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Efficacy of fungicides in *in-vitro* condition against *Fusarium graminearum* incited by head blight of wheat

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Abstract
Fusarium head blight (FHB) or scab is one of the major fungal diseases of cereals worldwide. This disease can cause severe damage to many economically important crops such as wheat, barley, corn and oats. Among those *Fusarium graminearum* is a seed and soil borne fungus causing the head blight disease in wheat? Most of the promising wheat cultivars are under a great threat for profitable cultivation due to the attack of Fungi, bacteria, virus and nematodes. A various bio-agents, botanicals and fungicide were applied for integrated management of pathogen. So, aim of this research efficacy of fungicides in different concentration the minimum growth of mycelium was found in T4- Carbendazim (12.00mm) in all concentration at 7th DAI followed by T5 Tebuconazole (19.00, 17.00 and 16.66 mm) at 50 ppm, 100 ppm and 150ppm in T2 Azoxytrobin (38.33, 34.00 and 32.66 mm) at 50, 100 and 150ppm respectively and highest growth of mycelium was recorded the untreated control growth of 82.33 mm.

Keywords: efficacy *Fusarium* fungicides head blight and wheat

Introduction
Fusarium head blight (FHB) or scab is one of the major fungal diseases of cereals worldwide. This disease can cause severe damage to many economically important crops such as wheat, barley, corn and oats. In North America, *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch) is considered to be the major causative agent of FHB although other Fusarium species are also implicated (McMullen *et al.*, 1997; Gilbert & Tekauz, 2000) [7, 4]. Since 1980, FHB has caused major yield and quality losses in the Prairies and in eastern Canada (Gilbert *et al.*, 2001) [5]. Fusarium spp. have both sexual and asexual life cycles and follow a generalized life cycle. Haploid mycelial structures are formed in both developmental stages (Ma *et al.*, 2013) [6]. During the asexual life cycle, the mycelial structures produce three types of mitotic spores, *viz*. micro conidia produced from conidiophores, macro conidia produced from sporodochium and chlamydospores produced on and within hyphae and macro conidia. Mycelia can either form through apomixes, which is restricted to homothallic species, or self-sterile heterothallic species. Both sexual orientations result in airborne spores which infect the floral tissues and contaminate the grain with mycotoxins (Ma *et al.*, 2013) [6]. It still remains unclear whether *F. graminearum* is a true hemibiotroph or not. Such information will help researchers to give management recommendations that minimize overwintering of the pathogen.
Fungicides have been widely employed, providing limited disease protection, because even the best fungicides are not fully effective in controlling FHB. These factors that contribute to the effectiveness of fungicides need to be considered, (i) cultivar resistance, (ii) climate, (iii) economic returns or yield gain, (iv) fungicide type and dose and (vi) management inputs, which in turn include timing and frequency of application. A number of fungicides including carbendazim, hexaconazole, mancozeb, benomyl, prochloraz, propiconazole, tebuconazole and triadimenol are useful for FHB control. However, none of these chemicals has resulted in complete FHB control. Some countries like South Africa, have no chemicals registered for the control of FHB on wheat and barley. From recent reports, there is a high possibility of fungicide resistance development due to over-use of the same types of fungicides. This comes on the ground reports of a recently identified *F. graminearum* isolate that is resistant to the fungicide Tebuconazole in the USA (Spolti *et al.*, 2014) [12] and some strains that are resistant

to Benz imidazole based fungicide in China (Chen and Zhou, 2009) [2]. Therefore, for the ongoing search of effective fungicides to treat FHB, AHAS could be a potential for target-specific antifungal compounds. It would be of great interest to analyse this target in other major plant pathogenic fungi and attempt to develop a single antimicrobial compound which targets all major plant fugal pathogens.

Materials and Methods

The present study was done efficacy of fungicides against *F. graminearum* in six treatments with three replications. Five fungicides are used in different concentrations *viz*. 50, 100 and 150 ppm in laboratory. The study was conducted at laboratory of Plant Pathology, School of Agriculture, Uttaranchal University, Dehradun, India.

Maintenance of pure culture

The culture of *F. graminearum* was isolated and maintained on Potato Dextrose Agar medium by regular sub culturing. The culture of *Fusarium* specie was grown in sterilized Petri plates on potato dextrose agar (PDA) medium for 8 days. Single branched hyphae from the periphery of the growing colony was marked under low power (10x) of compound microscope and transferred to PDA slants for maintenance. These culture tubes was incubated at 24±1^{0}C for about a week and again sub-cultured on PDA medium before storing in a refrigerator at 05^0±1C for further use.

