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Integrated Management of Spot Blotch Disease of Wheat (Triticum aestivum L.) Caused by Bipolaris sorokiniana (Sacc.) Shoem

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Abstract

Spot blotch caused by Bipolaris sorokiniana has a worldwide distribution, however, it is most hostile when temperatures and relative humidity are high and soil fertility is poor. The disease causes a substantial loss in yield, particularly in India's North Eastern Plains Zone. Field investigations were carried out from December to April for two consecutive crop seasons, 2021–2022 and 2022–2023 for the evaluation of fungitoxicants, botanicals, bio-agents, and micronutrients against spot blotch of wheat (Triticum aestivum L.) under the field conditions. The wheat genotypes were inoculated with an aqueous solution of the pathogen Bipolaris sorokiniana, which contained approximately 105 conidia ml⁻¹. Two inoculations were made: one at the booting phase and another fifteen days later. Seven micronutrients-calcium chloride, zinc sulphate+lime, ferrous sulphate+lime, boron, sulfur, molybdenum, and manganese sulphate+lime-four botanicals-leaf extracts of tulsi, neem, mentha, and garlic clove extract-four bioagents-Trichoderma harzianum SV-7, T. harzianum SV-28, T. harzianum IRRI-1, Pseudomonas fluorescens-and six fungitoxicants-azoxystrobin, difenoconazole, propiconazole, tebuconazole, azoxystrobin+tebuconazole, and carbendazim+mancozeb-were assessed against spot blotch of wheat in artificially inoculated field conditions. Furthermore, 32 wheat genotypes were evaluated for resistance to spot blotch in addition to the above experiments. During two consecutive crop seasons, the study found that fungitoxicants (propiconazole and tebuconazole), botanicals (garlic clove extract), micronutrients (zinc sulphate and boron), and bioagents (Trichoderma harzianum-IRRI-1) were effective in decreasing the disease index and increasing grain yield.

Keywords: Bipolaris sorokiniana, bioagents, micronutrients, propiconazole, spot blotch, wheat

1. Introduction

Wheat (Triticum aestivum L.) is the second most important cereal food crop in India after rice, and it has played a major role in stabilizing grain production in the country (Kumar et al., 2011). Several diseases that have affected wheat crops have resulted in significant yield loss. Foliar blights and rusts comprise the majority of fungal diseases that inflict damage on wheat crops. Due to a recent shift in cropping patterns; foliar blight, which comprises spot blotch (Bipolaris sorokiniana) and leaf blight (Alternaria triticina), has expanded. Previously, it caused 2.72 to 36.24% yield loss across different agro-climatic zones in India (Parashar et al., 1995) and presently, it has become a significant concern in hot, humid places where wheat is grown (Van and Rajaram, 1998). Spot blotch is a devastating wheat disease that causes significant losses in

the North Eastern Plain Zone (NEPZ) of India and other South Asian countries, especially in vulnerable cultivars. (Singh et al., 2014; Joshi et al., 2007). According to Van and Rajaram (1998), spot blotch affects 25 mha of wheat land worldwide while, about 40% of the world's wheat is grown in the Indian subcontinent (Joshi et al., 2007a), where spot blotch is estimated to cause crop losses of between 15% and 25% (Dubin and Van, 1991; Duveiller and Sharma, 2009). Because of the many environmental factors, local cultivars, fertigation schedules, and tactics used to combat the destructive disease, the estimation of losses from spot blotch differs from place to place. (Duveiller and Sharma, 2009; Pandey et al., 2005; Vaish et al., 2011). In India, yield loss in the Eastern Gangetic Plains can vary from 10% to 50%, contingent on the cultivar's tolerance to leaf blight and weather factors. Under extreme



circumstances, yield losses in eastern India can amount to 100% (Pandey et al., 2005). The disease becomes severe, especially during the grain-filling stage (Joshi and Chand, 2002). Symptoms of spot blotch are typically seen on leaves, seeds, glumes, awns, stems, and sub-crown internodes. The initial lesions on leaves are small, dark brown, and 1–2 mm long. In the latter stages, small lesions grow quickly and eventually reach a length of several centimeters (Acharya et al., 2011). Symptoms of spot blotch are typically seen on seeds, glumes, awns, stems, leaves, and sub-crown internodes. The initial lesions on leaves are small, dark brown, and 1–2 mm long. In the latter stages, little lesions grow quickly and eventually reach a length of several centimeters (Kumar et al., 2002).

