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Management of Leaf Blight of Wheat (*Triticum aestivum* L.) with Bio-Agents, Neem Leaf Extract and Fungicides

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A B S T R A C T

The experiment was conducted under *in vitro* and field conditions to observe the effect of bio-agents, botanical and fungicides against *Alternaria triticina*. Eight treatments were taken up with three replications and data collected was analyzed using CRD. Maximum inhibition per cent mycelia growth was observed in Propiconazole (89.72%), Hexaconazole (88.44%), Vitavax (87.70%), followed by *Trichoderma harzianum* (85.50%), *Trichoderma viride* (83.30%), *Pseudomonas fluorescens* (80.73%) and neem leaf extract (73.57%) as compared to control (0). An experiment was conducted under field condition to observe the effect of bio-agents, neem leaf extract and fungicides against *Alternaria triticina*. Eight treatments were taken up with three replications and data collected was analyzed using RBD. Minimum disease intensity per cent and production of wheat was recorded in treatment Propiconazole @ 0.1% (18.24% and 37.00q/ha respectively) followed by *Pseudomonas fluorescens* @ 0.5% (20.51% and 30.44q/ha), as compared to control (43.18% and 20.41q/ha). Propiconazole was significantly superior as compared to other treatments.

Introduction

The bread wheat (*Triticum aestivum*) is the most important spices accounting to a little over 87% of the total wheat production of India followed by the *Triticum durum* wheat 12% and *Triticum dicoccum* wheat 1%. The *Triticum dicoccum* wheat is grown only on limited acreage in Tamil Naidu, Andhra Pradesh, Maharashtra and Gujarat. Some wheat varieties (e.g. *Triticum aestivum*) are suitable for bread making while others (e.g. *Triticum durum*) are suitable for biscuits and cooking making (Sapirstein *et al.*, 2007). Gluten, the protein component of flour which gives the dough elasticity and strength, can be defined as the rubbery mass that remains when wheat dough is washed to remove starch granules and water soluble constituents (Wieser, 2007; Kaushik, *et al.*, 2013). In India, foliar blight of wheat had been noticed as early as 1924 (Kulkarni, 1924), but it was not of much consequence till recently. In the recent past, with the change in cropping system, foliar blight has now become a major disease far and wide in our country causing 2.72 to 36.24% yield losses under different agro climatic zones. In India, foliar blights of wheat are considered as one complex, which includes leaf blight caused by *Alternaria*

triticina Prasada and Prabhu and spot blotch caused by *Bipolaris sorokiniana*. In the Indogangetic plains, covering the entire north-western and north-eastern plains, rice-wheat rotation is the dominant cropping sequence. It has been commonly observed that the intensity of foliar blights has considerably increased in the rice-wheat system (Nagarajan and Kumar, 1998). During last decade multilocational surveys for determining incidence of foliar blights of wheat have been conducted in eastern U.P, Bihar, Haryana, Punjab, Delhi, Gujrat and Rajasthan (Singh *et al.,* 2004) In view of the growing concern about foliar blight of wheat, it was considered desirable to study the incidence of the disease and the causal organisms in Allahabad region where wheat (*Triticum aestivum* L.) is main food crop. Leaf blight caused by *Alternaria triticina* is the major disease in irrigated wheat in Vidarbha region of Maharashtra. Alternaria leaf blight was first reported from Maharashtra in 1924 (Kulkarni, 1924). The disease initially appears as small and irregularly scattered chlorotic lesions on the leaves in last week of December. As the disease progresses, several spots coalesce and cover the whole or part of the leaf giving it a blighted' appearance. Heavily infected fields show a burnt appearance (Sokhi and Joshi, 1972). The normal sown as well as late sown irrigated wheat varieties were found heavily infected with Alternaria leaf blight during January and February in Vidarbha region, causing considerable losses in the grain yield of irrigated wheat. Therefore, this trial was formulated to estimate the losses caused due to leaf blight disease (Shivankar *et al.,* 2000). The application of two irrigations reduced the severity of foliar blight as compared to no irrigation (Shrestha *et al.,* 1998) reported that low or imbalance soil nutrient levels predispose plants to more severe leaf blight attack found low incidence of disease when wheat crop was sowing on 30th November as compared 20th December.

