Mycopath (2015) 13(2): 81-88 **Research Article**

Effect of fungicides against *Bipolaris sorokiniana* isolates collected from different wheat growing regions of *Bangladesh*

T. Samia, *S. Sultana, S. K. Adhikary and K. G. Quddus

Agrotechnology Decipline, Khulna University, Khulna, Bangladesh
Corresponding author's email: hure_jannat888@yahoo.com

Abstract

In vitro experiments were conducted in the Plant Pathology Laboratory, Agrotechnology Discipline, Khulna University, to evaluate the variable effects of some selected fungicides against some *Bipolaris sorokiniana* isolates collected from different regions of Bangladesh. Four fungicides namely mancozeb, carbendazim, propiconazoole and copper oxychloride was used in the study. No mycelial growth was found even at the lowest concentration of propiconazole after 7 days of inoculation in all isolates of *B. sorokiniana.* Application of 300 ppm of copper oxychloride, carbendazim and mancozeb decreased radial mycelial growth after 7 and completely inhibited at higher concentrations after 12 days of inoculation. The results revealed that inhibition percentages were increase with the increased in concentration of all fungicides used in this study.

Keywords: Fungicide, *Bipolaris sorokiniana*, different location, pathogenic variation.

Introduction

Wheat is the second most important crop cultivated in Bangladesh in rotation with rice (Meisner *et al.,* 1992; Siddique, 2002) and wheat production is increasing day by day. In the last 11 years wheat production was highest in Bangladesh, however per unit yield is much lower than expected and one of the important causes is occurrence of diseases (Siddique *et al.,* 2002; Sultana, 2012). Leaf spot/leaf blotch/leaf blight caused by *Bipolaris sorokiniana* (Sacc.) *Shoemaker*, teleomorph *Cochliobolus sativus* is the most important one. *B. sorokiniana* also causes seed rot, common root rot, seedling blight, head blight, black point of wheat and barley and reduces seedling emergence ultimately causes the yield loss of the subsequent crop (Kumar *et al.,* 2002; Malaker, 2007). The diseases caused by *B. sorokiniana* may cause yield loss up to 80% (Alam *et al.,* 1998) due to wide distribution of pathogen in the warm humid growing regions (Kulkarni and Nargund, 1986; Kumar *et al.,* 2002; Kendra *et al.,* 2006). The fungus multiply asexually (Malaker *et al.,* 2007), while sexual propagation is rare in nature or occur occasionally and it create genetic variation among *B. sorokiniana* isolates. Variability in the isolates of *B. sorokiniana*, both morphological (Mishra, 1981; Maraite *et al*., 1998) and pathological (Nelson, 1960; Marait *et al.,* 1998). The fungus is transmitted by seed (Sultana and Rashid, 2012) and attacks the necrotic tissue of wheat plant and develop oval to round brown blotch surrounded by yellow halo (Acharya *et al*., 2011). High temperature and high relative humidity favor the outbreak of the disease, in particular in South Asia's 'intensive irrigated wheat–rice production systems' rice favors the rapid multiplication of this foliar blight pathogens and rice stubble plays its role as a substrate for the fungi after rice harvest (Kumar *et al*., 2002).

Fungicide can be an effective agent to control this disease. They are chemical compound that controls fungal disease by specifically inhibiting or killing the fungus causing the disease (McGrath, 2004; Singh, 2009). Fungicides that have curative properties reduce disease severity and increase the productivity of crop. Cultural practices often do not provide adequate disease control and resistant cultivars are not available or not acceptable in mark, certain high value crops have an extremely low tolerance for disease symptoms. In those case using fungicide is the most suitable to fight against diseases. It effectively controls the sudden attack of any disease so epidemic can be avoided easily (McGrath, 2004). The isolates of *B. sorokiniana* collected from various cities of Bangladesh were examined for their sensitivity. The conventional breeding has little impact on cultivar development in wheat. Almost all the cultivars are derived from seedlings (Litz *et al.,* 1994). The problem is intensified due to lack of resistance in available local and exotic cultivars. *In vitro* studies against *B. sorokiniana* are barely sufficient and even precise information on determination of efficacy,

sensitivity types of fungicides with minimum inhibitory concentration have not been characterized against *B. sorokiniana* (Iqbal, 2010). Research is needed to control this disease. Chemical control is the most preferable one in respect of Bangladesh.

