Research Article:

EFFICACY OF FUNGICIDAL FORMULATIONS ALONG WITH BOTANICALS AND BIO-CONTROL AGENTS AGAINST WHEAT SPOT BLOTCH (*Bipolaris sorokiniana*) UNDER LABORATORY AND FIELD CONDITIONS

Ritu Rani Poudel*, Ritesh Kumar Yadav, Hira Kaji Manandhar, and Sunar Man Shrestha
Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal

*Corresponding author: ritupoudel345@gmail.com
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ABSTRACT

The present investigation was conducted to identify suitable fungicide formulation along with botanicals and bio-control agents (BCAs) against spot blotch disease (*Bipolaris sorokiniana*) under laboratory and field conditions. To test the fungi toxicity laboratory experiment (in-vitro) was conducted using fungicides (nine) and BCA (two) at different levels of concentrations following the poisoned food technique. Similarly, A field experiment was conducted to test the efficacy of fourteen disease-controlling agents (fungicides, botanicals, and BCAs) under natural epiphytotic conditions for spot blotch of wheat (variety-Gautam). Under in-vitro conditions, fungicides Nativo 75WG (trifloxystrobin 25% + tebuconazole 50%), DILT (propiconazole 25% EC), and ZOLE PLUS (hexaconazole 5% SC) were highly effective against the mycelia growth of spot blotch fungi in all the concentrations (25, 50 and 100 mg/L). Likewise, under epiphytotic conditions highest efficacy against spot blotch as lower percent disease control (PDC) was observed as 75.41% followed by 74.60 % and 68.32 % in the plot having seed treated with Vitavax and plants sprayed with Nativo 75WG (trifloxystrobin 25% +tebuconazole 50%) followed by plot having seed treated with Vitavax and plants sprayed with DILT (propiconazole 25% EC) and plots having seed treated with Vitavax and plants sprayed with ZOLE PLUS (hexaconazole 5% SC), respectively. The present study showed that seed treatment with Vitavax followed by the spraying one of the following fungicides, i.e., Nativo 75WG, DILT, and ZOLE PLUS @ 0.05, 0.1, and 0.1 % respectively could be effective for the management of spot blotch in wheat.

Key words: *Bipolaris sorokiniana*, fungicides, genotypes, wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major cereal crops of Nepal, grown on 0.71 million hectares of land with a production of 2.13 million tons and productivity of 3.09 tons ha-1 (MOALD, 2021). In the past years, the annual productivity increment rate of wheat was 0.003 tons ha-1 (Joshi, 2017). It is indicated that the productivity of wheat in Nepal is much lower than in any other neighboring wheat-growing country. The production of wheat is constrained by various biotic and abiotic stresses. Among the biotic factors, spot blotch is one of the most serious biotic constraints. It occurs as a complex caused by *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur (anamorph: *Bipolaris sorokiniana*) and Tan spot (caused by *Pyrenophora tritici repentis* (Died.) Drechsler (anamorph: *Drechslera tritici repentis*) in warm wheat-growing areas of Nepal (Sharma & Duveiller, 2003) however, between the two pathogens, *Bipolaris sorokiniana* is appreciably dominant in terai regions of Nepal and Indo-Gangetic plains (Saari, 1998). It has been estimated that approximately 15-25% of the wheat has been lost due to infection of spot blotch by affecting about 25 million ha of wheat-growing area (40% of which is in the Indian subcontinent) (Dubin & van Ginkel, 1991; van Ginkel & Rajaram,

1998; Joshi et al., 2007). In Nepal infection of this disease has been recorded up to 85% in susceptible genotype RR-21 (Shrestha et al., 1997) while, the reduction of yield caused by this disease ranged from 23-40% depending on genotypes and environmental conditions (Bhandari & Tripathi, 2005; Sharma & Duveiller, 2006). At the same time, yield loss in individual fields is sometimes much higher when the post-anthesis period of the crop coincides with the high relative humidity and high temperature.

This pathogen attacks seedlings, leaves, roots, nodes, spikes, and grains during various stages of development. The rate of infection and pathogen growth relatively increases with high relative humidity and wet canopy for a prolonged period (Acharya et al., 2011). In Nepal, wheat is generally raised (late sowing) after harvesting rice which causes high relative humidity and temperature during the post-anthesis period. This often leads to a loss in productivity due to a combination of spot blotch and terminal heat stress (Joshi et al., 2007).

