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Evaluation of Micronutrients, Botanicals, Bioagents and Fungitoxicants against *Bipolaris sorokiniana* Causing Spot Blotch Disease in Wheat

Deepak Kumar[1], Durga Prasad[2], Ramji Singh[1] and Ajay Tomar[3]

[1]Dept. of Plant Pathology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh (250 110), India
[2]Dept. of Plant Pathology, College of Agriculture, Mandor, Agriculture University, Jodhpur, Rajasthan (342 007), India
[3]Dept. of Plant Pathology, Amity Institute of Organic Agriculture, Amity University, Noida, Uttar Pradesh (201 313), India

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Corresponding vats77360@gmail.com

0000-0002-0678-0859

ABSTRACT

Investigations were conducted during the rabi (November–April), 2021–22 and 2022–23 at the Bio-control Laboratory, Department of Plant Pathology, SVPUAT, Meerut, Uttar Pradesh, India to assess the effects of micronutrients, botanicals, bioagents and fungitoxicants against *Bipolaris sorokiniana* causing spot blotch disease in wheat. Using the poison food technique; 7 micronutrients (manganese sulphate, zinc sulphate, ferrous sulphate, boron, sulphur, molybdenum, and calcium chloride), 4 botanicals (extracts of garlic clove, tulsi leaf, neem leaf, and mentha leaf), 4 bioagents (*Pseudomonas fluorescens* and three strains: SV-7, SV-28, and IRRI-1 of *Trichoderma harzianum*) and 6 fungitoxicants (azoxystrobin, difenoconazole, propiconazole, tebuconazole, azoxystrobin+tebuconazole, carbendazim+mancozeb) were assessed. The inoculation plates were incubated at 25±1 0C until the fungus covered the control plate completely. After incubation, the colony's radial growth was measured at regular intervals for one week at 3, 4, and 7 days. The percentage inhibition of each treatment was then computed after the seventh day of incubation. The study revealed that the mycelial growth of *Bipolaris sorokiniana* was significantly inhibited by the following: botanicals (garlic clove and neem leaf extracts), bioagents (*Trichoderma harzianum*: Strain- IRRI-1 and SV-7), micronutrients (ferrous sulphate, zinc sulphate, and boron), and fungitoxicants (propiconazole and tebuconazole). Based on the findings mentioned above, and the final recommendation given to the farmers; these potential elements can be assessed for the effectiveness of their individual and combined application against spot blotch disease of wheat under field conditions.

KEYWORDS: *Bipolaris sorokiniana*, bioagents, micronutrients, fungitoxicants, spot blotch, wheat

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1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most extensively grown crops worldwide and a staple sustenance for people. In India, it is the second-most important cereal food after rice, and it has played a major role in stabilizing grain production in the country. Wheat grain has a strong nutritional profile with an average of 14.70% protein, 2.10% fat, 1.5% ash, 1.4% reducing sugars, 73.90% starch, and 78.10% total carbohydrates and offers 314 kcal/100g of food. It is a good source of minerals and vitamins (Kumar et al., 2011). The wheat crop is susceptible to several diseases, which are primarily responsible for the reduction in overall productivity. This is because the plant is exposed to various biotic and abiotic stimuli that disrupt healthy metabolism throughout its life cycle and under all environmental circumstances. One of the elements that leads to low yield is the frequency of diseases invading the crop. Following the Green Revolution, wheat production in the warmer and humid North Eastern Plain Zone (NEPZ) increased dramatically. Nevertheless, this crop has experienced severe yield losses due to a variety of fungal, bacterial, viral, nematode, and mycoplasma disease problems. In diseases, foliar blights and rusts comprise the majority of fungal diseases that damage 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 Ginkel 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 million hectares 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). 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). Scientists are now primarily concerned with using host resistance to manage wheat spot blotch. 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). An attractive substitute and environmentally responsible approach to controlling plant diseases is biological control, 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, the investigations were carried out to find the in vitro effect of micronutrients, botanicals, bioagents, and fungitoxicants on *Bipolaris sorokiniana* causing spot blotch disease in wheat.

