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INTERACTIVE INFLUENCES BETWEEN SPOT BLOTCH PATHOGEN AND SAPROPHYTIC FUNGI OF WHEAT AT THE MOST HOT SPOT LOCATION OF SOUTH ASIA

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Abstract

Micro-organisms living on wheat phylloplane exert different kinds of interactions with each others. This study was conducted to find out the nature of interactions existing between spot blotch causal organism [*Bipolaris sorokiniana* (Sacc.) Shoem.] and well adapted saprophytic fungi of wheat (*Triticum aestivum* L.) leaves at the most hot spot location of spot blotch disease. Four most abundant saprophytic fungi were identified and evaluated for their interactions with *B. sorokiniana*. Pure cultures of selected fungi were procured from the spot blotch Infected wheat leaves following the single spore isolation method. Separate *in-vitro* dual culture studies in completely randomized design were carried out to assess the interactions between each pair of *B. sorokiniana* and selected rival fungus. The effects of interaction on radial growth of both fungi were measured and percent growth inhibition was calculated. Morphological study, viability test of the mycelium and sporulation ability of the fungi at the interface of dual culture were carried out. *B. sorokiniana* significantly inhibited the growth of *Alternaria alternata* and *Nigrospora* sp. in dual culture. In contrast, the interactions of *B. sorokiniana* with *Epicoccum* sp. and *Curvularia* sp. resulted in the suppression of mycelial growth of both fungi of each pair. The rates of growth inhibition during 1st and 2nd weeks of interaction were different in some tested fungi. The hyphael morphology of both fungi at the interface was normal in all pairs of fungi. The mycelia of all the fungi were viable at the interface of dual culture with *B. sorokiniana*. The sporulation ability of the tested fungi wasn't affected by the interactions with *B. sorokiniana* except the *Nigrospora* sp. The well adapted saprophytic fungi of wheat leaves interact differently with spot blotch fungus *B. sorokiniana.*

Key Words: Wheat, *Bipolaris sorokiniana*, Spot blotch, Interactions, Saprophytes.

Introduction

Bipolaris sorokiniana [teliomorph; *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur] is a causal agent of spot blotch disease. Spot blotch is the most dominant and one of the economically most important diseases of wheat in all the warm wheat growing areas of south Asia (1). It occurs every year in moderate to severe form causing yield losses up to 25% in many regions of indo-gangetic plains including Nepal (2, 3). The recurrent epidemics of spot blotch disease in South Asia is due mainly to the lack of durable resistant genotypes, high cost of fungicides and less effective available cultural control measures (3,4,5). Use of available resistant genotypes, seed treatment and foliar sprays with triazole fungicides reduce the disease severity (6, 7); however, except resistant genotypes, the other methods are not practicable for poor and marginal farmers of South Asia.

The micro-organisms existing on phylloplane of plants exert various kinds of interactions with each others (8, 9). Several pathogenic and non-pathogenic micro-organisms reside on wheat phylloplane (10, 11, 12). Some interaction studies revealed the presence of synergistic or antagonistic or neutral interactions between various micro-organisms of wheat phylloplane (13, 14, 15, 16, 17). Saprophytes *Alternaria alternata* and *Cladosporium herbarum* had suppressed the mycelial growth of *Fusarium culmorum* of wheat *under both in-vitro* and *in-vivo* conditions (18). Similarly, some saprophytes were effective against obligate parasites of wheat. For example, *Cladosporium tenuissimum* reduced both the number and the longevity of wheat rust spores (19), and sterile fungus strain, Kyu-W63, isolated from wheat leaves inhibited the growth of wheat powdery mildew fungus *Erysiphe graminis tritici* (20). Likewise, *Chaetomium* sp., *Paecilomyces* sp. and some other saprophytes possessed antagonistic ability against spot blotch pathogen *B. sorokiniana* (21, 22, 23*);* however, detail studies on mutual and reciprocal relationships between predominant saprophytic fungi of wheat leaf and *B. sorokiniana* are still deficit.

Interactions between *B. sorokiniana* and major saprophytic fungi growing together in the same leaf may influence the development of spot blotch

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disease. The knowledge on existing interactions between *B. sorokiniana* and well adapted saprophytic fungi of wheat leaves will serve as a marker in the development of biological control measures for spot blotch disease, because the adaptability of the antagonists on plant system is a crucial hitch for several antagonists. Therefore, this study was conducted to partially depict the interactions present between *B. sorokiniana* and well adapted saprophytic fungi of wheat leaves at the most hot spot location of spot blotch disease (24).

