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Effect of *Trichoderma harzianum* and Selected Botanicals on Brown Leaf Spot of Paddy (*Oryza sativa* L.) Caused by *Helminthosporium oryzae* (Breda de Haan)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Paddy or rice crop (*Oryza sativa* L.) is the most important cereal crop next to wheat in area and production. The present study was investigated to evaluate the effect of *Trichoderma harzianum* and selected botanicals on brown leaf spot of Paddy (*Oryza sativa* L.) caused by *Helminthosporium oryzae* (Breda de Haan) under field condition. Three replications of paddy were planted in a randomized block design at the research plot of the Central Research Field, Department of Plant

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Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj during *Kharif* season of 2023. The minimum per cent disease intensity was recorded in T5- *Trichoderma harzianum* @ 0.1% (S.D) + Garlic bulb extract @ 10% (F.S) (33.06) followed by by *Trichoderma harzianum* @ 0.1% (S.D) + Neem leaf extract @ 10% (F.S) (37.81). Maximum plant height 102.34 (cm), number of tillers (27.00), panicle length 20.91 (cm), and yield (5.13 t/ha) were recorded in *Trichoderma harzianum* @ 0.1% (S.D) + Garlic bulb extract @ 10% (F.S) whereas maximum cost benfit ratio (1: 2.67) was recorded in T1- *Trichoderma harzianum* @ 0.1% (S.D) + Neem leaf extract @ 10% (F.S) when compared to untreated check.

Keywords: Bio agent; botanicals extract; brown spot; Helminthosporium oryzae; paddy.

1. INTRODUCTION

Paddy (*Oryza sativa* L.) is a staple food of 65 per cent of the total population in India. Rice (*Oryza sativa* L.) is a crucial staple food belonging to the grass family *Poaceae*. The cultivated rice falls under the genus *Oryza*, with approximately 24 species distributed in tropical, sub- tropical, and warm temperature regions worldwide.

India is the world's second-largest rice producer after China [1]. Rice is a staple for over 3.5 billion people globally, particularly in Asia, Latin America, and parts of Africa. Rice cultivation spans over 160 million hectares worldwide, thriving in diverse climates. In India, rice is a staple for 800 million people, contributing nearly 40% of the total food grain yield. The country dedicates 43 million hectares to rice cultivation, producing 112 million tons of milled rice with an average yield of 2.6 tons per hectare [2]. Rice is grown in almost every Indian state. Major rice-producing states were West Bengal (13.79%), Uttar Pradesh (13.34%), Andhra Pradesh (12.84%), and Punjab (11.01%) [3].

Rice contributes approximately 10% to the agricultural GDP and generates 3.5 billion days of employment in India [4]. In Uttar Pradesh, rice is a major crop, covering about 5.70 million hectares (12.29% of the total area). The state ranks second in the country for rice production, with 15.27 million tonnes produced (11.72% of the national production) and a productivity rate of around 1.94 tonnes per hectare (Agriculture Statistics at a Glance 2022).

During Great Bengal Famine 1942 to 1943, 50 to 90% in yield reduced [5]. It has been also reported that yield losses due to brown spot disease near about the world is 5 to 45% and in Asia losses is 6% to 90% [6]. In India estimated yield loss is about 45 percent and major producing state West Bengal is up to 29.13 percent and 44 percent were observed in Utter Pradesh [7].

The yield of basmati rice in India is significantly lower compared to other developed countries around the world. This reduced production can be attributed to various biotic and abiotic factors. One of the most critical factors contributing to the low productivity of both regular and Basmati rice is brown leaf spot, caused by *Helminthosporium oryzae* [8]. Among all the diseases, brown leaf spot disease is the most destructive and widespread in basmati rice. It typically leads to yield losses of 10 to 20 percent, and in severe cases, losses can escalate to as much as 80 percent. It affects the quality and the number of grains per panicle and reduces the kernal weight [9].