The most successful fungicide applications are those that target eradication; contemporary systemic fungicides, like as propiconazole and tebuconazole, are particularly effective against a variety of foliar diseases, including wheat (Dubin and Duveiller, 2000). Farmers and small businesses can readily develop botanical fungicides, which makes them special (Roy et al., 2005). Biological control is an attractive substitute and environmentally responsible approach to controlling plant diseases, which also reduces the need for chemical fungicides and the associated health hazards (Xu et al., 2011; Mishra and Singh, 2012). In biological control, microbial antagonists from the genera Bacillus, Pseudomonas, and Trichoderma are among the most often utilized bacteria and fungi, respectively (Bettiol and Morandi, 2009). Micronutrients can impact the biochemistry and physiology of plants, which in turn can affect their defense system against diseases (Romheld and Marschner, 1991; Reuveni and Reuveni, 1998; Yadav et al., 2013). With these details in view, the investigations were carried out to find the in vivo effect of micronutrients, botanicals, bioagents, and fungitoxicants on Bipolaris sorokiniana causing spot blotch disease in wheat.

2. Materials and Methods

2.1. Experimental site and location

The field experiments were carried out in two crop seasons, 2021-2022 and 2022-2023 at the Crop Research Centre, Chirodi, Sardar Vallabhbhai Patel University of Agriculture & Technology (SVPUAT), Meerut (U.P.), India. The location of the research center is 237 m above mean sea level and is situated between 290 4' N latitude and 770 42'E longitudes. Laboratory experiments were conducted at the biocontrol laboratory, Department of Plant Pathology, SVPUAT, Meerut (U.P.), India. This district has a subtropical, semi-arid climate with extremely hot summers and chilly winters. Summertime highs reach up to 46°C, while wintertime lows drop to as low as 6 to 8°C. The southwest monsoon, which lasts from July to September, accounts for 78-80% of the 864 mm of rainfall that falls on average each year. Summertime does, however, bring with it sporadic bouts of rain. The methods employed and the experiments carried out are detailed below.

2.2. Isolation, multiplication and preparation of spore suspension of Bipolaris sorokiniana

The culture of Bipolaris sorokiniana used in the present investigation was isolated from leaf samples collected from the wheat fields of the Crop Research Centre, SVPUAT, Meerut (U.P.), India during March, 2021 and 2022, adopting a standard procedure for isolation and purification. The pathogen was subcultured aseptically on the potato dextrose agar (PDA) medium and slants in test tubes by using a single hyphal-tip culture technique (Brown, 1924). Based on colony morphology, colony color, spore septation, and other distinguishing characteristics, the identity of the causative organism, B. sorokiniana, was verified following the standard description given by Aggarwal et al. (2002). Pure culture of the pathogen was maintained on PDA for further various studies including pathogenicity test, *In-vitro* evaluation, etc. On wheat grains, B. sorokiniana was mass-multiplied. To avoid saprophytic bacterial contamination, wheat grains were thoroughly mixed with 1 mg 500 g⁻¹ of chloramphenicol after being soaked in tap water for the entire night and dried under a fan. 50 g of wheat grains were placed into 250 ml conical flasks, which were then sealed with non-absorbent cotton and autoclaved (15 lb pressure) at 121°C for 20 minutes to ensure full sterilization. After allowing the sterilized grain to cool, a 5 mm piece of B. sorokiniana PDA culture was added to it under the laminar airflow chamber. The flasks were incubated in the BOD incubator at 25±1°C for 20 days following inoculation to allow the pathogen to multiply in bulk. To ensure optimal colonization and sporulation, the flask was shaken daily to break up any clumps and mix the wheat grains. Using a spray atomizer, the inoculum grown on wheat grains was used for inoculation. The sporulated wheat grains were filtered using muslin cloth in distilled water to harvest spores of B. sorokiniana and to make aqueous solution which was adjusted to spore suspension (approximately 10⁵ conidia ml⁻¹). The experimental wheat field was uniformly inoculated twice in the evening time with spore suspension. The wheat crop was first inoculated during the booting stage, and a second inoculation was performed 15 days later.

