Research Article

IN-VITRO EVALUATION OF COMMERCIALLY AVAILABLE FUNGICIDES AGAINST *Bipolaris sorokiniana*, THE CAUSE OF SPOT BLOTCH OF BARLEY

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ABSTRACT

A laboratory experiment was done to evaluate the effectiveness of commercially available fungicides against spot blotch pathogen of barley, *Bipolaris sorokiniana,* at Nepal Polytechnic Institute (NPI), Chitwan, Nepal by employing food poisoned technique. Fungicides such as Uthane-M45 (mancozeb 75% WP), Blutoxx (copper oxychloride 50% WP), Bavistin (carbendazim 50% WP), and Thiram (thiram 75% WS) with three concentrations (100 ppm, 200 ppm and 400 ppm) were included in the experiment. Experiment was arranged in a completely randomized design with four replications for each treatment. The mycelial growth of *B. sorokiniana* was recorded at 2, 4, 6, 8, and 10 days after inoculation. All the fungicides significantly reduced the mycelial growth of the pathogen as compared to control (without any fungicide) in poisoned culture. Copper oxychloride at 400 ppm inhibited 83% growth of mycelium of the fungus after 10 days. All the concentrations of copper oxychloride and 400 ppm of mancozeb were able to inhibit more than 50% of mycelial growth of the fungus. The results revealed that inhibition percentages were increased with the increase in the concentrations of all fungicides used in this study. The present results thus suggest that the current recommended doses of copper oxychloride and mancozeb for foliar application purposes may not be sufficient to manage the disease under field conditions and also for seed treatment. The recommended doses may need to be re-evaluated.

Key words: Food poisoned technique, fungicide, spot blotch,

INTRODUCTION

Barley (*Hordeum vulgare* L. 2n=14, sub family Poaceae), a crop of winter season, is grown eco-friendly worldwide for food, feed and forage under various agro-climatic situations (Kavita et al., 2017). Spot blotch caused by *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*) is one of the most important fungal diseases of barley (*Hordeum vulgare*). The global yield losses of barley caused by foliar diseases are estimated to the range of 10-40%, amounting to billions of dollars per season (Sharma & Duveiller, 2006). Disease severity up to 82% was reported in barley in Nepal due to *B. sorokiniana* (Subedi et al., 2020). Spot blotch of barley produces a broad range of symptoms, such as foot and root rot, leaf blotch and black point of seeds, and infected seeds can be the main source of inoculums, especially in newly cultivated areas. Diseased plants occur randomly, or in irregular patches and appear stunted and chlorotic (Usman & Salami, 2008). The pathogen can survive in seeds, soils, infected crop residues, and weed hosts (Iftikhar et al., 2009). Warm temperature more than 17°C, high humidity of 85% along with long wetness period of 12 hours caused by rainfall, or dews favor the disease (Burlakoti et al., 2013).

The disease affected all plant parts, and can cause up to 100% damage. Management of *B. sorokiniana* is possible through cultural practices, use of chemicals, biological practices and host resistance. Among them, fungicides can be an effective to control this disease. They are chemical compounds that control fungal disease by specifically inhibiting or killing the fungus causing the disease. Both seed treatment and foliar application of fungicides have been evaluated to control foliar blight (de Viedma & Kohli, 1997). Several chemicals are used for the management of *B. sorokiniana* in *in-vitro* (Kumar et al., 2019). Under this context, an experiment was done under the laboratory conditions with the objective to determine effectiveness of common fungicides and their potential concentrations against *B. sorokiniana*.

MATERIAL AND METHODS

The experiment was conducted at the Plant Pathology Laboratory of Nepal Polytechnic Institute, Bharatpur, Chitwan, Nepal during 30th March to 6th July, 2019.

