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Impact of Fungicides on *Bipolaris sorokiniana* causing Spot Blotch Disease of Wheat under *in Vitro* and *in Vivo* Condition

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ABSTRACT: Spot blotch of wheat is caused by *Bipolaris sorokiniana* and it is considered as one of the emerging disease. They affect the crop at any stage right from beginning to maturity of cropand cause 25 to 35 per cent loss in yield. In present study efforts were made to find out suitable management measures through various chemicals. Under *in vitro* condition, propiconazole and tebuconazole gave 100 per cent growth inhibition of *B. sorokiniana* even at lower concentration (25 ppm). Azoxystrobin, thiram and carboxin were also found effective as compared to control Under field condition, ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by secondspray at 20 days interval (two sprays) proved to be best in reducing spot blotch disease at harddough stage. Maximum seed germination per cent (96.00%) was recorded in three treatments*viz.*, ST by thiram + carboxin @ 2.5 g/kg of seed, ST by thiram + carboxin @ 2.5 g/kg of seed + FS sprays of propiconazole @ 0.1% (one) at boot leaf stage on Flag-1 leaf and ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second spray at 20 days interval (two sprays) followed (93 %) by ST by thiram @ 3 g/kg of seed. Maximum disease incidence (14%) was recorded in control while minimum disease incidence (5%) was recorded in ST by thiram + carboxin @ 2.5 g/kgof seed + FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf followed by second spray at 20 days interval (two sprays).

Keywords: Spot blotch, fungicides, propiconazole, tebuconazole, *Bipolaris sorokiniana.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered as one of the most widely grown and consumed food crop of the world. Presently, it provides 20 per cent of food calories to mankind in the world and it is staple food for nearly 40 per cent of world population (Pingali, 1999). There are several constraints to the world production of wheat. The crops suffers from at least 17 different diseases in the country (Ahmed and Hossain, 1985), fungi cause twelve of them. Among the fungal diseases, five are considered economically important because of their damaging nature and wide distribution throughout the wheat growing areasof the country. These diseases are spot blotch, foliar leaf blight, seedling blight, leaf rust, foot and root rot and black point of wheat, of which spot blotch is economically most important one (Ahmed and Hossain, 2005). Bipolaris leaf spot/blight caused by *Bipolaris sorokiniana* (Syn. *Cochliobolus sativus*) is the major and devastating disease of wheat in India (Hossain and Azad, 1992). The pathogen is considered to contribute significantly in lowering average yield of cereals in many developing countries and is one of the constraintsfor crop in warmer growing areas (Bhatti, 1988). Zhang and Yuen, (1999) assessed the lossof yield of wheat in China and found that grain yield of wheat reduced significantly by spotblotch. Not a single cultivar in the country is found to free from this disease (Hossain and Azad, 1992). The yield loss in wheat due to spot blotch disease in the country has been reported to the country due to 20% in variety Sonalika, 14 and 8% in Akbar and Kanchan, respectively (Razzaque and Hossain, 1991). In farmers field the yield loss was estimated to 14.97 per cent (Alam *et al.*, 1995). This disease reduced yield up to 40% in field conditionbut yield reduction of wheat due to spot blotch caused by *Bipolarisis sorokiniana* 57 and 65% in Kanchan and Solanika varieties, respectively (Hossain *et al.*, 1998). So, we have needed to know the effective fungicides to manage the disease.

MATERIALS AND METHODS

The *in vitro* experiments were conducted at PG laboratory of the Department of Plant Pathology and Field experiments were conducted in Rabi season in the year 2017 at Bihar Agricultural University farm, Sabour that is situated at an altitude of 45 meter above mean sea

level, lies between 24°30" and 25°30" at North latitude and 86°3" and 87°3" East longitude to find out the effective fungicides for effective management against *Bipolarisis sorokiniana*.