2.3. Collection and multiplication of bioagents

Pure cultures of biocontrol agents *viz. Trichoderma harzianum* SV-7, *Trichoderma harzianum* SV-28, *Trichoderma harzianum* IRRI-1 and *Pseudomonas fluorescens* were obtained from the Biocontrol lab of Department of Plant Pathology, SVPUAT, Meerut. Pure culture of bioagents (*Trichoderma harzianum*-SV-7, SV-28, IRRI-1) was maintained on Trichoderma selective medium (TSM) and *Pseudomonas fluorescens* on King's B agar medium slants. TSM was made by mixing Magnesium sulphate heptahydrate (0.2 g), Dipotassium hydrogen phosphate (0.9 g), Ammonium nitrate (1 g), Potassium chloride (0.15 g), Dextrose (3 g), Rose Bengal (0.15 g), Agar (20 g) and distilled water (1000 ml). King's B broth was made by mixing 20 g of peptone, 1.50 g of magnesium sulphate, and 1.50 g of potassium hydrogen phosphate, with one litre of distilled water and 10 ml of

glycerol in a container. These biocontrol agents were evaluated against B. sorokiniana in vivo and in vitro conditions. Grain sorghum was soaked in water for the entire night to facilitate the mass growth of isolates (SV-7, SV-28, and IRRI-1) of T. harzianum. The excess water was then drained off. Afterward, 250 ml conical flasks containing 100 g of sorghum grain were sterilized in an autoclave for 20 minutes at 121°C. Three 5 mm mycelial bits, cut from the edge of 5-day-old TSM cultures of the individual isolate of Trichoderma under aseptic conditions, were added to the grains after they had cooled. The flasks were then sealed with a sterilized cotton plug and kept in a BOD incubator at 25±10 C for 20 days, with periodic handshaking to ensure uniform growth. Following the incubation period, the Trichoderma inoculum was removed from the flasks, allowed to air dry in a ventilated clean room for up to two days, and then pulverized with a mortar and pestle (Naeimi et al., 2020). Two 1-liter conical flasks were filled with 500 ml of prepared King's B broth each, and these were autoclaved at 121°C for 15-20 minutes at 15 pressure to ensure sterilization in preparation for the mass multiplication of Pseudomonas fluorescens. Once the broth had cooled to room temperature, the isolates of *P. fluorescens* were inoculated with loop-full bacteria from freshly prepared King's B agar cultures (King's et al., 1954). The inoculation process was carried out under aseptic conditions, and the samples were incubated for 48 hours at 26±2°C. Following the collection of the Pseudomonas broth from the flasks, which had a colony count of at least 2×10^7 CFU, the broth was combined with the talc at a ratio of 400ml broth kg⁻¹ talc. For future research, the final mixture was stored at 4°C in a polythene bag after being shade-dried (Maurya et al., 2016).

2.4. Evaluation of micronutrient application on spot blotch under field conditions

The study was carried out between December and April 2021–2022 and 2022–2023. Its goal was to determine how different micronutrient combinations-such as calcium chloride (CaCl₂) @ 0.3%, zinc sulphate (ZnSO₄)+lime @ 0.5%, ferrous sulphate (FeSO₂)+lime @ 0.5%, boron @ 0.2%, sulphur @ 0.2%, molybdenum @ 0.2%, and manganese sulphate (MnSO₄)+lime @ 0.5%-affected wheat spot blotch. With three replications for each treatment, the experiment was conducted in RBD using a 4×3 m² plot. Standard agronomical practices were followed in the experiment, with the wheat cultivar WH 147, which is susceptible to spot blotch disease, serving as the test variety. Micronutrients were applied foliar in the evening at the tillering and boot leaf stages with the help of a knapsack sprayer. Water only was sprayed in the check plot.