Pure culture of *B. sorokiniana* was isolated from the infected leaf of barley plants grown at National Maize Research Program, Rampur, Chitwan. The pathogen was identified on the basis of black velvet like colonies, short conidiophores, black and shiny conidia (Morejon et al., 2006). Pure culture of *B. sorokiniana* was maintained in potato dextrose agar (PDA) slants in test tubes and transferred to petri plates containing PDA for fresh growth of the fungus for inoculation purpose.

Four chemicals mancozeb (Uthane M-45 75% WP), copper oxychloride (Blutoxx 50% WP), carbendazim (Bavistin 50% WP) and Thiram (thiram 75% WS) each with three concentrations (100 ppm, 200 ppm and 400 ppm active ingredient) were taken as treatments. They were tested *in-vitro* by using poisoned food technique (Shravelle, 1961) against *B. sorokiniana* to evaluate their efficacy in inhibiting the mycelial growth of the fungus. Chemicals to obtain specified concentrations were mixed into the medium in separate conical flasks for each fungicide before pouring into the petri plates. Twenty mL of the poisoned PDA was poured into the 90 mm sterilized petri plates and were left to solidify. Five mm discs of actively growing *B. sorokiniana* (one week old culture on PDA plate) were then placed at the centre of each petri plate and incubated at 27±2 degree Celsius in an incubator. Total of the five observations were taken at 2, 4, 6, 8 and 10 days after inoculation (DAI). Percent inhibition of fungal growth was calculated using the following formula (Vincent, 1947):

$$\text{Percent growth inhibition (\%)} = \frac{A-B}{A} \times 100$$

Where

A= colony growth of the *B. sorokiniana* in control plate

B = colony growth of the *B. sorokiniana* in treated plate

The experiment was conducted by using a completely randomized design (CRD), each treatment was organized with four replications.

The data were processed to fit into R-studio, analyses were conducted using R 3.0.3 and the agricolae version 1.1-8 package (Mendiburu, 2014).

RESULTS

Radial mycelial growth and fungicides

On 2 DAI, radial growth of mycelium ranged from 0.57 cm to 1.65 cm in which control plates (without fungicides) had the highest value. The lowest radial mycelium growth was observed in the treatment with 400 ppm copper oxychloride which was statistically similar ($p>0.05$) with 200 ppm copper oxychloride (Table 1). On 4 DAI, radial growth of mycelium ranged from 0.77 cm to 3.50 cm in which control plates had the highest value. The lowest radial mycelium growth was observed in 400 ppm copper oxychloride. On 6 DAI, radial growth of mycelium ranged from 0.99 cm to 4.48 cm in which control plates had the highest value. The lowest radial mycelium growth was found in 400 ppm copper oxychloride. On 8 DAI, radial growth of mycelium ranged from 1.05 cm to 5.82 cm in which control plates had the highest value. The lowest radial mycelium growth was found in 400 ppm copper oxychloride. On 10 DAI, radial growth of mycelium ranged from 1.15 cm to 6.76 cm in which control plates had the highest value and the lowest radial mycelium growth was observed for the treatment with 400 ppm copper oxychloride (Table 1).

Inhibition percentage of mycelial growth

In this study all four fungicides of different concentrations (100, 200 and 400 ppm) visibly inhibited mycelial growth over the control. Among the fungicides, copper oxychloride in 400 ppm had the highest inhibition percentage leaving all the concentrations of other fungicides behind (Table 2). Inhibition percentage of mycelial growth by copper oxychloride 200 ppm was at par with copper oxychloride 400 ppm. The lowest inhibition percent (19.50) was obtained from carbendazim 100 ppm. The inhibitory effect of all the fungicides taken was found to increase with increased concentrations (Table 2).

The real time pictures of the inhibition of *B. sorokiniana* by the tested fungicides at different concentrations are shown in figures (1, 2, 3, & 4) and the growth of the fungus in PDA without fungicide in figure (5).

