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In-Vitro Efficacy of Commercial Fungicides against *Bipolaris Sorokiniana*: Induced Spot Blotch Disease of Wheat

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***Abstract*— *Spot blotch caused by Bipolaris sorokiniana is an important disease of wheat. A laboratory experiment was conducted at Plant Pathology Division, Nepal Agriculture Research Council, Khumaltar, Nepal to evaluate the efficacy of commercially available fungicides viz, Saaf (Carbendazim 12%+ Mancozeb63%), Sectin (Fenamidone 10%+ Mancozeb 50%), Angel (Metalaxyl 8%+ Mancozeb 64%), Diathane M-45 (Mancozeb 75%), G-MIL(Cymoxanil 8%+ Mancozeb 64%) tested in two different concentration i.e. 50ppm and 100 ppm except Curex (Copper oxychloride 50%) tested in the concentration of 100ppm and 200ppm and Tilt (propiconazole 25%) tested in 15ppm and 30 ppm concentration with a control test by employing poisoned food technique against spot blotch pathogen of wheat Bipolaris sorokiniana. The experiment was laid out in Completely Randomized Design (CRD). The result revealed that all concentrations of different fungicides successfully inhibited the radial mycelial growth of the pathogen under in vitro condition. Based on the measurement of fungal radial growth, fungicide Tilt of 30ppm and 15 ppm concentration were the most effective followed by Sectin 100ppm, Diathane M-45 100ppm, Angel 100 ppm and Saaf 100ppm. G-MIL 50 ppm in poisoned food technique was the least effective. Use of safer and economical chemical fungicides can provide an effective and long-term solution against plant diseases in agricultural farming.*
Keywords— ***Spot blotch, Tilt, Trichoderma, efficacy, fungicides, pathogen.***

I. INTRODUCTION

After rice and maize, wheat (*Triticum aestivum*) is Nepal's third most significant cereal crop. Wheat is produced from the Terai to the high alpine regions, and wheat consumption is on the rise. However, the yield per unit is much lower than anticipated. The disparity between the maximum observed yield and the national average yield, as well as the declining yield trend, necessitate immediate research. The low yield is due to a number of factors, including the scarcity of improved varieties, the occurrence of various wheat diseases, a lack of reliable irrigation, inclement weather, a lack of improved technology, and biotic and abiotic stress factors, all of which result in significant yield losses each year (Joshi et al., 2007). Fungal diseases in wheat cultivation are more important among biotic stress factors because they produce a significant drop in yield as well as deterioration in grain quality. In the humid subtropics of South Asia, where the irrigated rice-wheat rotation covers more than 12 million ha, there is growing evidence that stress conditions are increasing the severity of foliar diseases (Dubin *et al.*, 1994) because rice serves as a host for the spot blotch fungi and rice stubble plays its role as a substrate for the fungi after rice harvest (Saari, 1998). Therefore, the fungal pathogen, *Bipolaris sorokiniana* (Sacc.) Shoemaker (teleomorph *Cochliobolus sativus*) induced spot blotch disease of wheat has emerged as one of the prime diseases for profitable wheat production in different zones of Nepal. Spot Blotch is caused by *Bipolaris sorokiniana* in Mega Environment 5A (ME5A), characterized by high temperature (coolest month greater than 17ºC) and high relative humidity (RH) (Dubin *et al.*, 1991; Rajaram *et al.*, 1993). It is a seed borne fungal. Spot blotch (oval to round brown blotch encircled by yellow halo) is the pathogen's principal symptom. The pathogen's continuing growth and development results in increasing leaf and spike damage, leading in yield loss. The disease occurs every year in Nepal in moderate to severe form (Duveiller *et al.*, 2005). *Bipolaris sorokiniana* was also identified as a main contributing factor in a study undertaken in Nepal in 1996 to investigate and identity the national issue of "low germination in wheat' (Shrestha et al., 1997). The losses due to spot blotch in warm regions of Nepal ranged from 23 –40% depending on the genotypes and other environmental factors (Tripathi *et al.*, 2005; Sharma *et al.*, 2006).

To lessen the losses caused by the disease, a range of syste mic fungicides with various modes of action and targets ha ve been developed (Pasquer et al., 2005). However, in vitro evaluation against *Bipolaris sorokiniana* are barely sufficient and even accurate information on determining efficacy, sensitivity of different fungicides with minimum inhibitory concentrations have yet to be defined against *Bipolaris sorokiniana* (Iqbal, 2010). It is important to examine the effect of different concentration of fungicides commonly available in market to control this disease.

