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RESEARCH ARTICLE

Impact of Arbuscular Mycorrhizal Fungi on the Growth and Yield of Rice (Oryza Sativa L.) Affected by Magnaporthe Oryzae (B. COUCH)

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ABSTRACT

An essential staple meal for millions of people worldwide, rice is an annual grass that is a member of the genus Oryza and family Poaceae. This study was carried out to assess how Arbuscular Mycorrhizal Fungi (AMF) affected the growth and yield of rice that had Magnaporthe oryzae infection. Three types of rice (YARDAS, FARO 44, and FARO 52) infected with Magnaporthe oryzae were subjected to evaluations using eight distinct concentrations of Arbuscular Mycorrhiza. Three replications of the experiment were conducted using a Randomized Complete Block Design (RCBD). After planting, growth data was gathered 30, 60, and 90 days later. Ninety days after planting, however, data on yield metrics were gathered. An analysis of variance (ANOVA) was performed on the acquired data, and means were separated using Tukey at 5% significance level. The result showed that 7g of Arbuscular Mycorrhiza significantly increase Chlorophyll content across all the varieties evaluated in this study. Similarly treating the infected rice varieties with 7g of the Mycorrhiza significantly increase the yield and yield parameters. While the disease incidence was only significantly reduced when the varieties were treated with 7g of Arbuscular Mycorrhiza; disease severity was decreased when the varieties were treated with 5-7g of Arbuscular Mycorrhiza. Treating rice varieties infected with *Magnaporthe oryzae* with Arbuscular Mycorrhiza increase both growth and yield, and reduce disease incidence and severity. It could therefore be recommended that farmers could use Arbuscular Mycorrhiza in mitigating the infection of *Magnaporthe oryzae*.

Key words: Arbuscular Mycorrhiza, Magnaporthe oryzae, Oryza sativa.

INTRODUCTION

One of the most important staple crops in the world, rice (Oryza sativa L.) provides a substantial portion of calories for almost half of the world's population (FAO, 2019). However, rice production faces significant challenges from a variety of biotic and abiotic factors. The fungus Magnaporthe oryzae causes rice blast disease, one of the most damaging biotic stresses that causes large production losses annually (Liu et al., 2020). About 80 countries worldwide are affected by the illness, which is common in both paddy fields and upland rice agriculture (Talbot, 2019). Chemical fungicides and resistant cultivars have long been the mainstays of rice blast management. But given M. oryzae's capacity for quick evolution and resistance gene eradication, as well as the expense and ecological effects of fungicides, other management approaches must be investigated (Kang et al., 2018). Arbuscular mycorrhizal fungi (AMF), especially Rhizophagus irregularis, have symbiotic interactions with a variety of plant species, including rice. By enhancing phosphorus and other nutrient uptake, these fungi can promote plant growth and stress tolerance (Smith & Read, 2018). It has been shown that AMF colonisation boosts resistance to a range of plant diseases, while the precise mechanisms are unclear (Pozo & Azcón-Aguilar, 2007). It is promising to see research on AMF's potential to mitigate the effects of rice blast disease, particularly in light of sustainable and eco-friendly farming practices. The purpose of this study is to assess how Rhizophagus irregularis affects the growth and yield of Magnaporthe oryzae-infected rice plants. The study aims to provide light on how AMF

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contributes to increased resistance to rice blast disease by evaluating the growth characteristics, yield, and disease severity of different rice cultivars. This study offers a viable substitute for chemical fungicides in the management of rice blast disease and adds to the expanding corpus of research on biological control techniques in agriculture.

MATERIALS AND METHODS

Experimental Site

This study was conducted in the screen house and plant pathology laboratory of the Department of Plant Biology, Bayero University, Kano, Nigeria, located at coordinates 11°57'56.27"N 8°25'51.22"E to 11°58'50"N 8°28'39"E.

Collection and Viability Testing of Arbuscular Mycorrhizal Fungi (AMF)

Collection of Fungal Isolates

Pure isolates of *Rhizophagus irregularis* were sourced from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (7°30′5.1264″N, 3°35.712″E). These isolates were used to treat rice seedlings.