2. MATERIALS AND METHODS

2.1. Isolation, purification, and identification of Bipolaris sorokiniana

The experiments were conducted during the rabi (November–April), 2021–22 and 2022–23 at the Bio-control Laboratory, Department of Plant Pathology, SVPUAT, Meerut, Uttar Pradesh, India. 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, adopting a standard procedure for isolation and purification. The pathogen was sub-cultured 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.

2.2. Collection and multiplication of bio-agents

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 ± 2^0 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 400 ml 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).

2.3. Evaluation of micronutrients on mycelial growth of Bipolaris sorokiniana

The "Poison Food Method" described by Grover and Moore (1962) was used to evaluate the effects of seven micronutrients on the growth of *Bipolaris sorokiniana*: manganese sulfate, zinc sulphate, ferrous sulphate, boron, sulphur, molybdenum, and calcium chloride. A stock solution of 10,000 ppm strength of each fungicide was made for the poison food technique by dissolving 1.0 g or 1.0 ml in 100 ml of sterilized distilled water. Using a standardized methodology, the amount of stock solution to be added to oatmeal agar was estimated to achieve the necessary concentration of fungicides in the medium. Oatmeal (60 g), Agar (15 g), and distilled water (1000 ml) were combined to prepare oatmeal agar medium. To obtain the final concentration of 1000 and 2000 ppm of each micronutrient, the necessary volume of stock solutions of different chemicals was added to 100 ml of sterilized melting oatmeal agar. Twenty ml of oatmeal agar containing the appropriate micronutrients was added to sterilized petri dishes. Control was provided by the unaltered medium. Five-millimeter mycelial discs of the pathogen-fungi obtained from cultures cultivated on oatmeal agar for seven days-were used to inoculate each plate. The inoculation plates were incubated at 25±1°C until the control plate was completely covered in fungus. After incubation, the colony's radial growth was measured at regular intervals for one week at 3, 4, and 7 days. The percentage inhibition of each treatment was then computed after the seventh day of incubation using the formula given by Vincent (1947).

2.4. Preparation and evaluation of plant extracts against mycelial growth of Bipolaris sorokiniana

Four plant extracts were produced for their in vitro assessment against *B. sorokiniana*: clove extract from garlic (*Allium sativum*), leaf extracts from tulsi (*Ocimum sanctum*), neem (*Azadirachta indica*), and mentha (*Mentha* spp.). 200g of garlic cloves were gathered, and they were first cleaned using tap water and then distilled water. After that, it was processed in a 1:1 ratio (200 g tissue in 200 ml distilled water) using distilled water. Using a mortar and pestle, the garlic clove was smashed and then strained through two layers of muslin cloth. Thus, the standard extract solution (100%) was prepared. The resulting filtrate was used as an aqueous solution at the appropriate concentration for later usage. After washing 200 g of freshly harvested tulsi, neem, and mentha leaves under the tap and then with distilled water, the leaf tissues were individually ground with a mortar and pestle in distilled water at a ratio of 1:1 w/v (Magar et al., 2020). A double-layered muslin cloth filter was then used to filter the extract. The filtrate so obtained was used as a stock solution for further studies.

Plant extracts were tested in vitro using the previously described poisoned food technique against *B. sorokiniana*. Three concentrations-5%, 10%, and 15%-of the plant extracts were added to the oatmeal agar medium. To achieve 5, 10 and 15% concentrations of plant extracts in the medium, 100 ml of oatmeal agar was filled with 5, 10, and 15 ml of plant extracts, respectively. To stop bacterial contamination, 30 ppm of chloramphenicol was also added to the medium before it was poured into the Petri plates. As a control, oatmeal agar plates devoid of plants were utilized. The amended oatmeal agar (20 ml $plate^{-1}$) was added to 90 mm sterilized Petri dishes under aseptic conditions. There were three plates poured for every treatment. After mixing oatmeal agar with botanicals in a laminar airflow incubator, the pathogen's circular mycelial disc (5 mm) was transferred to the plates followed by incubation at 25±1°C. After

incubation, colony diameter, or the radial mycelial growth of the fungus, was measured after 3, 5 and 7 days. The percentage of mycelial growth inhibition was then computed after 7 days, following the procedure previously outlined under micronutrients.

