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In Vitro and in Vivo Efficacy of Fungicides against *Alternaria triticina* Causing Leaf Blight of Wheat

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A B S T R A C T

Leaf blight of wheat caused by *Alternaria triticina* is a major constraint in production of wheat. Management of diseases using fungicides is a common and economical practice. Different types of chemical fungicides are available to control leaf blight disease. But there is a lack of awareness regarding the use of suitable fungicides on wheat plants affected with *Alternaria triticina*. The experiment was conducted under *in vitro* conditions to observe the effect of fungicides like Propiconazole (25%EC), Mancozeb (75WP) and Carbendazim (50% WP) against *Alternaria triticina* by Poisoned Food Technique. Propiconazole showed the highest range of (94.93%) inhibition followed by Carbendazim (90.85%) and Mancozeb (90.65%). The research findings in the present study suggest Propiconazole (25EC) as most effective fungicide for the treatment of leaf blight disease of wheat. Propiconozole 25% EC was found to be the best among all treatments for inhibiting the growth of mycelium of *Alternaria triticina* under *in vitro* condition. Propiconozole 25EC @ 0.1% was the superior spray solution in comparison to all fungicide for controlling leaf blight disease of wheat.

Key words: *Alternaria triticina,* Fungicides, Leaf blight, Wheat, *In vitro*

Wheat is the most staple food crop of India belongs to family Poaceae. In, India wheat is cultivated in an area of about 29.58 million hectares with 99.70 million tonnes production during 2017-18 [1]. Bihar is the 6th largest wheat producer among all states in India, and accounts for around 5% of all of India's wheat production [2]. Basically, three cultivars *Triticum aestivum* L. (Bread wheat), *Triticum durum* Desf. (Macroni or durum wheat), and *Triticum dicoccum Shrank* (Emmer wheat) are commonly cultivated in India. Wheat is the rich source of carbohydrates, dietary fiber, fat, protein, thiamine, riboflavin, niacin, pantothenic acid, vitamin B_6, folate, calcium, iron, magnesium, phosphorus, potassium, zinc and manganese. It is consumed in the form of roti, bread cakes, biscuits, cookies and crackers. Due to increasing population of the country [3] sustainable wheat production has to be maintained. Several abiotic and biotic stresses are responsible for reduction of wheat yields. The leaf blight of wheat is a major constraint in reduced both quality and production caused by *Alternaria triticina*. In the recent past, with the change in cropping system, leaf blight of wheat has now become a major disease in our country, particularly in Indo-Gangetic plains including eastern U. P. and Bihar, causing [4] 2.72 to 36.24% yield losses under different agro climatic zones. In view of the growing concern about leaf blight of wheat causing yield losses, the present study was conducted to test the efficacy of certain fungicides Propiconazole 25% EC, Mancozeb 75% WP, and Carbendazim 50% WP against *Alternaria triticina*, the causing organism of leaf blight of wheat, under *in-vitro and in-vivo* conditions.

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MATERIALS AND METHODS

Isolate of *Alternaria triticina*, potato dextrose agar media and different chemical fungicides were taken. The *in vitro* trial was conducted in the Laminar Air Flow chamber in experimental lab and *in-vivo* trial was conducted in the pot under the campus of P.G. Department of Biotechnology of T. M. Bhagalpur University. The efficacy of fungicides, Propiconazole (25% EC), Mancozeb (75% WP), and Carbendazim (50%WP) against *Alternaria triticina* was tested using poisoned food technique and the *in-vivo* trial was made in terms of disease severity.

Isolation of pathogen, Alternaria triticina

The wheat plant crop leaf showing characteristic symptoms of leaf blight were collected from T.M. Bhagalpur University campus, Bhagalpur. Infected leaf blight samples were thoroughly washed in running tap water and cut into small pieces of 2-5 mm size having half healthy leaf tissues and further surface sterilized with mercuric chloride (0.1%) for 30-60 seconds, infected leaf blight pieces were thoroughly washed three times in sterilized water and blotted dry on clean sterile blotting paper to remove the moisture. Leaf pieces were transferred aseptically into petri plates containing potato dextrose agar (PDA) medium and the petri- plates were incubated in a B.O.D incubator at 28 ± 1°C. After 48 hours of incubation, mycelial growth developed at the margin was transferred to PDA slants and the pathogen culture was maintained on PDA in culture tubes and stored in refrigerator for further use.