II. MATERIALS AND METHOD

Subculture of pathogen was made by transferring cells from a previous year pure culture to petri-dishes containing fresh growth medium (Potato Dextrose Agar) and incubating at 25ºC for 6 days to prepare the test pathogen (*Bipolaris sorokiniana*). The pure culture was isolated from infected leaves of wheat crop showing characteristic blight symptoms in the research field of Plant Pathology Division under Nepal Agriculture Research Council.

Seven fungicides with two different concentrations were evaluated for their efficacy to inhibit the mycelium growth of *B. sorokiniana* under in vitro condition following poisoned food technique. Five fungicides (Saaf, Sectin, Angel, Diathane M-45, G-MIL) with two concentration viz., 50 and 100 ppm, one fungicide (Curex) with two different concentration viz., 100 and 200 ppm and one fungicide (Tilt) with two different concentration viz., 15 and 30 ppm were evaluated. Concentration (PPM) of the fungicide was calculated based on active ingredients (a.i.) of the pesticide provided by the company on each packet. Stock solution of each fungicide was prepared in distilled water and incorporated into Potato dextrose agar medium and mixed thoroughly before autoclaving. After autoclaving the medium was poured aseptically in sterilized petri-plates of 9 cm size under laminar air flow and allowed to cool. Five mm mycelial circular discs of pathogen excised with sterile cork borer from a seven days old culture of test pathogen was placed at the centre of each petri-plate and incubated at 25ºC. PDA with water or without chemical served as control. The experiment was arranged in complete randomized design (CRD) and there were four replications for each (15) treatments. The plates were incubated at 25 ºC inside BOD incubator. Measurement of the colony diameter of pathogens was taken after 48 hours for 10 days (i.e 2nd, 4th, 6th, 8th day and 10th day) after inoculation with the help of vernier caliper. Percent growth inhibition of the pathogen was calculated by using the following formula of Vincent (1947).

$$I\,\% = \frac{C-T}{C} \text{ X } 100$$

Where,

I= inhibition percentage

C= Colony diameter in control and T=Colony diameter in treatment

The data collection started from 48 hours after poisoned food technique i.e. 8th to 18th March 2021. The data were recorded by measuring the growth of the test pathogen after each 24 hours by using Vernier caliper in mm. The data obtained from the experiment were analyzed using the software Gen Stat for the analysis of variance (ANOVA) to test the significance of treatments effect on mycelial growth of *Bipolaris sorokiniana*. Means of significant treatments at 5% level of significance were compared following Duncan's Multiple Range Test (DMRT) and Microsoft Excels.

Table 1 Commercial agrochemicals (treatments) used in the study

SN	Commercial Name	Common Name	Active Ingredients (a.i.)	50 ppm (mg)	100 ppm (mg)
1.	Saaf	Carbendazim 12% +Mancozeb 63% WP	75	6.67	13.33
2.	Sectin	Fenamidone 10% + Mancozeb 50% EC	60	8.33	16.67
3.	Angel	Metalaxyl 8% + Mancozeb 64% WP	72	6.94	13.89
4.	Diathane M-45	Mancozeb 75 % WP	75	6.67	13.33
5.	G-MIL	Cymoxanil 8% + Mancozeb 64% WP	72	6.94	13.89

SN	Commercial Name	Common Name	Active Ingredients (a.i.)	15 Ppm	30 Ppm
6.	Tilt	Propiconazole 25% EC	25	6	12
SN	**Commercial Name**	**Common Name**	**Active Ingredients (a.i.)**	**100 Ppm**	**200 Ppm**
7.	Curex	Copper oxychloride 50% WP	50	20	40
8.	Control	Water (Distilled)	0	0	0

III. RESULTS AND DISCUSSION

3.1 Effect of commercial fungicides on the radial growth of mycelium of *Bipolaris sorokiniana* by poisoned food method

Chemical fungicides viz, Saaf (Carbendazim 12%+ Mancozeb63%), Sectin (Fenamidone 10%+ Mancozeb 50%), Angel (Metalaxyl 8%+ Mancozeb 64%), Diathane M-45 (Mancozeb 75%), G-MIL(Cymoxanil 8%+ MAncozeb 64%) were tested in two different concentration i.e. 50ppm and 100 ppm except Curex (Copper oxychloride 50%) which was tested in concentration of 100ppm and 200ppm and Tilt (propiconazole 25%) tested in 15ppm and 30 ppm concentration against *Bipolaris sorokiniana*. In the table below, data were transformed to standard unit (cm) to avoid statistical complications during analysis and data were and rounded to hundredth decimal unit. From the result it was observed that poisoned food method showed all fungicides tested with different concentration inhibited the radial growth of *Bipolaris sorokiniana* as compared to untreated control.