2.5. Evaluation of botanicals, bioagents and fungitoxicants on spot blotch under field conditions

The experiment was carried out from December to April 2021-2022 and 2022–2023 to evaluate the effectiveness of various botanicals, bioagents, and fungitoxicants on spot blotch disease. Botanicas, which included extracts from the leaves of tulsi, neem, and mentha at a rate of 5%; bioagents, such as T. harzianum SV-7, SV-28, IRRI-1, and P. fluorescens at a rate of 10 gm I⁻¹ water; and fungitoxicants, such as azoxystrobin 23% SC, difenoconazole 25% EC, propiconazole 25% EC, tebuconazole 25% EC, azoxystrobin 11%+tebuconazole 18.3% SC, and carbendazim 12% + mancozeb 63% WP @ 0.1%, were applied twice as foliar sprays at 10-day interval. After symptoms of the disease appeared, these molecules were applied manually in the evening using a knapsack sprayer. Water only was sprayed in the check plot. Similar to that stated in head 2.9, the test variety's cultivar, plot size, and agronomic practices were followed.

2.6. Calculation of percent disease index, disease control, and increase in yield

Fifty leaves per replication from each genotype were randomly selected in the experiment to record the spot blotch severity (percentage) and identify wheat genotypes resistant to spot blotch diseases. The overall response of the pathogen to a certain genotype was indicated by the replication mean. The observations of percent foliar infection were recorded after the disease appearance. In other experiments, in which micronutrients, botanicals, bioagents, and fungitoxicants were assessed against spot blotch disease under field conditions; the disease severity was visually scored by using a double-digit scale (Kumar et al., 1998). Spot blotch severity on the flag leaf and flag-1 leaf was recorded on 50 randomly selected leaves from each replication's treatment. Three times, the severity of the disease was assessed at 10-day intervals. Before the plant extract, bioagents, and fungitoxicants were sprayed foliar, the first two disease data were recorded, and the final disease data was recorded ten days following the second spray. Recorded observations included the disease severity, yield quintal ha-1 (Q ha⁻¹), and 1000 grain weight (g). Percent disease index (PDI) was calculated with the help of methods given by Wheeler (1969). Percent disease control (PDC) and percent increase in yield were also calculated. Using Clewer and Scarisbrick (2001) technique, the experimental data were statistically analyzed. The variance ratio test was used to determine the significance of the treatment difference at the 5% probability level.

3. Results and Discussion

3.1. Effect of micronutrient application on spot blotch and yield parameters

In crop season 2021–2022, it was observed that of the seven micronutrients sprayed twice at the tillering and boot leaf stages, zinc was the most effective against spot blotch, with two applications resulting in a 36.90% disease index with a 23.41% reduction, however, it was at par with the disease index of 37.40%, and a 22.37% reduction in the disease index recorded in the crop where boron was applied. It was followed by a 39.58% disease index with a 17.84% reduction in disease index resulting in the crop where application of Iron was done. For the 2022–2023 crop season, the application of boron gave

a 24.05% disease index with a 27.16% decrease, and it was at par with the disease index (24.30%) and 26.40% reduction observed in the crop sprayed with zinc. Application of Iron resulted in a 26.31% disease index and a 20.32% reduction followed by manganese. Significant differences were found among treatments concerning yield parameters obtained during crop season 2021–22. Among all the treatments, the highest yield of 3.93 t ha-1 with an 18.38% increase was recorded with the application of zinc, and it was followed by the application of boron and Iron yielding 3.87 q ha-1. and 37.82 q ha-1 with 16.63 and 13.98% increase, respectively, and were statistically superior to rest treatments. Application of zinc and boron resulted in 41.37 g and 40.51 g 1000-grain weight, respectively. It was followed by the application of Iron resulted in 39.89 g which was at par with the 39.22 g 1000grain weight recorded in the crop sprayed with manganese. During crop season 2022–23, the highest yield 4.22 t ha⁻¹ with a yield increase of 17.76% was recorded in the crop where boron was applied. It was significantly higher than the yield recorded in rest treatments. The second highest yield i.e. 4.18 t ha-1 with a yield increase of 16.75% was recorded in the crop where zinc was applied, and it was followed by the application of Iron and manganese yielding 4.04 t ha-1 and 3.98 t ha-1 with 12.73 and 11.11% increase, respectively. The application of boron and zinc resulted in 42.63 g and 42.20 g 1000-grain weight, respectively, followed by the application of Iron and manganese resulted in 41.22 g and 40.88 g