Practical management of spot blotch has been the most important concern to protect against losses due to the disease in recent years. The disease-affected areas have increased every year all over the world attributable to the lack of durable resistant cultivars and ineffective control measures. The spot blotch occurs in complex form and poses multiple challenges in management due to its seed-borne, soil-borne, and wind-borne inoculums, particularly in the rice-wheat cropping systems (Sharma et al., 2007). To overcome the incidence of this disease many alternative strategies have been used i.e., resistant varieties, fungicidal spray, sowing date, quality seeds, and many more (Devi et al., 2012; Kumar et al., 2019). In the case of fungicides, previous studies suggested that triazole fungicides inhibit the synthesis of sterols (building blocks of the membranes of fungal cells) and have antioxidant properties with a different mode of action which effectively control the Bipolaris fungal pathogen (Ansari et al., 2017; Navathe et al. 2019; Somani et al., 2019). Similarly, other controlling measures i.e., fungicides (Imidacloprid and difenoconazole (Shahbaz et al., 2018), fludioxonil and difenoconazole (Wei et al., 2021), Carboxin, Thiram, and Thiophanate methyl (Malaker & Mian, 2009), Carbendazim and Copper oxychloride (Tiwari et al. 2022)), biological agents ((*T. harzianum* and *T. koningii* (Mónaco et al., 2004), *Pseudomonas fluorescence* (Yadav et al. 2015)), botanicals ((Eucalyptus leaf extract, Garlic clove extract, Neem leaf extract (Yadav et al. 2015), Clove oil, Ginger oil, Eucalyptus oil, Til oil, and Neem oil (Debsharma et al. 2021), *Calotropis procera*, *Jacaranda mimosifolia*, and *Thevetia peruviana* extracts (Naz et al. 2018), Neem, Mehedi, Garlic clove, Rhizome of ginger, seeds of black cumin (Hossain et al. 2016), and organic fungicides (Gupt et al. 2020)) were used for the management of spot blotch of wheat. However, variable environmental conditions, low or unbalanced soil nutrient levels, poor management practices, high temperatures, and hot winds accelerate the incidence of spot blotch in wheat, making it difficult to manage (Sharma et al., 2007). Therefore, identification of the suitable composition of treatments is necessary to combat the incidence of spot blotch. Keeping the above under consideration the experiment was conducted to test the efficacy of potential fungicides, BCAs, and botanicals against spot blotch disease of wheat so that suitable management practices could be identified for the management of this disease.

MATERIALS AND METHODS

Research site experimental materials

In the present study, field experiments were conducted at the research field of the Faculty of Agriculture, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal (22.64768 °N 84.34750 °E; 171 m above sea level). Laboratory experiments were conducted at the Plant Pathology Laboratory of the Department of Plant Pathology, AFU. To check the efficacy of fourteen fungicides along with botanical and BCAs, wheat variety Gautam was used. The

formulation and concentrations of each fungicide, botanicals, and BCAs are presented in Table 1.

Table 1. Details of treatments used for the management of spot blotch disease in wheat during the crop season 2019.

Treatments	Details of the treatments
T1	Untreated control
T2	Seed treatment with Vitavax (carboxin 37.5% + thiram 37.5% DS) @ 3g/kg seed
T3	Seed treatment with Rovral (iprodione 80 WP) @ 2.5 g/ kg seed
T4	Seed treatment with Bavistin (carbendazim 50 % WP) @ 3g/kg seed
T5	Seed treatment (10g/kg seed) +foliar spray of (*Pseudomonas fluorescens formulation* (spores 1x10^9 cfu/ml) @1%)
T6	Seed treatment (4g/kg seed) + foliar spray of (*Trichoderma viride formulation* (spores 1x10^9 cfu/ml) @0.3%)
T7	Seed treatment with Vitavax @3g/kg seed + foliar spray of 10% (W/V) Onion extract
T8	Seed treatment with Vitavax @3g/kg seed + foliar spray of 10% (W/V) Garlic extract or what??
T9	Seed treatment with Vitavax@3g/kg seed +foliar spray of DILT 25% EC (propiconazole @ 0.1%)
T10	Seed treatment with Vitavax@3g/kg seed + foliar spray of Folicur 25 EC (tebuconazole) @ 0.05%
T11	Seed treatment with Vitavax@3g/kg seed +foliar spray of Nativo 75WG (trifloxystrobin 25% + Tebuconazole 50%) @ 0.05%
T12	Seed treatment with vitavax@3g/kg seed + foliar spray of ZOLE PLUS 5% SC (Hexaconazole @ 0.1%)
T13	Seed treatment with vitavax@3g/kg seed +foliar spray of Bavistin (carbendazim 50%WP @0.1%)
T14	Seed treatment with vitavax @3g/kg seed +foliar spray of P-OXYRIDE 50%WP (coper-oxychloride @ 0.25%)
T15	Seed treatment with vitavax @3g/kg seed + foliar spray of Indofil M-45 75 WP (mancozeb@ 0.3%)

Experimental Design and Layout of Field

The field experiment was laid out in a randomized complete block design (RCBD) with three replications. A recommended seed rate of 100 kg/ha was used to calculate the required seed for each plot area. The seeds were hand sown in a 4m^2 plot size keeping ten rows of 2m length with 20 cm row to row, and 50cm plot to plot spacing.

