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RESEARCH ARTICLE Agricultural Science Digest, Volume Issue : ()

Bio Efficacy of Fungicides against the Wheat Head Blight Causing Fungus, *Fusarium graminearum*

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10.18805/ag.D-5999

ABSTRACT

Background: Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, is a significant threat to global wheat production. It results in extensive yield losses and mycotoxin contamination. This study aims to evaluate various fungicides *in vitro* to determine their effectiveness in controlling FHB. Through a comprehensive analysis of the fungicides' *in vitro* efficacy against *F. graminearum*, this research endeavours to establish a connection between laboratory observations and real-world field applications.

Methods: The current investigation was conducted in the Plant Pathology laboratory of CSKHPKV, Palampur, during 2021-2022, wherein, nine fungicides *viz.,* trifloxystrobin 25% + tebuconazole 50% (Nativo 75WG), carbendazim 12% + mancozeb 63% WP (SAAF), difenoconazole 25 EC (Score), carbendazim 50 WP (Bavistin), Propiconazole 25 EC (Tilt), tebuconazole 250 EC (Folicur), mancozeb 75WP (Indofil), copper oxychloride 50 WP (Blitox) and carboxin 37.5% +thiram 37.5% WS (Vitavax power) were evaluated at different concentrations against *F. graminearum*. In the *in vitro* assay, *F. graminearum* was subjected to varying concentrations of fungicides, ranging from low to high, to determine their inhibitory effects on the growth of mycelia, which are thread-like structures that form the vegetative part of fungi. The assay involved carefully monitoring the development of *F. graminearum* under different conditions to obtain accurate results.

Result: The study's results revealed that carbendazim 50 WP, trifloxystrobin 25% + tebuconazole 50% WG, and tebuconazole 250 EC at concentrations of 100 ppm or higher were the most effective in inhibiting the growth of mycelium. The varying degrees of effectiveness among the fungicides tested indicate that *F. graminearum* responds differently to various chemical treatments. The findings from this *in vitro* evaluation will provide valuable data for the ongoing efforts to develop effective and sustainable fungicide-based strategies to combat Fusarium head blight in wheat crops.

Key words: Disease management, *Fusarium graminearum*, Fusarium Head Blight, Wheat.

INTRODUCTION

Wheat is one of the most important food grain crop in the world and it is affected by number of abiotic and biotic factors. The biotic stresses are most serious constraints to wheat production in India and would continue to be a major challenge in achieving the goal sustainable food security (Gangwar *et al.,* 2018). Among the biotic factors, Fusarium Head Blight is an emerging disease leading to quantity and quality loss in wheat (Yashwant *et al.,* 2015).

Several species of Fusarium cause the disease, but *Fusarium graminearum* (Schwabe) is one of the major contributors to the disease's development in many nations. In the agricultural and food sector, outbreaks of FHB not only lead to a decline in both grain yield and quality but also result in the contamination of grains with harmful secondary fungal byproducts, commonly referred to as mycotoxins (Kaur and Rana, 2022). Deoxynivalenol (DON) and nivalenol (NIV) are particularly noteworthy among these mycotoxins (Colombo *et al.,* 2020).

There has been a significant increase in the occurrence of Fusarium Head Blight (FHB) globally in recent years. This increase is attributed to various factors such as long-term wheat-maize rotation, reduced tillage practices, and the cultivation of highly susceptible wheat varieties (Zhu *et al.,* 2018). Internationally, a common approach to combat FHB involves breeding wheat varieties with resistance to the disease (Zhu *et al.,* 2019). To manage and control FHB, screening and extensive uses of fungicides are common strategies employed. Fungicides are typically administered within a narrow timeframe, usually during anthesis, which is a critical period when the fungus initiates infection leading to grain contamination (Caldwell *et al.,* 2017). However, for effective control, the

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How to cite this article: Kaur, G., Rana, S.K., Masurkar, P., Anand, S., Sahoo, M., Singh, S. and Sinha, P. (2024). Bio Efficacy of Fungicides against the Wheat Head Blight Causing Fungus, *Fusarium graminearum.* Agricultural Science Digest. DOI: 10.18805/ag.D-5999.