In view of the above facts, the present study was undertaken to evaluate the variable effects of some selected fungicides against some *B. sorokiniana* isolates collected from different regions of Bangladesh.

Materials and Methods

Collection, isolation, identification, purification of fungi

Infected wheat leaves containing typical symptom of spot blotch of *B. sorokiniana* were collected from different regions of Bangladesh in the month of February 2014. The basic Medium, PDA was prepared following standard procedure. The pathogen was isolated following tissue planting method (Mian, 1995). Then plates were incubated at 25 ± 2 °C for 4-5 days. Then a bit of mycelium was transferred another (PDA) plates for pure culture. Again the plates were incubated for 5 days at 25±2 °C for sporulation. Identification of *B. sorokiniana* was done and photograph was taken. (Plate 1). Purified isolates was obtained using the single spore method as stated by (Ruppel, 1972) with some modifications (Adhikary 2004). Single sporulated conidia were transferred onto PDA plates and incubated at 25 ± 2°C for 7 days. Then pathogenicity of each isolate was tested. Then test tube slants of monoconidial isolates of *B. sorokiniana* were prepared and stored in refrigerator at 4°C (Table 1).

Response of different fungicides to isolates of *B. sorokiniana*

Different fungicides were evaluated *in vitro* condition against *B. sorokiniana* following poison food technique (Dhingra and Sinclair, 1986). Fungicidal suspension of different concentration was prepared dissolving required quantities of fungicide in warm PDA using magnetic stirrer. With each treatment five replications were given. The entire process was done in aseptic condition and incubated at 25 ± 2 °C, and the growth of control plate was observed regularly. Information of the fungicide used in the study is given in Table 2.

Measurement of radial mycelia growth and growth inhibition of *B. sorokiniana*

Percent inhibition of growth was calculated using the following formula (Naz *et al.* 2006):

$$\text{Percent inhibition} = \frac{X-Y}{X} \times 100$$

Where, X=Average growth of the fungus in control Petri dishes; Y= Average growth of the fungus in fungicide treated Petri dishes

Measurement of sporulation inhibition of *B. sorokiniana*

For measuring sporulation twelve days old culture plate in each concentration was flooded with 10 mL of sterilized distilled water. Conidia along with mycelial mass were separated from the substratum by scrapping with a narrow edged sterilized glass slide. The suspension was sieved through double layer cheese cloth to discard mycelial mass. The volume was adjusted up to 100 mL using distilled water. It was then diluted 10 times by adding distilled water. The number of spore mL^{-1} was counted by using a Neubauer hemocytometer. Petri dish was replicated five times. Percent inhibition of sporulation was calculated.

Experimental design and data analysis

Both experiments were laid out under completely randomized design (CRD). Data were transformed by using Square Root Method (Zaman, 1982) for exactly analyzing the data avoiding statistical complication. The data were analyzed statistically using MSTAT-C computer program and means were compared for difference following Duncan's Multiple Range Test (DMRT).

Results

Effect of copper oxychloride against *B. sorokiniana* isolates

Maximum mycelial growth inhibition of 70-80% was observed at 300 ppm of copper oxychloride in case of all isolate of *B. sorokiniana*. After 12 days of inoculation, complete inhibition of sporulation was recorded in BS-1 and BS-5 due to effect of different concentrations. Complete inhibition was recorded at 100-300 ppm, 300 ppm and 200-300 ppm in BS-3, BS-2 and BS-4, respectively (Table 3). Regression equation between different concentrations of copper oxychloride and mycelial growth inhibition percentage revealed that more than 50% of the variations (Fig. 1). Likewise, regression equation between different concentrations of copper oxychloride and sporulation inhibition (%) revealed 36%, 68%, 65%, 71% and 36% of the variation with increase in concentration in the case

of BS-1, BS-2, BS-3, BS-4 and BS-5, respectively (Fig. 2).