Preparation of treatments and application

The fungicides used in the present investigation were prepared based on the recommended doses. The botanicals were prepared using two-hundred-gram fresh raw garlic clove and onion bulbs were chopped and ground to prepare a fine paste with the help of a mixer grinder. The paste was soaked overnight in 200 mL distilled water. This was filtered using a fine muslin cloth to get 100% concentration of the solution and sterilized by autoclaving at 110°C for 10 minutes, further 10% (garlic and onion) treatments were prepared by mixing 10 mL of the autoclaved solution and 90 mL of distilled water. The treatments were applied both as seed treatment and foliar spray. The treatments for foliar spray were applied at the first appearance of the spot blotch disease complex. Control plots were sprayed with plain water while fungicides, botanicals, and

biological agents were applied with the help of the Agricultural Knapsack Manual and Battery Sprayer with a 16 L capacity. The spray was conducted during the afternoon time (2.30 pm to 4.00 pm). A total of four foliar sprays were applied at an interval of 10 days from 1st to 30th March 2019.

Disease Assessment, scoring, and Data Recording

The first observation was taken at anthesis. Then data were taken at every 10 days interval before the application of the foliar spray. Disease incidence and severity (intensity) were recorded for each treatment. Percent disease incidence was calculated by counting the total number of diseased leaves in randomly selected and tagged 10 plants per treatment in each replication. The double-digit scale (00 to 99) developed by the modification of the scale (Saari & Prescott, 1975), measures overall foliar infection on the whole plant based on two digits (Manandhar et al., 2016).

Disease scoring was done on ten randomly selected and tagged plants using a 1-9 scale. Similarly, the grain yield (tons ha-1) of each treatment was estimated on the plot basis and the thousand-grain weight was estimated by the manual counting of seeds of each treatment.

$$\text{Percent disease incidence (\%)} = \frac{\text{Total no.of diseased leaves}}{\text{Total no.of leaves observed}} \times 100$$

Percent Disease control (PDC) or Percent disease intensity (PDI) was calculated using the following formula given by (Shivankar and Wangikar, 1993) and (McKinney, 1923) i.e.

$$\text{(PDI)(\%)} = \frac{\text{the sum of all numerical ratings}}{\text{total no.of plant observed} \times \text{maximum disease grade}} \times 100$$

$$\text{(PDC)} = \frac{\text{Percent disease incidence in control-Percent disease incidence in treatment}}{\text{Percent disease incidence in control}} \times 100$$

AUDPC is based on the estimates of disease severity at different growth stages (Das et al.,1992). The AUDPC value was calculated according to the following formula (Madden et al., 2007).

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left([\{(Y_i + Y_{i+1})/2\}] \times (t_{(i+1)} - t_i)\right)$$

Where, Y_i = disease level at time t_i; Y_{i+1} = disease level at time t_{i+1}; t (i + 1) - t_i = time (days) between two disease scores and n = number of dates on which spot blotch was recorded.

Isolation and maintenance of pathogen

Diseased leaf samples of wheat were collected from the experimental area and brought to the plant pathology laboratory of the Department of Plant Pathology, Agriculture and Forestry University, Rampur Chitwan, Nepal for the isolation of the pathogen. The leaves of wheat with young lesions showing the typical symptoms were cut into a small bit of size 2-3 mm along with healthy tissue with the help of a surface-sterilized blade. These bits were surface sterilized to remove the secondary infections by dipping in 1 % sodium hypochlorite (NaOCl) for about 30 seconds followed by three washings in sterilized distilled water. These bits were transferred to PDA medium in sterilized Petri plates after blot drying in the sterilized filter papers. Three such bits were placed in each Petri plate and incubated for three days at 25±1°C. The culture thus obtained was subjected to purification as described by Choi et al. (1999).

A small bit of actively growing hyphae from the sporulating fresh cultures was taken aseptically with the help of a sterilized needle and again transferred to the PDA medium in sterilized Petri

plates for the further purification and multiplication of the pathogen and incubated for 7 days at 25±1ºC. Petri plates were exposed to daylight (not direct sunlight) for 3-4 hrs. to induce sporulation. The sporulating cultures were then purified by single spore isolation and the pure culture was maintained on a PDA medium. Petri plates were examined under the compound microscope for sporulation and the single conidium/spore was then removed with the help of a 5 mm cork borer from the plates along with agar and transferred aseptically to PDA medium in a test tube. The slants were incubated at 25±1ºC in an incubator. The stock cultures were revived by sub-culturing after every month and maintained throughout the course of studies on PDA in sealed culture tubes at 5°C in the refrigerator.