1000-grain weight, respectively (Table 1). Zinc application may have reduced the disease index because it caused specific metabolic alterations that led to a reduction in the severity of the disease and its manifestation at the level of resistance to spot blotch disease. Zinc may also contribute to resistance against pathogen attack by improving membrane integrity and signaling several defense pathways, such as those that are dependent on ethylene and salicylic acid (Bastakoti, 2023). Antioxidative enzymes, such as tyrosine ammonia lyase, polyphenol oxidase, peroxidase, and phenylalanine ammonia lyase, are important for the metabolism of phenylpropanoid compounds and may have roles in defense. Since zinc is a cofactor for these enzymes, zinc supplementation may be associated with increased plant defense against pathogens. As a result, zinc may be utilized as a soil-nutritive agent to strengthen plant resistance to fungal disease (Wadhwa, 2014). Boron is directly involved in the stability and construction of cell walls and can help reduce the severity of disease (Tripathi et al., 2022). Plants' use of iron in disease resistance has not been thoroughly investigated. Wheat rust and smut disease in wheat are two examples of diseases that iron can prevent or reduce in severity. According to Graham and Webb (1991), soil bacteria can produce fungal antibiotics with the help of iron. The crop that received a zinc spray and then boron application had the lowest disease index for the 2021–2022 and 2022-2023 crop seasons. Zinc, boron, and manganese treatments decreased wheat spot blotch, according to similar

SI. No.	Treatments	Percent disease index			Per cent disease control			Cropping season- 2021–22			Cropping season- 2022–23		
		2021–	2022– 23	Aver- age	2021–	2022– 23	Aver- age	Yield (t ha ⁻¹)	1000 grain weight (g)	In- crease in yield (%)	Yield (t ha ⁻¹)	1000 grain weight (g)	In- crease in yield (%)
T ₁	MnSO ₄ + Lime	40.24	26.70	33.47	16.47	19.13	17.80	3.69	39.22	11.06	3.98	40.88	11.11
T ₂	ZnSO ₄ + Lime	36.90	24.30	30.60	23.41	26.40	24.90	3.93	41.37	18.38	4.18	42.20	16.75
T ₃	FeSO ₄ + Lime	39.58	26.31	32.94	17.84	20.32	19.08	3.78	39.89	13.98	4.04	41.22	12.73
$T_{_{4}}$	Boron	37.40	24.05	30.72	22.37	27.16	24.76	3.87	40.51	16.63	4.23	42.63	17.76
T ₅	Sulphur	44.39	30.72	37.55	7.86	6.96	7.41	3.51	37.90	5.84	3.82	39.20	6.75
T ₆	Molybde- num	41.52	27.82	34.67	13.82	15.74	14.78	3.62	39.04	9.10	3.91	40.23	9.21
T ₇	Calcium chloride	43.47	30.08	36.77	9.77	8.90	9.33	3.57	38.46	7.59	3.88	39.68	8.32
T ₈	Untreated control	48.18	33.02	40.60	0.00	0.00	0.00	3.32	36.42	0.00	3.58	38.00	0.00
CD (p=0.05)	1.22	1.30	-	1.32	1.45	-	1.79	1.39	1.25	1.34	1.66	1.25
SEm±		0.39	0.42	-	0.43	0.47	-	0.58	0.45	0.41	0.43	0.54	0.40

findings earlier reported by Yadav et al. (2013) and Kostas and Dordas (2005). According to Rahman et al. (2009), the application of chemical fertilizers at appropriate levels resulted in a considerable reduction in the severity of spot blotch. Manganese prevents the induction of pectin methylesterase, a fungal enzyme that breaks down host cell walls, and aminopeptidase, an enzyme that provides necessary amino acids for fungal development (Marschner, 1995). To boost a plant's resistance to diseases, chlorine ions can mediate the reduction of Mn+2, and +3 oxides and increase Mn for the plant (Dordas, 2008). According to Singh and Singh (2007), the application of zinc sulfate produced yields of 46.36 and 4.27 t ha⁻¹, respectively, and decreased spot blotch intensities by 45.9% in 2001–2002 and 45.5% in 2002–2003. As such, the current findings are strongly corroborated by the findings of previous researchers.