Identification of pathogen by cultural and morphological studies

Spore suspension was made from culture of the pathogen grown on PDA. One drop of the spore suspension was placed on a slide and morphological characters were examined under compound microscope. The network of hyphae called mycelium is hyaline in nature. After number of days passing, it becomes olive buff in appearance. The conidiophores found to be branched, erect, single emerged through the stomata. The conidiophores were septate and light brown in colour and the conidia were usually brownish to black in colour. The colour of conidium was light brown to olive and become darker with age. Conidium was of irregularly oval, ellipsoid conical, gradually tapering into a beak. After a long period, culture was appeared as a grayish-black with metallic seen in brown colour of lightening. On the basis of cultural and morphological characteristic of the colony, mycelium, conidiophores and conidia with their appearance, identified as *Alternaria triticina*.

Maintenance of the culture

The pathogen was sub-cultured on potato dextrose agar slants and allowed to grow at 27°C-28°C for a week under the incubator and growth culture slants were preserved in a refrigerator at 5°C and renewed once in 30 days.

Poisoned food technique

The Poisoned Food Technique [5] was followed to evaluate the efficacy of fungicides, Propiconazole (25 EC), Mancozeb (75% WP) and Carbendazim (50% WP) with concentration (10ppm, 20ppm, 50ppm, 100ppm, 200ppm) in laboratory against *Alternaria triticina* with three replications each. Mycelial disc of 5 mm size from seven days old cultures was cut out by a sterilized cork borer and one such disc was placed at the center of each agar plate. Control plate was also maintained. After incubation period for a week at room temperature, radial growth was measured till fungus attained maximum growth in check plates. The efficacy of the fungicides was expressed as percent inhibition of mycelial growth over control, which was calculated by using the formula by (Vincent [6]).

$$I = \frac{C - T}{C} \times 100$$

Where

I = Percent inhibition

C = Radial growth of fungus in controls

T = Radial growth of fungus in treatments

Pot experiment

A pot experiment was conducted during rabi crop season (2017-18). Wheat seed of variety K-0307 was sowing in pot in mid of November. Pots are amended with required NPK fertilizer with sterilized soil. The surface-sterilized eight seeds were sown in each pot. Microbial inoculum of *Alternaria triticinia* was prepared by scraping 10-12 old pathogen culture and dissolving in distilled water. Then, it was mixed by vertexing for avoiding the aggregation of conidial clumps and the concentration was made to 1 x10^6 conidia ml^{-1} by diluting with distilled water. Microbial inoculum of *Alternaria triticina* was spread on the wheat plant at the booting stage. Different fungicides at appropriate concentration was prepared like Propiconozole 25EC @ 0.1%, Carbendazim 50% WP @ 0.1% and Mancozeb 75% WP @ 0.25%. When the symptoms were appeared firstly then the prepared solutions of fungicides were sprayed separately after an interval of 20 days. Additionally, one control was also maintained treated with water. After the last spray of fungicide solution, the per cent disease intensity recorded after 20 days. The severity of Alternaria blight of wheat was recorded using 0-5 rating scale and per cent disease intensity was calculated. A statistical analysis of randomized block design (RBD) with three replications was made in polyhouse experimental trial.

RESULTS AND DISCUSSION

The result of effect of different chemical fungicides on mycelial growth inhibition of *Alternaria triticina* under *in vitro* condition were shown (Table 1).