Viability Assessment of AMF

Sterilized sand soil was aseptically packed into Petri dishes and moistened to maximum water-holding capacity with a 0.1% Trypan blue solution, which aids hyphal visibility. A nylon mesh (50 µm pore size) was placed over the soil, topped with 10 x 10 mm pieces of cellulose acetate membrane filter paper (0.45 µm pore size). The mesh and filter squares were sterilized by soaking in 70% ethanol for 5 minutes, then rinsed with distilled water. Single AMF spores were placed on each filter square, and the dishes were incubated in darkness at 20°C. Spore germination was observed daily for 5 days under a microscope. Viable spores were identified by germ tube lengths exceeding the spore diameter (Brundrett & Juniper, 1995).

Collection, Isolation, Purification, and Identification of Magnaporthe oryzae

Collection of Diseased Samples

Diseased rice samples showing symptoms of blast disease were collected using simple random sampling from five rice-growing blocks in Wudil during the rainy season of 2019. Diseased tissues (3-4 cm) were excised using sterilized scalpels and placed on sterilized moistened cotton in Petri dishes (TeBeest et al., 2014).

Isolation of the Pathogen

Small pieces of contaminated plant tissue (5 mm2) were removed from the borders of the lesions, surface-sterilized for two minutes in 1% NaOCl, rinsed twice in distilled water, and then put on wet, sterilized filter sheets. To encourage fungal growth and spore generation, these tissues were subsequently moved to Petri dishes with Potato Dextrose Agar (PDA) and cultured for 7–10 days at 27 ± 1°C with a 12-hour photoperiod (Fang et al., 2018).

Purification of the Pathogen

After sporulation, fungal spores and mycelia were transferred onto PDA using a sterile loop. After being spread out onto PDA plates and suspended in sterile water, the spores were cultured for two to three days. Isolated single mycelial colonies were stored at 4°C. Before being transferred to new Petri plates, the purified mycelium was cultivated for two more weeks on Whatman filter paper (70 mm diameter) on PDA (Jia, 2009).

Microscopic Identification

Spores were mounted on slides with Lactophenol Cotton Blue stain and observed under a light microscope at 400x magnification. Morphological characteristics were documented (Mew & Gonzales, 2002).

Sub-culturing of *M. oryzae*

Magnaporthe oryzae was sub-cultured on PDA medium, sterilized at 1.41 kg/cm² for 20 minutes. A 7 mm disc of the fungus was placed in the center of each Petri dish and incubated at 28 ± 2°C (Jia, 2009).

Agronomic Practices and Experimental Design

Varieties

Three rice varieties were used in this study: two improved varieties (FARO-52 and FARO-44) obtained from the Africa Rice Centre, and one local variety (YARDAS) sourced from farmers in Wudil. The local variety was suspected to be susceptible to *M. oryzae* infection (Shafullah et al., 2011).

Experimental Design and Treatments

The experiment employed a Randomized Block Design with three replications. Treatments included eight different concentrations of AMF (ranging from 1g to 7g and a control without AMF) applied to each of the three rice varieties. A total of 72 pots (25 cm in diameter, 22 cm depth) were filled with 5 kg of sterilized clay-loam soil.

Bioassay Procedures

Inoculation with AMF

The appropriate concentration of AMF was thoroughly mixed with sterilized soil before potting. Five grams of rice seeds were sown per pot, with seedlings thinned to five plants per pot two weeks after sowing (Lina & Michael, 2019).

Preparation of M. oryzae Conidia

Magnaporthe oryzae was cultured on PDA plates at 26°C for 10-12 days. The plates were then incubated under black fluorescent light at 24°C for 3 days to induce conidia formation. Conidia were harvested by adding 20 ml of sterilized water to the plates and collecting the suspension using a paintbrush. The concentration of conidia was adjusted to 1 x 10^5 conidia/ml using a hemocytometer (Chuwa et al., 2015).

Inoculation of Rice Varieties with M. oryzae

Two weeks after sowing, rice plants were inoculated by spraying a conidial suspension onto the plants until thoroughly wet. Plants were covered with black polythene bags to promote spore formation (Shafullah et al., 2011).

Data Collection

Growth Parameters

Plant height, number of tillers per pot, and number of leaves were recorded at 30, 60, and 90 days after sowing (DAS) (Bijay et al., 2018).

Physiological Parameters

Chlorophyll content was measured at 30, 60, and 90 DAS using a SPAD-502 Plus chlorophyll meter (Bijay et al., 2018).