For *in vitro* experiments

Experiments on the screening of fungicides were tested against *B. sorokiniana*, under laboratory conditions by employing Poisoned Food Technique (Sharvelle, 1961). Stock solution of each treatment (fungicides) was prepared by using following formula:

$$C_1V_1 = C_2V_2$$

Where, C_1 = Concentration of stock solution (gm/ml), C_2 = Desired concentration (gm/ml), V_1 = Volume (ml) of the stock solution to be added and V_2 = Measured volume (ml) of the PDA medium.

For *in vitro* experiments

Under field conditions, the disease data was recorded in three stages (flowering, dough and hard dough) from randomly selected 25 plants from each plot tagged. So, 25 plants plot-1 were tagged for disease rating using the double-digit (dd) scale.

In vitro* effect of different fungicides against *B. sorokiniana

Efficiency of six different fungicides *viz.*, Azoxystrobin, Carboxin, Propiconazole, Tebuconazoze, Mancozeb, Thiram against mycelial growth of *B. Sorokiniana* was studied under *in vitro* condition by Poisoned Food Technique (Sharvelle, 1961) at three concentrations *viz.* 25, 50, 100 ppm. Required quantity of each fungicide was added to Potato Dextrose Agar medium prior to solidification and thoroughly mixed them by shaking prior to pouring in sterilized Petri plates of 70 mm. The medium was allowed to solidify. 5 mm bits of fungus culture were cut with the help of sterilized cork borer from 20 days old culture and then put at the center of Petri plates with sterilized inoculation needle. The fungal bit was reversed so that the pathogen could come in direct contact with the medium. One set ofcontrol was maintained in which the medium will be not mixed with any fungicide, simply inoculated with the pathogen. Five replications of each treatment were maintained in incubator at 27 ± 1°C for 15 days.

Observations

Mycelial growth (mm) was taken 15 days after incubation. The per cent inhibition over control was calculated in both fungicides and nanoparticles by Vincent's (1947) formula I= [(C-T)/C] × 100

Where, I= Percent inhibition, C = Radial growth of fungus in control, and T = Radial growth of fungus in treatment.

In vitro* effect of different fungicides against *B. sorokiniana

The variety Agra local, which is highly susceptible to spot blotch, was used for the study in year 2017. Ten treatments of fungicides with one check were laid out in randomizedblock design (RBD) with three replications. The plot size was maintained at 3 rows of 5 m length, row to row distance 20 cm and recommended agronomic practices were followed to raise the crop. Four fungicides namely Thiram 37.5%, Propiconazole 25% EC, Tebuconazole 25% EC and Mancozeb 75% WP and another two fungicides mixture Carboxin 37.5% + Thiram 37.5% were applied in the field in different mode with a different spraying schedule. The ten different treatments mentioned below:

	Treatments
T1	ST by thiram @ 3 g/kg of seed
T2	ST by thiram + carboxin @ 2.5 g/kg of seed
T3	ST by thiram + carboxin @ 2.5 g/kg of seed + FS sprays of propiconazole @ 0.1% (one)at boot leaf stage on Flag-1 leaf
T4	ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leafstage on Flag-1 leaf followed by second spray at 20 days interval (two sprays)
T5	FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf
T6	FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second sprayat 20 days interval (two sprays)
T7	FS of tebuconazole @ 0.1% at boot leaf stage on Flag-1 leaf
T8	FS of tebuconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second sprayat 20 days interval (two spray
T9	Three FS of mancozeb @ 0.25% at boot leaf stage on Flag-2 leaf followed by secondand third spray at 10 days intervals each (three spray)
T10	Control

Observations

1. Seed germination at 20DAS.
2. Disease incidence.
3. Record of spot blotch at flowering, dough and hard dough stages
4. Seed weight/plot (Kg)
5. 1000 grain weight (gm).

RESULTS AND DISCUSSIONS

A. In vitro effect of different fungicides against *B. sorokiniana*

In the present study, six fungicides *viz.* azoxystrobin, carboxin, propiconazole, tebuconazole, mancozeb, thiram were evaluated at three different concentrations *viz.*, 25, 50 and 100 ppm under *in vitro* condition against mycelial growth of *Bipolaris sorokiniana*.