In vitro evaluation of fungicides

In vitro evaluation of five fungicides in three replications were evaluated *in vitro* at different concentrations (each @ 50, 100 and 150 ppm) against F. *graminearum* by applying Poisoned food technique (Nene and Thapliyal, 1993) [9] and using potato dextrose agar (PDA) as basal culture medium. Based on active ingredient, the requisite quantity of each test fungicide was calculated and mixed thoroughly with autoclaved and cooled (40^{0}C) PDA medium separately in conical flasks (250 ml / cap) to obtain desired concentrations of 50, 100 and 150 ppm. Fungicide amended PDA medium was then poured (20 ml/plate) aseptically in glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicide and its test concentrations, a triplicate set of petri plates/treatment/replication were maintained. After solidification of the medium, all the plates were inoculated aseptically with a 5 mm culture disc obtained from a week old actively growing pure culture of *F. graminearum* separately. The culture disc was placed on PDA in inverted position in the centre of the Petri plate and plates were incubated at 28 + 2 ^{0}C. Petri plates filled with plain PDA (without any fungicide) and inoculated separately with the culture disc of *F. graminearum* were maintained as untreated control. Observations on radial mycelial growth/colony diameter of the test pathogens were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogen, per cent mycelial growth inhibition of the test pathogens with the test fungicides over untreated control were calculated by applying following formula (Vincent, 1929) [14].

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

Statistical analysis

Data was analysed by using complete randomized design (CRD) with the help of analysis of variance table (ANOVA) wherever required. The F value will be calculated and critical difference (CD) was tested at five per cent level of significance for comparing treatment means (Steel, 1997) [13].

Results and Discussion

The experiments were revealed that out of six treatments using five fungicides in different concentration experiment was conducted during 2020-21 *in-vitro*. The treatment, Antracol, Azoxytrobin, Polyram, Carbendazim and Tebuconazole at different concentrations (each @ 50, 100 and 150 ppm) with Control (PDA) against *F. graminearum* by applying Poisoned food technique. The most effective treatment was found T4 (12.00) in 150 ppm at 7th days DAI followed by T5 (16.66 mm) 150 ppm result show in (Table: 1) & Plate-1.

After 3rd day, however the minimum growth of mycelium was found in Carbendazim (7.00 mm) at 50, 100 and 150ppm both are same followed by Tebuconazole (11.66, 10.33 and 9.00 mm) at 50 ppm, 100 ppm and 150ppm in Azoxytrobin (18.00, 16.33 and 14.00 mm) at 50, 100 and 150ppm respectively and highest growth of mycelium was recorded the untreated control growth of 29.66 mm.

After 5th day, however the minimum growth of mycelium was found in Carbendazim (9.00 mm) at 50, 100 and 150ppm both are same followed by Tebuconazole (13.66, 13.33 and 12.66 mm) at 50 ppm, 100 ppm and 150ppm in Azoxytrobin (25.33, 23.66 and 21.66 mm) at 50, 100 and 150ppm respectively and highest growth of mycelium was recorded the untreated control growth of 54.33 mm.

After 7th day, however the minimum growth of mycelium was found in Carbendazim (12.00 mm) at 50, 100 and 150ppm both are same followed by Tebuconazole (19.00, 17.00 and 16.66 mm) at 50 ppm, 100 ppm and 150ppm in Azoxytrobin (38.33, 34.00 and 32.66 mm) at 50, 100 and 150ppm respectively and highest growth of mycelium was recorded the untreated control growth of 82.33 mm.

The results obtained from the experiment suggest that the fungicides have been effective in inhibiting the mycelia growth of the pathogen. The percent mycelia inhibition was calculated using the formula, I = [C-T)/C] X 100, where,

I = % of growth inhibition

C=control plates growth

T=Treated plates growth.

After 7th day, mycelium inhibition was found in Carbendazim (85.42%) at 50, 100 and 150ppm both are same followed by Tebuconazole (76.92, 79.35 and 79.76%) at 50 ppm, 100 ppm and 150ppm in Azoxytrobin (53.44, 58.70 and 60.33%) at 50, 100 and 150ppm respectively.