Effect of propiconazole against *B. sorokiniana* isolates

After 7 days of inoculation no mycelia growth was found in all treated plate except control in all isolate of *B. sorokiniana*.

Effects of carbendazim against *B. sorokiniana* isolates

By applying carbendazim at different concentration 100% mycelial growth inhibitions was observed at 300 ppm in BS-2 and 75-77% in case of BS-1 and BS-5. So far 200 ppm was found effective for BS-3 and BS-4200 ppm. Counting of sporulation after 12 days of inoculation for all isolates showed no sporulation due to effect of 100-300 ppm concentration of the fungicides (Table 4). Regression analysis between different concentrations of carbendazim and mycelial growth inhibition % revealed over 43%, 63%, 64%, 66% and 44% of the variation in BS-1, BS-2, BS-3, BS-4 and BS-5, respectively with increased in fungicide concentration (Fig. 3). Regression assessment between various concentrations of copper oxychloride and sporulation inhibition (%) showed 60-70% of the variation due to increase of concentration (Fig. 4).

Effect of mancozeb against *B. sorokiniana* isolates

By using mancozeb at different concentration maximum mycelial growth inhibition was observed in 300 ppm in case of all isolate of *B. sorokiniana* [BS-1 (34 %), BS-2 (56.5 %), BS-3 (48 %), BS-4 (62 %), and BS-5 (30 %)]. Inhibition (%) was increase with the increase of concentrations of applying mancozeb. Counting of sporulation after 12 days of inoculation, maximum sporulation inhibition was found in 300 ppm in all isolates of *B. sorokiniana* [BS-1 (50.48 %), BS-2 (57.59%), BS-3 (46.14 %), BS-4 (41.33 %), and BS-5 (77.85 %)] over control (Table 5). Regression equation between different concentrations of mancozeb and mycelial growth inhibition % as well as spore inhibition % revealed that more than 85% variations in different isolates (Fig. 5 and 6).

Discussion

Pathogenic variations were observed with different concentration of each fungicide on different isolates**.** Again there were differences in temperature humidity in the area of collection isolates.

Higher concentration of copper oxychloride gave no sporulation in all cases and it may be effective to control pathogenic activity of *B. sorokiniana*. Sharma (2006) found, copper oxychloride inhibits mycelial growth mostly and successfully inhibits sporulation of *B. tetramera.* Complete inhibition of radial mycelial growth and sporulation of *B. sorokiniana* was found with Propiconazole, so it may be effective to complete control of pathogenic activity of *B. sorokiniana.* 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). Higher concentrations of Carbendazim inhibited the total redial mycelial growth and sporulation of all isolates of *B. sorokiniana.* Yamaguchi *et al.* (2010) observed severity of *Bipolaris sorokiniana* was very much less in media containing Carbendazim at low concentration and mostly suppressed at higher concentration. Giri *et al.* (2001) collected seed samples of different wheat varieties from *Bipolaris sorokiniana* leaf blight infected fields and found carbendazim failed to control seed borne infection. Mancozeb might be effective to control pathogenic activity of *B. sorokiniana* at higher concentration. Similarly, Hasan *et al.* (2012) found the highest control of the fungi due to application of Mancozeb (1.86 g). Sharma (2006) findings explored effectiveness of Mancozeb (Dithane M-45) against rot of *Coccinia indica* caused by *Bipolaris tetramera*. Giri *et al.* (2001) also demonstrated effectiveness of mancozeb (90.5%) to control infection of seeds caused by *Bipolaris sorokiniana*.

Acknowledgements

Authors greatly appreciate and thank S. Sultana, Dr. S. K. Adhikary and K. G. Quddus for their guidance and constructive criticism and plant pathology laboratory, Agrotechnology Decipline, Khulna University, Khulna, Bangladesh, lab assistant for supplement of different ingredients during the period of conducting this study.