Data pertaining to colony diameter and per cent inhibition of mycelial growth presented in Table 1, Fig. 1a & b revealed that all the fungicides were superior in inhibiting the mycelial growth of fungus over check. Among all treatments tebuconazole and propiconazol were found most effective giving 100 per cent inhibition even at lowest tested concentration i.e. 25 ppm. Among other fungicides at 25 ppm concentration, colony diameter of *Bipolaris sorokiniana* ranged from 16.00-35.32 mm being minimum (16.00 mm) in azoxystrobin and maximum (35.32 mm) in carboxin. After propiconazole and tebuconazole, azoxystrobin was found effective fungicides at 25 ppm by giving 64.25 per cent mycelial inhibition which was followed by thiram which inhibited 45.85 per cent of mycelial growth. Carboxin was found to be least effective giving 21.09

per cent mycelial inhibition. Same trend was observed at 50 and 100 ppm concentrations also where highest inhibition (100%) was in propiconazole and tebuconazole and lowest inhibition in carboxin. Both the triazoles gave 100 per cent growth inhibition at all concentrations and were found most effective followed by azoxystrobin, thiram, mencozeb and carboxin.

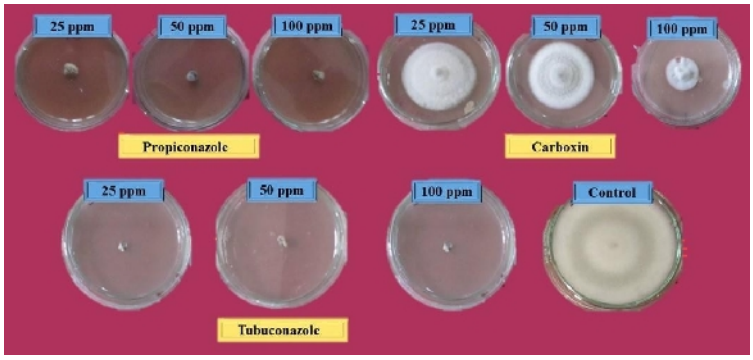
The results of present experiment are in complete agreement with Naresh *et al*., (2009) who reported that azoxystrobin, carboxin, propiconazole, tubuconazole, mencozeb and thiram were highly effective in controlling *Bipolaris sorokiniana.* Hasan *et al*., (2012) has reported 100 per cent growth inhibition of *B. sorokiniana*. Samia *et al.* (2015) has also studied the effect of propiconazole against *B. sorokiniana* and found that after 7 days of inoculation no mycelia growth was found in all treated plate except control in all isolate of

B. sorokiniana. Our findings match with these, but confront the finding of Giri *et al.* (2001) who reported that mancozeb were highly effective in inhibiting the mycelial growth (90.5%) of *B. sorokiniana.* Duveiller and Dubin, (2002) found that triazole group tebuconazole and propiconazole was most effective in inhibition of mycelial growth *B. sorokiniana* of wheat under *in vitro* condition. It can be concluded that all tested fungicides have greater effect on growth of *Bipolaris sorokiniana.* Propiconazole, tebuconazole and azoxystrobin were found best fungicides can contribute towards the management of disease caused by *Bipolaris sorokiniana.*

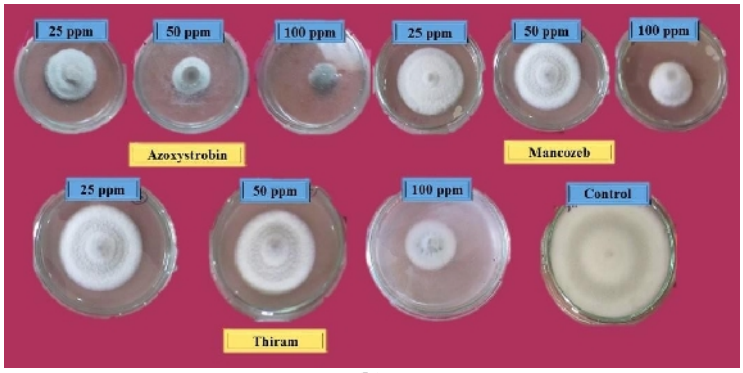
Table 1: Effect of fungicides on mycelial growth of *Bipolaris sorokiniana*.

