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EFFECT OF *TRICHODERMA* SPP. AND NEEM LEAF EXTRACT ON BROWN SPOT OF PADDY (*ORYZA SATIVA*) CAUSED BY *HELMINTHOSPORIUM ORYZAE* (BREDA DE HAAN)

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ABSTRACT

The current study was carried out for managing brown spot disease of paddy using biocontrol agents and botanical extract. *In vitro* and *in vivo* evaluation of *Trichoderma* spp. and Neem leaf extract against *Helminthosporium oryzae* was carried out. In *in vitro* condition, five species of *Trichoderma* (*T. harzianum, T. hamatum, T. asperellum, T. virens* and *T. reesei*) were taken following dual culture technique; neem leaf extract, carbendazim and mancozeb were taken following poison food technique. This study evaluated chemical and biological control strategies during *Kharif* 2024 in Prayagraj, Uttar Pradesh. The combined treatment of Carbendazim @0.2% (seed treatment) and Mancozeb @0.2% (foliar spray) showed the highest efficacy, with 98.52% mycelial growth inhibition, lowest disease intensity (39.24%) and maximum yield (3.63 kg/plot). Among bio-control options, *Trichoderma harzianum* [(ST)+(SD)] with Neem leaf extract @10% (foliar spray) achieved 86.48% pathogen inhibition and significantly improve plant performance (yield: 3.23 kg/plot). The results suggest that integrated use of *T. harzianum* and Neem extract offers a promising eco-friendly alternative to chemical fungicides.
***Keywords*:** Brown spot· *Helminthosporium oryzae*· Neem leaf extract· Paddy· *Trichoderma* spp.

Introduction

Oryza sativa L., widely known as rice or paddy, is a cultivated species belonging to the genus *Oryza* and the Poaceae family, which includes the "true grasses." Archaeological findings indicate that rice has been preserved in China since around 3000 B.C. While *Oryza sativa* is native to the tropical and subtropical regions of southern Asia, another species, *Oryza glaberrima* (African rice), originated in West Africa (Gutaker *et al*., 2020). Rice is a staple food for much of the world's population, providing approximately 20% of the global caloric intake (Monika *et al*., 2022).

Among the major fungal diseases affecting rice, Brown Leaf Spot (BLS) commonly referred to as brown spot is particularly destructive and contributes significantly to yield losses. The disease was first reported in Japan around 1900 and is also known by various names such as *nai-yake* (meaning "seedling blight"), sesame leaf spot, and Helminthosporiosis. It has a global distribution and has been observed in nearly all rice-growing countries, including Japan, China, Myanmar, Sri Lanka, Bangladesh, Iran, and regions across Africa, South and North America, Russia, the Philippines, Saudi Arabia, Australia, Malaysia, and Thailand. In India, Brown Spot was first documented by Sundararaman in 1919 in Madras (present-day Chennai). Under favorable conditions for the pathogen, the disease can become severe, leading to extensive leaf spotting and yield losses of up to 90%.

One of the most devastating historical impacts of brown spot disease was observed during the Great Bengal Famine of 1942. The disease tends to thrive under conditions of water scarcity and poor soil fertility, particularly when nitrogen levels are deficient (Sunder *et al*., 2014). Brown spot of rice, caused by the fungal pathogen *Helminthosporium oryzae*, poses a serious threat to rice cultivation by reducing grain

quality, limiting tiller formation, and significantly decreasing yield.

The pathogen primarily spreads through airborne conidia (spores), which, under high humidity, germinate and infect plant tissues by forming specialized penetration structures called appressoria. Initial symptoms typically appear as small reddish-brown lesions on the leaves, which can later develop into more extensive necrotic areas, resulting in seed discoloration, stunted growth, and poor plant vigor (Akbar *et al*., 2023). Microscopically, the fungus produces curved, septate conidia and forms grey to dark brown mycelial mats on infected tissues (Sunder *et al*., 2014).

Management strategies for brown spot commonly involve the use of resistant rice cultivars, chemical fungicides, and integrated disease management (IDM) approaches. However, the continuous emergence of new pathogen races has raised concerns about the long-term effectiveness and environmental sustainability of chemical control. Consequently, there is increasing interest in eco-friendly alternatives. Among these, biological control agents such as *Trichoderma*, *Bacillus*, and *Pseudomonas* species, along with botanical extracts from *Azadirachta indica* (neem) and *Calotropis gigantea*, have demonstrated promising antifungal activity and plant growth-promoting properties (Mau *et al*., 2022; Abdul-Halim *et al*., 2022; Shaheen *et al*., 2024).