Preparation of fungicides for poison culture

Nine chemical fungicides viz., Vitavax (Carboxin 37.5% + Thiram 37.5% DS), Iprodione 80 WP (Rovral), Carbendazim 50 % WP (Bavistin), Propiconazole 25% EC (DILT), Tebuconazole 0.05% (Folicur 25 EC), Nativo 75WG (Trifloxystrobin 25% + Tebuconazole 50%), Hexaconazole 5% SC (ZOLE PLUS), Coper-oxychloride 50% AI (P-OXYRIDE 50%WP), and Indofil M-45 75 WP (Mancozeb) were selected for the experiment (Supplementary Table 1). They were tested in three different levels of concentrations i.e., 25 ppm, 50 ppm, and 100 ppm except for Indofil M-45 75 WP (Mancozeb) which was used in 50 ppm, 100 ppm, and 200 ppm.

In-vitro evaluation

The experiment was done in a completely randomized design (CRD) by the poisoned food technique (Schmitz, 1930). PDA media in the lukewarm stage were amended with the desired concentration of different fungicides before pouring into 9 cm Petri plates under laminar air flow to avoid bacterial contamination. PDA plates without fungicide were used as control. Then, inoculation of 5 mm circular discs of the pathogen (*B. sorokiniana*) (24 hours) after pouring amended PDA media in Petri plates) was done with the help of a sterile cork borer from the one-week-old culture at the center of each PDA plate under aseptic conditions. The experiment was done in three replications and the plates were incubated at 25°C inside the incubator. Colony diameter i.e., radial mycelial growth of the fungus was recorded daily for 10 days by the regular scale, and percent growth inhibition (I %) of *B. sorokiniana* was calculated by using the formula given by Vincent in 1947.

I% = (C-T)/C×100 Where, C = Colony diameter in control plates (PDA only) T = Colony diameter in treated plates (PDA with plant extracts or chemicals)

Statistical analysis

R Studio software version 1.4.1103 and the agricolae package ver. 1.3-3 (Mendiburu 2020, R Core Team 2020) were used for the analysis of variance (ANOVA) to test the significance of the treatment's effect on different experiments conducted in the present study. Before analysis arcsine square root transformation (Steel and Torrie 1980) was applied to the percent data. LSD test was performed to compare the means of significant treatments @1% and 5% levels of significance.

RESULTS

The performance of fourteen different fungicides in controlling the spot blotch in wheat was evaluated along with untreated control under natural epiphytotic conditions on the Gautam variety of wheat during the crop season 2019. Analysis of variance showed significant ($P<0.01$) variations among the treatments. The coefficient of variance (CV) ranged from 3.24% for grain yield (t ha-1) to 19.28% for thousand-grain weight (Table 2). While the mean performance showed that grain yield and thousand-grain weight (TGW) were significantly increased by all treatments (except for one treatment seed treated with Vitavax and plants sprayed with onion

extract) compared to untreated control (Table 2). The highest grain yield (4.84 tons ha-1) and TGW (48.63) was recorded in plots having seed treated with Vitavax and plants sprayed with DILT (propiconazole 25% EC) followed by plots having seed treated with Vitavax and plants sprayed with Nativo 75WG (trifloxystrobin 25%+tebuconazole 50%) and plot having seed treated with Rovral (iprodione 80 WP) (Table 2).

The lowest PDI (9.72%) and AUDPC (96.30) were observed in plots having seed treated with Vitavax and plants sprayed with Nativo 75WG (trifloxystrobin 25%+tebuconazole 50%) followed by plots having seed treated with Vitavax and plants sprayed with DILT (propiconazole 25% EC) (10.00%) and plots having seed treated with Vitavax and plants sprayed with ZOLE PLUS (hexaconazole 5% SC) (12.13%). Likewise, the highest PDI (Fig. 1A) and AUDPC were recorded in the untreated control plot (42.77% and 429.63) followed by the plot having seed treated with Vitavax and plants sprayed with 10% onion (39.07% and 400.00) and the plot having seed treated and plants sprayed with *Pseudomonas fluorescens* (36.48% and 374.07) (Table 2). The grain yield/ha of wheat has a highly significant negative correlation (r=-0.943, p-value: 0.001) with AUDPC. The predicted linear regression line also displayed a downward slope i.e., y=-0.0048.467x +4.9002, where 'Y' denoted predicted crop yield of wheat and 'X' stood for the AUDPC showed R^2= 0.8898 (p-value: 0.01) for explaining variation (Fig. 1B).