Table 1. Effect of fungicides at different concentrations on the radial growth of mycelium of *B. sorokiniana* by poisoned food technique, 2019

Fungicides	Concentration (ppm)	Mean radial mycelial growth (cm)				
		Day 2	Day 4	Day 6	Day 8	Day 10
Carbendazim	100	1.42^{bc}	2.96^{b}	3.63^{cd}	4.58^{def}	5.45^{b}
	200	1.35^{bcd}	2.82^{bcd}	3.67^{cd}	4.85^{bcd}	5.15^{b}
	400	1.25^{d}	2.70^{d}	3.60^{cde}	4.45^{ef}	5.15^{b}
Copper oxychloride	100	0.92^{f}	1.66^{f}	2.13^{f}	2.81^{g}	3.21^{d}
	200	0.65^{g}	1.16^{g}	1.45^{g}	1.70^{i}	2.00^{e}
	400	0.57^{g}	0.77^{h}	0.97^{h}	1.05^{j}	1.15^{f}
Thiram	100	1.46^{b}	2.95^{b}	3.98^{b}	4.97^{bc}	5.60^{b}
	200	1.31^{cd}	2.73^{cd}	3.81^{bc}	4.88^{bcd}	5.55^{b}
	400	1.11^{e}	2.51^{e}	3.43^{de}	4.61^{cde}	5.23^{b}
Mancozeb	100	1.36^{bcd}	2.88^{bc}	3.87^{bc}	5.10^{b}	5.55^{b}
	200	0.96^{f}	2.35^{e}	3.32^{e}	4.22^{f}	4.61^{c}
	400	1.01^{ef}	1.61^{f}	2.00^{f}	2.17^{h}	2.48^{e}
Control		1.66^{a}	3.50^{a}	4.48^{a}	5.82^{a}	6.76^{a}
Grand Mean		1.15	2.36	3.11	3.94	4.46
CV		8.01	5.32	6.90	6.57	8.20
LSD		0.13	0.18	0.31	0.37	0.52
SEm(±)		0.07	0.09	0.15	0.18	0.26
P-value		<0.001	<0.001	<0.001	<0.001	<0.001

Note: CV: Coefficient of variation, LSD: Least significant difference, Means followed by the same letter in a column are not significantly different by DMRT at 5 and 1% level of significance, SEm (±) indicates standard error of the mean

Table 2. Effect of fungicides at different concentrations on the inhibition percentage of *B. sorokiniana* by poisoned food technique, 2019

Fungicides	Concentration (ppm)	Mycelial growth inhibition (%)				
		Day 2	Day 4	Day 6	Day 8	Day 10
Carbendazim	100	14.20	15.28	19.09	21.34	19.50
	200	18.88	19.20	18.01	16.73	23.88
	400	24.70	22.85	19.75	23.62	23.91
Copper Oxychloride	100	44.38	52.49	52.38	51.72	52.51
	200	60.90	66.77	67.69	70.82	70.43
	400	65.36	77.85	78.26	81.97	83.00
Thiram	100	11.92	15.69	11.11	14.60	17.22
	200	21.02	21.75	14.99	16.09	17.94
	400	32.96	28.21	23.39	20.82	22.58
Mancozeb	100	18.10	17.53	13.66	12.46	17.98
	200	42.08	32.78	25.82	27.45	31.81
	400	39.17	53.92	55.45	62.67	63.25
Control (without fungicide)		0	0	0	0	0