Brown spot is still widely reported across India [10] and more generally in south and South - East Asian countries [11]. The pathogen Attacks the crop from seedling to milk stage. The symptoms appear as minutes spots on the coleoptiles, leaf blade, leaf sheath panicle branches glumes and spiklets which were appeared a minute spots on leaves typical spots were brown in colour with grey or whitish centre resembling sesame seed with typical yellow halo over spots [12]. Thus, the present study aimed to evaluate the effect of selected botanicals on brown leaf spot of Paddy (*Oryza sativa* L.) caused by *Helminthosporium oryzae* (Breda de Haan).

2. MATERIALS AND METHODS

The experiment was carried out at the Central Research Field, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj (U.P) India. during *Kharif* season 2023. The study was laid-out with Randomized Block Design (RBD) with three replications. *Trichoderma harzianum* was used as a seedling treatment. It is a broad-spectrum antimicrobial agent with antifungal properties that may have inhibited the growth of fungi. Whereas, botanicals extract which was applied as a foliar

spray to inhibit the spore germination and decreasing fungal growth (*Helminthosporium oryzae*). Three sprays of all treatments were given at an interval of 15 days. Treatments were imposed after appearance of the first disease symptoms. Observations on disease intensity (%) of brown leaf spot, plant height (cm), number of tillers, and panicles length (cm) of paddy were recorded. Yield (t/ha) and B:C ratio data were obtained after the harvest on physiological maturity. The treatments comprised of application of selected botanicals extract viz., Neem, Eucalyptus, Turmeric, Lantana camara, Ginger, @ 10% and Propiconazole 25 EC @ 0.1% (treated check) and control (untreated). The prepared botanicals extracts was sprayed at three times at 60, 75, and 90 DAT of interval. The disease intensity of brown leaf spot was recorded after ten days of spray. Per cent disease intensity (PDI) was calculated after each spray by using 0-9 disease rating scale on the basis of percentage area of foliage infected by the pathogen.

2.1 Isolation and Identification of the Pathogen

Diseased samples collected from different areas during the season and isolation of pathogen was carried out in the laboratory. Firstly collected diseased samples washed thoroughly under the tap water and then cut into small pieces 2-4 mm in size with the help of a sterilized blade in such a way that the sample contained a 50 per cent healthy portion as well as a 50 per cent diseased portion. The surface of the pieces was sterilized by using 1 per cent Sodium hypochlorite solution for 30 seconds to 1 minute, then finally wash well with the three changes of sterilized distilled water and to remove excess water then pieces was placed on blotter paper. With the help of a sterilized inoculating needle place the sample pieces on petri plates containing potato dextrose agar medium under the aseptic conditions in the laminar airflow chamber. Five pieces on PDA media on each plate. Inoculated petri plates kept in an incubator at 25°±2°C and examine at frequent intervals to check the growth of the target fungal pathogen.

Morphological characters like mycelial colour, culture characters, conidial characters and spore size were recorded.

2.2 Purification and Maintenance of the Pathogen

The culture obtained was purified once after the pathogen identity confirmed. Periodic sub culturing on PDA slants was done to keep the pure culture. Throughout the investigation, this pure culture employed. The cultures of the fungus was sub-cultured on PDA slants and kept in laboratory at 28±1 °C for 15 days. Further, these cultures was sub-cultured once in a month and used for future purpose [13].

2.3 Preparation of Botanicals Extracts

Botanicals extracts was prepared from, leaves of various plants *viz*, Neem (*Azadirachta indica), Eucalyptus (Eucalyptus globulus), Lantana (Lantana camera), and rhizome of* Turmeric (*Curcuma longa*), bulb of Garlic (*Allium sativum*), Washing of the materials with running tap water was followed by sterile distilled water, air dried at 27^0C and ground to obtain extracts of each plant species. The extraction was done by means of pestle and mortar. Water extract was obtained by adding one gm of tissue in one ml of water (1:1w/v) and filtered through double layers of muslin cloth. This forms the standard solution (100%). The botanical extract were sprayed at the rate of 10% prepared from standard solution. All the treatments were given as foliar spray. botanicals extract was sprayed @ 100 ml/liter of water, and propiconazole @ 1 ml/ liter of water.