Table 1: Isolates of *Bipolaris sorokiniana* used in the study.

Isolates	Regional Origin		Wheat Cultivars	Pathogenic Reaction	February Monthly average *		
	Districts	Thana			Maximum T (◦C)	Minimun T (◦C)	Humidity %
BS-1	Jessore	Sador	Sourov-64	+	29.5	14.4	72
BS-2	Kurigram	Vurungomari	Sonalika	+	26	12.7	75
BS-3	Gazipur	Joydebpur	Barigom-25	+	28.1	15.5	64
BS-4	Faridpur	Boalmari	Sonali	+	27.6	14.6	72
BS-5	Dinajpur	Sador	Triticale	+	26.4	12.5	70

BS = *Bipolaris sorokiniana*
+ = Pathogenic
*Bangladesh Meteorological Department

Table 2: List of fungicides that were used in the experiment

Chemical name	Mode of action	Trade name	Concentration(ppm)
Carbendazim	Systemic	Noin 50 Wp	50,100,200,300
Mancozeb	Contact	Diathen M-45 80WP	50,100,200,300
Propiconazole	Systemic	Sunconazole 250EC	50,100,200,300
Copper oxychloride	Contact	Sulcox 50wp	50,100,200,300

Table 3: Effect of copper oxychloride on radial mycelial growth inhibition and sporulation inhibition (%) of some isolates of *B. sorokiniana.*

Conc. (ppm)	BS-1		BS-2		BS-3		BS-4		BS-5	
	Radial growth inhibition %	Sporulation inhibition %	Radial growth inhibition %	Sporulation inhibition %	Radial growth inhibition %	Sporulation inhibition %	Radial growth inhibition %	Sporulation inhibition %	Radial growth inhibition %	Sporulation inhibition %
0	0 d	0	0 d	0 d	0 d	0 c	0 d	0 d	0 e	0
50	56.54 c	100	64 c	64 c	55 c	34 b	59 c	64 c	59.28 d	100
100	75.89 c	100	70 b	71.4 b	68 ab	100 a	67 b	72 b	73 c	100
200	74.53 b	100	70.2 b	70.4 b	65 b	100 a	68 b	100 a	76 b	100
300	82.40 a	100	76.6 a	100 a	72 a	100 a	73 a	100 a	81 a	100
CV%	2.88	0	3.43	1.29	6.66	0.82	3.76	0.18	2.4	0
	**	NS	**	**	**	**	**	**	**	NS

Level of significance at 1% level as **

Table 4: Effect of carbendazim on radial mycelial growth inhibition and sporulation inhibition percentage of some isolates of *B. sorokiniana.*

Conc. (ppm)	BS-1		BS-2		BS-3		BS-4		BS-5	
	Radial growth inhibition %	Sporulation Inhibition %	Radial growth inhibition %	Sporulation Inhibition %	Radial growth inhibition %	Sporulation Inhibition %	Radial growth inhibition %	Sporulation Inhibition %	Radial growth inhibition %	Sporulation Inhibition %
0	0 d	0 c	0 d	0 d	0 d	0 c	0 d	0 d	0 d	0 e
50	69.58 c	59.98 b	69 c	66.28 c	68.95 c	36.94 b	67.8 c	65.96 c	70.7 c	75.5 d
100	70.65 bc	100 a	77.7 b	67.48 c	81.7 b	100 a	78.8 b	74.84 b	72.9 bc	79.44 c
200	73.12 ab	100 a	77.6 b	79.24 b	100 a	100 a	100 a	100 a	75.5 ab	83.34 b
300	75.59 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	77.9 a	100 a
CV %	3.78	5.65	3.16	1.67	1.74	1.66	1.97	2.42	5.13%	0.45

Level of significance at 1% level

Table 5: Effect of Mancozeb on radial mycelial growth, inhibition percentage and sporulation of five isolates of *B. sorokiniana*.