Table 1 Effect of different chemical fungicides on mycelial growth inhibition of *Alternaria triticina* under in vitro condition

Fungicides	Growth inhibition (%)						Growth inhibition (%)
	10ppm	20ppm	50ppm	100ppm	200ppm	Control	
Propiconozole (25% EC)	8.49 (16.89)	10.73 (19.08)	85.82 (67.87)	88.1 (69.82)	94.93 (77.08)	0.00 (14.04)	48.01 (44.13)
Carbendazim(50%WP)	6.75 (17.26)	11.55 (63.18)	79.54 (64.74)	81.79 (72.62)	90.85 (8.76)	0.00 (12.15)	45.08 (39.78)
Mancozeb (75%WP)	2.32 (19.63)	4.63 (44.31)	11.55 (60.59)	48.83 (0.00)	75.89 (0.00)	0.00 (0.00)	23.87 (20.75)
Mean	5.85 (17.92)	8.97 (42.19)	58.97 (64.40)	72.90 (47.48)	87.22 (28.61)	0.00 (8.73)	

	Between fungicides (F)	Within fungicides concentration ©	F × C
S.Em. ±	0.96	1.36	2.36
C.D at 5%	2.77	3.92	6.80
S.Ed±	1.36	1.92	3.33

Mean of three replications

Value in parenthesis indicated angular transformed values

All three chemical fungicides were evaluated against *Alternaria triticina* by poisoned food technique *in vitro* condition. And the fungicides were tested at five different concentrations; 10ppm, 20ppm, 50ppm,100ppm, and 200ppm. All three fungicides showed significant mycelial inhibition of *Alternaria triticina* under poisoned food technique. Out of three different fungicides, Propiconozole 25% EC showed significant mycelial growth inhibition of 8.49, 10.73, 85.82, 88.1, 94.93 percent at concentration of 10ppm, 20ppm, 50ppm, 100ppm, 200ppm, respectively as compared to other fungicide Carbendazim 50% WP and Mancozeb 75% WP *in vitro*. Mancozeb 75% WP showed 2.32, 4.63, 11.55, 48.83, and 75.89 percent mycelial growth inhibition of the fungus at 10ppm, 20ppm, 50ppm, 100ppm and 200ppm concentration, respectively whereas, Carbendazim 50% WP showed 6.75, 11.55, 79.54, 81.79, and 90.85 percent mycelial growth inhibition at different concentrations respectively. The concentration difference of fungicide between 20ppm and 10ppm was non-significant in comparison to other concentrations. The cumulative mycelial growth inhibition was increased with increased concentration of fungicides. The effectiveness of Mancozeb in inhibiting the growth of *Alternaria triticina* [7]. Mancozeb showed mycelial growth inhibition of *Alternaria triticina* [8-9]. Mancozeb showed 87.74% of efficacy against *Alternaria triticina* at 500ppm and Propiconozole showed 84.00% growth inhibition of pathogen @ 50 ppm and showed complete inhibition at higher concentration above 50 ppm [10]. Similar trend was also reported by earlier workers [11]. Among different fungicides tested, Propiconozole 25% EC was found to be the best among all treatments for inhibiting the growth of mycelium of *Alternaria triticina* under *in vitro* condition. This finding is in conformity with earlier studies of [12-13] reported cent percent inhibition of radial growth of fungus *Alternaria alternata*, with Propiconazole. Propiconozole showed 89.72% of inhibition but Hexaconozole and Vitavex showed 88.44% and 87.70% of inhibition against *Alternaria triticina* [14]. Overall fungicide Propiconozole 25% EC, Carbendazim 50% WP and Mancozeb 75% WP proved the most effective with 94.93, 90.85 and 75.89 percent mean fungal growth inhibition. In all the fungicide bioassay studies, with increase in the concentration there was corresponding decrease in the growth of fungus.

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

The result was presented in (Table 2) revealed that all the treatments of chemical fungicides differed in respect of wheat disease severity in different wheat plant growth stage.

