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Difenoconazole: A new seed dressing molecule for effective management of flag smut (*Urocystis agropyri*) of wheat

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Abstract

Flag smut (FS) caused by *Urocystis agropyri* (Preuss) Schroet., is one of the major wheat yield limiting factors throughout the world. The chemical fungicides play a key role in effective and timely management of FS disease. To identify the suitable chemicals and appropriate dosages, five different fungicides Carboxin 17.5% + Thiram (17.5%), Tebuconazole (5.36%), Difenoconazole (3%), Flutriatol (25g L^{-1}) and Carbendazim (50%WP) were evaluated at two different concentrations as seed treatment under field conditions in FS sick plots for two *rabi* seasons. The seed treatment with Difenoconazole 3% (2.5 g kg^{-1} seed) provided complete protection (100%) against flag smut disease. Further, it was observed that the fungicide seed treatment not only controlled smut severity, but also avoided the yield losses and enhanced the wheat grain yield as compared to the control. Therefore, adoption of this fungicide as seed treatment to combat *U. agropyri* in absence of resistant cultivars in FS affected areas would benefit the farmers.

Keywords: Flag smut, fungicides, seed treatment, *Urocystis agropyri,* yield

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most vital staple food crops in the world, but its production is adversely affected by a number of plant pathogens from sowing to harvest. The seed- and soil-borne fungus *Urocystis agropyri* responsible for flag smut is one of the important diseases of wheat. It was first reported from South Australia in 1868 and was subsequently observed in various other countries, including Chile, Egypt, China, Japan, South Africa, Mexico, Pakistan, India and USA (Savchenko *et al.,* 2017; Toor *et al.,* 2013). The pathogen causes systemic infection on host plants, forming sori in leaves, as narrow stripes between the leaf veins (Mordue and Walker, 1981). As a result, infected plants fail to produce any seeds and support malformed inflorescences due to sorus formation (Purdy, 1965). The seed and soil-borne nature of the disease results in gradual built up of the inoculum in soil (Ram and Singh, 2004). Under congenial environmental conditions, pathogen may cause complete yield loss (Hori, 1907). Several reports indicated that the incessant nature of the pathogen and the cultivation of susceptible varieties, flag smut may become a serious threat for sustainable wheat cultivation (Shekhawat and Majumdar, 2013; Singh and Singh, 2011). Up to 20% of crop loss was reported in the USA, Iran, Italy and Egypt (Purdy, 1965). Yu *et al.* (1936) reported 90–94% infection in China. A yield loss of 5% was reported in India by Padwick (1948). Flag smut incidence has been reported from different states of India, such as Punjab, Haryana, Himachal Pradesh, Madhya Pradesh, Uttar Pradesh, Delhi, Bihar and Rajasthan (Goel *et al.,* 1977).

Effective control of FS originating from both seed-borne and soil-borne inoculum have been reported through seed treatment with Copper carbonate (Fischer and Holton, 1957), Quintozene (Yasu and Yoshino, 1963), Benomyl, Carboxin and Oxycarboxin (Metcalfe and Brown 1969; Goel *et al.,* 2001). In addition, Fenfuram, Triadimefon, Triadimenol and Tebuconazole provided control of *U. agropyri* (Goel and Jhooty, 1985; Tariq *et al.,* 1992; Singh and Singh, 2011; Shekhawat and Majumdar, 2013). However, in past several years, fungicide chemistry has evolved rapidly and at present, there are several new fungicides in the market. The continuous use of similar type of fungicide molecules may develop resistant strains of pathogens. Thus, it is needed to evaluate new fungicides for efficacy against seed and soil borne diseases of wheat. Therefore, the objectives of this study were to evaluate the efficacy of fungicides in controlling FS of wheat under field conditions to avoid losses caused by this fungus.