Materials and Methods

In-situ experiment

In-situ experiment (field) was laid out in randomized block design (RBD) with eight treatments (Table 1) Propiconazole (Tilt 25 EC) @ 0.1%, hexaconazole @ 0.5% and vitavax @ 0.25% *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and neem leaf extract (*Azadirachta indica)* and three replication including inoculated check in the experimental field of Sam Higginbottom Institute of Agriculture Technology and Sciences (Deemed to-be University) Allahabad, India in *Rabi* season (December 2015). Each replication consisted of 24 plots of 2 × 1 m^2 each. The seeds of variety PBW-343, which is highly susceptible to leaf blight were collected from (SHIATS), Allahabad was sown in December with spacing 22.25 cm bio-agents and botanicals were sprayed just after initiation of disease and repeated thrice at 15 days interval observations were recorded in five selected tagged plants three days after last sprays of bio- agents as well as botanicals using 0 to 9 grade scale (Kapadiya and Dhruj, 1999).

The data were subjected to the statistical analysis

Preparation of fungicidal spray solution

The spray solution of a desired concentration was prepared by adopting the following formula:

$$V = \frac{C \times A}{\% \text{ a.i.}}$$

Where,

V = Volume/weight of commercial fungicide ml or g
C = Concentration required

A = Volume of solution to be prepared
% a.i. = percentage of active ingredient in commercial product

Disease intensity (%) was calculated by using the following formula

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of leaves/plant x Maximum disease grade}} \times 100$$

***In vitro* experiment**

The *in vitro* trial was laid out in completely randomized design (CRD) with three replications and eight treatments including check in the experimental laboratory of department of Plant Pathology. The management of leaf blight of wheat, with bio-agents, neem leaf extract and fungicides was tested applying poison food and dual culture techniques against *Alternaria triticina.* The observation of the mycelia growth inhibition per cent was recorded at 1 to 7 days.

Efficacy of fungicides

Efficacy of three fungicides Propiconazole (Tilt 25 EC) @ 0.1%, hexaconazole @ 0.5% and vitavax @ 0.25% against mycelia growth of *Alternaria triticina* was tested by poisoned food technique (PFT).The required quantity of each fungicide was added separately to sterilized medium mixed thoroughly and poured in sterilized 9 cm diameter glass. Three replications were maintained for each treatment. A control was also maintained where medium was not supplemented with any fungicides. Each plate was inoculated with 5 mm discs with the help of sterilized cork borer from the edge of the fungal culture and incubated at 25+1ºC for 7 days. The linear growth of the test fungus was recorded and per cent growth inhibition was calculated by following formula (Vincent, 1927):

$$\text{Per cent inhibition (I)} = \frac{C-T}{C} \times 100$$

Where,

I = % inhibition
C = growth in control
T = growth in treatment

Efficacy of plant extracts

Following plant extracts neem leaf extract (*Azadirachta indica*) were used in vitro (poisoned food techniques). The effect of each plant extract was tested at two different concentrations 10, 20%. Following the method used with slight modification to get these the required plant part was thoroughly washed with sterilized water and was ground separately in electric grinder the 100 g washed leaves using equal amount of 100ml sterilizer distilled water. The mixture was squeezed with double layered sterilized muslin cloth. The extracts thus obtained were considered as of 100% concentrations. Require amount of stock solution was added to PDA to get desired concentrations. Required quantity of each plant extract was mixed thoroughly in melted PDA, to get desired concentration, just before pouring in sterilized 9 cm diameter glass petri plates and was allowed to solidify for 12 hours. Each plate was inoculated with 5 mm disc of mycelia bit taken with the help of sterilized cork borer from the periphery of 7 days old cultures of *Alternaria triticina* growing on PDA. The inoculated petri plates were incubated at $25+1^0$ C four petri plates were used for each treatment serving as three replications. A control was also maintained where medium was not supplemented with any fungicides. Each plate was inoculated with 5 mm discs with the help of sterilized cork borer from the edge of the fungal culture and incubated at $25+1^0$ C for 7 days. The linear growth of the test fungus was recorded

and per cent growth inhibition was calculated by following formula (Vincent 1927).