2.4 Recording the Disease Intensity (%)

After transplanting, five plants per treatment per replication were randomly selected. Regularly watched for first appearance of disease. The observation on disease intensity was recorded using a progressive 0-9 scale, as given in [14]. Numerical rating grade was given on the basis of percentage of area affected.

Table 1. Disease rating scale

Grade	Leaf area infected
0	Absolutely free from infection
1	<1% area of infection
3	4-5% area of infection
5	11-15% area of infection
7	26-50% area of infection
9	76-100% area of infection

2.5 Per Cent Disease Intensity (PDI)

Per cent disease intensity was recorded at 60, 75 and 90 days after incidence of brown leaf spot of paddy. Percentage of disease intensity will be calculated in accordance with following formula [15].

Disease intensity (%) = Sum of all individual disease rating / Total number of Plant assessed × Maximum rating ×100

2.5.1 Economics analysis

Cost of cultivation, gross return, net return and benefit cost ratio was worked out to evaluate the economics of each treatment, based on the existing market prices of input and output.

2.5.2 Cost of cultivation

The cost of cultivation for each treatment was work out separately, taking into consideration all the cultural practices followed and costs of inputs used in the cultivation.

2.5.3 Gross return

The gross return from each treatment was calculated by using the following formula: Gross return (ha-1) = Yield (q/ha) × Price (Rs/q).

2.5.4 Cost benefit ratio

The benefit cost ratio was calculated by using the following formula [16]. Benefit Cost ratio = Gross return / Total cost of cultivation.

3. RESULTS AND DISCUSSION

3.1 Effect of *Trichoderma harzianum* and Selected Botanicals on Per Cent DiseaseIntensity of Brown Spot of Paddy

The minimum disease intensity of brown spot of paddy was recorded in T_5 -*Trichoderma harzianum* @ 0.1% (S.D) + Garlic bulb extract @ 10% (F.S) (33.06), followed by T_1 – *Trichoderma harzianum* @ 0.1% (S.D) + Neem leaf extract @ 10% (F.S) (37.81), T_2- *Trichoderma harzianum* @ 0.1% (S.D) + Eucalyptus leaf extract @ 10% (F.S) (40.08), T_3- *Trichoderma harzianum* @ 0.1% (S.D) + Turmeric rhizome extract @ 10% (F.S) (41.15), T_4- *Trichoderma harzianum* @ 0.1% (S.D) + Lantana camara leaf extract @ 10% (F.S) (42.86), as compared to T_6 – Propiconazole @ 0.1% (F.S) (27.32), and T_0 -Control (untreated check) (45.20) as presented in Table 2.

3.2 Effect of *Trichoderma harzianum* and Selected Botanicals on Plant Height

The maximum plant height (cm) of paddy was recorded in T_5 -*Trichoderma harzianum* @ 0.1% (S.D)+ Garlic bulb extract @ 10% (F.S) 102.34 (cm), followed by T_1 – *Trichoderma harzianum* @ 0.1% (S.D) + Neem leaf extract @ 10% (F.S) 101.45 (cm), T_2- *Trichoderma harzianum* @ 0.1% (S.D) + Eucalyptus leaf extract @ 10% (F.S) 100.82 (cm), T_3- *Trichoderma harzianum* @ 0.1% (S.D) + Turmeric rhizome extract @ 10% (F.S) 99.95 (cm), T_4- *Trichoderma harzianum* @ 0.1% (S.D)+ Lantana camara leaf extract @ 10% (F.S) 99.23 (cm), as compared to T_6 – propiconazole @ 0.1% (F.S) 103.81 (cm), and T_0 -Control (untreated check) 97.08(cm) as presented in Table 2.