Yield Attributes

Yield attributes, including the number of effective tillers per pot, panicle length, number of spikelets (fertile and sterile), spikelet sterility percentage, spikelet fertility percentage, and the number of grains per panicle, were recorded (Onoriode, 2020; Bijay et al., 2018; Sangita et al., 2018).

Disease Incidence and Severity

Leaf blast incidence and severity were assessed at 60 and 90 DAS. Disease severity was rated on a scale from 0 (no lesion) to 9 (≥75% affected area) (Shafullah et al., 2011; Obilo et al., 2012).

Statistical Analysis

Two-way Analysis of Variance (ANOVA) was used to examine the data, and Tukey's test was used to identify significant differences between treatment means at a 5% significance level. Genstat statistics software version 6 was used for all statistical studies.

RESULTS

Microscopic Analysis of Magnaporthe oryzae

The study of the cultural and morphological characteristics of *Magnaporthe oryzae* on Potato Dextrose Agar (PDA) showed significant growth within 3 to 4 days. The colonies exhibited a white to ash-colored cottony appearance, with septate, branched, and hyaline mycelium. Conidiophores were slender and straight, bearing clusters of pyriform conidia with 2-3 septations. Tri-septate conidia were observed under a light microscope using Lacto phenol cotton blue stain.

Effect of Arbuscular Mycorrhizal Fungi on Chlorophyll Content of Rice

The application of Arbuscular Mycorrhizal Fungi (AMF) significantly impacted the chlorophyll content in rice plants infected with *M. oryzae*. At 30 days after sowing, plants treated with 7g of AMF and the control group exhibited the highest chlorophyll content, with SPAD values of 36.96 and 39.19, respectively. Chlorophyll content increased over time, with significant differences observed at 60 and 90 days after sowing among the treatments. Notably, the local variety YARDAS recorded the highest chlorophyll content at 30 days, while FARO 52 had the highest content at 90 days.

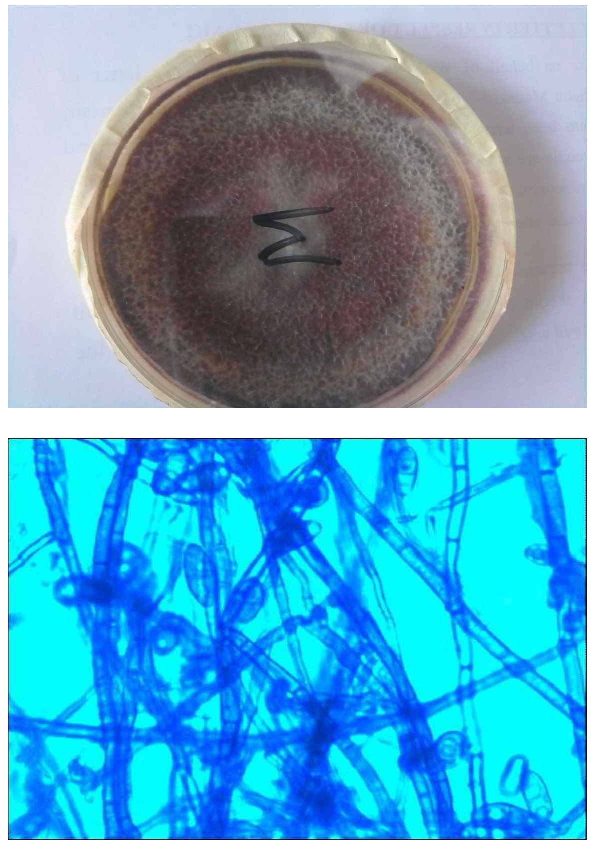

Plate 1 & 2: Cultural and Microscopic appearance of *M. oryzae* under light microscope x 400.

Effect of Arbuscular Mycorrhizal Fungus on Plant Height of Rice Infected with *Magnaporthe oryzae*

Table 1 shows the impact of arbuscular mycorrhizal fungi on rice plant height. The results showed that the treatments and varieties differed significantly (P ≤ 0.05). Plants treated with 7g of Arbuscular Mycorrhizal Fungi had the highest plant heights (40.26) 30 days after planting. This treatment outperformed the other therapies assessed in the trial by a significant margin (P<0.05). Plants treated with 6g of Arbuscular Mycorrhiza were not far from plants treated with 4g of AM. Plants infested with 7g, 6g, and 5g had significantly greater plant heights (P≤0.05) at 60 days following planting, with respective plant heights of 88.83, 88.63, and 84.54. However, the control group's plant height was significantly lower (P<0.05). Plants treated with 3–7g of Arbuscular Mycorrhizal Fungi had considerably (P≤0.05) increased plant heights 90 days after planting.