Submitted: 18-03-2024 **Accepted:** 21-05-2024 **Online:** 23-07-2024

application of fungicides may need to extend up to 11 days after anthesis to prevent the accumulation of deoxynivalenol (DON) in the seeds (Freije and Wise, 2015). According to earlier research, triazoles, specifically prothioconazole, demonstrated efficacy against FHB by impeding the germination of ascospores and the growth of mycelium in *Fusarium graminearum* (Klix *et al.,* 2007). Additionally, certain benzimidazole fungicides, including carbendazim, have been employed in FHB control for an extended period (Liu *et al.,* 2014).

We have conducted research on the occurrence of Fusarium Head Blight (FHB) in open fields located in various districts of Himachal Pradesh, India. Our study aims to establish a foundation for future wheat FHB management in this region. The main objective is to identify the causal agent and test fungicides *in vitro* to find effective treatments against the pathogen. By thoroughly examining the *in vitro* performance of fungicides against *Fusarium graminearum*, our study bridges the gap between laboratory findings and practical field applications. Our insights could lead to better FHB control measures, ensuring the yield and quality of wheat crops.

MATERIALS AND METHODS

Collection, isolation and identification of the pathogen

Infected earheads displaying typical symptoms of Fusarium Head Blight (FHB) were collected from the wheat-growing districts *viz.*, Kangra, Una, Sirmour, Hamirpur, Mandi and Bilaspur of Himachal Pradesh, India and transported to the laboratory. The associated pathogen was isolated using the Agar plate method with Potato Dextrose Agar (PDA) as the basal medium. A pure culture of the pathogen was obtained using the single hyphal tip method (Dhingra and Sinclair, 1985). The pure cultures were preserved in a refrigerator at 4°C for further studies. The pathogen was identified based on morphological and cultural parameters as Booth (1971) and Leslie and Summerell (2006) described. The *in vitro* studies were conducted in the laboratory of Plant Pathology, CSKHPKV, Palampur, India during 2021-2022.

In vitro evaluation of fungicides

Nine fungicides *viz.*, trifloxystrobin 25% + tebuconazole 50% (Nativo 75WG), carbendazim 12% + mancozeb 63% WP (SAAF), difenoconazole 25 EC (Score), carbendazim 50 WP (Bavistin), Propiconazole 25 EC (Tilt), tebuconazole 250 EC (Folicur), mancozeb 75WP (Indofil), copper oxychloride 50 WP (Blitox) and carboxin 37.5% +thiram 37.5% WS (Vitavax power) were evaluated at 50, 100, 250, 500, 750, 1000, 1500 and 2000 ppm concentrations against test pathogen by poisoned food technique (Nene and Thapliyal, 2000).

Double strength PDA medium was prepared in distilled water and sterilized in an autoclave at 1.5kg/cm^2 pressure (121±1°C) for 20 minutes. Simultaneously, double strength concentrations of fungicides were also prepared in sterilized distilled water and mixed individually with the double strength medium to achieve the desired concentrations. Then the contents were poured into Petri plates and allowed to solidify. Mycelial bits (5 mm dia) were cut with the help of a sterilized cork borer from the margin of an actively growing colony and each placed in the centre of media plates. A control treatment was also maintained wherein only plain sterilized distilled water was added to double strength medium. Each treatment was replicated thrice in CRD design. The inoculated plates were incubated at 25±1°C in a BOD incubator.

Regular observations were made for the growth of *F. graminearum* and finally, colony diameters were measured with a scale when the control plates were completely covered by the pathogen and per cent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947).

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

Where,
C= Mycelial growth of fungus in control (mm).
T= Mycelial growth of fungus in treatment (mm).

RESULTS AND DISCUSSION

Isolation and identification of *Fusarium* species causing FHB of wheat

The fungi that were obtained from the diseased earheads of wheat were kept as pure cultures on PDA slants. To identify the fungus, its morpho-cultural characteristics like colony growth, colour, shape and size of spores were analyzed and compared with standard descriptions/ keys. (Plate 1 was used for comparison and confirmation). The fungus produced mycelium in shades of white, yellow and pale pink colours. Its macroconidia were canoe-shaped with tapered apical cells and foot-shaped basal cells, while microconidia were absent. The conidia measured 5-6 septate and 45.0-68.0 µm in length and 4.1-4.3 µm in width. Based on these characteristics, the pathogen was identified as *Fusarium graminearum*.