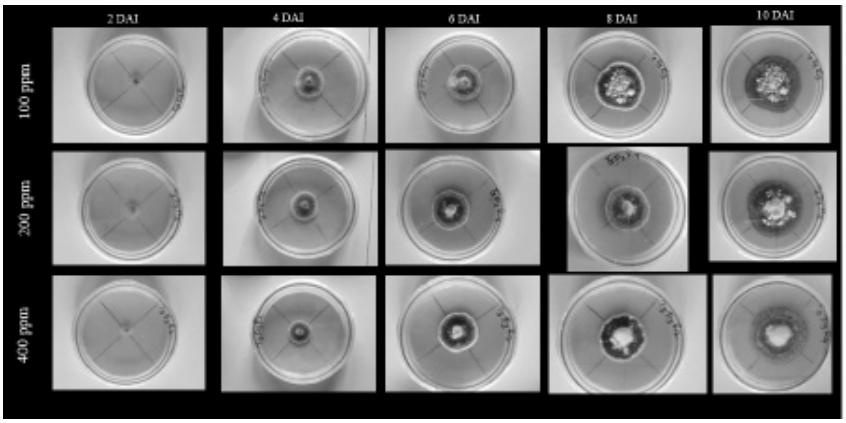

Figure 1. **Mycelial growth of *Bipolaris sorokiniana* at 100, 200 and 400 ppm carbendazim on 2, 4, 6, 8 and 10 days after inoculation**

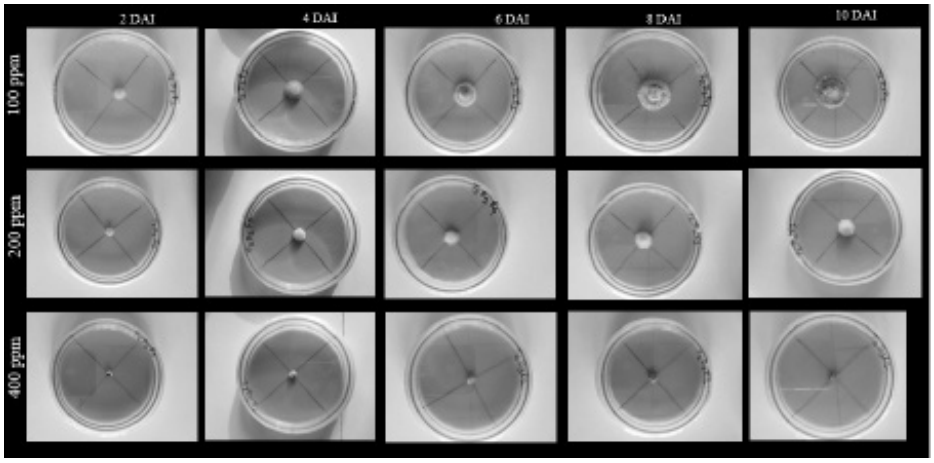

Figure 2. **Mycelial growth of *Bipolaris sorokiniana* at 100, 200 and 400 ppm copper oxychloride on 2, 4, 6, 8 and 10 days after inoculation**

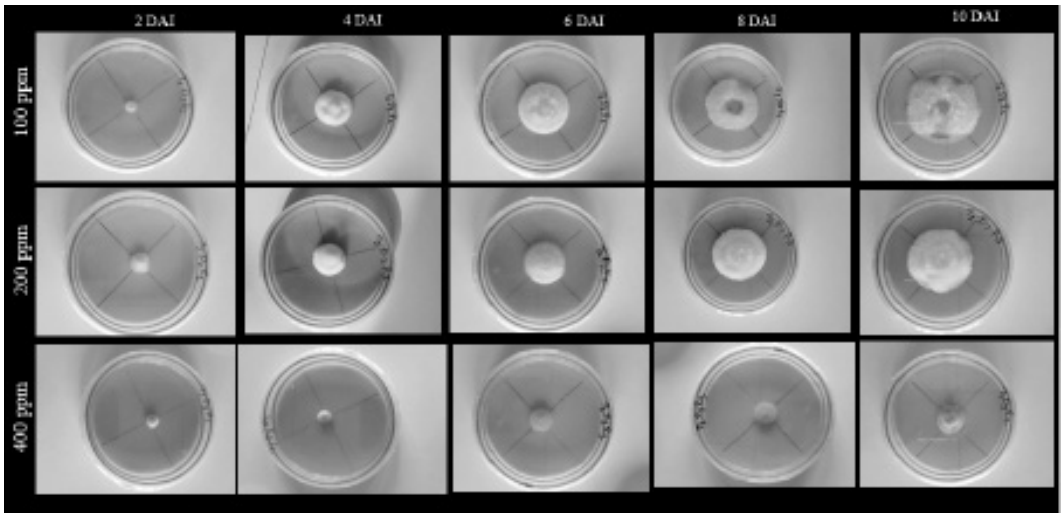

Figure 3. Mycelial growth of *Bipolaris sorokiniana* at 100, 200 and 400 ppm mancozeb on 2, 4, 6, 8 and 10 days after inoculation