Materials and methods

Observation of prevalence and Isolation of major saprophytes

Two hundred spot blotch infected and blighted wheat leaf samples were randomly collected from different wheat genotypes grown at National Wheat Research Center, Bhairahawa, Nepal during January to March, 2009. The fresh leaf samples were kept in Petri plates containing 3 layered moistened filter papers. The Petri plates were then incubated at 25 ± 1^0 Celsius temperatures for seven days. Saprophytic fungi having high Intensity (frequency in tested samples) and severity (density of saprophytes on each sample) on tested wheat samples were observed and recorded, and of which the four most abundant saprophytes were selected for the study. Pure cultures of selected saprophytes were prepared through single spore isolation method. In which, few spores of the saprophytes grown on incubated leaf samples were carefully taken with the help of a needle and transferred to plain agar plates. The spores were gently dispersed on plane agar with the help of a L shaped glass rod. The plates were then incubated at 25 ± 1^0 Celsius temperatures for 24 hours. Single germinating spore with a small disk of plane agar was cut and transferred to PDA slants.

Dual culture of B. sorokiniana and the selected saprophytes

Separate *in-vitro* dual culture studies as suggested by (25), were carried out to assess the interactions between each pair of selected saprophyte and *B. sorokiniana*. Each study having 4 treatments was conducted in Completely Randomized Design (CRD) with five replications. The treatments were: i) Contender saprophyte in dual culture with *B. sorokiniana* ii) Contender saprophyte (sole culture) *iii) B. sorokiniana* in dual culture with contender saprophyte, *and iv) B. sorokiniana* (sole culture)

Seven mm diameter mycelial plugs taken from the growing edges of one week old pure cultures of *B. sorokiniana* and the selected saprophytic fungi were inoculated in Petri plates having potato dextrose agar (PDA). The two plugs were placed with up side down position on opposite sides in a 90 mm diameter Petri-dish at equal distance from each other and from the periphery.

All the inoculated Petri-dishes were incubated at 25 ± 1^0 Celsius temperatures. The interactions between each saprophyte and *B. sorokiniana* were studied and radial growths of *B. sorokiniana* and contender saprophytic fungi were measured at seven and 14 days of incubation with the help of a scale (mm). Percent growth inhibition in radial growth of *B. sorokiniana* and rival fungi was calculated using the following formula of (26):

$$Percent\ inhibition = \frac{colony\ radius\ of\ tested\ fungi\ (alone) - colony\ radius\ of\ fungi\ (in\ presence\ of\ contender)}{colony\ radius\ of\ tested\ fungi\ (alone)} \times 100$$

Effects of interaction on morphology and viability of the mycelium

After 14 days of incubation, pieces of disc from the interactive zone of each pair of dual cultures were cut and transferred to glass slide to observe and examine under a microscope. The physical interactions between hypha of two fungi were observed.

To examine the viability of mycelium, 10 small mycelial discs of each fungus from the margin of interface of each pair of fungi were taken after 14 days of incubation. These discs were transferred to plane agar plates and viability was examined under a microscope by evaluating the growth and development of hypha and sporulation of fungi after ten days of incubation.

Effects of interaction on sporulation of fungi

The experiment was conducted in completely randomized design with five replications. The dual culture and control plates were incubated for three weeks at 25 ± 1^0 Celsius temperatures. Sixteen hours dark and eight hours light regime was maintained during the incubation period. After three weeks, 11 mm diameter plugs of *B. sorokiniana* and contender saprophytes were cut from the interface of two fungi. The plugs were also obtained from control plates. Each plug was transferred to separate test tubes containing one ml sterilized distilled water, and the test tubes were shaken for 10 minutes to make suspension of spores. A small drop of the suspension was mounted on a haemocytometer and numbers of spores were counted.

Data Analysis: Normal colony growth data were analyzed directly using AOV. The percent data were transferred in to arc sign values and analysis of variance (AOV) was performed (27). Means were compared using least significant differences.