2.5. Evaluation of bio-agents against mycelial growth of Bipolaris sorokiniana

Using the dual culture technique, four distinct bio-control agents-*Pseudomonas fluorescens*, *Trichoderma harzianum* (SV-7), *Trichoderma harzianum* (SV-28), and *Trichoderma harzianum* (IRRI-1)-were evaluated in vitro against *B. sorokiniana* (Maurya et al., 2014; Bastakoti et al., 2017). The mycelial disc of the test fungus from the 7-day-old pure culture was inoculated at one end of the petri plate containing PDA medium and that of fungal bioagent at the opposite end whereas in the case of bacterial bioagent, streaking of bacterial suspension at the opposite end of the petri plate (one cm away from the edge) was done (Bastakoti et al., 2017). For every treatment, three replications were maintained. PDA plates that were solely inoculated with the test pathogen's culture disc were kept as an untreated control. The plates were incubated at 26±1°C, and the test pathogen's radial growth was measured until the control plates' mycelial growth covered the entire plate. Using the previously indicated formula, the percent inhibition was computed.

2.6. Evaluation of fungitoxicants against mycelial growth of Bipolaris sorokiniana

Using the poison food technique, six different fungicides-azoxystrobin 23% SC, difenoconazole 25% EC, propiconazole 25% EC, tebuconazole 25% EC, azoxystrobin 11%+tebuconazole 18.3% SC, carbendazim 12%+mancozeb 63% WP-were evaluated against *B. sorokiniana* at three different concentrations: 25 ppm, 50 ppm, and 75 ppm. The necessary concentration of several fungicides was added to the lukewarm oatmeal agar medium using a micropipette and aseptic procedures. Conical flasks containing fungicide-amended oatmeal agar medium were topped up with a pinch of antibiotic chloramphenicol powder before being transferred into Petri plates to prevent bacterial contamination. Aseptically, 20 ml of amended oatmeal agar medium was added to 90 mm Petri dishes, and they were left undisturbed until the media solidified. Five-millimeter mycelial discs were cut using a cork borer from a seven-day-old *B. sorokiniana* culture. These discs were then inoculated into the center of Petri plates and incubated at 25±1°C. Each treatment was maintained in three replications, and the untreated control set consisted of oatmeal agar plates that had been inoculated with the pathogen but had not been altered. Three, five, and seven days after incubation, the radial growth of mycelia was observed and measured at regular intervals for a week. Using the previously mentioned formula (Vincent, 1947), the percentage of inhibition was computed after 7 days of incubation.

3. RESULTS AND DISCUSSION

3.1. Effect of micronutrients on mycelial growth of Bipolaris sorokiniana

Among the several micronutrients tested in vitro against *B. sorokiniana*, ferrous sulphate (0.00 mm) at 3000 ppm concentrations showed essentially no mycelial development, which was statistically distinct from other treatments. The concentration of ferrous sulphate showed the highest inhibition of mycelium's radial growth, at 3000 ppm. This was followed by boron, and zinc sulphate, which showed 88.74 and 82.11% inhibition, respectively, and manganese sulphate, which showed 44.33% inhibition at 3000 ppm concentration (Table 1 and Figure 1). Similar results have also been reported by Naga et al. (2022), who found that, among the four micronutrients tested against *Bipolaris oryzae*, zinc sulphate (at 0.3%), copper sulphate, ferrous sulphate, and manganese sulphate showed the greatest inhibition. During the current experiment, we also noticed that higher inhibition corresponded with increased concentrations of micronutrients. According to Jaiganesh et al. (2019), at 3000 ppm concentration, $ZnSO_4$ showed the greatest suppression of mycelial development (83.30%) among the different macro-micro nutrients, followed by ferrous sulphate (65.50%) and manganese sulphate (61.10%).