Means with the different letters are significantly different at 5% level of significance using LSD. In (Table 2), different letters in a column signifies that the treatment means are significantly different with each other at P-value <0.001.

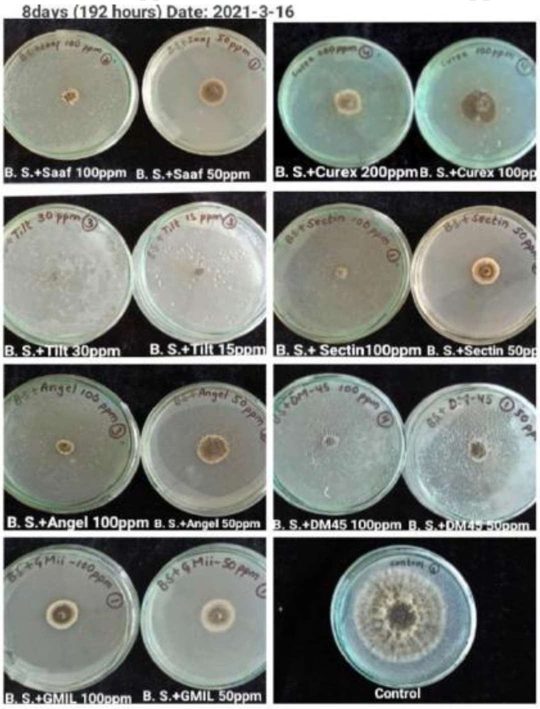
Table 2 Mean radial growth of mycelium of Bipolaris sorokiniana in presence of commercial fungicides

Fungicides	***Mean radial mycelial growth (cm)***				
	Day 2	*Day 4*	*Day 6*	*Day 8*	*Day 10*
Saaf 100ppm	0.00 g	0.99 gh	1.25 f	1.55 f	2.03 hi
Saaf 50ppm	0.90 d	1.46 d	2.07 cd	2.75 bc	3.37 bc
Sectin 100ppm	0.00 g	0.00 i	0.74 g	0.88 g	1.06 k
Sectin 50ppm	0.84 de	1.37 de	2.01 cd	2.34 d	2.60 ef
Angel 100ppm	0.63 f	0.85 h	1.08 f	1.59 f	1.82 ij
Angel 50ppm	0.86 de	1.45 d	2.10 cd	2.60 c	2.88 de
Diathane M-45 100ppm	0.69 f	0.83 h	1.09 f	1.35 f	1.54 j
Diathane M-45 50ppm	0.83 de	1.14 fg	1.73 e	2.05 e	2.44 fg
G-MIL 100ppm	0.74 ef	1.21 ef	1.94 de	2.67 c	3.20 c
G-MIL 50ppm	0.88 d	1.49 d	2.25 c	2.99 b	3.63 b
Curex 200ppm	1.52 c	1.93 c	1.95 de	2.18 de	2.24 gh
Curex 100ppm	1.76 b	2.50 b	2.62 b	2.81 bc	3.08 cd
Tilt 30ppm	0.00 g	0.00 i	0.00 h	0.00 h	0.00 l
Tilt 15ppm	0.00 g	0.00 i	0.00 h	0.00 h	0.00 l
Control	2.15 a	4.77 a	6.92 a	8.36 a	9.00 a
Grand Mean	0.78	1.33	1.85	2.27	2.59
LSD	0.1137	0.1636	0.2299	0.243	0.2827
CV	10.1%	8.6%	8.7%	7.5%	7.7%
SEm	0.0399	0.0574	0.0807	0.0853	0.0992