Compared to plants treated with 3g of the treatment, which is significantly higher than the untreated plants, plants treated with 4g, 5g, 6g, and 7g of AM are significantly higher (P<0.05). According to the influence of variety, YARDAS and FAR0-44 had the largest plant heights 30 days after sowing, and they were substantially greater (P<0.05) than FAR0-52. For YARDAS and FAR0-52, plant height was considerably higher (P<0.05) 60 days after sowing. However, the three types' plant heights varied dramatically 90 days after sowing, with YARDAS yielding statistically larger plant heights. Compared to FAR0-44, FAR0-52 was considerably (P<0.05) greater.

Table 1: Effect of Arbuscular Mycorrhizal Fungus on Chlorophyll Content of Rice Infected with *Magnaporthe oryzae*

	Chlorophyll Content (SPAD)		
	Day after Planting		
TREATMENTS (g)	30	60	90
-AMF	39.19^{a}	36.61^{e}	41.77^{e}
AMF1	33.88^{cd}	39.00^{de}	55.99^{d}
AMF2	33.20^{cd}	46.87^{cde}	64.91^{c}
AMF3	31.17^{d}	51.84^{bcd}	70.81^{bc}
AMF4	35.66^{bc}	58.12^{abc}	71.80^{bc}
AMF5	33.00^{cd}	57.79^{abc}	71.42^{bc}
AMF6	31.96^{d}	64.00^{ab}	74.34^{b}
AMF7	36.96^{ab}	69.31^{a}	84.99^{a}
LSD	1.899	9.50	4.777
VARIETIES			
YARDAS	38.03^{a}	56.03^{a}	72.18^{b}
FAR0-44	34.28^{b}	44.36^{b}	49.65^{c}
FAR0-52	30.81^{c}	58.44^{a}	79.19^{a}
LSD	1.163	5.82	2.925
INTERACTION (VarxTrt)	S (P=.001)	S (P=.001)	S (P=.001)

Key: Means along column for both treatment and variety with different letters are significantly different at P ≤ 0.05. AMF – Arbuscular Mycorrhizal Fungi

Table 2: Effect of Arbuscular Mycorrhizal Fungus on Plant Height of Rice Infected with *Magnaporthe oryzae*

	Plant Height (cm)		
	Days after Planting		
Treatments (g)	30	60	90
-AMF	32.62^{e}	65.02^{e}	73.49^{c}
AMF1	31.72^{e}	72.20^{d}	85.42^{b}
AMF2	35.31^{d}	79.21^{c}	86.48^{b}
AMF3	35.66^{cd}	81.21^{bc}	94.04^{ab}
AMF4	37.07^{bc}	82.52^{bc}	98.25^{a}
AMF5	37.83^{b}	84.54^{abc}	100.15^{a}
AMF6	37.08^{bc}	86.63^{ab}	103.12^{a}
AMF7	40.26^{a}	88.83^{a}	99.95^{a}
LSD	1.084	3.641	6.084
VARIETIES			
YARDAS	38.97^{a}	82.06^{a}	99.83^{a}
FAR0-44	38.65^{a}	76.55^{b}	84.67^{c}
FAR0-52	30.22^{b}	81.44^{a}	93.34^{b}
LSD	0.664	2.230	3.726
INTERACTION (VarxTrt)	S(P = .001)	L(P = 0.107)	S(P = .001)

Key: Means along column for both treatment and variety with different letters are significantly different at P ≤ 0.05, AMF – Arbuscular Mycorrhizal Fungi

Effect of Arbuscular Mycorrhizal Fungi on Yield Parameters

Yield parameters, including panicle length, number of grains per panicle, number of effective tillers, and percentage of fertile and sterile spikelets, were significantly affected by AMF treatment. Plants treated with 7g of AMF produced the longest panicles (29.52 cm) and the highest number of grains per panicle. The improved varieties FARO 44 and FARO 52 outperformed YARDAS in panicle length and other yield parameters.