3.3 Effect of *Trichoderma harzianum* and Selected Botanicals on Number of Tillers

The maximum number of tiller of paddy was recorded in T_5 -*Trichoderma harzianum* @ 0.1% (S.D) + Garlic bulb extract @ 10% (F.S) (27.00), followed by T_1 – *Trichoderma harzianum* @ 0.1% (S.D) + Neem leaf extract @ 10% (F.S) (26.37), T_2- *Trichoderma harzianum* @ 0.1% (S.D) + Eucalyptus leaf extract @ 10% (F.S) (25.22), T_3- *Trichoderma harzianum* @ 0.1% (S.D) + Turmeric rhizome extract @ 10% (F.S) (24.15), T_4- *Trichoderma harzianum* @ 0.1% (S.D) + Lantana camara leaf extract @ 10% (F.S) (23.16), as compared to T_6 – propiconazole @ 0.1% (F.S) (29.03), and T_0 -Control (untreated check) (21.03) as presented in Table 2.

3.4 Effect of *Trichoderma harzianum* and Selected Botanicals on Panicle Length

The maximum panicle of paddy was recorded in T_5 -*Trichoderma harzianum* @ 0.1% (S.D) + Garlic bulb extract @ 10% (F.S) 20.91 (cm), followed by T_1 – *Trichoderma harzianum* @ 0.1% (S.D) + Neem leaf extract @ 10% (F.S) 19.63 (cm), T_2- *Trichoderma harzianum* @ 0.1% (S.D) + Eucalyptus leaf extract @ 10% (F.S) 18.58 (cm), T_3- *Trichoderma harzianum* @ 0.1% (S.D) + Turmeric rhizome extract @ 10% (F.S) 18.23(cm), T_4- *Trichoderma harzianum* @ 0.1% (S.D) + Lantana camara leaf extract @ 10% (F.S) 17.89 (cm), as compared to T_6 – propiconazole @ 0.1% (Foliar spray) 22.06 (cm), and T_0 -Control (untreated check) 14.93 (cm) as presented in Table 2.