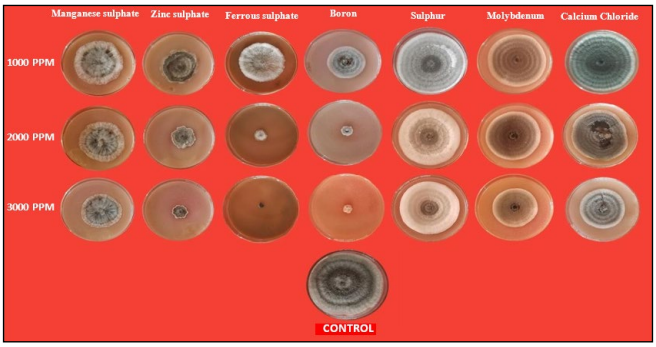

Figure 1: *In vitro* evaluation of micronutrients against mycelial growth of *Bipolaris sorokiniana*

3.2. Effect of plant extracts on mycelial growth of Bipolaris sorokiniana

After seven days of inoculation, garlic showed the highest growth inhibition (55.50, 64.33, and 75.46%) at 5, 10, and 15% concentrations. Neem showed 49.02, 57.88, and 66.67% inhibition, and mentha showed 37.94, 48.95, and 59.94% inhibition of *B. sorokiniana* at 5, 10 and 15% concentrations, respectively. The findings showed that, among the four plant extracts, neem and garlic extracts were

Table 1: Effect of micronutrients on mycelial growth of *Bipolaris sorokiniana*

Sl. No.	Treatments	Mycelial growth of pathogen (mm)									Percent growth inhibition of mycelium after 7 days inoculation			Mean
		3* days after inoculation			5* days after inoculation			7* days after inoculation						
		1000 ppm	2000 ppm	3000 ppm	1000 ppm	2000 ppm	3000 ppm	1000 ppm	2000 ppm	3000 ppm	1000 ppm	2000 ppm	3000 ppm	
T_1	Manganese sulphate	30.20	26.57	21.42	50.30	44.28	35.71	70.10	62.08	50.10	22.11	31.02	44.33	32.48
T_2	Zinc sulphate	18.10	11.14	5.34	30.10	18.57	11.42	42.16	26.10	16.10	53.15	71.00	82.11	68.75
T_3	Ferrous sulphate	22.28	6.14	0.00	37.22	8.57	0.00	52.12	12.20	0.00	42.08	86.44	100	76.17
T_4	Boron	19.81	6.85	0.00	32.85	11.42	7.14	46.23	16.06	10.13	48.60	82.15	88.74	73.16
T_5	Sulphur	37.54	31.71	30.85	64.10	52.85	51.42	90.00	74.14	72.21	0.00	17.62	19.76	12.46
T_6	Molybdenum	31.71	28.28	25.71	52.85	47.12	42.85	74.15	66.21	60.11	17.61	26.43	33.21	25.75
T_7	Calcium chloride	37.00	30.15	28.28	64.00	50.00	47.14	90.00	70.24	66.22	0.00	21.95	26.42	16.12
T_8	Control	38.57	38.57	38.57	64.28	64.28	64.28	90.00	90.00	90.00	-	-	-	-
CD (p=0.05)		1.50	1.45	1.43	1.19	1.34	1.11	1.14	1.18	1.00	-	-	-	-
SEm±		0.49	0.48	0.47	0.39	0.44	0.37	0.37	0.39	0.33	-	-	-	-

*Average of three replication

more successful in inhibiting the pathogen's growth than mentha and tulsi extract (Table 2). Similar findings were also reported by Magar et al. (2020), who found that, among the five botanicals tested, 15% of garlic clove extract exhibited the greatest mycelial growth inhibition (52.85%), followed by bojho (*Acorus calamus*) resulted in 52.48% of growth inhibition. According to Jatoi et al. (2019), out of five plant extracts, *Zingiber officinale* and *Allium sativum* were found to be more effective against *Helminthosporium oryzae*.