$$\text{Per cent inhibition (I)} = \frac{C-T}{C} \times 100$$

Efficacy of bio agent

In vitro efficacy of three bio-control agents *viz, Trichoderma harzium, Trichoderma viride* and bacterial antagonist *viz, Pseudomonas fluorescens were* tested by using dual culture plate method on PDA medium (singh *et.al* 2005). After 12 hours of pouring these petri plates were inoculated with 5mm discs with the help of sterilized cork borer from the edge taken from 7 days old culture of *Alternaria triticina* and antagonistic agents *viz, Trichoderma harzium, Trichoderma viride* and bacterial antagonist *viz, Pseudomonas fluorescens.* The linear growth of the test fungus was recorted and per cent growth inhibition was calculated by (Arora and upadhyay 1978).

Results and Discussion

Radial mycelial growth of fungus was recorded at 1 an7 DAI. Percent inhibition of growth was calculated using the following formula as suggested by Sundar *et al.*, (1995):

$$\text{Inhibition (\%)} = \frac{X-Y}{X}$$

Where,

X = Mean mycelial growth (radial) of pathogen in control plate;
Y = Mean mycelial growth (radial) of pathogen in treatment

Growth of *Alternaria triticina*

Antagonistic activity of *Trichoderma viride, Trichoderma harzianum* and *Pseudomonas fluorescens* and were investigated by dual culture method on PDA. Data reveals that, *Trichoderma viride, Pseudomonas fluorescens* were potential antagonists of *Alternaria triticina* forming a clear zone of inhibition. On microscopic examination hyphae of antagonists were observed coiling and oppressed around hyphae of *Alternaria triticina*, *T. harzianum* (85.50 %) was most effective over other treatments followed *by Trichoderma vridie* (83.30%) and *Pseudomonas fluorescens* (80.73 %) were least effective (Table 2 and Figure 1) The efficacy of different fungicides and Neem leaf extracts against *Alternaria triticina* were assayed *in vitro*. Observations were recorded on growth of the test fungus. Under various treatments, the observations recorded on per cent inhibition of growth are presented in table 2 and figure 1 that all the treatment at all the concentration inhibited the fungal growth and the data also revealed that the all the fungicides and neem leaf extracts were significantly superior over check at all the treatment.

Propicanazole, hexaconazole, vitavax and neem leaf extract gave complete growth inhibition of *Alternaria triticina.* A significant difference in data presented on inhibition per cent of mycelium growth was observed among the treatment. Maximum inhibition per cent was recorded on Propicanazole (89.72%) except at it was followed by Hexaconazole (88.44%), Vitavax (87.70%) and Neem leaf extract (73.57 %). Besides the agricultural practices, physical and biological methods used for the management of diseases caused by *Alternaria triticina*, chemical fungicides are most commonly adopted by the growers. Fungicides like; Propicanazole, carbendazim, hexaconazole, ridomil and topsin etc. have been recommended against Alternaria. Such synthetic fungicides bring about the inhibition of pathogens either by destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogen and hence are extremely effective.

Effect of fungicides on the disease intensity of leaf blight of wheat

The result presented in table 3 revealed that all the treatments were statistically significant and increased plant height (cm) as compared to control. Among the bio-agents, botanical and fungicides used the maximum plant height (cm) was recorded in T_4-*Trichoderma viride*@2.5% (78.82cm), as compared to untreated control (70.04cmT_0), respectively. T_4-*Trichoderma viride*@2.5% (78.82cm), treatment was followed by T_6 -*Trichoderma harzianum*@2.5% (78.27cm), T_3-vitavax@ 0.25% (77.24cm), T_5 -*Pseudomonas flourescens*@2% (76.45cm), T_1- Tilt@0.1% (74.37cm), T_2-Hexaconazole @0.5% (73.46cm) and T_7- Neem leaf extract@ 10% (73.25cm). As compared to T_0 control (70.04 cm). Among the treatments maximum plant height (cm) was recorded in T_4-*Trichoderma viride* (78.82cm) and T_6 -*Trichoderma harzianum* (78.27cm) also reported similar funding.