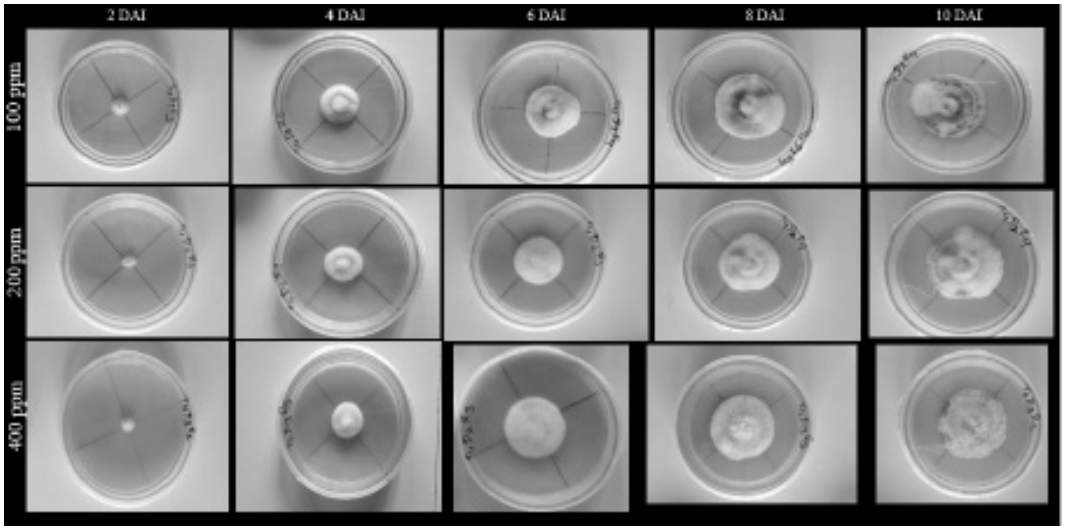

Figure 4. **Mycelial growth of *Bipolaris sorokiniana* at 100, 200 and 400 ppm Thiram on 2, 4, 6, 8 and 10 days after inoculation**

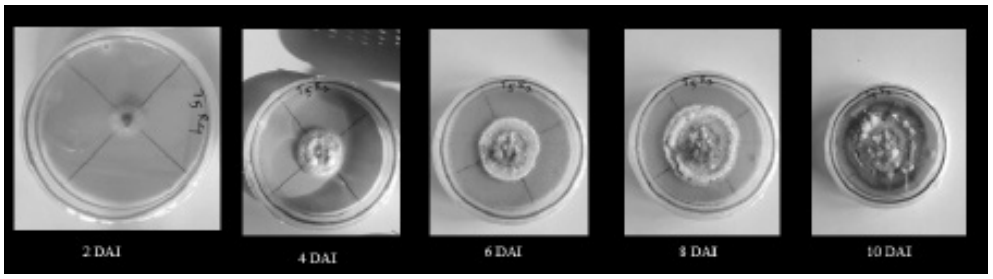

Figure 5. **Mycelial growth of *Bipolaris sorokiniana* in control plates (without fungicide) on 2, 4, 6, 8 and 10 days after inoculation**

DISCUSSION

The variations were observed in different concentrations of selected fungicides in mycelial growth of the pathogen. Among the selected fungicides, copper oxychloride (400 ppm) was the most effective against *B. sorokiniana*, the pathogen causing spot blotch of barley. Sharma (2006) reported that copper oxychloride was the most effective and had successfully inhibited the mycelial growth of the fungus *Bipolaris tetramera*. Samia et al. (2015) reported 70-80% mycelial growth inhibition in isolates of *Bipolaris sorokiniana*, collected from different region of Bangladesh, at 300 ppm concentration of copper oxychloride. 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).