Materials and Methods

Geographical location

The experiment was conducted at the Central Research Field of Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj, during the *Kharif* season of 2024. The field experiment was laid out in a Randomized Block Design (RBD)**,** while the laboratory experiment was conducted using a Completely Randomized Design (CRD). Both experiments included six treatments and were replicated three times.

Isolation and purification of the pathogen

Diseased rice leaves showing typical brown spot symptoms were collected from infected fields and initially washed under running tap water to remove surface debris. Under aseptic conditions, the symptomatic leaf portions were cut into small segments and surface-sterilized using 0.1% sodium hypochlorite solution for one minute. These segments were then rinsed three times with sterile distilled water to eliminate any residual disinfectant.

The sterilized leaf bits were aseptically transferred onto Petri plates containing Potato Dextrose Agar (PDA) medium. To prevent bacterial contamination, streptomycin was added to the medium at a concentration of 100 ppm during the lukewarm stage, prior to pouring it into sterile Petri dishes. The inoculated plates were sealed and incubated at 27±2°C in a BOD incubator.

For obtaining pure cultures of the pathogen, the hyphal tip method was employed, followed by sub-culturing onto fresh PDA slants or Petri plates. Cultures were maintained by periodic transfer onto fresh media, as recommended by Tuite (1969). Additionally, the single spore isolation method was used for purification of the culture, following the procedure described by Toussoun and Nelson (1976). The resulting pure cultures were maintained on PDA slants and stored at 4ºC for further use.

Procurement of *Trichoderma* spp.

Trichoderma spp. (*T. harzianum, T. hamatum, T. asperellum, T. virens* and *T. reesei*) used in this study were obtained from the division of Plant Pathology, ICAR-IARI, Pusa, New Delhi. The fungal species were identified based on the colony morphology and microscopic examination.

Mass multiplication of *Trichoderma* spp.

For mass multiplication sorghum grains were taken, cleaned properly and soaked overnight. Next day, grains were boiled for 20 to 25 minutes to soften the grains (not mushy). After that excess water was drained out and the grains were spread to decrease the moisture content. To remove the excess moisture, 2g of calcium carbonate was added per 100g of parboiled semi-dried sorghum seeds. The seeds were then transferred to polypropylene bags or 250 ml conical flasks and autoclaved twice at 121°C at 15 minutes. After cooling, the sorghum seeds were aseptically inoculated with the *Trichoderma* spp. grown in PDA media and incubated at room temperature for 5–7 days (Sinha *et al*. 2022). After seven days the colonized grains were air-dried in open shade. After that grinded well to get a fine powder and passed through 50 and 80 mesh sieves to obtain pure spore powder (Singh *et al*., 2012). 1g spore powder was taken and thoroughly mixed with sterile distilled water. The concentration of spores was determined by hemocytometer and adjusted to 10^6 conidia ml^{-1} (2×10^6 cfu/g) (Chaudhary *et al*., 2020).

Dual culture technique

Sterilized and cooled potato dextrose agar medium (20ml) was poured into sterilized Petri plates.

Fungal antagonists *i.e. Trichoderma* spp. was evaluated by inoculating *Helminthosporium oryzae* at one side of the Petri plate and the *Trichoderma* sp. at exactly opposite side of the same plate by leaving about 4 cm gap. After required period of incubation *i.e.*, when the growth in control plate records 90 mm in diameter, the radial growth of the pathogens was measured (Vincent 1947).

Preparation of neem leaf extract

Fresh neem leaves (*Azadirachta indica*) were washed thoroughly with tap water followed by sterile distilled water. The leaves were then homogenized in sterile distilled water at a ratio of 1 ml per gram using a sterile mortar and pestle. The homogenate was filtered through double-layered muslin cloth and subsequently through Whatman No. 1 filter paper to obtain a 100% stock extract. A 10% extract was prepared by grinding 10 g of leaf material in 100 ml of sterile distilled water, following the procedure outlined by Nene and Thapliyal (1993).