Results and discussion

Major saprophytic fungi of wheat leaf

The intensity and severity of major saprophytic fungi on tested leaf samples are presented on Table 1. The pathogen *B. sorokiniana* was found in all the samples with very high severity. The result agrees with previous workers, who disclosed that *B. sorokiniana* is the most dominant pathogen of wheat in warm areas of South Asia (1). Saprophyte *Alternaria alternata* prevailed on wheat leaves with the highest intensity (92.5%) and with very high severity than the other tested saprophytes. *Epicoccum* species prevailed with slightly lower frequency (88.5%) and severity than the *Alternaria alternata*. *Curvularia* species were found on 71% leaf samples with moderate severity in each prevailed sample. The intensity (48.5%) and severity of *Nigrospora* species were low as compare to the other selected saprophytes. The results reveal that *Alternaria alternata* and *Epicoccum* spp. are the most dominant saprophytes of wheat leaves based on their frequency and severity. Similarly, the result suggests that *Curvularia* spp. is a frequent, but moderately prevailing saprophyte of wheat leaves. Likewise, the results signify that *Nigrospora* spp. is comparatively a less common saprophyte of wheat as compare to the other procured saprophytes.

Table 1. *B. sorokiniana* and major saprophytic fungi with their intensity on wheat leaf samples and severity on single leaf at Bhairahawa, Nepal during 2009.

S/N	Name of the fungi	Frequency of fungi	Percent frequency	Mean severity on each leaf
1	*Bipolaris sorokiniana*	200	100.0	Very high
2	*Alternaria alternata*	185	92.5	Very high
3	*Epicoccum* species	177	88.5	High
4	*Curvularia* species	142	71.0	moderate
5	*Nigrospora* species	97	48.5	low

Interaction between Bipolaris sorokiniana and major saprophytes

The effects of interaction on radial growths of *B. sorokiniana* and its contender saprophytic fungi are summarized on Table 2. There were significant ($p<0.05$) inhibitions in colony growth of either fungus of a pair or both fungi of a pair in dual cultures. Similarly, the extents of interaction between each pair of fungi during the 1st and 2nd week of incubation periods are summarized in Table 3. This is the first interaction study between spot blotch fungus *Bipolaris sorokiniana* and major foliar saprophytic fungi of wheat prevailed on the most hot spot location of spot blotch disease.

Table 2. Mean radial growth of Bipolaris sorokiniana and selected foliar saprophytes of wheat in in-vitro interaction studies conducted at Bhairahawa, Nepal in 2009

S/N	Pair of fungi in dual culture	Radius of fungal colony growth, mm			
		1st week		2nd week	
		Sole culture	Dual culture	Sole culture	Dual culture
1	*B. sorokiniana* +	16.40	13.60	19.20	16.40
	A. alternata	23.80	17.40**	41.20	21.00**
2	*B. sorokiniana* +	15.40	10.80**	21.60	11.80**
	Epicoccum sp.	13.60	12.90	26.20	15.60**
3	*B. sorokiniana* +	15.00	11.00**	19.00	12.20**
	Curvularia sp.	19.80	15.80**	25.60	19.75**
4	*B. sorokiniana* +	16.00	14.60	20.80	21.10
	Nigrospora sp.	27.60	8.40**	35.80	9.20**

For the means with in a horizontal line of each week's observation, the double asterisks ** indicate significance at 1%

Interactions between B. sorokiniana and Alternaria alternata

There was a significant inhibition ($p<0.01$) in mycelial growth of *Alternaria alternata* due to interactions with *B. sorokiniana* in dual culture, but the inhibition in mycelial growth of *B. sorokiniana* due to interactions with *Alternaria alternata* was non-significant (Table 2). The significantly lower radial growth of *Alternaria alternata* on dual culture indicates the antagonistic ability of *B. sorokiniana* over *Alternaria alternata*. Similarly, the significant ($p<0.01$) antagonistic ability of *B. sorokiniana* in both weeks indicates that the antagonistic ability become active in early stage of dual culture and remain active during the whole periods of dual culture. However, despite the antagonistic effect of *B. sorokiniana, Alternaria alternata* is the most dominant saprophyte of wheat foliages (Table 1).

On the other hand, the non significant ($p<0.05$) differences between radial growth of *B. sorokiniana* on dual culture and the radial growth of *B. sorokiniana* in control plates indicate the absence of any antagonistic effect of *Alternaria alternata* on growth of *B. sorokiniana*. The result differs from the findings of some previous workers, who revealed the antagonistic ability of *Alternaria alternata* on *Plasmopara viticola* and *Fusarium* species (18, 28).