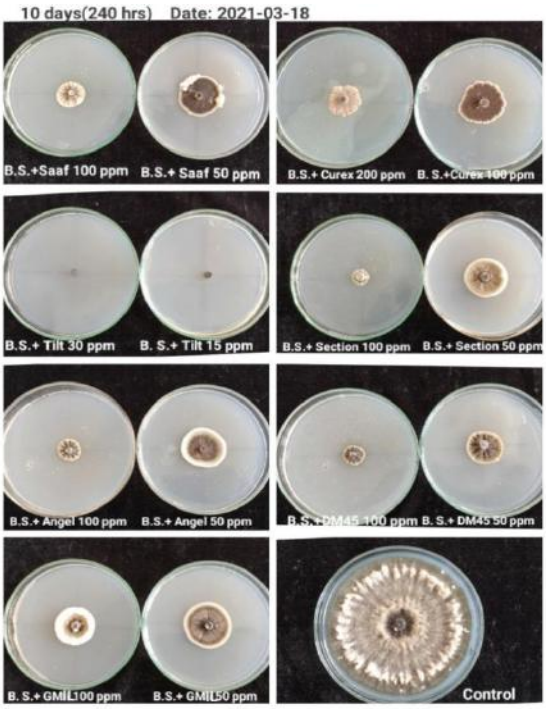
P-value	<0.001	<0.001	<0.001	<0.001	<0.001
Sedm	0.0564	0.0812	0.1141	0.1206	0.1402

CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by Duncan's Multiple Range Test at 5% level of significance, SEm (±) indicates standard error of the mean, cm is centimeters

Data of Day 10 shows that the pathogen covered the entire petri-plate of 9cm in the control treatment. Tilt 15 ppm and Tilt 30 ppm made lowest or no growth of the pathogen. The lowest mean radial growth was observed in the treatment Sectin 100ppm (1.06cm) being the most effective fungicide treatment after Tilt 15 and 30 ppm. The radial growth of the pathogen was highly influenced by Tilt (30 and 15ppm) and Sectin 100ppm. While significant treatments, G-MIL 100ppm (3.2cm) and Curex 100 ppm (3.08cm) were least effective followed by significant treatments G-MIL50 ppm (3.63cm), Saaf 50ppm (3.37cm) as compared to other treatments. The results clearly demonstrated that the fungicide Tilt (Propiconazole 25%) of both 30 and 15 ppm concentration followed by Sectin (Fenamidone 10% + Mancozeb 50%) of 100 ppm were individually effective against the pathogen by maximum inhibiting the mycelia growth in all days of data collection.



Radial mycelial growth of pathogen on Day 8



Radial mycelial growth of pathogen on Day 10

Table 3 Effect of commercial fungicides on the inhibition percentage of Bipolaris sorokiniana by poisoned food method

Fungicides	**Mycelial growth inhibition (%)**				
	Day 2	**Day 4**	**Day 6**	**Day 7**	**Day 10**
Saaf 100ppm	100.00	79.21	81.88	81.49	77.48
Saaf 50ppm	58.24	69.24	70.04	67.12	62.50
Sectin 100ppm	100.00	100.00	89.29	89.38	88.13
Sectin 50ppm	60.90	71.29	70.93	72.06	71.11
Angel 100ppm	70.77	82.08	84.33	80.90	79.72
Angel 50ppm	60.09	69.66	69.63	68.85	68.00
Diathane M45 100ppm	67.98	82.61	84.14	83.80	82.83
Diathane M45 50ppm	61.60	76.05	75.05	75.49	72.91
G-MIL 100ppm	65.55	74.60	72.01	68.01	64.44
G-MIL 50ppm	59.16	68.73	67.44	64.22	59.67

Curex 200ppm	29.58	59.56	71.77	73.84	75.11
Curex 100ppm	18.21	47.61	62.21	66.38	65.78
Tilt 30ppm	100.00	100.00	100.00	100.00	100.00
Tilt 15ppm	100.00	100.00	100.00	100.00	100.00
Control	0.00	0.00	0.00	0.00	0.00

Irrespective of the low concentration, at the end of tenth day maximum inhibition of mycelial growth for the control of *Bipolaris sorokinina* was recorded in Tilt 30ppm (100%) and Tilt 15ppm (100%) which was significantly superior than other treatments followed by Sectin 100ppm (88.13%) , Diathane M-45 100ppm (82.83%), Angel 100ppm (79.72%), Saaf 100ppm (77.48%) and Curex 200ppm (75.10%), Diathane M-45 50ppm (72.90%) and Sectin 50ppm (71.10%). The mycelial growth inhibition percentage ranged between 59.6 to 100 % on tenth day of poisoned food experiment. The inhibitory effect of G-MIL50 ppm was the lowest i.e. 59.6%. Looking at the percentage inhibition between same fungicides of different concentrations, the results revealed that inhibition percentages increased with the increase in concentration of fungicides. Most of the reports also concluded increase in concentration to be directly proportional to the increase in inhibition potential (Maitlo *et al.*, 2014; Rafique *et al.*, 2016). Among the fungicides, Tilt (Propiconazole 25%) in almost all days of data collection was found to be significantly superior showing highest mycelial growth inhibition throughout the whole experiment.

