from HCM Mycorrhizal to promote early and effective AMF colonization. The control plots (T2) did not receive any AMF inoculation but were otherwise maintained under the same management conditions.

Nursery preparation and transplantation

A raised nursery bed was prepared following standard rice nursery practices. For T1, HCM Mycorrhizal was incorporated into the nursery soil at the recommended rate prior to sowing. Pre-germinated paddy seeds were sown treatment-wise. Seedlings were uprooted 8–10 days after sowing and transplanted into the main field. In the treated plots, seedlings required for transplanting one acre were subjected to a root-dip treatment using an HCM Mycorrhizal suspension prepared by dispersing 4 kg of the formulation in water for 20–30 minutes immediately before transplanting to promote early mycorrhizal colonization, whereas control seedlings were transplanted without AMF treatment.

Mycorrhizal assays

Mycorrhizal assays are used to evaluate the efficiency of biofertilizers, improve agriculture sustainability and aid in the restoration of degraded soil. Root and soil samples were collected at different crop growth stages to assess AMF colonization. Spore density in soil was determined using the wet-sieving and decanting technique described by Gerdemann and Nicolson (1963) [24]. The number of AMF spore in 1gm of the rhizosphere was calculated under a stereo microscope 40× magnification according to this formula:

$$TSN = \frac{SN \times W}{S}$$

Where, TSN- Total AMF spores in 1gm of rhizosphere, SN - AMF spore number in 1ml of spore suspension, W- amount of water used [ml], S- amount of soil used [g].

Root samples were washed under tap water to remove soil debris and fixed in formalin–aceto–alcohol (FAA). The roots were then cleared and stained following the modified method of Kormanik and McGraw (1984) [25]. AMF colonization (%) was determined microscopically following the method of Giovannetti and Mosse (1980) [26], by calculating the proportion of colonized root segments relative to the total number of root segments examined. Molecular confirmation of AMF was performed using ITS.

Measurement of plant growth and yield

Three rice plants from each treatment were randomly collected during transplanting, tillering, flowering, and harvesting stages. The plants were washed thoroughly, oven-dried at 80°C until constant weight, and the dry matter was recorded in g/plant.

At maturity, rice plants from each treatment plot were harvested and threshed manually. The total grain yield per plot was recorded, and the mean yield was expressed in tonnes per acre.

Statistical analysis

The experimental data were analyzed using the Randomized Complete Block Design (RCBD). Treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% significance level, following the statistical procedures of Gomez and Gomez (1984) [27].

Results and Discussion

Molecular identification of AMF consortium (HCM Mycorrhizal)

PCR amplification of the ITS region from the HCM Mycorrhizal formulation produced clear and specific amplicons, confirming successful DNA extraction and amplification. BLASTn analysis of the ITS sequences revealed high similarity with NCBI GenBank reference sequences, identifying *Glomus intraradices* (accession no. PX691472), *Glomus mosseae* (PX691473), *Glomus aggregatum* (PX691474), and *Rhizophagus clarus* (PX691475). These results confirm the molecular identity of the arbuscular mycorrhizal fungal consortium present in the HCM Mycorrhizal product.

AMF colonization in rice roots

The rice plants treated with HCM Mycorrhizal (T1) showed higher root colonization by arbuscular mycorrhizal fungi compared to the control (T2). In T1, colonization increased from 15.2% at transplanting, 29.7% at tillering and 33.4% at flowering, while the control remained very low with 1.1%, 2.2%, and 2.4% at the same stages (Figure 1). In another study, AMF colonization at the nursery stage improved rice growth. The DN + AMF (Dry Nursery with AMF inoculation) treatment produced 29 effective tillers per pot, showing a 99% increase over the control [28]. Moreover, the AMF inoculum study demonstrated a marked increase in root colonization in rice plants following inoculation, with treated plants showing 90% mycorrhizal colonization compared to only 10% in the control [29].

AMF spore count in soil

The spore count of AMF in the rice rhizosphere was higher in T1 compared to the control. In T1, spores increased from 10.7 spores/g soil at transplanting to 17.9 spores/g at tillering, and

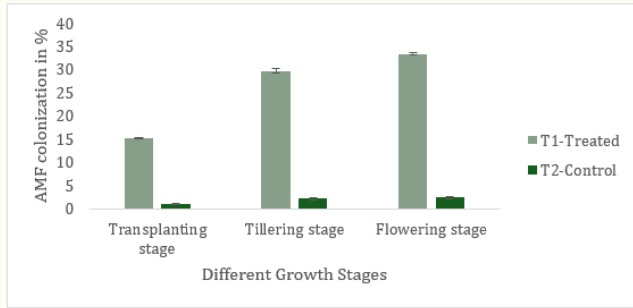


Figure 1: Effect of HCM Mycorrhizal on AMF Colonization (%) in Paddy.

slightly decreased to 15.2 spores/g at flowering. In the control, spore counts were negligible (0.0, 1.0, and 1.0 spores/g soil). This shows that HCM Mycorrhizal effectively multiplied in the soil and supported root colonization (Figure 2). Previous study has shown that nine weeks after planting, rice plants treated with AMF showed much higher spore density in the soil than the controls. In soil, AMF biofertilizer recorded 51.50 ± 3.31 spores/g, compared to 26.00 ± 2.38 spores/g in the control. In sterilized soil, AMF treatment had 46.00 ± 6.49 spores/g, while the control showed only 0.15 ± 0.30 spores/g, demonstrating effective impact of AMF under both soil conditions [30].