Poison food technique

To prepare a 10% neem-amended medium, 10 ml of the aqueous extract was mixed with 90 ml of autoclaved Potato Dextrose Agar (PDA) cooled to 40–45°C, and thoroughly mixed. The medium was poured into sterile 9 cm Petri plates. A 5 mm mycelial disc from a one-week-old culture of *Helminthosporium oryzae* was placed at the center of each plate. Plates without neem extract served as control. Each treatment was replicated three times, and plates were incubated at 28 ± 2°C until full mycelial growth was observed in the control. PDA medium (90ml) were dispensed in 150 ml capacity conical flasks and autoclaved at 120°C at 15 lbs. pressure/square inch for 15 minutes, after cooling down at 40-45^{0}C the required dose of the test fungicides i.e. Carbendazim and Mancozeb (0.2%) were added and gently shaken in circular motion for homogeneous mixing of the chemicals. To obtain 0.2% concentration 2g of carbendazim and mancozeb were mixed in 100 ml distilled water in two different flasks and mixed thoroughly. The poisoned media were then poured in 9 cm diameter sterilized Petri plates and solidified. Medium without fungicide served as control. Mycelial disc (5mm) of pathogen culture taken from actively growing margin was inoculated at the centre with mycelium mat facing the media surface. For each treatment four replications were maintained. The inoculated plates were then incubated at 27±2ºC in BOD incubator. Observation on linear growth of fungus was carried out at 24 hours interval till the fungus in the control plates covered whole surface of the plate. Per cent growth inhibition was calculated by using the formula given by (Vincent 1947).

Percentage inhibition of mycelia growth of test pathogen was calculated using the formula:

$$I = C\text{-}T \times 100 / C$$

Where, I = Percent reduction in growth of test pathogen

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment

Field experiment

Paddy seeds of variety BPT 5204 were taken from department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj. The seeds were soaked overnight in clean water and packed in muslin cloth and kept in shade till getting sprout. On the other day the sprouted seeds were treated with the pure spore powder of *Trichoderma* spp. multiplied on sorghum grain. Then pre-sprouted seeds were uniformly sown on the well- prepared nursery bed. The nursery beds were saturated with water for first five days and the level of water was increased gradually. Transplanting of paddy was done after 21 days of nursery with spacing of 15 cm row to row and 10 cm plant to plant with dipping in the spore suspension of *Trichoderma* spp. The experimental plot size was 3m x 2m for each treatment uniformly across replications. The spore suspension was prepared by scraping the spores of *Trichoderma* spp. (2×10^6 CFU/g) (Khan *et al*., 2022) through a needle from cultures grown on potato dextrose agar plates (Chowdappa *et al*., 2013). Botanical extracts were prepared by using method of standard procedure given by Mahapatra and Das (2013). Matured leaves and other botanicals were collected and sterilized with distilled water; the leaves were homogenized in a pre-chilled pestle and mortar using chilled, sterilized distilled water. Aqueous extract of this botanical (1% w/v) was prepared by mixing 100g fresh leaves with 100 ml of sterile distilled water and crushing in warring blender. The extract was filtered through four layers of moist muslin cloth. The filtrate thus obtained was considered as 10% plant extract. Treatments were applied following the appearance of the initial disease symptoms. Observations were recorded on brown leaf spot disease intensity (%), plant height (cm), number of tillers per plant, and panicle length (cm). Yield per plot was obtained after harvesting at physiological maturity.

The intensity of disease was visually assessed in all the plots at weekly interval from first appearance of disease for each treatment. The data was analyzed statistically. Disease intensity was calculated by following the given formula (Wheeler 1969).

$$\text{Disease intensity}(\%) = \frac{\text{Sum of all disease ratings}}{\text{Total no. of rating} \times \text{Maximum disease grade}} \times 100$$

Disease rating scale:

Grade	Leaf area infected
0	No symptoms on the leaves
1	Small spots covering 1% or less area
3	Small spots (up to 5mm in size) covering 1-10% of leaf area
5	Spots enlarging and covering 26-50% of leaf area
7	Spot coalesce to form big patches covering 26-50% of leaf area
9	Big spot covering 51% or more of leaf area