From the results of our experiment and also as per the available literatures, it is known that with the increase in concentration of copper oxychloride the inhibition percentage is increased, but we were not able to get 100 percent inhibition even at 400 ppm. At 200 ppm the inhibition percentage was 70. Samia et al. (2015) also reported that the inhibition percentage at 300 ppm was 70-80 percent whereas inhibition percent at 400 ppm came to be 83 percent in our experiment.

Along with copper oxychloride, mancozeb at 400 ppm was also effective against the growth of mycelium of *B. sorokiniana*. The result was in agreement with the experiment reported by Hasan et al. (2012) in barley in which mancozeb at 400 ppm had resulted 70.83 percent inhibition in the mycelial growth of *B. sorokiniana*. Samia et al. (2015) also reported 34-62% radial growth inhibition of different isolates of *B. sorokiniana* at 300 ppm concentration. Giri et al. (2001) reported reduction of seed infection of *B. sorokiniana* by 90.5% with seed treatment by mancozeb. 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. Variation in the inhibition percentage of *B. sorokiniana* at different concentrations of mancozeb was reported in different experiments. There was 63 and 31 percent inhibition at 400 ppm and 200 ppm, respectively in our experiment whereas 70 percent inhibition at 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 *B. sorokiniana* and different quality of mancozeb used in these experiments and also could be due to different conditions for the growth of the fungus where the experiment was conducted.

In our experiment, carbendazim could not inhibit the growth of *B. sorokiniana*. This was in agreement with the research results reported by Kavita et al. (2017) as it was only 16.52 percent and 24.94 percent inhibition of the fungus at 500 and 1000 ppm concentrations of carbendazim, respectively. Giri et al. (2001) also reported the failure of carbendazim in the control of *B. sorokiniana*, but the finding did not agree with the report of Samia et al. (2015), as the authors had reported 100 percent and 77 percent mycelial growth inhibition at 300 and 200 ppm concentrations. There are some contradictions whether carbendazim can control the *B. sorokiniana* or not. Our results have clearly demonstrated that carbendazim does not inhibit *B. sorokiniana*. Farmers are using this fungicide against spot blotch in their fields and that should not be recommended.

Thiram has generally been reported effective against *B. sorokiniana* for seed treatment (Giri et al., 2001; Singh & Kumar, 2008). But we did not find it effective against the fungus under *in-vitro* evaluation. There could be some other unknown reasons behind this which we could not explain and it needs further investigation.

The recommended dose of above fungicide is generally 2-3 g (formulation rate) per litre water. Generally, if a fungicide at 100 ppm gives 100 percent in-vitro inhibition then the recommendation dose of the fungicide for application will be 1 g per litre water. Our results suggest that higher concentration (more than 400 ppm) is required for 100 percent inhibition even in the case of copper oxychloride. That means the dose of the fungicides may need to be increased for an effective management of the disease under field conditions. On the other hand if the dose of a fungicide is increased that can be phytotoxic. Fungicide such as copper oxychloride at higher concentration is toxic to plant because of copper ion. So a lot of work is required to find and recommend an effective and safe dose for management of diseases in crop plants.

CONCLUSION

From the results of this experiment, it can be concluded that copper oxychloride and mancozeb are effective against *B. sorokiniana* at higher doses. These fungicides are commonly available and used; and they are comparatively less hazardous. However, the results indicates that the current recommended doses of copper oxychloride (2 g/litre of water) and mancozeb (2-3 g/litre of water) for foliar application purposes may not be sufficient to manage the disease under field conditions. Also, mancozeb is recommended for seed treatment and the current recommended dose (2-3 g/kg seed) may need to be re-evaluated for effective control of seed-borne infection.

ACKNOWLEDGEMENT

Authors greatly appreciate and thank Nepal Polytechnic Institute for laboratory access and financial support.

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