Table.1 Different treatments

Treatments	Treatment name
T_0	Control
T_1	Propiconazole @ 0.1% (FS)
T_2	Hexaconazole @ 0.5% (FS)
T_3	Vitavax @ 0.25% (FS)
T_4	*Trichoderma viride* @ 2.5% (FS)
T_5	*Trichoderma harzianum* @ 2.5% (FS)
T_6	*Pseudomonas fluorescens* @ 2% (FS)
T_7	Neem leaf extract(*Azadirachta indica)* @10% (FS)

FS = foliar spray

Table.2 Effect of different fungicides, bioagents and botanicals against on mycelia growth of *alternaria triticina*

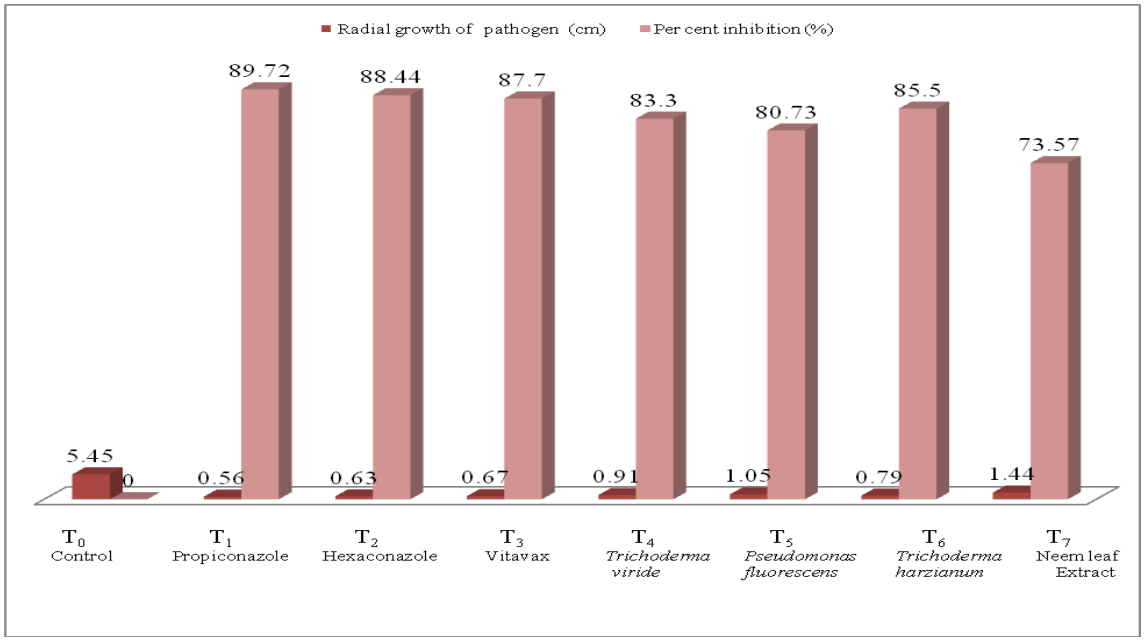
S.N	**Treatments**	**Radial growth of pathogen (cm)**	**Per cent inhibition (%)**
T_0	Control	5.45	0
T_1	Propiconazole	0.56	89.72
T_2	Hexaconazole	0.63	88.44
T_3	Vitavax	0.67	87.70
T_4	*Trichoderma viride*	0.91	83.30
T_5	*Pseudomonas fluorescens*	1.05	80.73
T_6	*Trichoderma harzianum*	0.79	85.50
T_7	Neem leaf extract	1.44	73.57
Mean		11.5	
F-test		S	
SEd (±)		0.10	
CD (5%)		0.21	

Table.3 Effect of foliar spray with BAU-fungicides and botanicals on disease intensity and plant growth of leaf blight of wheat

Treatment (%)	Plant height (cm) 90 DAS	PDI		Yield (Q\ha)	C:B Ratio
		45 DAS	90 DAS		
Control	70.04	33.00	43.18	20.41	1:1.03
Propiconazole@ 0.1%	74.37	16.18	18.24	37.00	1:1.42
Hexaconazole@ 0.5%	73.46	17.92	19.94	34.63	1:1.36
Vitavax@ 0.25%	77.24	22.13	24.55	32.20	1:1.52
Trichoderma viride@2%	78.82	22.26	25.51	28.89	1:1.43
Pseudomonas fluorescens@2.5%	76.45	19.15	20.51	30.44	1:1.38
Trichoderma harzianum@2%	78.27	23.03	25.76	27.04	1:1.34
Neem leaf extract@ 10%	73.25	23.50	28.48	26.37	1:1.22
S.Ed. (+)	0.60	0.78	0.73	0.50	…….
C.D. (P=0.05%)	1.29	1.67	1.56	1.07	…….