Conc. (ppm)	BS-1		BS-2		BS-3		BS-4		BS-5	
	Radial growth inhibition %	Sporulation Inhibition %	Radial growth inhibition %	Sporulation Inhibition %	Radial growth inhibition %	Sporulation Inhibition %	Radial growth inhibition %	Sporulation Inhibition %	Radial growth inhibition %	Sporulation Inhibition %
0	0 c	0 e	0 d	0 e	0 d	0 e	0 d	0 e	0 d	0 e
50	0.27 c	19.81 d	4.2 d	12.71 d	7 c	9.51 d	5 d	9.2 d	2 d	3.32 d
100	7.3 b	32.42 c	10.7 c	24.44 c	11 c	27.06 c	15 c	16.87 c	8 c	3.316 b
200	9.8 b	43.13 b	31.2 b	47.94 b	18 b	39.87 b	26 b	21.25 b	12	22.73 c
300	34 a	50.48 a	56 a	57.59 a	48 a	46.14 a	62 a	41.33 a	30 a	77.85 a
CV%	23.12	2.41	17.11	2.11	26.5	2.85	23.87	4.01	21.9	1.57
Level of significance at 1% level										

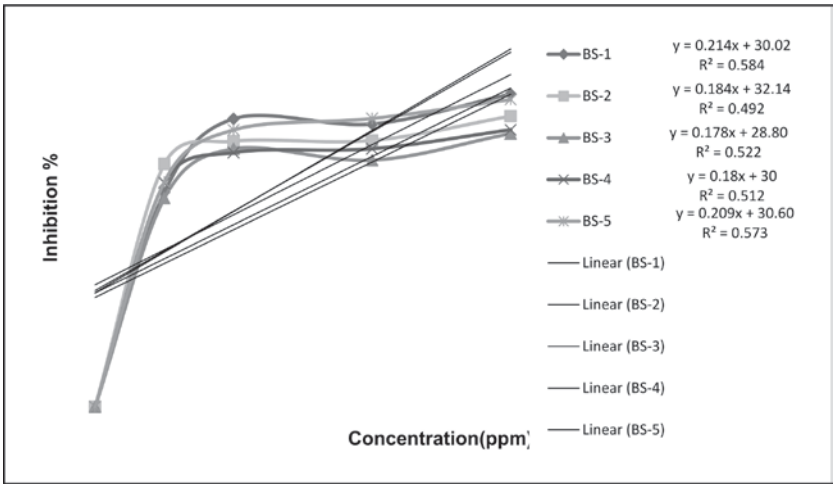

Fig. 1: Functional relationship between concentration and mycelial growth inhibition % by copper oxychloride.

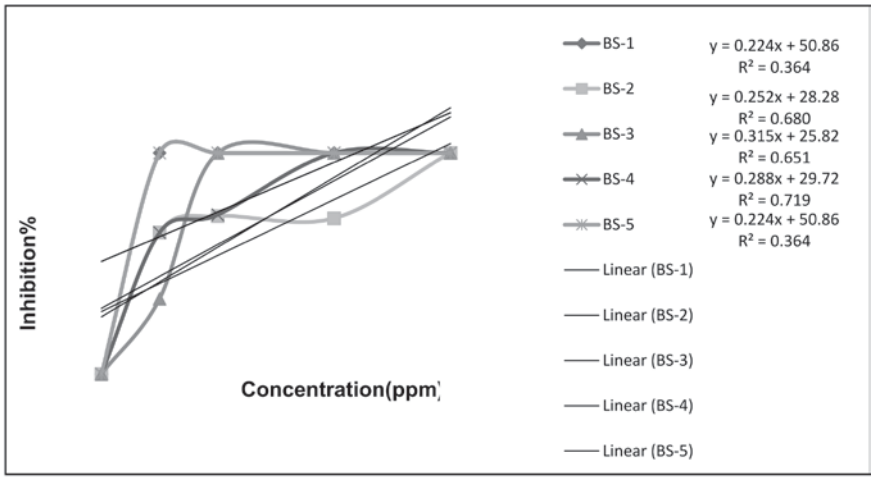

Fig. 2: Functional relationship between concentration and sporulation inhibition % by copper oxychloride.