Table 3: Effect of Arbuscular Mycorrhizal Fungus on Yield Parameters of Rice Infected with *Magnaporthe oryzae*

	Yield Attributes								
Treatments(g)	PNL (cm)	NGP	NTP	PFS (%)	PSS (%)	WIG (g)	W1000G (g)	YPP (g)	YPH (ton)
-AMF	20.90^{e}	88.0^{e}	13.67^{d}	80.56^{d}	19.76^{a}	1.0022^{a}	24.27^{c}	31.08^{e}	6.71^{e}
AMF1	21.82^{e}	86.1^{de}	22.00^{c}	84.17^{c}	15.82^{a}	0.5522^{b}	25.85^{bc}	58.45^{d}	13.15^{d}
AMF2	23.63^{d}	97.3^{cd}	24.00^{bc}	89.07^{b}	10.93^{bc}	0.3822^{c}	26.02^{bc}	66.64^{bcd}	15.00^{bcd}
AMF3	24.51^{cd}	99.8^{c}	21.44^{c}	89.11^{b}	11.49^{b}	0.3789^{c}	27.39^{bc}	60.43^{cd}	13.60^{cd}
AMF4	25.39^{c}	105.8^{bc}	25.00^{bc}	91.38^{ab}	8.61^{bc}	0.3200^{c}	28.84^{ab}	77.36^{bc}	17.41^{bc}
AMF5	27.43^{b}	103.8^{c}	26.11^{b}	90.69^{ab}	9.31^{bc}	0.2833^{cd}	29.18^{ab}	78.63^{b}	17.69^{b}
AMF6	27.90^{b}	115.2^{ab}	24.33^{bc}	93.05^{a}	6.95^{c}	0.2856^{cd}	29.63^{ab}	83.56^{b}	18.80^{b}
AMF7	29.52^{a}	119.2 a	30.44^{a}	92.25^{ab}	9.25^{bc}	0.1522^{d}	31.76^{a}	115.88^{a}	a 26.07^{a}
LSD	0.6604	7.076	2.437	2.177	2.557	0.0997	2.412	11.00	2.458
Varieties									
YARDAS	24.39^{b}	93.6^{b}	18.25^{c}	84.85^{c}	15.67^{a}	0.6629^{a}	26.36^{b}	51.85^{c}	c 11.63^{c}
FAR0-44	25.35^{a}	105.3^{a}	28.71^{a}	93.38^{a}	6.78^{c}	0.3421^{b}	28.35^{a}	90.13^{a}	20.21^{a}
FAR0-52	25.67^{a}	106.9^{a}	23.17^{b}	88.12^{b}	12.10^{b}	0.2537^{c}	28.90^{a}	72.53^{b}	16.32^{b}
LSD	0.4044	4.333	1.492	1.333	1.566	0.0610	1.477	6.74	1.505
Interaction (VarxTrt)	L(P=.005)	L(P=.019)	S(P=.001)	S(P=.001)	S(P=.001)	S(P=.002)	S(P=.002)	S(P=.001)	S(P=.001)

Key: Means along column for both treatment and variety with different letters are significantly different at P ≤ 0.05. PNL – Panicle Length, NPG- Number of Grain Per Panicle, NTP – Number of effective Tillers Per Pot, PFS – Percentage of Fertile Spikelets per Pot, PSS – Percentage of Sterile Spikelets per Pot, WIG- Weight Infected Grains per pot, W1000G- Weight of 1000 Grains per pot, YPP- Yield Per Pot, YPH – Yield Per Hectare, AMF – Arbuscular Mycorrhizal Fungi.