	25 ppm		50 ppm		100 ppm	
Treatments	**Mycelial Growth (mm)***	**Growth inhibition(%)**	**Mycelial growth(mm)***	**Growth inhibition(%)**	**Mycelial growth(mm)***	**Growth inhibition(%)**
Carboxin	35.32	21.09	29.52	34.05	25.10	43.92
Azoxystrobin	16.00	64.25	14.00	68.72	12.00	73.19
Propiconazole	0.00	100.00	0.00	100.00	0.00	100.00
Tubuconazole	0.00	100.00	0.00	100.00	0.00	100.00
Mencozeb	28.94	35.35	25.40	43.25	20.28	54.69
Thiram	24.24	45.85	20.28	54.69	16.52	63.09
Control	44.76	0.00	44.76	0.00	44.76	0.00
CD @ 1%	1.74	-	2.13	-	0.55	-
CV	4.66	-	6.38	-	1.85	-
SEM±	0.44		0.55		0.14	

* Mean of five replications.



(a)



(b)

Fig. 1. Effect of fungicides on mycelial growth of *Bipolaris sorokiniana.*

B. In vivo effect of different fungicides against *B. sorokiniana*

Effect of fungicides on seed germination and disease incidence. The data on seed germination and disease incidence of wheat presented in Table 2 and showed that all the fungicides reduced the disease incidence and enhancing seed germination significantly in comparison to untreated control irrespective of their mode of applications. Seed germination at 20 days after sowing (DAS) ranged from 80-96 per cent. Minimum seed germination (80.00%) was observed in control while maximum seed germination per cent (96.00%) was recorded in three treatments *viz.*, ST by thiram + carboxin@ 2.5 g/kg of seed, ST by thiram + carboxin @ 2.5 g/kg of seed + FS sprays of propiconazole@ 0.1% (one) at boot leaf stage on flag-1 leaf and ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf followed by second spray at 20 days interval (two sprays) followed (93 %) by ST by thiram @ 3 g/kg of seed.

Disease incidence at 20 days after sowing (DAS) ranged from 5-14 per cent.Maximum disease incidence (14%) was recorded in control while minimum disease incidence (5%) was recorded in ST by thiram + carboxin @ 2.5g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf followed by second spray at 20 days'interval (two sprays) which was followed by 6% in case of ST by thiram + carboxin @ 2.5 g/kg of seed (6%) and ST by thiram + carboxin @ 2.5 g/kg of seed + FS sprays of propiconazole @ 0.1% (one) at boot leaf stage on flag-1 leaf. Our observations are in accordance with the findings of the numerous workers. Selvakumar *et al.*, (2015); Kumar *et al.*, (2014); Singh *et al.*, (1997); Maity *et al*., (2000).

Table 2: Effect of fungicides on disease incidence and seed germination at 20 DAS.

	Treatments	Seed germination %	Disease incidence %
T1	ST by thiram @ 3 g/kg of seed	93	11
T2	ST by thiram + carboxin @ 2.5 g/kg of seed	96	6
T3	ST by thiram + carboxin @ 2.5 g/kg of seed + FS sprays of propiconazole @ 0.1% (one) at boot leaf stage on Flag-1 leaf	96	6
T4	ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second spray at 20 days interval (two sprays)	96	5
T5	FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf	91	9
T6	FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second spray at 20 days interval (two sprays)	91	9
T7	FS of tebuconazole @ 0.1% at boot leaf stage on Flag-1 leaf	91	9
T8	FS of tebuconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second spray at 20 days interval (two spray	91	9
T9	Three FS of mancozeb @ 0.25% at boot leaf stage on Flag-2 leaf followed by second and third spray at 10 days intervals each(three spray)	91	10
T10	Control	80	14
	CD @ 5%		2.56
	CV		17.15
	SEM±		0.86