Efficacy of fungicides against *F. graminearum* reported earlier by several workers the DMI (sterol biosynthesis inhibitors) or triazoles (tebuconazole, metconazole and prothioconazole) fungicides are reported to be the most effective chemical compounds against Fusarium spp. to reduce FHB incidence and DON accumulation (Edwards *et al.*, 2001; Simpson *et al.*, 2001; Pirgozliev *et al.*, 2002; Mesterházy *et al.*, 2003) [3, 1, 10, 8]. Several triazole group of fungicides which includes metconazole, prothioconazole, tebuconazole, prothioconazole + tebuconazole, and propiconazole are effectively managing fusarium head blight. While, application of strobilurin have mixed response. Early application may help to improve agronomic traits and spraying at the time of anthesis or grain filling will reduce the infection but increase the DON accumulation (Blandino *et al.*, 2009) [1]. Zafar *et al.*, (2020) [15] evaluated four fungicides

(Nativo, Amistar, Carbandazim and Topsin-M) in i*n-vitro* condition were used to check mycelial growth inhibition. After investigation Nativo found to be most effective against mycelial growth followed by Topsin M, Carbendazim and Amistar.

Conclusion

Fusarium species may cause severe plant diseases and produce mycotoxins, which have a serious impact on human and animal health. The fungicides inhibited the growth of fungi and reduced mycotoxin production. The results suggest the possibility of pesticides application is a major, in case of apply as a seed treatment intended for sowing, which may contribute to increased resistance of plants to Fusarium. In my research found that fungicide carbendazim best for the control of Fusarium species.

Table 1: Evaluation of different fungicides against *Fusarium graminearum* in *in-vitro* condition

Treatment	3rd day			5th day			7th day			Inhibition % at 7th days		
	50 PPM	100 PPM	150 PPM	50 PPM	100 PPM	150 PPM	50 PPM	100 PPM	150 PPM	50 PPM	100 PPM	150 PPM
T_1-Antracol	37.00	26.00	22.66	43.00	37.33	37.66	52.66	43.33	40.33	36.03	47.37	51.01
T_2-Azoxytrobin	18.00	16.33	14.00	25.33	23.66	21.66	38.33	34.00	32.66	53.44	58.70	60.33
T_3-Polyram	41.66	31.33	29.00	52.66	41.00	36.66	57.66	57.33	51.66	29.96	30.36	37.25
T_4-Carbendazim	7.00	7.00	7.00	9.00	9.00	9.00	12.00	12.00	12.00	85.42	85.42	85.42
T_5-Tebuconazole	11.66	10.33	9.00	13.66	13.33	12.66	19.00	17.00	16.66	76.92	79.35	79.76
T_6-Control (PDA)	29.66	29.66	29.66	54.33	54.33	54.33	82.33	82.33	82.33	0	0	0
CD at 5%	5.87	4.17	4.00	3.69	2.36	3.25	4.60	3.76	3.14	-	-	-
CV	13.51	11.54	11.98	6.25	4.40	6. 27	6.01	4.9	4.44	-	-	-

* Mean of Three Replications

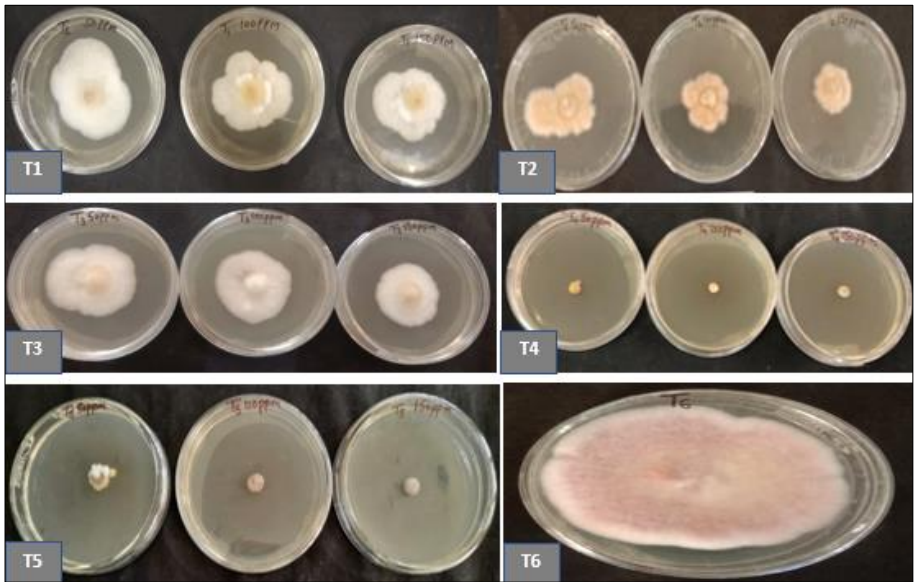

Plate 1: Effect of different fungicides on growth of mycelium of *Fusarium graminearum*.

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