DISCUSSION

In this study, *Magnaporthe oryzae*, the fungal pathogen, was isolated from the rice field. The fungal isolates' morphological traits showed that the colonies have a cottony, white to ash look. The mycelium of the *Magnaporthe oryzae* colony was branching, septated, and hyaline. It was discovered that the coniodiophores were thin, straight, and had clusters of conidia with two to three septations and pyriform forms. Under a light microscope, tri-septate conidiation was seen after staining with Lactophenol Cotton Blue Stain. Mew and Gonzales (2002) and Getachew et al. (2014) noted similar results. The study showed that AMF had a positive impact on the growth and yield of *M. oryzae*-infected rice plants. AMF administration led to a greater chlorophyll content, which is a sign of improved photosynthetic efficiency, especially at higher dosages (5-7g). Significant gains were seen 60 and 90 days following planting, with this effect becoming more noticeable as the plants became older. When rice infected with *Magnaporthe oryzae* was treated with Arbuscular Mycorrhizal Fungi (AMF), the amount of chlorophyll increased considerably. The plants' levels of chlorophyll rose dramatically when Arbuscular Mycorrhiza was treated. This is supported by the findings of Elahi et al. (2010) and Elhindi et al. (2017). Plants inoculated with Arbuscular Mycorrhiza may have increased chlorophyll content since magnesium is essential for chlorophyll production and mycorrhizal plants have higher Mg uptake (Mathur et al., 2018).

The greater nutrient intake made possible by the symbiotic interaction between the fungi and rice plants is responsible for the improved growth parameters seen in AMF-treated plants. The increased number of leaves, which in turn supported higher chlorophyll content and better photosynthetic activity, was probably facilitated by this interaction. Additionally, AMF treatment had a positive impact on yield measures. AMF appears to improve the overall reproductive success of rice plants under biotic stress circumstances, as evidenced by the increase in panicle length, number of grains per panicle, and number of effective tillers. By encouraging spikelet fertility, the decrease in sterile spikelets bolsters the idea that AMF can increase rice yield. In this study, rice infected with Magnaporthe oryzae showed a substantial increase in yield and yield parameters (Panicle Length, Number of Grain per Panicle, Number of Effective Tillers per Pot, Weight of I000 Grains, Yield per Pot, and Yield per Hectare) when arbuscular mychorrizal fungi were present. These results align with the findings of Kamali et al. (2020), Lina and Micheal (2020), and Merina et al. (2017). Arbuscular mycorrhizal fungi increase agricultural yields by enhancing nutrient and water absorption (Rezaie et al., 2020). AMF also improves plant resistance to biotic stress, which supports plant growth and yield, according to a number of studies (Bernaola & Stout, 2020). They may be able to absorb large amounts of nitrogen from decaying and dead matter, which will aid in their growth and prolong their lifespan. They are crucial to the nitrogen cycle because they can transfer 20% to 75% of the absorption of Arbuscular Mycorrhiza (AM) plants to their hosts (Begum et al., 2019). The diverse ways that the rice varieties responded to AMF treatment emphasise how crucial genetic factors are in deciding how successful AMF is. When compared to the native variety YARDAS, the improved varieties FARO 44 and FARO 52 shown larger yield improvements, suggesting that they may be more appropriate for AMF-based therapies in the management of M. oryzae infections. Overall, the results highlight how AMF can be used as a sustainable rice blast disease management technique, improving rice plant development and productivity by improving nutrient uptake and stress tolerance.

Conclusion

Magnaporthe oryzae was successfully isolated and identified, revealing septated, branched, and hyaline mycelial hyphae. The application of Arbuscular Mycorrhizal fungi resulted in increased growth and enhanced yield across all three varieties of rice examined. Notably, the variety FARO-44 exhibited the most robust performance following the treatment. Furthermore, the application of Arbuscular Mycorrhizal fungi contributed to a reduction in the incidence and severity of *Magnaporthe oryzae*. Specifically, the application of 7g of Arbuscular Mycorrhizal Fungi demonstrated the most favourable outcomes in terms of both yield improvement and disease incidence reduction. The disease incidence reduced up to 60 % at treatment of 7g of Arbuscular Mycorrhizal Fungi.

Recommendations

1. Farmers are recommended to consider using Arbuscular Mycorrhiza as bio-control agents to mitigate the infection of *Magnaporthe oryzae*.
2. Further studies are recommended to investigate the effects of Arbuscular Mycorrhizal Fungi on rice infected with *Magnaporthe oryzae* under water stress conditions.
3. It is recommended that Arbuscular Mycorrhizal Fungi be recognized as effective bio-fertilizers and bio-control agents in mitigating rice blast disease. Additionally, the widespread adoption of Arbuscular Mycorrhizal Fungi as seed treatment before planting is encouraged.

Farmers are encouraged to adopt improved varieties of rice to enhance disease resistance and overall crop performance.

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