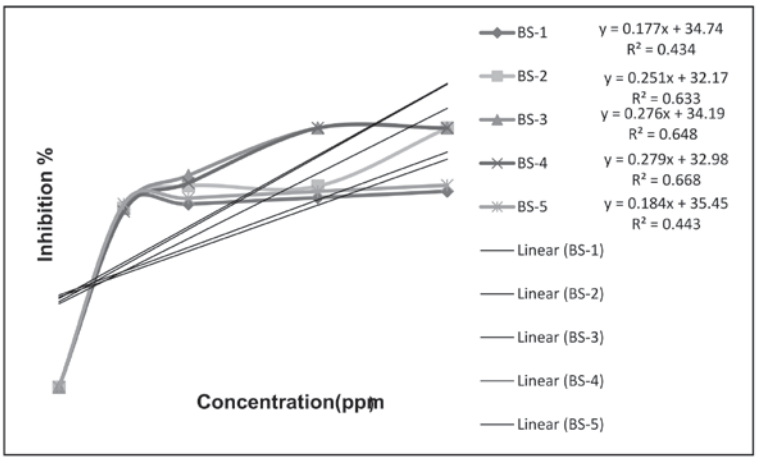

Fig. 3: Functional relationship between concentration and mycelial growth inhibition % carbendazim.

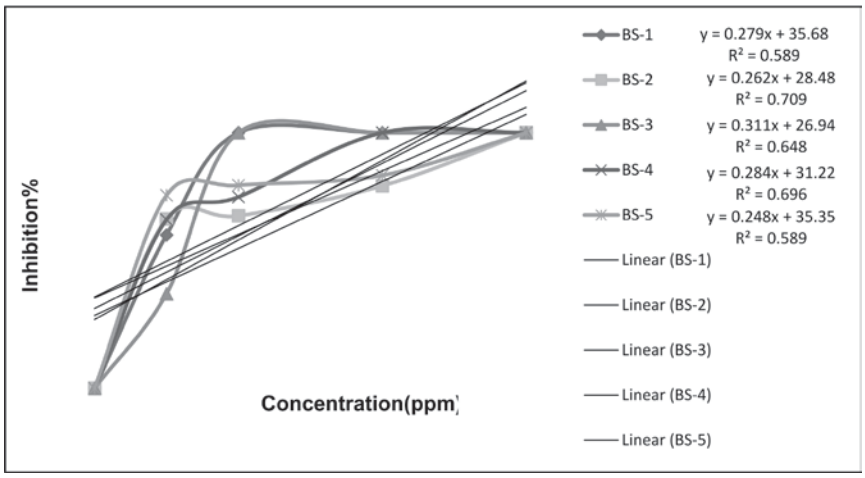

Fig. 4: Functional relationship between concentration and sporulation inhibition % carbendazim.

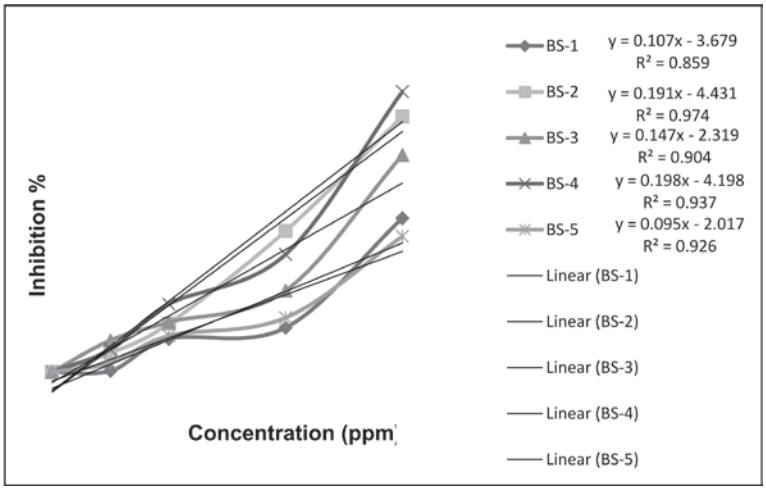

Fig. 5: Functional relationship between concentration and mycelial growth inhibition % by mancozeb.