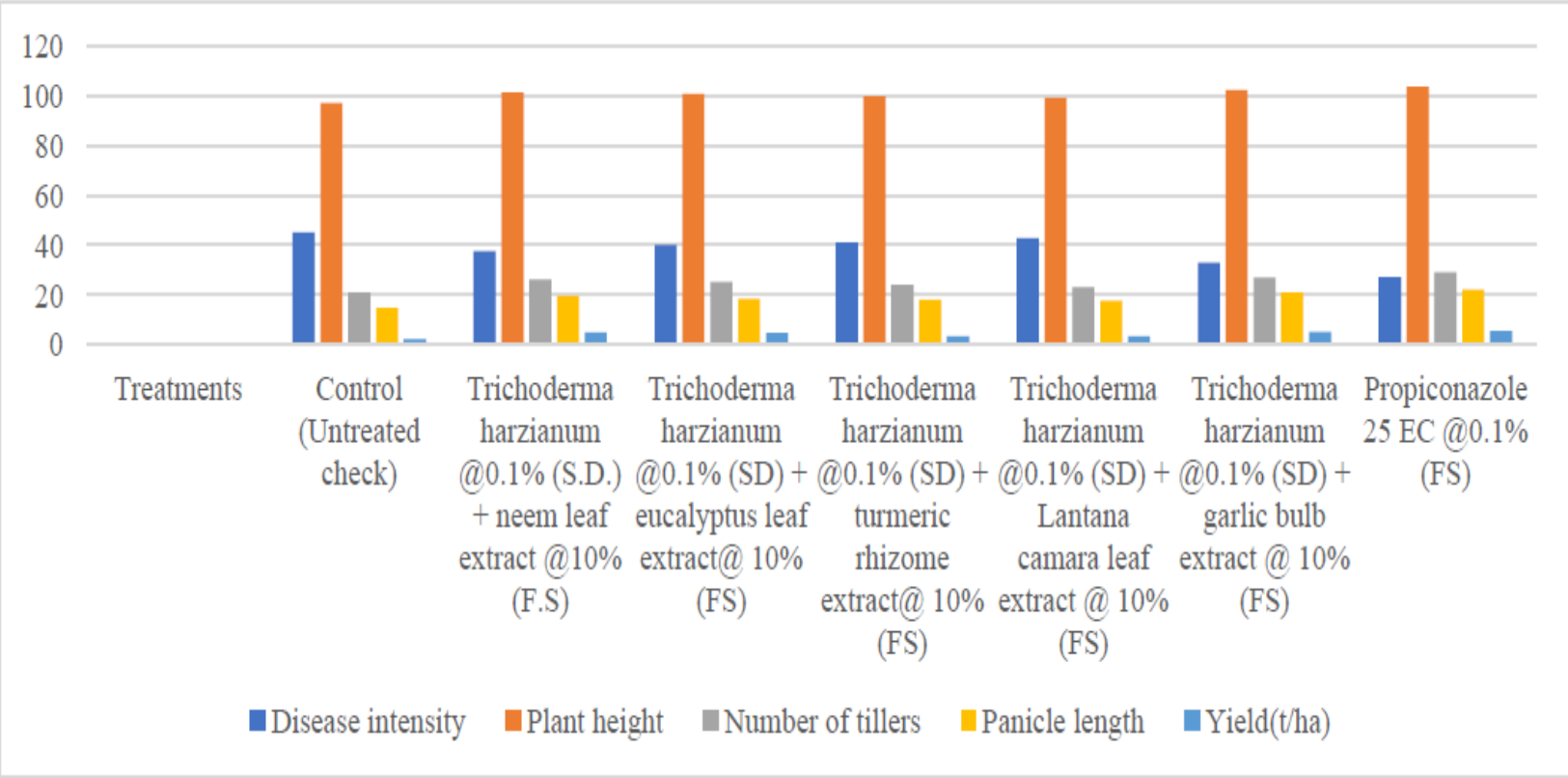

Fig. 1. Effect of selected treatments on disease intensity (%) of brown leaf spot,growth parameters and yield (t/ha) of Paddy