Interactions between B. sorokiniana and Epicoccum sp.

There were significant inhibitions ($p<0.01$) in mycelial growth of both *Epicoccum* sp. *and B. sorokiniana* due to interactions with each other in dual culture (Table 2). The significantly ($p<0.01$) lower radial growth of both fungi on dual culture indicates the presence of mutual antagonism between the two fungi. Similarly, the non significant antagonistic ability of *B. sorokiniana* against *Epicoccum* sp. in 1st week and significant antagonistic ability on 2nd week suggests that the antagonistic ability of *B. sorokiniana* against *Epicoccum* sp. activates either after 1st week of growth period or increased rapidly as the colony become matured. The results accord with the outcomes that *Epicoccum* species have antagonistic ability against *Pyrenophora tritici repentis* (29) and *Sclerotinia sclerotiorum* under natural conditions (30, 31).

Epicoccum species are the second most dominant saprophytes of wheat leaves, and are highly abundant on wheat phylloplane during whole crop season (Table 1). It signifies the high adaptability of the fungi on wheat foliages, and high tolerability against the changing environmental conditions within a season. These characteristics indicate its high potential to be used and developed as a bio-control agent of *B. sorokiniana*.

Interactions between B. sorokiniana and Curvularia sp.

There were significant inhibitions ($p<0.01$) in mycelial growth of both *Curvularia* sp. *and B. sorokiniana* due to interactions with each other in dual culture (Table 2). The significantly ($p<0.01$) lower radial growths of both fungi on dual culture indicate the presence of mutual antagonisms between the two fungi. The significant mutual antagonisms between two fungi on both 1st and 2nd weeks of dual culture suggest that the antagonistic ability is initiated at early growth periods and remain active as the fungi become matured. The result clears that *Curvularia* sp. has antagonistic ability against *B. sorokiniana;* however, (32) reported absence of any antagonistic effect of *Curvularia lunata* on a fungus *Sclerotium rolfsii*.

Interactions between B. sorokiniana and Nigrospora sp.

There were significant ($P<0.01$) reductions in radial growth of *Nigrospora* sp. in dual culture with *B. sorokiniana* as compare to its sole culture (Table 2). The significantly lower radial growth of *Nigrospora* sp. in dual culture than that in control plates indicates the strong antagonistic ability of *B. sorokiniana* on *Nigrospora* sp. under *in-vitro* conditions.

The lower frequency and severity of *Nigrospora* sp. on spot blotch infected wheat leaves may be partially due to the strong antagonistic ability of *B. sorokiniana* (Table 1). On the other hand, the non significant differences in radial growth of *B. sorokiniana* on dual culture and on control plates indicate the lack of any antagonistic effects of *Nigrospora* sp. on growth of *B. sorokiniana*. The result doesn't accord the previous findings of (21), who revealed that *Nigrospora sphaerica* had strong inhibitory effects against *B. sorokiniana*.

Table 3. Mean percent growth inhibition of selected fungi in the 1st and 2nd weeks of interactions in *in-vitro* studies conducted at Bhairahawa, Nepal in 2009

S/N	Pair of fungi in dual culture	Mean percent growth inhibition (arc sine value)	
		1st week	2nd week
1	*B. sorokiniana* +	18.6 (15.37) †	16.8 (13.46)
	A. alternata	30.9 (26.64)	44.2 (48.60)
2	*B. sorokiniana* +	29.40 (29.02)	40.10 (42.91)
	Epicoccum sp.	10.10 (5.05)	39.50** (40.47)
3	*B. sorokiniana* +	30.98 (26.55)	36.13 (34.98)
	Curvularia sp.	26.36 (19.95)	28.38* (22.65)
4	*B. sorokiniana* +	8.50 (1.70)	2.34 (-2.72)
	Nigrospora sp.	56.70 (69.71)	59.50 (74.09)

Within a horizontal line the single asterisk * indicates significance at 5% and double ** indicate significance at 1%

† The figures in parenthesis are actual percentage data

Percent growth inhibition and ages of colony

There were significant differences ($P<0.05$) in percent growth inhibition between one- and two-week old colonies of *Epicoccum* sp. and *Curvularia* sp. in the dual culture with *B. sorokiniana* (Table 3). The significantly different percent growth inhibitions

In contrast, there were non significant differences in percent growth inhibition between one- and two-week old colony of *Alternaria alternata* and *Nigrospora* sp. in dual culture with *B. sorokiniana* (Table 3). It point outs that the extent of inhibitory effect of *B. sorokiniana* on these two fungi remains equal through out the growth period, and is unchanged due to the age of colony.