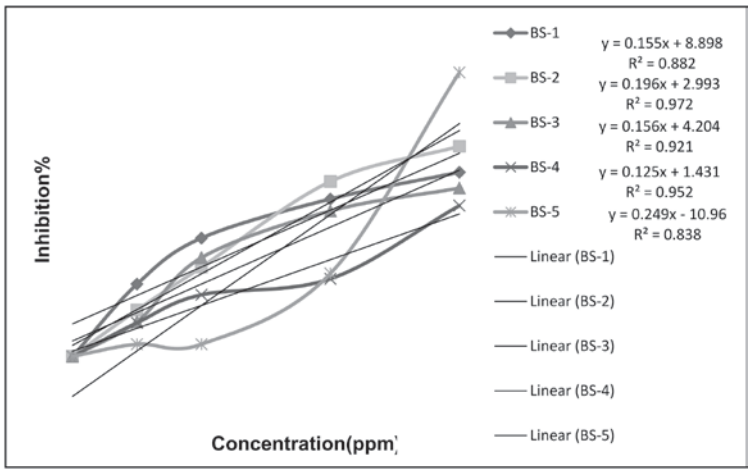

Fig. 6: Functional relationship between concentration and sporulation inhibition % by mancozeb.

References

Acharya K, Dutta AK and Pradhan P, 2011. *Bipolaris sorokiniana* (Sacc.) Shoem. The most destructive wheat fungal pathogen in the warmer areas. *Aust. J. Crop Sci*., **5**: 1064-1071.

Adhikary SK and Mian IH, 2004. Reaction of some plant species and rice germplasms to *Bipolaris sorokiniana. Indian J. Agric. Res*., **38**: 116-120.

Alam KB, Banu SP, Shaheed MA, 1998. Occurrence and significance of spot blotch in Bangladesh. Bangladesh Meteorological Department. Mexico, D.F. CIMMYT. p. 376.

Dhingra OD, Sinclair JB, 1986. Basic Plant Pathology Methods. CRC Press, Inc. Boca Raton, Florida. USA.

Giri GK, Gade RM, Patil CU, 2001. Seed borne *Bipolaris sorokiniana* in wheat and its chemical control. *J. Soils Crops*, **11**: 109-112.

Hasan MM, Ahmed F, Islam MR, Murad KFI, 2012. *In vitro* effect of botanical extracts and fungicides against Bipolaris sorokiniana, causal agent of leaf blotch of barley. J. *Agrofor. Environ*., **6**: 83-87.

Iqbal Z, Pervez MA, Ahmad S, Iftikhar Y, Yasin M, Nawaz A, Ghazanfara MU, Dasti IA, Saleem A, 2010. Determination of minimum inhibitory concentrations of fungicides against fungus *Fusarium mangiferae. Pak. J. Bot.*, **42**: 3525-3532.

Kendra RM, Heloiza MD, Moraes, Bach EE, 2006. Identification of *Bipolaris bicolor* and *Bipolaris sorokiniana* on wheat seeds *(Triticum aestivum* L.) in Brazil. *Braz. J. Microbiol*., **37**: 247-250.

Kulkarni S, Nargund VB, 1986. Soil borne disease of wheat. In Problems and Progress of Wheat Pathology in South Asia. Malhotra Publishing House, New Delhi. p. 216- 229.

Kumar J, Schafer P, Huckelhoven R, Langen G, Baltruschat H, Stein E, Nagarajan S, and Kogel K, 2002. Pathogen profile Bipolaris sorokiniana, a cereal pathogen of global concern: cytological and molecular approaches towards better control. *Mol. Plant Pathol.*, **3**: 185-195.