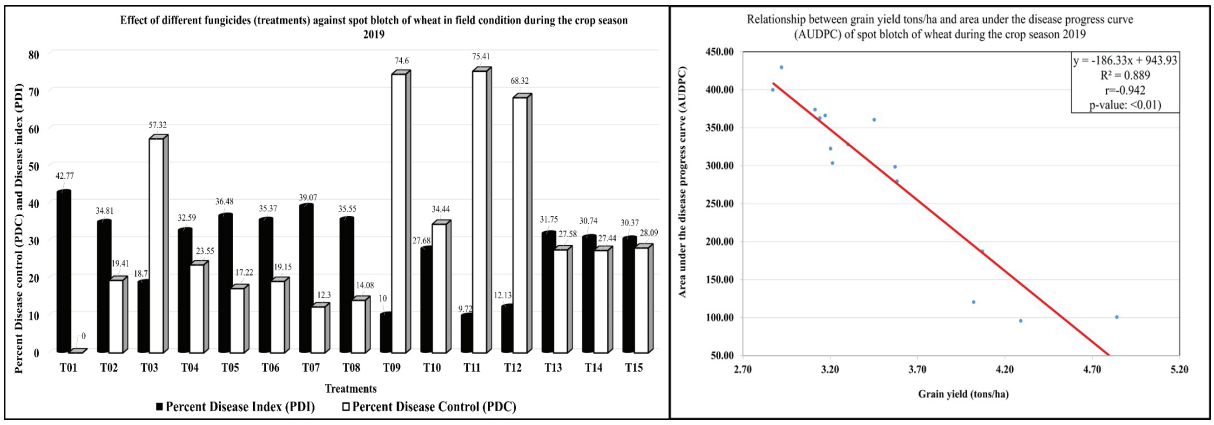
At the same time, most of the fungicides used in field experiments were further evaluated under in-vitro conditions using poisoned food techniques to validate their efficacy. A total of nine fungicides (Vitavax Power, Rovral, Bavistin, DILT, Folicur 25 EC, Nativo 75WG, ZOLE PLUS, P-OXYRIDE, and Indofil M-45) were evaluated at different concentrations. Out of them, seven systemic (Vitavax Power, Rovral, Bavistin, DILT, Folicur 25 EC, Nativo 75WG, and ZOLE PLUS) and one none-systemic (P-OXYRIDE), fungicides were tested at the concentrations of 25, 50, 100 ppm, while another non-systemic fungicide (Indofil M-45) was tested at the concentration of 50, 100, 200 ppm. The results showed that the tested fungicides at various concentrations gave outstanding results for inhibiting the mycelia growth of *B. sorokiniana* and showed their toxic potential. Among all nine fungicides, Bavistin (carbendazim 50 %WP) comparatively showed the highest mycelium growth at all the concentrations with very low percent inhibition in reducing the mycelia growth of test fungi (Table 3 and Fig. 2). While the fungicides Nativo 75WG (trifloxystrobin 25% + tebuconazole 50%), DILT (propiconazole 25% EC), and ZOLE PLUS (hexaconazole 5% SC) were highly effective and significantly superior in reducing the mycelia growth of spot blotch fungi in all the concentrations with the minimum mycelial growth (0.0 cm) followed by Rovral (iprodione 80 WP) at 100 ppm (0.76 cm), 50 ppm (0.88 cm), and 25 ppm (1.01 cm), whereas untreated control recorded high mycelial growth (6.55 cm) followed by Bavistin (carbendazim 50 % WP) at 25 ppm (4.11 cm) and 50 ppm (3.53 cm),

Table 2. Effect of different treatments on spot blotch disease and grain yield of wheat at Rampur, Chitwan during the crop season 2019