Microscopic observation and viability of hypha

The hyphae of both fungi of each pair were not touched by each other until 14th day in the dual culture. The hyphae of tested fungi at interactive zone in dual culture was intact and no physical assail on hypha of one fungus by another fungus was observed under a microscope. The mycelial viability of none of the tested fungi was lost at the interface areas in dual culture. The significant inhibition in growth without direct contact of mycelia suggests that the prevailed antagonisms may be due to the production of inhibitory substances by the

in the two weeks suggest that the extent of inhibitory effects of *B. sorokiniana* on these two fungi differs in different ages. Similarly, the higher inhibition in 2nd week as compare to the 1st week suggests that the extent of antagonistic ability is increased as the fungal colonies matured.
fungi or due to the competition for nutrients or both (33). However, the mechanisms of inhibition in colony growth of the tested fungi were not addressed in our studies.

Effect of interactions on sporulation of the tested fungi

The effect of interactions on sporulation of either fungi of a dual culture is presented in Table 4. There were non significant ($p<0.05$) differences in number of spores of *B. sorokiniana* between sole culture and dual culture with *A. alternata, Curvularia* sp. *and Nigrospora* sp. The results specify that *A. alternata, Curvularia* sp. *and Nigrospora* sp. could not influence on sporulation of *B. sorokiniana*. However, the spores of *B. sorokiniana* were significantly ($p<0.01$) lower in dual culture with *Epicoccum* sp. than in the sole culture. The result reveals that *Epicoccum* sp. inhibits the sporulation of *B. sorokiniana* under *in-vitro* conditions

Table 4. Sporulation of *B. sorokiniana* and major saprophytic fungi of wheat leaf under *in-vitro* conditions at Bhairahawa, Nepal in 2009

S/N	Pair of Fungi	Mean spore production, '000 number of spores/ml	
		Sole culture	Dual culture
1	*B. sorokiniana* +	100.00	84.40
	A. alternata	210.80	173.20
2	*B. sorokiniana* +	65.20	27.60**
	Epicoccum sp.	0.00	0.00
3	*B. sorokiniana* +	81.20	62.00
	Curvularia sp.	59.20	46.80
4	*B. sorokiniana* +	67.60	58.80
	Nigrospora sp.	3.60	15.20*

For the means with in a horizontal line, the single asterisk * indicates significance at 5% and double asterisks ** indicate significance at 1%*

Similarly, the number of spores of *Nigrospora* sp. was significantly ($P<0.05$) higher in dual culture with *B. sorokiniana* than in sole culture. The result suggests that *B. sorokiniana* enhances the sporulation of *Nigrospora* sp. The reasons of enhanced sporulation were not addressed in our work. However, the higher sporulation of *Nigrospora* sp. might be due to the limitation of nutrients in dual culture with *B. sorokiniana*, because sporulation of some fungi is enhanced under low nutrients conditions (34). Likewise, the non significant differences in number of spores of *Curvularia* sp., *A. alternata* and *Epicoccum* sp. between their sole and dual culture with *B. sorokiniana* point out that *B. sorokiniana* can't influence on the sporulation of these fungi under *in-vitro* conditions.

Conclusion

Spot blotch disease causal organism *B. sorokiniana* interacts differently with the well adapted saprophytes of wheat leaves. *Alternaria alternata*, *Epicoccum* species, *Curvularia* species and *Nigrospora* species are the major and well adapted saprophytes of wheat leaves. *B. sorokiniana* exerts antagonistic relationship with densely prevailed saprophyte *Alternaria alternata* and sparsely prevailed saprophyte *Nigrospora* sp. Similarly, *Curvularia* sp. hinders only the mycelial growth of *B. sorokiniana*, whereas *Epicoccum* sp. hinders both the mycelial growth and sporulation of *B. sorokiniana*. *Epicoccum* species and *Curvularia* species abundantly present and naturally adapted on wheat leaf surface, so they could be exploited in development of bio-control strategies against *B. sorokiniana.* However, the enhancement in their efficiency and verification under field conditions are must before such applications in the field.

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