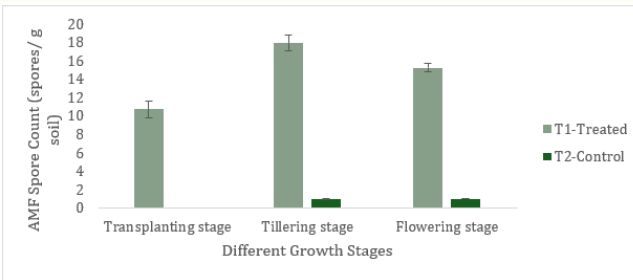


Figure 2: Effect of HCM Mycorrhizal on AMF Spore Density (spores/g) in Paddy.

Dry matter production

Dry weight of rice plants was higher in T1 compared to the control at all growth stages. In T1, dry weight noted 10.54 g/plant at transplanting, 19.9 g/plant at tillering and 76.8 g/plant at flowering, while T2 had 6.90, 8.81, and 43.12 g/plant respectively (Figure 3). According to another study, rice seedlings treated with

AMF had higher shoot and root growth than untreated plants after transplanting in paddy soil (PS). The total dry matter of the treated plants was 38.2 g/plant, leading to the highest grain yield under continuously flooded (CF) conditions at 105 days [31]. The Application of 45 g AMF enhanced biomass accumulation in rice. Treated plants recorded a shoot dry matter of 43.30 ± 0.02 g and a root dry matter of 22.16 ± 0.08 g, indicating a positive effect of AMF fertilization on both shoot and root growth [32].

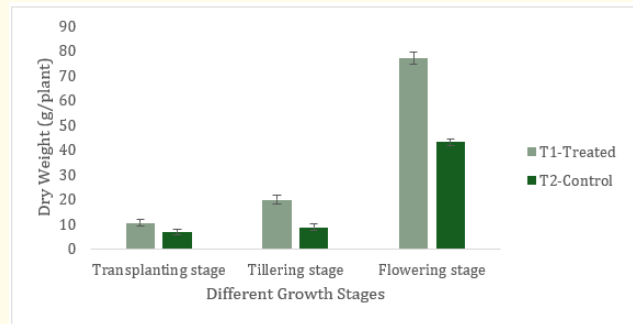


Figure 3: Effect of HCM Mycorrhizal on Rice Dry Matter Accumulation.

Grain yield at harvest

The grain yield of rice at 110 days was higher in T1 (2.29 tonnes/acre) than in the control (1.88 tonnes/acre) (Figure 4). This demonstrates that application of HCM Mycorrhizal improved overall rice productivity by supporting better root colonization, nutrient uptake, and plant growth in comparison with control (Figure 5). The application of mycorrhizal bio-fertilization enhanced rice productivity, mycorrhizal treatment recorded a grain yield of 3.15 tonnes/fed, which was higher than the 2.81 tonnes/fed obtained in the uninoculated control [33]. In another study, paddy plants inoculated with *in vitro*-produced AMF showed an improvement in grain yield. Transplanted paddy treated with the AMF consortium recorded a yield of approximately 3.40 tonnes/acre, while direct-seeded paddy produced about 2.79 tonnes/acre, demonstrating the positive influence of AMF on rice productivity [34].

Conclusion

The results of the study demonstrated that the application of HCM Mycorrhizal positively impacted the growth and productivity

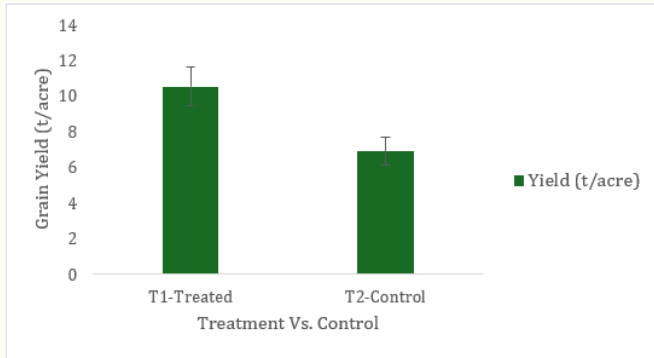


Figure 4: Effect of HCM Mycorrhizal on Rice Grain Yield.



Figure 5: Effect of HCM mycorrhizal treated vs control paddy plant.

of rice. Higher AMF colonization and spore density in treated plants are related to higher dry matter accumulation and grain yield. These outcomes indicate that HCM Mycorrhizal serves as an effective bio-inoculant, enhancing root development, nutrient uptake, and overall paddy productivity under field conditions. Additionally, it improves soil structure, promotes beneficial microbial activity, and increases plant tolerance to abiotic stresses such as drought and salinity. The application of AMF-based biofertilizers provides an efficient, sustainable, and environmentally friendly approach to boosting rice yield, reducing reliance on chemical fertilizers, and maintaining long-term soil health.

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