3.3. Effect of bio-agents on mycelial growth of Bipolaris sorokiniana

Trichoderma harzianum (IRRI-1) was the most effective bioagent against *B. sorokiniana*, showing the highest mycelial inhibition (76.53%) of the pathogen after seven days of inoculation. *T. harzianum* (SV-7) and *T. harzianum* (SV-28) followed with 72.88 and 70.58% inhibition, respectively (Table 3). The current results corroborated those of Khan et al. (2021), who assessed the effectiveness of bioagents against the mycelial growth of *B. sorokiniana* and discovered that *T. harzianum*-1 was the most effective, exhibiting 77.90% pathogen inhibition, followed by *T. harzanium*-2 (74.80%) and *T. viride*-1 (74.41%). *P. fluorescence* showed the lowest level of inhibition. Hasan (2013) assessed the effectiveness of *Trichoderma harzianum* in the management of wheat spot blotch infections. Following an eight-day incubation period, these investigations unmistakably demonstrated that *T. harzianum* isolate RUT-103 inhibited the radial growth of *B. sorokiniana*. It is well known that *T. harzianum* exhibits antagonistic characteristics against most fungi. Trichoderma uses its main tool, mycoparasitism, to lyse fungal cell walls by coiling its hyphae around the hyphae of the target pathogen. This is accomplished by enzymatic activity employing chitinase and cellulase enzymes produced by the same. The dual culture method was used in experiments to demonstrate this. This could be the cause of the inhibition observed in the *B. sorokiniana* growth in the culture plate when *Trichoderma harzianum* was grown using the dual culture technique. The synthesis of volatile and non-volatile secondary metabolites is another way that *Trichoderma harzianum* inhibits *B. sorokiniana*. Targeted organism growth may be inhibited by these secondary metabolites because of their toxicity to pathogenic fungi.

3.4. Effect of fungitoxicants on mycelial growth of Bipolaris sorokiniana

Six fungicides were evaluated for their effectiveness against *B. sorokiniana*, and all of them markedly reduced the pathogen's ability to develop mycelia. After seven days of inoculation, propiconazole at 75 ppm showed the maximum inhibition (100%) of mycelial growth, followed by 92.97% and 84.27% at 50 and 25 ppm, respectively. Tebuconazole was discovered to be the second most potent fungitoxicant, exhibiting 100%, 91.79%, and 78.04% inhibition at 75, 50, and 25 ppm, in that order. Propiconazole, tebuconazole, azoxystrobin+tebuconazole, and difenoconazole at 75 ppm

Table 2: Effect of plant extracts on mycelial growth of *Bipolaris sorokiniana*

Sl. No.	Treatments	Mycelial growth of pathogen (mm)												Mean
		3* days after inoculation			5* days after inoculation			7* days after inoculation			Percent growth inhibition of mycelium after 7 days of inoculation			
		5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	
T_1	Garlic clove extract	17.20	13.78	9.47	28.68	22.93	15.77	40.05	32.10	22.08	55.50	64.33	75.46	65.09
T_2	Tulsi leaf extract	25.62	22.20	17.08	42.64	36.97	28.46	59.70	51.76	39.85	33.66	42.48	55.72	43.95
T_3	Neem leaf extract	19.68	16.25	12.86	32.77	27.07	21.42	45.88	37.90	30.00	49.02	57.88	66.67	57.85
T_4	Mentha leaf extract	23.94	19.70	15.45	39.89	32.81	25.75	55.85	45.94	36.05	37.94	48.95	59.94	48.94
T_5	Control	38.58	38.58	38.58	64.28	64.28	64.28	90.00	90.00	90.00	-	-	-	-
CD (p=0.05)		1.90	1.45	1.68	1.63	1.13	1.35	1.62	1.05	1.04	-	-	-	-
SEm±		0.59	0.45	0.52	0.51	0.35	0.42	0.50	0.33	0.32	-	-	-	-