In vitro evaluation of fungicides against *Fusarium graminearum*

A perusal of the data presented in Table 1 indicated that all the fungicides and combination formulations except copper oxychloride gave complete inhibition of mycelial growth at ≥500 ppm concentrations. At 250 ppm, propiconazole 25 EC, tebuconazole 250 EC, carbendazim 50 WP, trifloxystrobin 25% + tebuconazole 50% WG and carboxin 37.5% +thiram 37.5% WS all showed cent per cent inhibition. At 100 ppm concentration, tebuconazole 250 EC, carbendazim 50 WP and trifloxystrobin 25% + tebuconazole 50% WG gave complete inhibition of mycelial growth (Plate 2). At 50 ppm carbendazim 50 WP gave the maximum inhibition of 87.77 per cent followed by trifloxystrobin 25% + tebuconazole 50% WG, tebuconazole 250 EC, carboxin 37.5% + thiram 37.5% WS and propiconazole 25 EC with 85.33, 83.84, 80.74 and 77.41 per cent inhibition. Copper oxychloride 50 WP, a non-systemic fungicide was found effective only at higher

concentrations *i.e.* 2000, 1500, 1000 and 750 ppm giving 90.96, 80.01, 71.16 and 61.23 per cent mycelial inhibition.

The effectiveness of some of the above tested fungicides has also been reported by earlier workers (Ivic *et al.,* 2011; Avozani *et al.,* 2014). Jones (2000) reported that *F. graminearum* isolates were sensitive to benomyl, tebuconazole, fludioxonil and mancozeb at concentrations of 3.5×10^{-1}, 9.9×10^{-1}, 7.4×10^{-3} and 7.3 mg a.i./ L, respectively

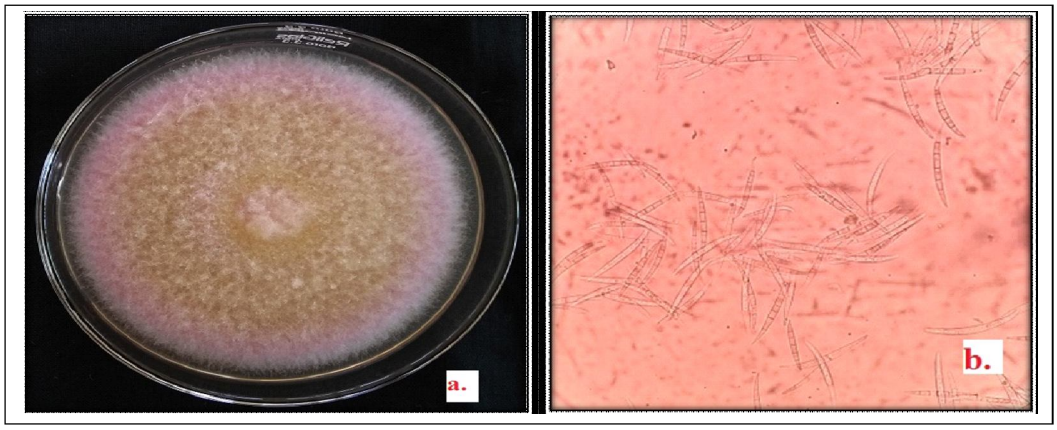

Plate 1: Morphocultural characteristics of *Fusarium graminearum* (a. mycelium and b. macroconidia).