Results

Dual culture

The *in vitro* evaluation of different *Trichoderma* species against *Helminthosporium oryzae* revealed significant suppression of fungal radial growth compared to the control (Table 1). Among the treatments, *Trichoderma harzianum* (T_3) exhibited the most pronounced antifungal activity, with a radial growth of 12.16 mm and an inhibition percentage of 86.48%. This was followed by *T. hamatum* (T_2), which showed a radial growth of 12.83 mm and 85.74% inhibition. Other treatments, including *T. asperellum* (T_1), *T. reesei* (T_4), and *T. virens* (T_5), also significantly inhibited the radial growth, with inhibition values ranging from 82.41% to 83.88%. The formation of a clear zone in the antagonism test indicates the antagonistic activity of *Trichoderma* spp. against *Helminthosporium oryzae*, likely due to mycoparasitism and the production of antifungal metabolites (Siahaan *et al*., 2024). *T. harzianum* showed the highest efficacy, consistent with reports of its ability to produce lytic enzymes (e.g., chitinases, glucanases) and secondary metabolites that inhibit pathogen growth through mycoparasitism and competition (Makhlouf *et al*., 2020).

Table 1 : Dual culture assay of bioagents and test fungi on 5th day

Treatments	Treatment details	Radial growth (mm)	Mycelial inhibition (%)
T_0	Control	90^{a}	0
T_1	*Trichoderma asperellum* against *Helminthosporium oryzae*	14.5^{bc}	83.88
T_2	*T. hamatum* against *H. oryzae*	12.83^{c}	85.74
T_3	*T. harzianum* against *H. oryzae*	12.16^{de}	86.48
T_4	*T. reesei* against *H. oryzae*	14.66^{ef}	83.71
T_5	*T. virens* against *H. oryzae*	15.83^{f}	82.41
		CD (0.05) = 0.84	

Poison food technique

After 120 hours of incubation, significant differences were observed in the radial growth and inhibition percentage of *Helminthosporium oryzae* across treatments (Table 2). The control (T_0) showed maximum radial growth (90 mm), indicating uninhibited fungal development. In contrast, all treatments significantly suppressed fungal growth. Carbendazim exhibited the highest antifungal efficacy, recording the lowest radial growth (1.33 mm) and the greatest inhibition (98.52%), consistent with its systemic, broad-spectrum activity (Das *et al.,* 2022; Xu *et al*., 2018). Mancozeb followed with a radial growth of 2.17 mm and an inhibition rate of 97.58%, reflecting its multi-site action on fungal enzymes. Neem leaf extract also demonstrated considerable antifungal activity, reducing radial growth to 4.17 mm and achieving 95.36% inhibition. This effect is attributed to bioactive compounds such as azadirachtin, known for their antifungal properties (Narayanaswamy *et al*., 2021; Kumar and Simon, 2021).

Table 2 : Poison food assay of neem leaf extract and test fungi on 5th day

Treatments	Treatment details	Radial growth (mm)	Mycelial inhibition (%)
T_0	Control	90^{a}	0
T_1	Neem leaf extract against *Helminthosporium oryzae*	4.17^{b}	95.36
T_2	Carbendazim against *H. oryzae*	1.33^{c}	98.52
T_3	Mancozeb against *H. oryzae*	2.17^{cd}	97.58
		CD (0.05) = 1.06	