*Average of three replication

Table 3: Effect of bioagents on mycelial growth of *Bipolaris sorokiniana*

Sl. No.	Treatments	Mycelial growth of pathogen after 7-day inoculation (mm)	Percent growth inhibition
T_1	*Trichoderma harzianum* SV-7	23.32	72.88
T_2	*Trichoderma harzianum* SV-28	25.30	70.58
T_3	*Trichoderma harzianum* IRRI-1	20.18	76.53
T_4	*Pseudomonas fluorescens*	44.62	48.11
T_5	Control	86.00	-
CD (p=0.05)		1.85	-
SEm±		0.58	-

Table 4: Effect of fungitoxicants on mycelial growth of *Bipolaris sorokiniana*

Sl. No.	Treatments	Mycelial growth of pathogen (mm)												Mean
		3* days after inoculation			5* days after inoculation			7* days after inoculation			Percent growth inhibition of mycelium after 7 days of inoculation			
		25 ppm	50 ppm	75 ppm	25 ppm	50 ppm	75 ppm	25 ppm	50 ppm	75 ppm	25 ppm	50 ppm	75 ppm	
T_1	Azoxystrobin	26.61	22.35	18.12	44.35	37.22	31.86	62.10	52.12	44.60	27.24	38.94	47.75	37.97
T_2	Difenoconazole	13.14	6.44	0.00	21.91	10.73	0.00	30.68	20.08	0.00	64.05	76.47	100	80.16
T_3	Propiconazole	5.75	2.67	0.00	9.58	4.28	0.00	13.42	6.00	0.00	84.27	92.97	100	92.41
T_4	Tebuconazole	8.47	2.95	0.00	14.10	4.92	0.00	18.74	7.00	0.00	78.04	91.79	100	89.94
T_5	Azoxystrobin+ Tebuconazole	8.62	4.00	0.00	18.83	14.40	0.00	26.37	15.05	0.00	69.10	82.36	100	83.82

Table 4: Continue...

Sl. No.	Treatments	Mycelial growth of pathogen (mm)												Mean
		3* days after inoculation			5* days after inoculation			7* days after inoculation			Percent growth inhibition of mycelium after 7 days of inoculation			
		25 ppm	50 ppm	75 ppm	25 ppm	50 ppm	75 ppm	25 ppm	50 ppm	75 ppm	25 ppm	50 ppm	75 ppm	
T_6	Carbendazim+ Mancozeb	21.50	13.12	6.02	35.08	21.85	10.02	50.12	30.59	14.04	41.28	64.16	83.55	63.00
T_7	Control	37.00	37.00	37.00	60.97	60.97	60.97	85.36	85.36	85.36	—	—	—	—
CD (p=0.05)		0.30	0.17	0.11	0.26	0.17	0.11	0.23	0.23	0.12	—	—	—	—
SEm±		0.94	0.54	0.34	0.82	0.53	0.33	0.72	0.71	0.37	—	—	—	—

*Average of three replication

concentrations were found to cause a 100% inhibition of mycelial growth (Table 4). The current results corroborated those of Khan et al. (2021), who found that of the six fungicides tested, propiconazole, tebuconazole, azoxystrobin, and azoxystrobin+difenoconazole completely inhibited the pathogen's mycelial growth followed by difenoconazole. Similar results were reported by Magar et al. (2020), who found that out of 8 fungicides tested, propiconazole was the most effective fungicide against *B. sorokiniana*, it fully inhibited mycelial growth at all concentrations. Mehboob et al. (2015) found that the combination of azoxystrobin+difenoconazole performed the best in preventing the mycelial growth of *Drechslera sorokiniana*, followed by difenoconazole and thiophanate methyl.

4. CONCLUSION

Ferrous sulfate, propiconazole, garlic extract, and *Trichoderma harzianum* (IRRI-1), respectively, exhibited the highest mycelial inhibition of *Bipolaris sorokiniana* causing spot blotch disease in wheat.

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