3.2. Effect of fungitoxicants, botanicals and bioagents on spot blotch and yield parameters

The results of the trial, which was carried out during the 2021-2022 crop season, demonstrated that, in comparison to the untreated control, every treatment considerably decreased the disease. The magnitude of reduction differed, although, between the treatments. Among the fungitoxicants, botanicals and bioagents evaluated against spot blotch; the highest disease reduction (54.62%) was recorded with foliar application of propiconazole exhibited the lowest disease index (23.00%) which was significantly less than the rest treatments followed by tebuconazole exhibiting 24.51% disease index with 51.64% reduction. Next to them was the foliar application of difenoconazole and it was statistically superior to the rest of the treatments exhibiting a 50.06% reduction in disease. During crop season 2022–23; the highest reduction in disease (62.86%) was noticed due to the foliar application of tebuconazole which was significantly superior to the rest of treatments followed by propiconazole exhibiting a 61.39% reduction. Ergosterol production is inhibited in biological systems by propiconazole, tebuconazole, and difenoconazole; yet, ergosterol is a chemical required for the development of fungal organelles. During crop season 2021-22, the highest yield (3.98 t ha-1) and yield increase (27.62%) were recorded due to application of propiconazole. It was significantly higher than the yield recorded in the rest treatments. The second highest yield (3.94 t ha⁻¹) with a yield increase of 26.05% was recorded in crop sprayed with Tebuconazole, and it was followed by application of difenoconazole, azoxystrobin+ tebuconazole and carbendazim+mancozeb yielded 3.89 t, 3.84 t and 3.75 t ha⁻¹ with 24.55, 22.91 and 20.03% increase in yield, respectively. Application of propiconazole and tebuconazole resulted in 41.13 g and 40.95 g 1000-grain weight respectively. Application of difenoconazole resulted in 40.32 g 1000-grain weight and it was at par with the 1000- grain weight recorded due to crop sprayed with azoxystrobin+tebuconazole. Other treatments that followed descending order of yield and 1000-grain weight were the

application of carbendazim+mancozeb, azoxystrobin, garlic clove extract, neem leaf extract, T. harzianum (IRRI-1), tulsi leaf extract, T. harzianum (SV-28), mentha leaf extract, T. harzianum (SV-7) and P. fluorescens. Minimum 1000-grain weight (36.26 g) was recorded in untreated control where only plain water was sprayed. In crop season 2022-23, among all the treatments evaluated against spot blotch; the highest yield of 4.16 t ha⁻¹ with a 25.72% increase in yield was recorded due to the application of tebuconazole, and it was followed by the application of propiconazole and azoxystrobin+ tebuconazole yielded 4.13 t ha-1 and 4.08 t ha-1 with 24.81 and 23.21% increase in yield, respectively and were significantly superior comparatively other treatments. Application of Tebuconazole and propiconazole resulted in 42.55 g and 42.18 g 1000-grain weight, respectively. Application of azoxystrobin+tebuconazole resulted in 41.70 g 1000-grain weight and it was at par with 41.00 g 1000- grain weight recorded in the crop sprayed with difenoconazole. Other treatments that followed descending order of 1000grain weight were the application of carbendazim + mancozeb, azoxystrobin, garlic clove extract, Neem leaf extract, tulsi leaf extract, T. harzianum (IRRI-1), mentha leaf extract, T. harzianum (SV-7), T. harzianum (SV-28) and P. fluorescens (Table 2). The results of this study are consistent with the findings of previous researchers. Mahapatra and Das (2013) reported that the best results were obtained from twice applying foliar sprays of propiconazole 25% EC @ 0.1%, which also decreased wheat spot blotch and raised 1000-grain weight and yield. Because allicin (diallyl-thiosulfinate), an organosulfur component found in fresh garlic tissue, has antifungal properties against plant pathogenic fungi, garlic extract has been shown to reduce plant disease (Sarfraz et al., 2020). Garlic clove and Neem leaf extracts were found to be effective against wheat spot blotch under field conditions (Yadav et al., 2015). Yadav et al. (2015) observed that T. harzianum and P. fluorescence reduced the spot blotch of wheat under field conditions but both were comparatively less effective than fungicides. Hasan (2013) reported that applying T. harzianum as a foliar spray after seed treatment decreased spot blotch and increased yield. The existence of certain plant exudates on the leaf surface and other microorganisms that may be the food source for *T. harzianum* enabled luxuriant population dynamics, which in turn may have suppressed Bipolaris sorokiniana through mycoparasitism and antibiosis on the leaf surface, potentially provide circumstantial evidence for the herb's effectiveness against foliar pathogens. Stresstolerant genes, proteases, chitinases, glucanase, tubulins, and cell adhesion proteins are among the key gene types found in *Trichoderma* spp. that are involved in biocontrol action. Stress tolerance, hyphal development, cell wall disintegration, and parasite activity are all regulated by these genes. As per Pokhrel et al. (2022), chitinase is accountable for hydrolyzing glycosidic linkages, while xylanase breaks down hemicellulose.