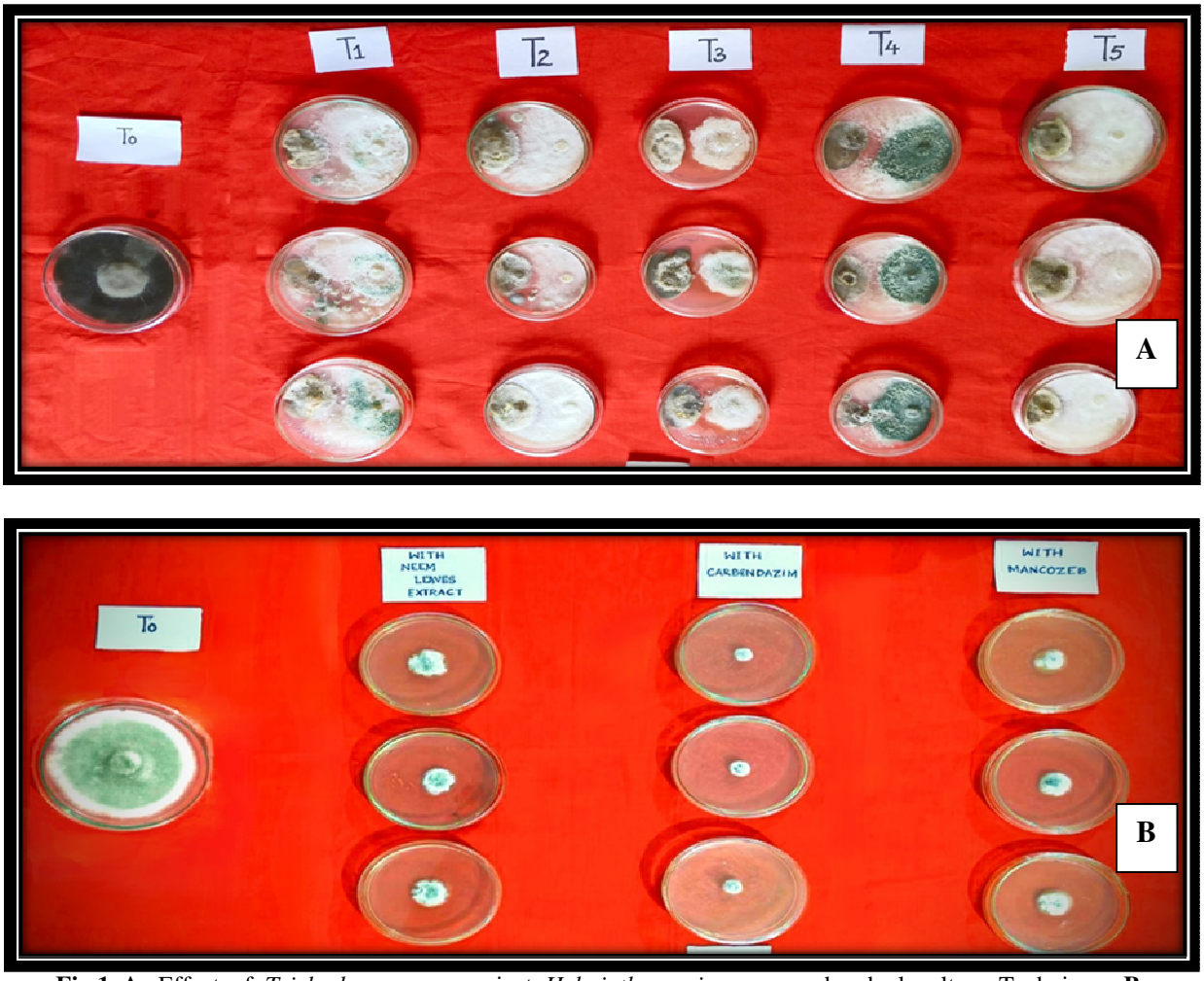

Fig.1 A. Effect of *Trichoderma* spp. against *Helminthosporium oryzae* by dual culture Technique, **B.** Efficacy of botanical extract and selected fungicides against *Helminthosporium oryzae* carried out by poison food technique. (T_0- *Helminthosporium oryzae* , T_1- *T. asperellum*+ *H. oryzae* , T_2- *T. hamatum*+ *H. oryzae*, T_3- *T. harzianum*+ *H. oryzae*, T_4- *T. reesei*+ *H. oryzae* and T_5- *T. virens*+ *H. oryzae*)

Per cent disease intensity

The minimum disease intensity was recorded in the chemical treatment T_6- Carbendazim @0.2% (FS) + Mancozeb @0.2% (FS) with 39.24%. Among the bio-control treatments, T_3- *Trichoderma harzianum* [(ST)+(SD)] + Neem leaf extract @10% (FS) showed the lowest disease intensity (41.03%), followed by T_1- *T. asperellum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (42.06%), T_2- *T. hamatum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (43.97%), T_4- *T. reesei* [(ST)+(SD)] + Neem leaf extract @10% (FS) (44.73%) and T_5- *T. virens* [(ST)+(SD)] + Neem leaf extract @10% (FS) (47.31%) as compared to T_0- Control (48.55%) as provided in Table 3.