Treatment Details	GY	TGW	PDC(Arcsine transformation)	PDI	AUDPC	Yield increment over control (%)
Control	2.82^{g}	31.33^{e}	0.00 (0.00)^{j}	42.04	419.75	
Seed treatment with Vitavax (carboxin 37.5% + Thiram 37.5% DS) @ 3g/kg	3.45^{de}	34.33^{bcde}	19.41(0.41)^{i}	31.76	322.84	22.34
Seed treatment with Rovral (iprodione 80 WP) @ 2.5 g/ kg	4.07^{c}	46.47^{abc}	57.32(0.52)^{fgh}	18.70	187.65	44.33
Seed treatment with Bavistin (carbendazim 50 % WP)	3.30^{ef}	33.43^{cde}	23.55(0.74)^{d}	36.85	371.60	17.02
Seed treatment (10g/kg) +1% foliar spray (*Pseudomonas fluorescens* spores 1x10^{9} cfu/ml)	3.11^{f}	31.60^{e}	17.22(0.65)^{e}	27.69	279.63	10.28
Seed treatment (4g/kg) + 0.3% foliar spray (*Trichoderma viride* spores 1x10^{9} cfu/ml)	3.17^{f}	32.10^{de}	19.15(0.65)^{e}	30.93	300.00	12.41
Seed treatment with Vitavax + 10% Onion	2.97^{g}	31.10^{e}	12.30(0.45)^{hi}	39.07	400.00	5.32
Seed treatment with Vitavax + 10% Garlic	3.14^{f}	32.00^{de}	14.08(0.59)^{ef}	35.56	369.14	11.35
Seed treatment with Vitavax + DILT (propiconazole 25% EC)	4.84^{a}	48.6^{3}a	74.60(0.53)^{fg}	9.72	96.30	71.63
Seed treatment with Vitavax + Folicur 25 EC (tebuconazole 0.05%)	3.58^{d}	34.90^{bcde}	34.44(0.65)^{e}	36.85	379.01	26.95
Seed treatment with Vitavax + Nativo 75WG (trifloxystrobin 25% + tebuconazole 50%)	4.29^{b}	46.83^{ab}	75.41(0.49)^{ghi}	10.00	100.00	52.13
Seed treatment with Vitavax + ZOLE PLUS (hexaconazole 5% SC)	4.02^{c}	45.57^{abcd}	68.32(1.06)^{c}	12.13	120.37	42.55
Seed treatment with Vitavax + Bavistin (carbendazim 50% WP)	3.20^{f}	33.10^{de}	27.58(1.26)^{b}	34.81	360.49	13.48
Seed treatment with Vitavax + P-OXYRIDE 50%WP (coper-oxychloride 50% AI)	3.57^{d}	37.70^{abcde}	27.44(1.54)^{a}	32.59	328.40	26.60
Seed treatment with Vitavax + Indofil M-45 75 WP (Mancozeb)	3.21^{f}	32.37^{de}	28.09(1.47)^{a}	30.37	303.70	13.83
F-test	***	***	***			
Mean	3.521	36.76	0.733			
CV	3.24	19.28	6.63			
LSD	0.191	11.68	0.0814			

*** Significant at (P< 0.001) per cent LSD, data in parenthesis arcsine square root transformation

CV: Cofficient of Variation, **LSD:** Least significant difference (Superscripts), **PDI:** Percent disease intensity, **PDC:** Percent disease control, **AUDPC**: Area under disease progress curve, and **GY**: Grain yield tons ha-1, **TGW**: Thousand grain weight



[A] [B]

Fig. 1. A) Per cent disease index (PDI) and per cent disease control (PDC) of various fungicides against spot blotch (The treatments details were presented in Table-1), and B) Relationship between area under disease progress curve (AUDPC) of spot blotch and grain yield of wheat under different treatments of wheat in field condition during the crop season 2019

P-OXYRIDE 50%WP (Copper-oxychloride 50% AI) at 25 ppm (3.95cm) and Indofil M-45 75 WP (Mancozeb) at 50 ppm (3.36 cm) after 10 days of the incubation period.

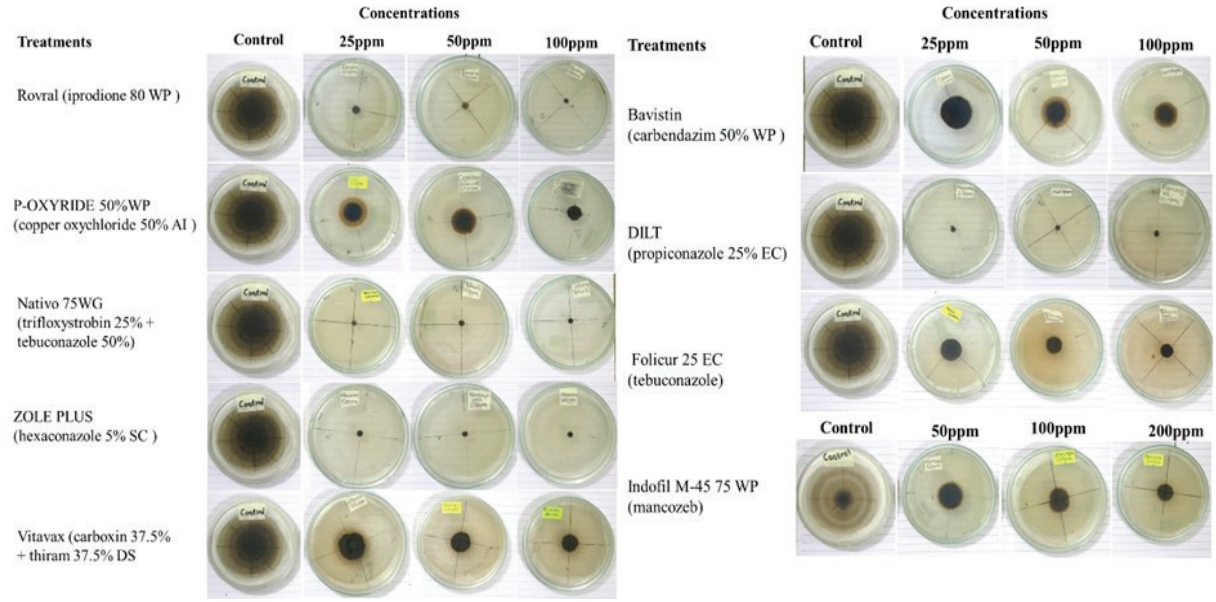


Fig. 2. Effect of various fungicides on mycelial growth of spot blotch fungus of wheat, *Bipolaris sorokiniana*, at different concentrations after 10 days period of incubation.