SI. No.	Treatments	Percent disease index			Per cent disease control			Cropping season- 2021–22			Cropping season- 2022–23		
		2021– 22	2022– 23	Aver- age	2021–	2022– 23	Aver- age	Yield (t ha ⁻¹)	In- crease yield (%)	1000 grain weight (g)	Yield (t ha ⁻¹)	In- crease yield (%)	1000 grain weight (g)
T ₁	Trichoderma harzianum SV-7	36.47	29.08	32.77	28.05	30.14	29.09	3.32	6.40	38.00	3.57	7.85	38.26
T ₂	Trichoderma harzianum SV-28	35.74	28.63	32.18	29.49	31.22	30.35	3.39	8.38	38.42	3.54	6.97	38.04
T ₃	Trichoderma harzianum IRRI-1	33.10	27.24	30.17	34.70	34.56	34.63	3.44	9.98	38.80	3.68	11.05	38.71
T ₄	Pseudomonas fluorescens	40.63	31.59	36.11	19.84	23.46	21.65	3.29	5.28	37.14	3.48	5.13	37.46
T ₅	Garlic clove extract	32.18	25.10	28.64	36.51	39.70	38.10	3.53	12.83	39.08	3.87	16.96	39.76
T ₆	Tulsi leaf extract	35.92	27.91	31.91	29.13	32.95	31.04	3.38	8.13	38.51	3.72	12.40	39.00
T ₇	Neem leaf extract	34.05	26.11	30.08	32.82	37.28	35.05	3.51	10.92	39.00	3.83	15.48	39.25
T ₈	Mentha leaf extract	36.06	28.14	32.40	28.86	32.40	30.63	3.35	7.17	38.12	3.66	10.59	38.49
T ₉	Azoxystrobin	29.54	22.25	25.89	41.72	46.55	44.13	3.69	18.05	39.11	3.90	17.81	40.53
T ₁₀	Difenocon- azole	25.31	19.80	22.55	50.06	52.43	51.24	3.90	24.55	40.32	3.99	20.44	41.00
T ₁₁	Propiconazole	23.00	16.07	19.53	54.62	61.39	58.00	3.99	27.62	41.13	4.13	24.81	42.18
T ₁₂	Tebuconazole	24.51	15.46	19.98	51.64	62.86	57.25	3.94	26.05	40.95	4.16	25.72	42.55
T ₁₃	Azoxystrobin+ Tebuconazole	25.79	18.11	21.95	49.12	56.49	52.80	3.84	22.91	40.01	4.08	23.21	41.70
T ₁₄	Carbendazim +Mancozeb	26.94	20.42	23.68	46.85	50.94	48.89	3.75	20.03	39.89	3.93	18.68	40.92
T ₁₅	Untreated control	50.69	41.63	46.16	0.00	0.00	0.00	3.12	0.00	36.26	3.31	0.00	36.81
CD (p=0.05)	1.79	1.81	-	1.12	1.23	-	1.62	1.43	1.27	1.49	1.18	1.44
SEm±		0.61	0.62	-	0.38	0.42	-	0.55	0.49	0.43	0.51	0.40	0.49

4. Conclusion

During both crop seasons, propiconazole and tebuconazole demonstrated the greatest reduction in spot blotch disease with increased yield among the fungitoxicants, botanicals, and bioagents. Comparing treated and untreated control, garlic clove extract and *T. harzianum* (IRRI-1) both decreased spot blotch and increased yield. Twice applications of zinc sulphate reduced spot blotch significantly and enhanced yield and it was followed by the boron application.

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