Plant height

The maximum plant height was recorded in the chemical treatment T_6- Carbendazim @0.2% (FS) + Mancozeb @0.2% (FS) with 98.25cm. Among the bio-control treatments, T_3- *Trichoderma harzianum* [(ST)+(SD)] + Neem leaf extract @10% (FS) showed the highest plant height (97.30cm), followed by T_1- *T. asperellum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (96.45cm), T_2- *T. hamatum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (95.77cm), T_4- *T. reesei* [(ST)+(SD)] + Neem leaf extract @10% (FS) (94.8cm) and T_5- *T. virens* [(ST)+(SD)] + Neem leaf extract @10% (FS) (94.13cm) as compared to T_0- Control (93.3cm) as provided in Table 3.

Number of tillers

The maximum number of tillers was recorded in the chemical treatment T_6- Carbendazim @0.2% (FS) + Mancozeb @0.2% (FS) with 27.00. Among the bio-control treatments, T_3- *Trichoderma harzianum* [(ST)+(SD)] + Neem leaf extract @10% (FS) showed

the highest number of tillers (26.06), followed by T_1- *T. asperellum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (25.06)**,** T_2- *T. hamatum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (24.46), T_4- *T. reesei* [(ST)+(SD)] + Neem leaf extract @10% (FS) (24.13) and T_5- *T. virens* [(ST)+(SD)] + Neem leaf extract @10% (FS) (23.8) as compared to T_0- Control (23.2). The details are given in Table 3.

Panicle length

The maximum panicle length was recorded in the chemical treatment T_6- Carbendazim @0.2% (FS) + Mancozeb @0.2% (FS) with 22.76cm (Table 3). Among the bio-control treatments, T_3- *Trichoderma harzianum* [(ST)+(SD)]+ Neem leaf extract @10% (FS) showed the highest panicle length (22.01cm), followed by T_1- *T. asperellum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (21.57cm)**,** T_2- *T. hamatum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (20.85cm), T_4- *T. reesei* [(ST)+(SD)]+ Neem leaf extract @10% (FS) (20.31cm) and T_5- *T. virens* [(ST)+(SD)] + Neem leaf extract @10% (FS) (19.8cm) as compared to T_0- Control (19.38cm).

Yield

Maximum yield was recorded in the chemical treatment T_6- Carbendazim @0.2% (FS) + Mancozeb @0.2% (FS) with 3.63kg/plot. Among the bio-control treatments, T*3- Trichoderma harzianum* [(ST)+(SD)] + Neem leaf extract @10% (FS) showed the highest yield (3.23kg/plot), followed by T_1- *T. asperellum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (2.76kg/plot)**,** T_2- *T. hamatum* [(ST)+(SD)] + Neem leaf extract @10% (FS) (2.62kg/plot), T_4- *T. reesei* [(ST)+(SD)] + Neem leaf extract @10% (FS) (2.52kg/plot) and T_5- *T. virens* [(ST)+(SD)] + Neem leaf extract @10% (FS) (2.26kg/plot) as compared to T_0- Control (1.57kg/plot). Treatment wise results are given in Table 3.