* Mean of three replications.
* FS-Foliar spray
* ST-Seed treatment

Effect of fungicides on spot blotch severity at Flowering, Dough and Hard Dough stage. Severity of spot blotch per plot was screened under field conditions against *B. sorokiniana.* The disease was scored on 0-9 dd scale. The progress of the disease in susceptible variety Agra local was monitored from 2nd fortnight of March and disease severity at flowering, dough and hard dough stage during 2016-17 crop seasons. Data presented in Table 3.

At flowering stage disease severity ranged from 12-36 in dd scale. Minimum spot blotch severity (12) in dd scale was recorded in three treatments like ST by thiram + carboxin@ 2.5 g/kg of seed, ST by thiram + carboxin @ 2.5 g/kg of seed FS sprays of propiconazole@ 0.1% (one) at boot leaf stage on flag-1 leaf and ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf followed by second spray at 20 days interval (two sprays) which was followed by FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf whereas maximum leaf blight/blotch were recorded in control i.e. 36 in dd scale.

At dough stage spot blotch severity ranged from 23 to 47 in dd scale. Minimum spot blotch severity (23) was recorded in ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf followed by second spray at 20 days'interval (two sprays) which was at par with ST by thiram + carboxin @ 2.5 g/kg of seed and ST by thiram + carboxin @ 2.5 g/kg of seed + FS sprays of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf i.e. 24 in dd scale whereas maximum spot blotch severity (47) were recorded in control. At hard dough stage spot blotch severity ranged from 34 to 69 in dd scale. Minimum spot blotch severity (34) was recorded in ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf followed by second spray at 20 days' interval (two sprays) which was at par with ST by thiram + carboxin

@ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf i.e. 35 in dd scale whereas maximum spot blotch severity (69) were recorded in control. Our observations are in accordance with the findings of the numerous workers. Goulart *et al.*, (1995); Malaker and Mian (2009); Pandey and Tewari (1999).

Table 3: Evaluation of fungicidal on spot blotch of wheat.

	Treatments	Flowering	Dough	Harddough
T1	ST by thiram @ 3 g/kg of seed	23	34	57
T2	ST by thiram + carboxin @ 2.5 g/kg of seed	12	24	46
T3	ST by thiram + carboxin @ 2.5 g/kg of seed + FS spraysof propiconazole @ 0.1% (one) at boot leaf stage on Flag-1 leaf	12	24	35
T4	ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second spray at 20 days interval (two sprays)	12	23	34
T5	FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf	23	34	47
T6	FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second spray at 20 days interval (two sprays)	23	34	46
T7	FS of tebuconazole @ 0.1% at boot leaf stage on Flag-1 leaf	24	35	47
T8	FS of tebuconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second spray at 20 days interval (two spray	23	34	46
T9	Three FS of mancozeb @ 0.25% at boot leaf stage on Flag-2 leaf followed by second and third spray at 10 days intervals each (three spray)	23	35	57
T10	Control	36	47	69
	CD @ 5%	1.04	0.56	0.87
	CV	2.84	1.01	1.04
	SEM±	0.35	0.24	0.29

* Mean of three replications.
* FS-Foliar spray
* ST-Seed treatment.

Seed weight/plot (Kg) and 1000 grain weight (gm)

The effect of different fungicides also reflected on yield attributes like grain yield as well as 1000 grain weight. Data pertaining to Seed weight/plot (Kg) and 1000 grain weight(gm) presented in Table 4.

All the treatments showed increase in the yield attributes as compared to control. Seed weight/plot varied from 2.91-4.48 kg. Maximum Seed weight/plot (4.48 kg) was recorded in ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf followed by second spray at 20 days' interval (two sprays) which was at par with FS of propiconazole @ 0.1% at boot leaf stage on flag-1 leaf (4.07 kg) whereas minimum Seed weight/plot (2.91 kg) was recorded in control.