Table 3. **Effect of different fungicides at different doses on radial mycelial growth of *Bipolaris sorokiniana***

Treatments	Concentrations (ppm)	Radial growth (cm)	Inhibition percentage
Rovral (iprodione 80 WP)	**25**	1.01^{k}	$84.50(1.16)^{d}$
	50	0.88^{k}	$86.56(1.19)^{c}$
	100	0.76^{k}	$88.35(1.22)_{b}$
P-OXYRIDE 50%WP (Coper-oxychloride 50% AI)	**25**	3.95^{bc}	$39.72(0.68)^{n}$
	50	3.05^{e}	$53.41(0.81)^{k}$
	100	2.51^{fg}	$64.98(0.93)^{h}$
Nativo 75WG (trifloxystrobin 25% + tebuconazole 50%)	**25**	0.0^{l}	$100.0(1.57)^{a}$
	50	0.0^{l}	$100.0(1.57)^{a}$
	100	0.0^{l}	$100.0(1.57)^{a}$
ZOLE PLUS (Hexaconazole 5% SC)	**25**	0.0^{l}	$100.0(1.57)^{a}$
	50	0.0^{l}	$100.0(1.57)^{a}$
	100	0.0^{l}	$100.0(1.57)^{a}$
Vitavax (carboxin 37.5% + thiram 37.5% DS)	**25**	2.28^{fghi}	$65.11(0.93)^{h}$
	50	2.26^{fghi}	$65.41(0.94)^{h}$
	100	2.15^{ghi}	$67.16(0.96)^{g}$
Bavistin (carbendazim 50 % WP)	**25**	4.11^{b}	$37.09(0.65)^{o}$
	50	3.53^{cd}	$45.96(0.74)^{m}$
	100	2.48^{fg}	$62.02\ (0.90)^{ij}$
DILT (propiconazole 25% EC)	**25**	0.0^{l}	$100.0(1.57)^{a}$
	50	0.0^{l}	$100.0(1.57)^{a}$
	100	0.0^{l}	$100.0(1.57)^{a}$
Folicur 25 EC (Tebuconazole 0.05%)	**25**	2.45^{fgh}	$62.68(0.91)^{i}$
	50	1.86^{ij}	$71.44\ (1.00)^{f}$
	100	1.58^{j}	$75.78\ (1.05)^{e}$
Indofil M-45 75 WP (Mancozeb)	**50**	3.36^{de}	$48.44\ (0.76)^{l}$
	100	2.58^{f}	$60.42\ (0.89)^{j}$
	200	2.03^{hi}	$68.85(0.97)^{g}$
Control		$6.5^{5}a$	$0.00(0.00)^{p}$
F-test		***	***
LSD (≤0.05)		0.429	0.018
CV (%)		14.86	1.01

LSD: Least significant difference (Superscripts), *** Significant at (P< 0.001) percent LSD, Data in parenthesis are arcsine square root transformation,

DISCUSSION

Spot blotch (*Bipolaris sorokiniana*) is one of the major diseases of wheat in warm and humid climates in South Asian countries which affects the production and productivity of wheat. The resistant sources of this disease have been identified however, the level of resistance in the popular varieties is still not sufficient to avoid yield and quality of the seed produced (Singh et al. 2014). The identification of an effective fungicidal formulation for the management of this disease would be beneficial for wheat growers. In the present study efficacy of fourteen fungicides along with botanicals against spot-blotch was tested under field conditions. The results revealed that most of the systemic fungicides performed well as compared to the non-systemic fungicides. Similar results were previously reported that fungicides, especially the triazole group, have shown good effects in reducing the disease severity and maintaining it