Table 3 : Effect of treatments on growth parameters of paddy

		Per cent disease intensity			Plant height			Number of tillers			Panicle length	Yield
Treatments	**Treatment details**	**60 DAT**	**75 DAT**	**90 DAT**	**30 DAT**	**60 DAT**	**90 DAT**	**30 DAT**	**60 DAT**	**90 DAT**	**At the time of harvest**	**In Kg/plot**
T_0	Control	23.54^{a}	29.47^{a}	48.55^{a}	40.15^{f}	60.76^{f}	93.30^{g}	8.2^{f}	18.66^{e}	23.2^{f}	19.38^{g}	1.57
T_1	*Trichoderma asperellum* + Neem leaf extract	18.81^{b}	24.57^{b}	42.06^{ab}	42.56^{bc}	62.31^{c}	96.45^{c}	10.33^{c}	20.33^{c}	25.06^{c}	21.57^{c}	2.76
T_2	*Trichoderma hamatum* + Neem leaf extract	19.54^{bc}	25.81^{c}	43.97^{c}	42.32^{c}	61.88^{d}	95.77^{d}	9.93^{cd}	20.00^{c}	24.46^{d}	20.85^{d}	2.62
T_3	*Trichoderma harzianum* + Neem leaf extract	17.79^{cd}	22.28^{cd}	41.03^{cd}	43.06^{b}	62.72^{b}	97.30^{b}	11.2^{b}	21.06^{b}	26.06^{b}	22.01^{b}	3.23
T_4	*Trichoderma reesei* + Neem leaf extract	20.47^{de}	27.22^{de}	44.73^{de}	41.46^{d}	61.93^{d}	94.80^{e}	9.4^{de}	19.4^{d}	24.13^{de}	20.31^{e}	2.52
T_5	*Trichoderma virens* + Neem leaf extract	21.18^{e}	27.8^{e}	47.31^{ef}	40.76^{e}	61.25^{e}	94.13^{f}	9.06^{e}	19.06^{d}	23.8^{e}	19.8^{f}	2.26
T_6	Carbendazim + Mancozeb	16.3^{f}	21.6^{ef}	39.24^{fg}	43.76^{a}	63.79^{a}	98.25^{a}	11.86^{a}	21.8^{a}	27.00^{a}	22.76^{a}	3.63
	CD (0.05)	1.51	1.49	2.2	0.52	0.35	0.55	0.54	0.38	0.51	0.32	0.32

Discussion

A clear zone was formed in antagonism test of *Trichoderma* spp. This may be due to its antagonistic activity against *Helminthosporium oryzae*, such as mycoparasitism and metabolite compound activity. The metabolite compounds play an important role in the antagonistic ability of *Trichoderma* spp. (Siahaan *et al*., 2024). The superior performance of *T. harzianum* is consistent with previous studies highlighting its capacity to produce lytic enzymes (e.g., chitinases, glucanases) and antifungal metabolites, which may disrupt pathogen development through mycoparasitism and competition for space and nutrients (Mazrou *et al*. 2020). Similarly, Iwuagwu *et al*. (2020) reported that Neem extracts significantly inhibited the radial growth of *H. oryzae in vitro*. The use of neem-based treatments could be a sustainable approach in integrated disease management strategies for rice cultivation. Kumar and Simon (2021) observed that Neem leaf extract at 10% concentration effectively reduced brown leaf spot severity and improved plant growth parameters in rice. The use of neem-based treatments could be a sustainable approach in integrated disease management strategies for rice cultivation. Among the biological treatments, T_3 (*T. harzianum* + neem leaf extract) and T_1 (*T. asperellum* + neem leaf extract) showed notable reductions in per cent disease intensity along with increased plant height, number of tillers, panicle length and yield compared to the control (T_0). The synergistic effect of *Trichoderma* spp. and neem has been documented in various studies by Motlagh and Mohammadian (2017),

Jatoi *et al*. (2015) and Mahmud *et al*. (2020). These findings suggest that integrating biological agents like *Trichoderma* spp. with botanical extracts such as neem can be an effective strategy for disease management, offering a more sustainable and environmentally friendly alternative to chemical fungicides.

Conclusion

The present study clearly demonstrates that among the bio-control treatments, *Trichoderma harzianum* [(ST)+(SD)] combined with Neem leaf extract @ 10% (FS) (T_3) emerged as the most promising alternative. This combination exhibited substantial antifungal activity, resulting in 86.48% inhibition of mycelial growth of *Helminthosporium oryzae*, the causal organism of brown spot disease in paddy. It showed significantly reduced per cent disease intensity of Brown spot of paddy caused by *Helminthosporium oryzae* under field conditions. Furthermore, it enhanced plant growth and productivity, recording maximum plant height (cm), number of tillers, panicle length (cm), and yield (kg/plot). This combination treatment consisting of *T. harzianum* and neem extract gave the maximum yield advantage (105.73%) over the control as compared to other combinations of biological agents and neem extract. The chemical treatment consisting of Carbendazim (0,2%) and Mancozeb (0.2%) though provided the best protection with 131.21% yield advantage over the control, it suffers from negative environmental impacts. In this context, the result obtained with the best combination of biological and botanical treatment in this study as described above has significance. However, it is required to validate the results on a larger plot for at least three seasons/years prior to its recommendation for management of brown spot disease in rice.

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Declarations

Conflict of interest on behalf of all authors, the corresponding author states that there is no conflict of interest.

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