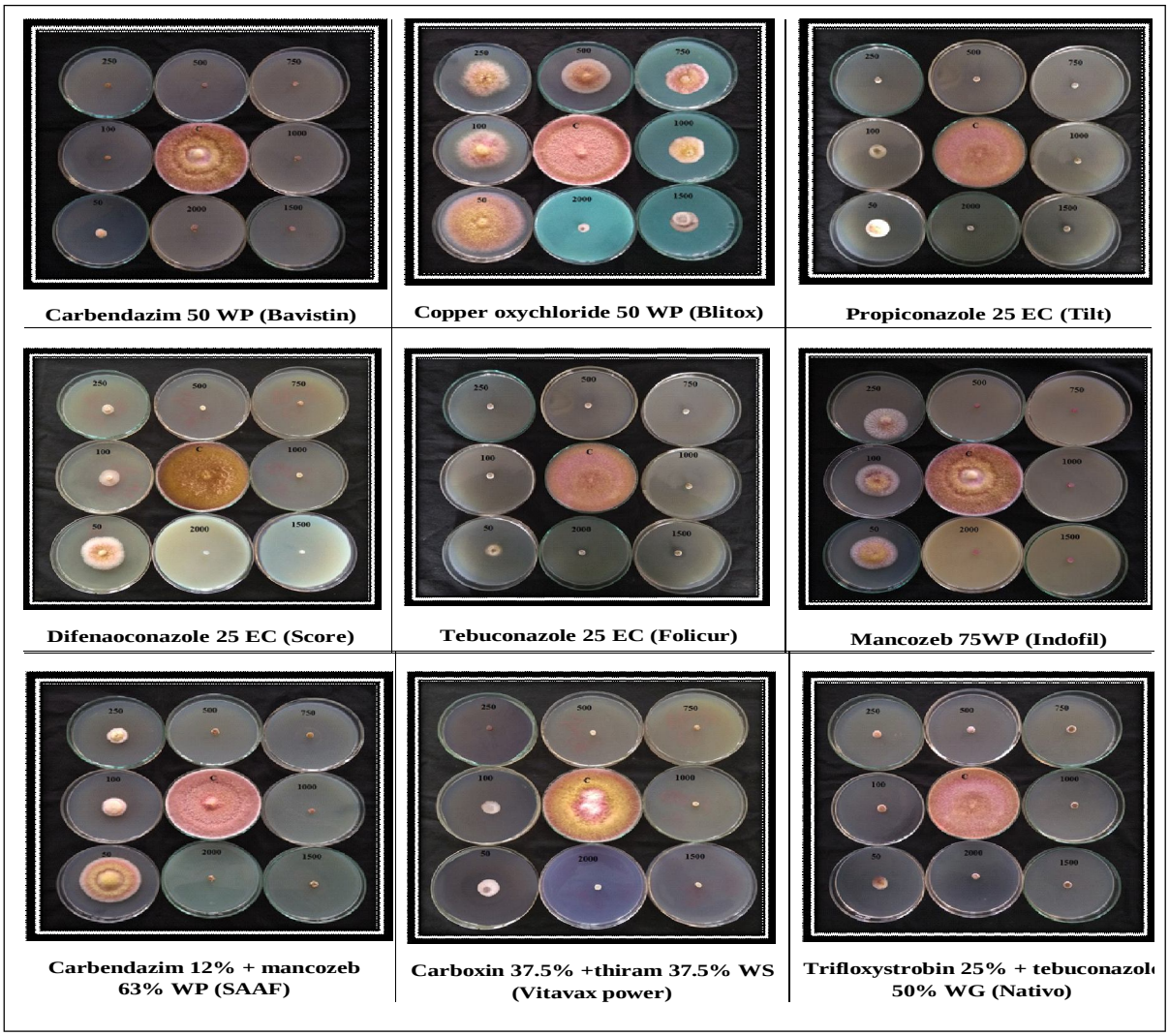

Plate 2: *In vitro* evaluation of fungicides against *Fusarium graminearum.*

Table 1: *In-vitro* evaluation of fungicides against *Fusarium graminearum.*

Fungicide	Mycelial growth (mm) at different concentration (µg/ ml)								Mycelial inhibition (%) at different concentration (µg/ ml)							
	50	100	250	500	750	1000	1500	2000	50	100	250	500	750	1000	1500	2000
Difenoconazole	44.23	20.48	14.15	0.00	0.00	0.00	0.00	0.00	50.86	77.24	84.28	100.00	100.00	100.00	100.00	100.00
25 EC (Score)	(41.66)*	(4.63)**	(3.87)**	(1.00)**	(1.00)**	(1.00)**	(1.00)**	(1.00)**								
Propiconazole	20.33	13.09	0.00	0.00	0.00	0.00	0.00	0.00	77.41	85.46	100.00	100.00	100.00	100.00	100.00	100.00
25 EC (Tilt)	(26.78)	(3.75)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)								
Tebuconazole	14.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	83.84	100.00	100.00	100.00	100.00	100.00	100.00	100.00
25 EC (Folicur)	(22.39)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)								
Carbendazim	11.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	87.77	100.00	100.00	100.00	100.00	100.00	100.00	100.00
50 WP (Bavistin)	(19.35)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)								
Trifloxystrobin	13.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	85.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00
25%+ tebuconazole	(21.28)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)								
50% WG (Nativo)																
Mancozeb	51.11	45.62	39.33	0.00	0.00	0.00	0.00	0.00	43.21	49.31	56.30	100.00	100.00	100.00	100.00	100.00
75WP (Indofil)	(45.61)	(6.82)	(6.35)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)								
Carbendazim	53.33	25.04	19.33	0.00	0.00	0.00	0.00	0.00	40.74	72.18	78.52	100.00	100.00	100.00	100.00	100.00
12% + mancozeb	(46.89)	(5.10)	(4.50)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)								
63% WP (SAAF)																
Carboxin 37.5%	17.33	10.67	0.00	0.00	0.00	0.00	0.00	0.00	80.74	88.15	100.00	100.00	100.00	100.00	100.00	100.00
+thiram 37.5%	(24.59)	(3.41)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)								
WS (Vitavax power)																
Copper oxychloride	81.33	60.78	50.67	45.25	34.89	25.96	17.45	8.14	9.63	32.47	43.70	49.73	61.23	71.16	80.61	90.96
50 WP (Blitox)	(64.37)	(7.86)	(7.18)	(6.79)	(5.98)	(5.18)	(4.29)	(3.01)								
Control	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(71.53)	(9.53)	(9.53)	(9.53)	(9.53)	(9.53)	(9.53)	(9.53)								
CD (p=0.05)	1.28	0.13	0.12	0.11	0.13	0.10	0.12	0.10	-	-	-	-	-	-	-	-

*Figures within parentheses are arc sine transformed value.