Complete inhibition of radial mycelial growth and sporulation of *Bipolaris sorokiniana* was found with Tilt (Propiconazole), so it may be effective to completely control the pathogenic activity of *Bipolaris sorokiniana*. The result agrees with the results of various previous workers. Chattopadhyay *et al.,* (2013) found out that Tricyclazole inhibited the melanin production and reduced the sporulation, spore size and number of septa in conidia of *Bipolaris sorokiniana* under in vitro condition. Triazole fungicides (e.g., propiconazole and tebuconazole) inhibit the synthesis of sterols, which are building blocks of the membranes of fungal cells. Response of *Bipolaris sorokiniana* on media containing fungicide of Triazole group (e.g.- Tebuconazole and Propinazole) reduced the growth of fungus successfully (Pannu *et al.,* 2006; Sooväli and Koppel, 2009; Yamaguchi *et al.*, 2010; Acharya *et al.*, 2011; Rahman *et al.*, 2013). Singh and Gupta (2000) studied the bioassay of fungicides against *Dreschlera sativus* causing leaf blight of wheat and the result recordes tilt to be the most effective fungicide in inhibiting the mycelial growth.

Diathane M-45 (Mancozeb) 100ppm (82.83%) also successfully inhibited the mycelial growth. Giri *et al.* (2001) also demonstrated effectiveness of mancozeb (90.5%) to control infection of seeds caused by *Bipolaris sorokiniana*. Mancozeb, belonging to the dithiocarbamate family, disrupt the metabolism of fungi by inhibiting either glucose oxidation, or nucleic acid synthesis, or by degradation of fatty acids (Angdembe *et al.*, 2019). Mancozeb has direct effect upon the core biochemical processes within the fungus which results in inhibition of spore germination (Wong and Wilcox, 2001). Variation in the inhibition percentage of *Bipolaris sorokiniana* at different concentrations of mancozeb was reported in different experiments. There was higher (82.83%) percent inhibition at 100 ppm in our experiment whereas only 70 percent inhibition even at higher concentration of 400 ppm (Hasan *et al.*, 2012) and 34 percent inhibition at 300 ppm (Samia *et al.,* 2015) are also reported. These differences in inhibition rates may be due to different strains of *Bipolaris sorokiniana* and different quality of mancozeb used in these experiments. Sharma (2006) findings explored effectiveness of Mancozeb (Dithane M-45) against rot of *Coccinia indica* caused by *Bipolaris tetramera*.

Mancozeb alone highly inhibited the growth of *Bipolaris sorokiniana*. However, it showed variations when used as combination fungicides. Mancozeb with Fenamidone inhibited the mycelial growth by 88.13 % at 100 ppm. Mancozeb with carbendazim at 100ppm also showed better results i.e. 77.48% mycelial inhibition. Likewise Mancozeb with metalalxyl at 100 ppm showed 79.72% mycelial growth inhibition. But Mancozeb with Cymoxanil at 100 ppm (64.4%) and 50 ppm (59.6%) was lower as compared to other combinations.

It was reported that copper oxychloride showed 75.1% mycelial growth inhibition at 200 ppm and 65.7% at 100ppm. The mode-of-action of copper fungicides is the nonspecific denaturation of cellular proteins. It disrupts the function of proteins and enzymes after absorption and results in cell damage and membrane leakage (Husak, 2015). Samia *et al.* (2015) also reported 70-80% mycelial growth inhibition in isolates of *Bipolaris sorokiniana*, collected from different region of Bangladesh, at 300 ppm concentration of copper oxychloride.

IV. CONCLUSION

Among the tested fungi-toxicants, Propiconazole (15 and 30 ppm) gave 100% mycelial inhibition of the test pathogen being the most effective fungicide followed by other single composition fungicides as well as combination fungicides viz., Fenamidone + Mancozeb 100ppm (88.13%), Mancozeb (82.83%), Metalaxyl + Mancozeb 100ppm (79.72%), Carbendazim + Mancozeb 100ppm (77.48%). The experiment showed variable effect of different fungicides of similar concentration. The effective treatments from the result of this study under in vitro conditions are only indicative and can be used to test for further trial under natural field condition (in pots or fields) for the confirmation of their efficacy against *Bipolaris sorokiniana*.

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