From the initial 45 days, the highest disease severity was recorded in control (40.18%), followed by Mancozeb (39.56%) @ 0.25%, Carbendazim (38.09%) @ 0.1% and Propiconozole (35.12%) @ 0.1%. At 65 days, the lowest (20.24%) disease intensity was recorded in Propiconozole in comparison of Carbendazim (23.18%) and Mancozeb (25.45%). At the last 85 days the lowest disease intensity was observed in Propiconozole while the highest (90.82%) was recorded in control pot. Among three treatments of chemical fungicide Propiconozole @ 0.1% showed the best in comparison to another. Propiconozole reduces the percent disease intensity maximum with percent disease reduction (92.32%). The percent disease intensity was significantly differed from each other by all three fungicides in concern of effect of treatment and days. It means three times spray of chemical fungicides solution at the different stages of wheat plant growth reduces the percent disease intensity continuously. Propiconozole was the superior spray solution in comparison to all fungicide for controlling leaf blight disease of wheat.

Table 2 Effect of foliar spray with fungicides on disease severity and percent disease reduction of leaf blight disease of wheat in pot experiment (2017-2018)

Treatments	PDI (Disease Severity)				PDR (Percent Disease Reduction			
	45 Days	65 Days	85 Days	Mean	45 Days	65 Days	85 Days	Mean
T_0: Control	40.18 (39.31)	71.24 (57.54)	90.82 (72.38)	67.41 (56.41)	62.22 (52.08)	35.86 (36.76)	12.68 (20.84)	36.92 (36.56)
T_1: Propiconozole (25% EC) @ 0.1%	35.12 (36.32)	20.24 (26.72)	12.14 (20.36)	22.5 (27.80)	65.18 (53.81)	80.44 (63.73)	92.32 (73.94)	79.31 (63.83)
T_2: Carbendazim (50% WP) @ 0.1%	38.09 (38.09)	23.18 (28.76)	21.15 (27.36)	27.47 (31.40)	61.20 (51.45)	73.21 (58.81)	86.24 (68.20)	73.55 (59.49)
T_3: Mancozeb (75%WP) @ 0.25%	39.56 (38.95)	25.45 (30.28)	26.12 (30.72)	30.37 (33.31)	63.81 (52.99)	71.14 (57.48)	78.18 (62.14)	71.04 (57.54)
Mean	37.59 (38.17)	46.70 (35.83)	37.55 (37.70)		63.10 (52.58)	65.16 (54.20)	67.35 (56.28)	
	Effect of treatment		Effect of days	T×D	Effect of treatment		Effect of days	T×D
S.Em. ±	0.20		0.23	0.41	0.38		0.44	0.76
C.D at 5%	0.60		0.70	1.21	1.13		1.30	2.26
S.Ed±	0.29		0.33	0.58	0.54		0.62	1.08

Mean of three replications
Value in parenthesis indicated angular transformed values

Disease severity of fungal infection by the application of foliar fungicides and the disease severity was lower 17% by the application of foliar chemical fungicides in comparison of control 36% of wheat variety ARF [15]. In all the fungicide bioassay studies, with increase in the concentration there was corresponding decrease in the growth of fungus. Besides the agricultural practices, physical and biological methods used for the management of disease caused by *Alternaria triticina*, chemical fungicides are most commonly adopted by the growers. For controlling plant disease, inhibition of spore germination and appressorium formation are required [16]. Chemical fungicides able to inhibit growth and development as well as spore germination by the accumulation of toxicants in fungal cell this may result in death of cell [17]. Also, cell death may also result due to leakage of cellular constituents from cell membrane. Fungicides like, Propiconozole, Carbendazim, Mancozeb, Hexaconazole, Ridomil and Topsin etc. have been recommended against Alternaria. Chemical 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 [17]. From the farmers point of view the economics of disease management is essential. Using of fungicides not only helps in reducing the disease severity but also helps in increasing wheat yield giving high benefit to the farmers. So, the fungicides should be cost effective.