Litz RF, Ploetz RC, Zentmyer GA, Nishijima WT, Rohrbach KG, Ohr HD, 1994. Mango. In: *Compendium of tropical fruit diseases*. (Eds.). The APS Press, St. Paul, Minn., U.S.A.

Malaker PK, Mian IH, Md. Maniruzzaman K, Reza MMA, 2007. Survival of *Bipolaris Sorokiniana* (Sacc.) Shoemaker in soil and residue of wheat. *Bangl. J. Bot*., **36**: 133-137.

Maraite H, Dizinno T, Longree HV, Daumerie and Dueveiller E, 1998. Fungi association with foliar blight of wheat in warm areas. In: Duveiller, E., H. J. Dubin, J. Reeves, A. McNab(eds.):Helminthosporium blights of wheat: spot blotch and tan spot, Mexico, D. F. CIMMYT. p. 293-300.

McGrath MT, 2004. What are Fungicides. The Plant Health Instructor. DOI: 10.1094/PHI-I-2004-0825-01.

Meisner CA, 1992. Impact of Crop Management Research in Bangladesh. Wheat Special Report No.3. CIMMYT, Mexico, D.F.

Mian IH, 1995. Methods in Plant Pathology, IPSA-JICA project publication. Institute of

Postgraduate Studies in Agriculture, Gazipur, Bangladesh. No.24 Microspora spores. *J. Theoret. Biol.*, **142**: 223-235.

Mishra AP, 1981. Variability, physiologic specialization and genetics of pathogenesity in *Graminicolus helminthosporium* affecting cereal crops. *Indian Phytopath*., **34**: 1-22.

Naz F, Rauf CA, Haque IU Ahamed I, 2006. Management of *Fusarium oxysporum* with plant diffustates and chemicals. *Pak. J. Phytopathol*., **18**: 36-43.

Nelson RR, 1960. Evolution of sexuality and pathogenicity I. Inter specific crosses in the genus *Helminthosporium*. *Phytopathology*, **50**: 375-377.

Pannu PPS, Chahal SS, Sharma VK, Kaur M, Bagga PS, 2006 .Occurrence of brown leaf spot of rice in Punjab, its effect on grain yield and its control. *Indian Phytopathol.*, **59**: 190-193.

Rahman MM, Ali MS, Nahar A, Karim MM, Begum K, 2013. Efficacy of fungicides in controlling leaf blight of wheat. *Int. J. Expt. Agric*., **3**: 1-3.

Ruppel EG, 1972. Variations among isolates of *Cercospora beiticola* from sugarbeet. *Phytopathology*, **62**: 134-136.

Sharma V, 2006. Chemical control of an unrecorded rot of *Coccinia indica* caused by *Bipolaris tetramera*. *Indian Phytopathol*., **59**: 161-163.

Siddique MAKS, Rashid AQMB, Hossain I, Khalequzzaman KM, Uddin MK, 2002. Reaction of some wheat varities to seed borne *Bipolaris sorokiniana* and *Fusarium moniliforme. Pak. J. Biol. Sci*., **5**: 1211-1213.

Singh RS, 2009. Plant Diseases. Oxford and IBH Publishing CO. PVT. LTD. New Delhi, Chapter 16. Plant Disease Management. p. 643-660.

Sooväli P, Koppel MP, 2009. Efficiency of fungicides tebuconazoe in barley varieties with different resistance level. *Agric. Food Sci*., **19**: 34-42.

Sultana A, Rashid AQMB, 2012. Effect of seed category as affected by *Bipolaris sorokiniana* on the germination of wheat Seeds. *J. Environ. Sci. Nat. Resour.*, **5**: 113-116.

Yamaguchi K, Mutsunobu M, 2010. A simple selective medium for the primary isolation of Bipolaris, Drechsleraand Exserohilumspecies. *Bull. Minamikyusu Univ. Japan*., **40**: 55-58 .

Zaman SMH, Rahim K, Howlader, 1982. Simple Lessions from Biometry. Bangladesh Rice Research Institute. Dhaka, Bangladesh.