below 10% (Roy et al. 2023; Sharma & Duveiller, 2006). In comparison to untreated control in the present study grain yield and thousand-grain weight were significantly increased in all treatments except seed treated with Vitavax and plants sprayed with onion extract. The most effective treatments in the present study were plots having seeds treated with Vitavax and plants sprayed with DILT (propiconazole 25% EC) followed by plots having seeds treated with Vitavax and plants sprayed with Nativo 75WG (trifloxystrobin 25%+tebuconazole 50%). Likewise, the highest PDC (Fig. 1) was observed in plots having seeds treated with Vitavax and plants sprayed with Nativo 75WG (trifloxystrobin 25%+tebuconazole 50%) followed by plots having seeds treated with Vitavax and plants sprayed with DILT (propiconazole 25% EC) and plots having seed treated with Vitavax and plants sprayed with ZOLE PLUS (hexaconazole 5% SC) indicating their higher disease reducing capability. A similar trend had been observed in the area under disease progress curve (AUDPC). Previous studies showed seed treatment with Vitavax 200 B and Bavistin reduced seedling infection (Sharma et al., 2005). The seed treatment with fungicidal formulation Vitavax 200 WS (Carboxin + Thiram 1:1) @ 2.0, 2.5, and 3.0 g per kg seed gave good results in reducing seedling mortality, the incidence of foliar diseases in wheat (Singh et al., 2007). Similarly, seed treatment with Corboxin (27.5WS) + Thiram (27.5WS) at the rate of 2.5gm per kg of seed with two sprays of Propiconazole (25EC) at the rate of 0.1% at boot leaf stage and 20 days after the first spray reduced disease incidence and severity. The thousand-grain weight and grain yield also increased in the above treatment (Kumar et al., 2014; Mahapatra & Das, 2013). The seed treatment with Vitavax power @ 3 g kg of seed followed by two sprays of propiconazole @ 0.1% at the time of disease initiation on flag -1 leaf and soft dough stage were best for the management of spot blotch in wheat (Singh et al. 2017). The lowest spot blotch infection could be achieved by the use of Vitavax power @ 2.5 g/kg of seed and two sprays of propiconazole (Tilt) @0.1%, as well as two sprays of propiconazole @ 0.1% (Singh et al. 2014).

Among the nine fungicides used under in-vitro conditions at various concentrations for the spot blotch toxicity using poisoned food technique, except Bavistin all of the systemic fungicides (Vitavax Power, Rovral, DILT, Folicur 25 EC, Nativo 75WG, and ZOLE PLUS) were effectively reduced the mycelia growth of test fungi. Based on growth inhibition, Nativo 75WG (trifloxystrobin 25%+tebuconazole 50%), DILT (propiconazole 25% EC), and ZOLE PLUS (hexaconazole 5% SC) were highly effective and significantly superior in reducing the mycelia growth of test fungi in all the concentrations. Previous findings showed propiconazole, hexaconazole, and difenoconazole + propiconazole had complete inhibition on the growth of spot blotch (Hasan et al. 2012). Similarly, TILT (propiconazole 25%) was the most effective (complete mycelial growth inhibition at all concentrations) against the pathogen (Magar et al. 2020). Out of fifteen fungicides tested against *B. sorokiniana* under in-vitro conditions in barley found that propiconazole at 0.1% and 0.05% was the most effective in controlling the mycelial growth of the pathogen (Pande et al. 2017). Our study as well as previous studies showed that most of the systemic fungicides particularly zoles fungicides were effective for the management of foliar blight in wheat. The regression analysis of grain yield and AUDPC showed it had a significant negative association with the applied treatments as compared to the control. A similar trend of relationship was previously reported in winter wheat (Wegulo et al. 2009). Likewise, in our study minimum inhibition percent for mycelial growth of spot blotch other than control has been recorded in Bavistin (carbendazim 50% WP) followed by P-OXYRIDE 50% WP (coper-oxychloride 50% AI) and Indofil M-45 75 WP (Mancozeb). Some of the previous studies suggested that Bavistin (carbendazim 50%) didn't inhibit the growth of the pathogen (Angdembe et al. 2019; Giri et al. 2001, Kavita et al. 2017) which is in agreement with our finding. In contrast, higher concentrations of 200 ppm and 300 ppm of Bavistin (carbendazim 50%) could inhibit the mycelial growth up to 77% and 100% which

indicated that the application of Bavistin at higher concentrations could be effective for the management of spot blotch (Samia et al. 2015).

CONCLUSION

In the present investigation, the efficacy of fungicides was tested under field as well as in-vitro conditions indicating that the systemic fungicides were more effective than non-systemic, botanicals, and biocontrol agents for the management of spot blotch. Among the systemic fungicides Nativo 75WG (trifloxystrobin 25% + tebuconazole 50%), DILT (propiconazole 25% EC), and ZOLE PLUS (hexaconazole 5% SC) were highly effective. In conclusion, the effective management of spot blotch in wheat could be achieved by seed treatment with Vitavax followed by the three sprays of one of the fungicides Nativo 75WG (trifloxystrobin 25% + tebuconazole 50%) @ 0.05%/litre, DILT (propiconazole 25% EC) @ 0.1%/litre, and ZOLE PLUS @ 0.1 %/litre respectively. The application of fungicides should be followed strictly at the required concentration as well as mixing of the different fungicides could be effective to avoid the development of resistance against fungicides. However, the present investigation was based on the one-season experiment; therefore, further validation of the efficacy of these fungicides would be beneficial for further recommendation.

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