PDI= Per cent disease intensity, DAS= Date after sowing, C: B= Cost Benefit ratio

Fig.1 Effect of different fungicides, bio agents and botanicals against on mycelia



BAU-Biofungicide and botanical differed in respect of leaf blight disease Intensity (%) at different growth stages (Table 3). At 45 DAS, the lowest (16.18%) disease intensity was recorded with Propiconazole@ 0.1% followed by Hexaconazole@ 0.5% (17.92%). The highest disease incidence was recorded in control (33.00%), followed by *Pseudomonas fluorescens* (19.15%). Vitavax@ 0.25% (22.13%), *Trichoderma viride* (22.26%), *Trichoderma harzianum* (23.03%) and neem leaf extract@10% (23.50%), At 90 DAS, the lowest (18.24%) disease intensity was recorded with Propiconazole@ 0.1%, while

the highest (43.18%) was recorded in control plot. Among the BAU fungicides and botanicals propiconazole@ 0.1% and *Pseudomonas fluorescen*, performed better than other BAU fungicides and botanicals to reduce per cent disease intensity of the leaf blight disease (Table 3).

Effect of BAU-fungicides and botanicals on the yield of leaf blight of wheat

Among the bio-agents, botanical and fungicides used the maximum grain yield was recorded in Propicanazole@ 0.1% (37.00) as compared to untreated control (20.41) followed by Hexaconazole@ 0.5% (34.63), vitavax@ 0.25% (32.20), *Pseudomonas flourescens* (30.44), *Trichoderma viride* (28.89), T_6 -*Trichoderma harzianum* (27.04) and Neem leaf extract @10% (26.37). Among the treatments most effective was Propicanazole@ 0.1% (37.00) and Hexaconazol@ 0.5% (34.63), However, the treatments Neem leaf extract and *Trichoderma harzianum* were non-significant and statistically at par with each other Copes, (2009). They reported that in the present result showed that all the treatments tested in this study gave satisfactory result against *Alternaria triticina*. Among all the treatment, systemic fungicides such as propicanazole and hexaconazole reduced that *Alternaria triticina* intensity and leaves become disease free. Healthy leaves have more photosynthetic activity, ultimately enhance the number of ears. But the efficacy of bio-agent such as *Pseudomonas fluorescens*, *Trichoderma viride* and *Trichoderma harzianum* were less as compared to the systemic fungicides. Results showed that the highest yield was recorded in Propicanzole (37.00q/ha), followed by *Pseudomonas flourescens*@0.5% (30.44), reported that use of chemical inducers had adverse effect on the plant growth. But given highest yield because chemicals attributed to elicitor's effect on physiological processes in plant such as ion uptake, cell elongation, cell divition, enzymatic activation and protein synthesis (Gharib and Hegazi, 2010) all treatments are significant to each other and statistically at par with each other. When cost benefit ratio was worked out, interesting result was achieved. Among the treatment studied, the best and most economical treatment was vitavax@0.25% (1:1.52), *Trichoderma viride* (1:1.43), Propicanazole @ 0.1%(1:1.42), Hexaconazole@0.5% (1:1.36) followed by *Pseudomonas fluorescens* (1:1.38), *Trichoderma harzianum* (1:6.48), and Neem leaf extract (1:1.22), as compared to control (1:1.03).

All the plant extracts and BAU-Biofungicide significantly inhibited mycelial growth of the pathogen. Maximum (89.72%) reduction of mycelial growth and based on the results, In case of plant height (cm) most effective was Trichoderma viridie@2.5%and *Pseudomonas fluorescens*@2.5% was found the most effective treatment which gave recorded minimum disease intensity (%) and yield (q/ha), as compared to other treatments except Tilt @ 0.1% (propiconazole) which was taken as treated control. so it may be concluded that bio-agents along with propiconazole @ 0.1% can be used for the management of leaf blight of wheat. The present research findings are limited to one crop season (December 2015) under Allahabad agro-climatic conditions as such more trials are required in future to validate the findings.

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