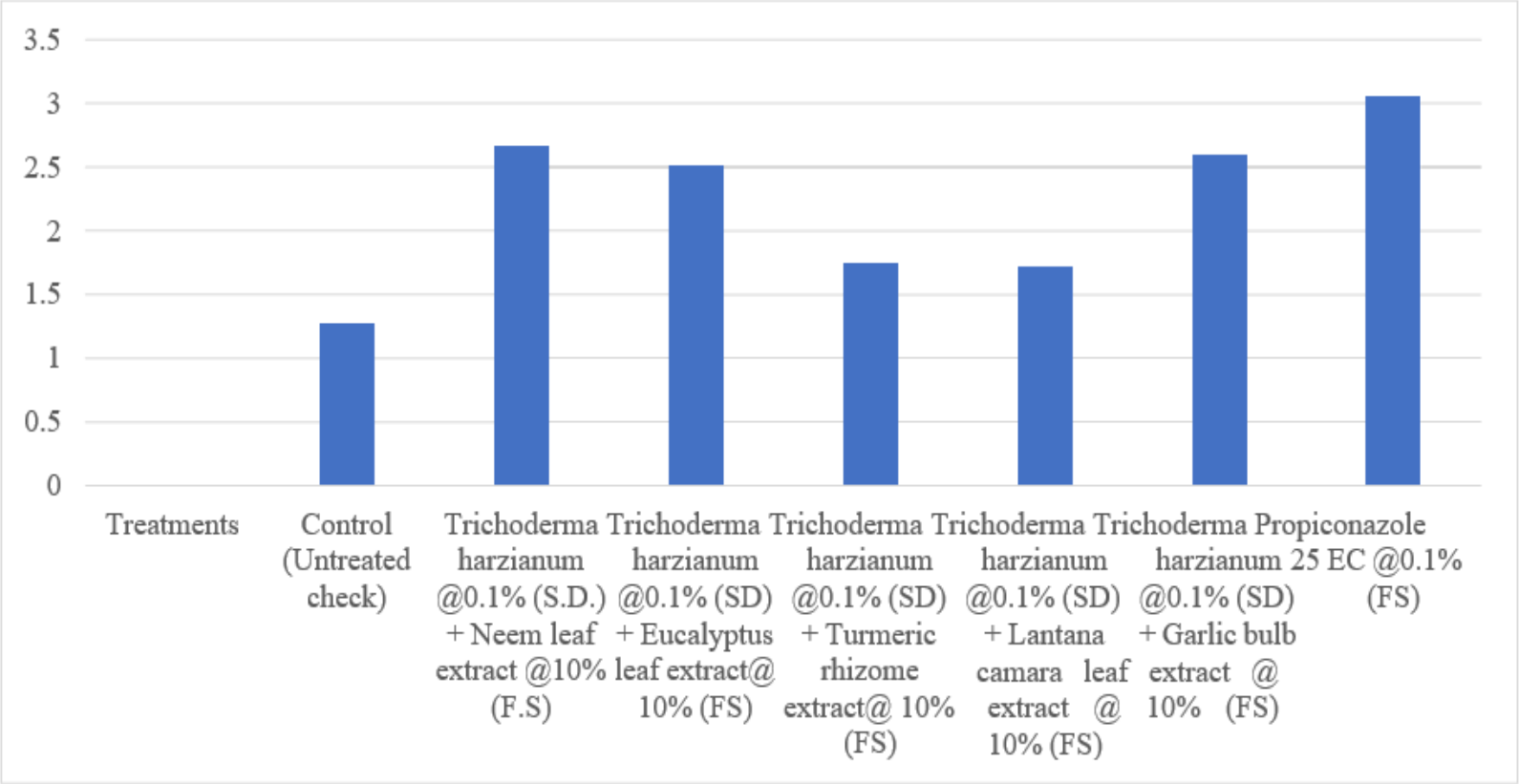

Fig. 2. Effect of selected treatments on cost benefit ratio of Paddy

Table 2. Effect of selected treatments on disease intensity (%) of brown leaf spot,growth parameters and yield (t/ha) of Paddy

S.No	Treatments	Disease intensity	Plant height(cm)	Numberof tillers	Panicle length(cm)	Yield(t/ha)	Cost benefitratio
T0	Control (Untreated check)	45.20[a]	97.08e	21.03[b]	14.93[e]	2.23[f]	1:1.27
T1	*Trichoderma harzianum* @0.1% (S.D.) + Neem leafextract @10% (F.S)	37.81[e]	101.45[c]	26.37[c]	19.63[c]	4.91[c]	1:2.67
T2	*Trichoderma harzianum* @0.1% (SD) + Eucalyptusleaf extract@ 10% (FS)	40.08[d]	100.82[c]	25.22[d]	18.58[d]	4.84[c]	1:2.51
T3	*Trichoderma harzianum* @0.1% (SD) + Turmeric rhizome extract@ 10% (FS)	41.15[c]	99.95[d]	24.15[e]	18.23[d]	3.35[d]	1:1.75
T4	*Trichoderma harzianum* @0.1% (SD) + Lantana camara leaf extract @ 10% (FS)	42.86[b]	99.23[d]	23.16[f]	17.89[d]	3.31[e]	1:1.72
T5	*Trichoderma harzianum* @0.1% (SD) + Garlic bulbextract @ 10% (FS)	33.06[f]	102.34[b]	27.00[b]	20.91[b]	5.13[b]	1:2.60
T6	Propiconazole 25 EC @0.1%(FS) (Treated check)	27.32[g]	103.81[a]	29.03[a]	22.06[a]	5.53[a]	1:3.06
	C. D. @ 5 %	0.74	0.78	0.75	0.91	0.08	

Where, S.D- (Seedling Dip), F.S- (Foliar spray)