CONCLUSION

The present study provides scientific information regarding effectiveness of chemical fungicides against leaf blight casual organism *Alternaria triticina* of wheat. The *in vitro* experimental results showed that all the applied fungicides inhibited the mycelial growth of *Alternaria triticina* but Propiconozole 25% EC fungicide was found most suitable for controlling the growth of *Alternaria triticina* under *in- vitro and in vivo* conditions compared to Mancozeb and Carbendazim. Further, it is advisable to make field study with these fungicides where several abiotic and biotic components could influence the host-pathogen interaction. The present study has application for farmers and provides primary information for control and management of leaf blight disease of wheat.

LITERATURE CITED

1. Anonymous. 2018. *Pocket Book of Agricultural Statistics*. Directorate of Economics and Statistics New Delhi. pp 75.
2. Bhotika A. 2011. Regulations on a flour milling industry in India.
3. Singh RP. 2003. Analysis of growth performance of wheat crop in Jharkhand. *Jr. Res. Birsa Agri. University* 15(2): 217-223.
4. Kakraliya SS, Zacharia S, Bajiya MR, Sheshma M. 2017. Management of leaf blight of wheat (*Triticum aestivum* L.) with bio-agents, neem leaf extract and fungicides. *Int. Jr. Current Microbiology Applied Science* 6(7): 296-303.
5. Nene YL, Thapliyal PN. 1993. *Fungicides in Plant Disease Control*. (3rd Edition). Oxford and IBH Publishing Co., Pvt. Ltd., New Delhi. pp 526-531.
6. Vincent JM. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 159(850): 57-64.
7. Hossain I, Rahman MH, Aminuzzaman FM, Ahmed F. 2001. Efficacy of fungicide and botanicals in controlling leaf blight of wheat and its cost analysis. *Pakistan Jr. Biol. Science* 4: 178-180.
8. Rahman MM, Nahar MS, Karim MM, Begum K. 2013. Efficacy of fungicides in controlling leaf blight of wheat. *Intern. Jr. Expt. Agriculture* 3: 1-3.
9. Sahu DK, Khare CP, Singh HK, Thakur MP. 2013. Evaluation of newer fungicide for management of early blight of tomato in Chhattisgarh. *The Bioscience* 8(4): 1255-1259.
10. Kommeta M, Kapadiya B, Akbari FL. 2019. Evaluation of different fungicides against leaf blight (*Alternaria triticina*) of wheat under *in vitro* condition. *Int. Jr. Curr. Microbiology and Applied Science* 8(6): 1751-1758.
11. Shivankar SK, Shivankar RS, Nagone AH. 2000. Evaluation of fungicides against Alternaria leaf blight of wheat. *Indian Jr. Science* 32: 243-249.
12. Patel RC. 2008. Studies on fruit rot (*Alternaria alternata* Fr. Keissler) of chilli (*Capsicum annum* L.) and its management. *M. Sc. (Agriculture) Thesis,* Anand Agricultural University, Anand, Gujarat, India.
13. Ginoya CM, Gohel NM. 2015. Evaluation of newer fungicides against *Alternaria alternata* (Fr.) Keissler causing fruit rot disease of chilli. *Int. Jr. Plant Protection* 8(1): 169-173.
14. Kakraliya SS, Choskit D, Pandit D, Abrol S. 2017. Effect of bio-agents, neem leaf extract and fungicides against Alternaria leaf blight of wheat (*Triticum aestivum* L.). *Nat. Prod. Chem. Research* 5: 295. doi: 10.4172/2329-6836.10002
15. Bhatta M, Regassa T, Wegulo SN, Baenziger SP. 2018. Foliar fungicide effects on disease severity, yield, and agronomic characteristics of modern winter wheat genotypes. *Crop Economics, Production, and Management Agronomy Journal* 110(2): 602-610.
16. Khan A, Hsiang T. 2003. The infection process of *Colletotrichum graminicola* and relative aggressiveness on four turfgrass species. *Can. Jr. Microbiology Ottawa* 49(7): 433-442.
17. Lukens RJ. 1971. *Chemistry of Fungicidal Action*. Champman and Hall Ltd. London. pp 56-66.