**Figures within parentheses are square root transformed values.

under laboratory conditions. Mullenborn *et al.* (2008) recorded high sensitivity of different *Fusarium* spp. *viz.*, *F. graminearum, F. culmorum* and *F. poae* to tebuconazole and prothioconazole as compared to fluoxastrobin and azoxystrobin under laboratory conditions. Gupta (2019) found that vitavax power and bavistin showed cent per cent mycelial inhibition of *F. graminearum* even at 50 ppm concentration, whereas tilt was found least effective (55.74% inhibition) at the same concentration. Khanna *et al.* (2021) evaluated six fungicides against *F. oxysporum* f. sp. *ciceris* and found that carbendazim 50 WP was the most toxic fungicide in restricting colony diameter of the pathogen with the least EC50 and EC90 values of 1.28 and 15.24 ppm a.i. followed by azoxystrobin 23 SC with corresponding values of 1.57 and 49.16 ppm a.i., respectively. Bhujbal *et al.,* (2021) reported that Carbendazim (0.1%) resulted in 92.13 per cent inhibition of pathogen *F. oxysporum* f.sp. *lycopersici.* Tang *et al.,* (2022) tested the bioefficacy of seven fungicides against *F. avenaceum* and found that carbendazim and Propiconazole exhibited the highest inhibition rate with an EC_{50} value of 2.1 mg/L and 2.6 mg/L, respectively.

CONCLUSION

Fusarium species can lead to significant plant diseases and the production of mycotoxins, posing a substantial threat to human and animal health. In conclusion, the *in vitro* evaluation of fungicides against Fusarium Head Blight in wheat represents a crucial step towards enhancing our understanding and management of this destructive disease. The results obtained from these controlled laboratory experiments provide valuable insights into the efficacy of various fungicides in inhibiting the growth and development of *Fusarium graminearum*, the primary causative agent of FHB. The findings underscore the significance of triazoles and benzimidazole fungicides as effective agents against FHB. Such knowledge is instrumental in guiding the selection of promising candidates for subsequent field trials and practical applications in agriculture. Moreover, the study sheds light on the potential mechanisms of action exhibited by the evaluated fungicides, offering a deeper understanding of their impact on the fungal pathogen. This information is pivotal for the development of targeted and sustainable strategies for FHB management. By bridging the gap between laboratory findings and on-field applications, this research contributes to the collective effort aimed at safeguarding wheat crops from the detrimental effects of Fusarium Head Blight, ensuring global food security and maintaining the quality of agricultural produce.

Conflict of interest

All authors declared that there is no conflict of interest.

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