Same trend was observed in case of 1000 grains weight per plot. 1000 grains weight per plot varied from 41.38-47.03 gm. Maximum 1000 grains weight per plot (47.03 gm) wasobserved ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at bootleaf stage on flag-1 leaf followed by second spray at 20 days' interval (two sprays) which was followed by FS of propiconazole @ 0.1% @ 0.25% at boot leaf stage on flag-1 leaf followed by second spray at 20 days' intervals (two sprays). While 1000 grains weight (41.38 gm) was recorded in control. Our observations are in accordance with the findings of the numerous workers. Mahapatra and Das 2013) and Singh *et al.* (1995).

Table 4: Effect of fungicide on yield of wheat.

	Treatments	Seed weight/plot(kg)	1000 grains weight (gm)
T1	ST by thiram @ 3 g/kg of seed	3.12	42.53
T2	ST by thiram + carboxin @ 2.5 g/kg of seed	3.55	43.80
T3	ST by thiram + carboxin @ 2.5 g/kg of seed + FS sprays of propiconazole @ 0.1% (one) at boot leaf stage on Flag-1 leaf	4.03	44.71
T4	ST by thiram + carboxin @ 2.5 g/kg of seed + FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf followedby second spray at 20 days interval (two sprays)	4.48	47.03
T5	FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf	3.90	44.00
T6	FS of propiconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second spray at 20 days interval (two sprays)	4.07	44.95
T7	FS of tebuconazole @ 0.1% at boot leaf stage on Flag-1 leaf	3.90	44.21
T8	FS of tebuconazole @ 0.1% at boot leaf stage on Flag-1 leaf followed by second spray at 20 days interval (two spray	4.01	45.12
T9	Three FS of mancozeb @ 0.25% at boot leaf stage on Flag-2 leaf followed by second and third spray at 10 days intervals each (three spray)	3.70	44.28
T10	Control	2.91	41.38
	CD @ 5%	0.24	0.32
	CV	3.76	0.42
	SEM±	0.82	0.10

* Mean of three replications.
* FS-Foliar spray
* ST-Seed treatment

CONCLUSION

♦ The present study revealed that under *in vitro* condition propiconazole and tebuconazole completely checked growth of *B. sorokiniana* even at 25 ppm. Azoxystrobin, thiram and carboxin were also found effective in checking the growth of *B. sorokiniana.* Among *in vitro* test of fertilizers maximum percent inhibition were recorded in urea at 1.5% i.e. 81.03%, which was at per with soluble potash (80.76%). Among *in vitro* test of nano compounds maximum percent inhibition (64.81%) were recorded in silver nanoparticles @ 100 ppm, which was at per with aluminium nanoparticles (55.34%). Among bio-agents *Trichoderma harzianum* (Th-b1), BAU isolate proved to be better in all respect over other bio-agents in dual culture technique.

♦ Under detached leaf technique maximum incubation period and maximum disease severity was found in case of PBW 660 i.e. 6.67 days and 10.00% respectively which was followed by HD 2967 i.e. 5.67 days and 20.33 respectively. Minimum average number of lesion per leaf (1.33) and necrotic lesion area (0.73 mm^2) was found in PBW 660 and HD 2967 respectively.

♦ Under field conditions seed treatment by carboxin @ 2.5g/kg of seed + foliar spray of propiconazole @ 0.1%at boot leaf or at the time of initiation of disease on flag-1 followed by second spray at 20 days interval (two spray) proved to be best in reducing spot blotch disease of wheat. Under field condition maximum seed wt/plot (4.48) and 1000 grain wt. (47.03g) was found in case of only one foliar spray of propiconazole @ 0.1% at boot leaf stage. Maximum seed germination (96.00%) and minimum disease incidence (5%) was found found in case only one foliar spary of propiconazole @ 0.1% at boot leaf stage.

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Conflict of interest. Nil.

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