3.5 Yield (t/ha)

The maximum yield of paddy was recorded in T_5 -*Trichoderma harzianum* @ 0.1% (S.D) + Garlic bulb extract @ 10% (F.S) 5.13 (t/ha), followed by T_1 – *Trichoderma harzianum* @ 0.1% (S.D) + Neem leaf extract @ 10% (F.S) 4.91 (t/ha), T_2- *Trichoderma harzianum* @ 0.1% (S.D) + Eucalyptus leaf extract @ 10% (F.S) 4.84 (t/ha), T_3- *Trichoderma harzianum* @ 0.1% (S.D) + Turmeric rhizome extract @ 10% (foliar spray) 3.35 (t/ha) , T_4- *Trichoderma harzianum* @ 0.1% (S.D) + Lantana camara leaf extract @ 10% (F.S) 3.31 (t/ha), as compared to T_6 – propiconazole @ 0.1% (F.S) 5.33 (t/ha), and T_0 - Control (untreated check) 2.23 (t/ha) as presented in Table 2.

3.6 Cost Benefit Ratio on Paddy

The maximum cost benefit ratio 1:2.67 was recorded in T_1 – *Trichoderma harzianum* @ 0.1% (S.D) + Neem leaf extract @ 10% (F.S), followed by T_5 -*Trichoderma harzianum* @ 0.1% (S.D) + garlic bulb extract @ 10% (F.S) (1:2:60), T_2- *Trichoderma harzianum* @ 0.1% (S.D) + eucalyptus leaf extract @ 10% (F.S) (1:2:51), T_3- *Trichoderma harzianum* @ 0.1% (S.D) + turmeric rhizome extract @ 10% (F.S) (1:1:75), T_4- *Trichoderma harzianum* @ 0.1% (S.D)+ lantana camera leaf extract @ 10% (F.S) (1:1:72), as compared to T_6 – propiconazole @ 0.1% (F.S) (1:3:06), and T_0 -Control (untreated check) (1:1:27) as presented in Table 2.

4. DISSCUSION

The probable reasons for such findings may be that *Trichoderma harzianum* was used as a seedling treatment. It is a broad-spectrum antimicrobial agent with antifungal properties that may have inhibited the growth of fungi. This biocontrol agent may have worked by competing with pathogens for a resource producing enzymes that may have degraded fungal cell walls, and enhanced the plant's own defense mechanism [17]. Whereas, garlic which was applied as a foliar spray, contains allicin, garlicin, and allylsulfides [18]. Allicin is a major component that may have undergone metabolic transformation to produce diallyl sulfide (DAS) and diallyl disulfide (DADS) [19]. These allicin-derived organosulfur compounds may have interfered with fungal pathogen by inhibiting spore germination and decreasing growth of *Helminthosporium oryzae* [20]. The combined use of *Trichoderma harzianum* and garlic treatment resulted in a comprehensive approach in managing plant disease. This synergy may have minimized plant disease intensity and promote better plant health, leading to improved crop yield and resilience. The use of biological and natural treatment like these offer a sustainable alternative to chemical pesticides, benefiting both the environment and agricultural productivity. Limited work on brown spot prompted a study on plant extracts. Indiscriminate chemical use results in toxicity, resistance, pollution, and increased costs. Plant pathologists focus on developing effective, safe biocontrol methods, with botanical extracts emerging as preferred for brown spot in paddy.

5. CONCLUSION

Helminthosporium oryzae (Breda de Haan) was found associated with brown leaf spot disease of rice (*Oryza sativa*. L). The disease intensity (%), plant height (cm), number of tillers, panicle length (cm) and yield (t/ha), overall results revealed that *Trichoderma harzianum* @0.1% (S.D) + Garlic bulb extraxt @10 % as foliar spray was significantly effective against *H. oryzae* (Breda de Haan). The maximum C:B ratio (1:2.67) was found in T_1 – *T. harzianum* @ 0.1% (S.D) + Neem leaf extract @ 10% (F.S) recorded the highest benefits as compared to treated and untreated control. It is worth mentioning that the conclusion drawn from this study were based on observations carried at Central Research field Department of Plant Pathology, SHUATS, Prayagraj U.P India. Therefore, to substantiate the present result more such trials are required for further recommendation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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