2 Materials and Methods

2.1 Field experiments and fungicide treatments: Field experiments were conducted during wheat growing seasons 2014-15 and 2016-2017 at Plant Pathological Research field at ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal. Each experiment was performed in randomized block design (RBD) with three replications. The fungicides were evaluated in disease sick plots created artificially by continuous supplementation of FS inocula. Wheat cultivar 'PBW343' was selected for the study. Seeds were sown in 6 m × 3 m plots with a row to row spacing of 22.5 cm. Sowing was done under moisture deficit conditions to provide predisposing for FS development. The seed rate (100 kg ha^{-1}), NPK fertilizer (120:60:40 kg ha^{-1}) and FYM (2 t ha^{-1}) were applied to the experimental fields. Sowing was done on the first week of November and crop was raised with standard agronomic practices. The seeds were treated with different chemical fungicides (Table 1) and seed treatments (ST) include: T1= Carboxin (17.5%) + Thiram (17.5%) @2.5 ml kg^{-1} seed; T2= Carboxin (17.5%) + Thiram (17.5%) @1.25 m1 kg^{-1} seed; T3=Tebuconazole (5.36%) @ 2.5 ml kg^{-1} seed; T4=Tebuconazole (5.36%) @ 1.25 m1 kg^{-1} seed; T5= Tebuconazole (5.36%) @ 2.5 g kg^{-1} seed; T6= Tebuconazole (5.36%) @1.25 g kg^{-1} seed; T7= Difenoconazole (3%) @ 2.5 g kg^{-1} seed; T8= Difenoconazole (3%) @ 1.25g kg^{-1} seed; T9= Flutriafol (25g L^{-1}) @ 2.5 ml kg^{-1} seed; T10= Flutriafol (25g L^{-1}) @ 1.25 ml kg^{-1} seed; T11= Carbendazim (50%WP)@ 2.5 g kg^{-1} seed; T12= Carbendazim (50%WP) @ 1.25 g kg^{-1} seed. Untreated seed without any fungicide served as a control.

2.2 Disease assessment and data recoding: Flag smut (FS) disease recordings were made ten weeks post wheat sowing. The percentage of smutted plants and of smutted tillers per plant in each plot was recorded. A tiller was considered infected, if disease symptoms have appeared on any part of the stem, culm and leaf. Second and third assessments were made at fortnightly intervals to record further changes in flag smut development and combined for final analysis. The yield data (1000 grain weight) was also recorded on whole plot basis and converted to quintal per hectare after harvesting. Flag smut incidence (FSI) was calculated for each plot by using following formula:

2.3 Statistical analysis: The disease and yield data was subjected to analysis of variance (ANOVA) and pair-wise mean comparison was made by using Duncan's multiple range test (DMRT). Critical difference (CD) value at 0.05 probability levels were worked out for testing significance of differences among treatments.

Table 1: Effect of fungicide treatments on incidence of flag smut of wheat under disease sick plots during 2014-15 and 2016-17 rabi seasons

Fungicide treatment(s)	Concentration used (kg^{-1} seed)	* FSI (%)		Pooled Mean		Yield (q ha^{-1})		Pooled Mean (q ha^{-1})
		2014-15	2016-17	FSI (%)	**PDC (%)	2014-15	2016-17	
Carboxin (17.5%) + Thiram (17.5%)	2.5 ml	0.00^{a}	2.16^{b}	1.08^{a}	85.88	34.33^{a}	45.40^{b}	39.87^{b}
Carboxin (17.5%) + Thiram (17.5%)	1.25 m1	0.08^{a}	3.39c	1.74^{b}	77.25	34.67^{a}	46.30^{b}	40.49^{b}
Tebuconazole (5.36%)	2.5 ml	0.00^{a}	0.96^{a}	0.48^{a}	93.73	33.77^{b}	49.10^{a}	41.44^{a}
Tebuconazole (5.36%)	1.25 m1	0.00^{a}	2.40^{b}	1.20^{a}	84.31	34.37^{a}	46.40^{b}	40.39^{b}
Tebuconazole (5.36%)	2.5 g	0.20^{a}	0.00^{a}	0.13^{a}	98.30	34.53^{a}	51.00^{a}	42.77^{a}
Tebuconazole (5.36%)	1.25 g	0.06^{a}	0.00^{a}	0.03^{a}	99.61	34.67^{a}	47.20^{b}	40.94^{b}
Difenoconazole (3%)	2.5 g	0.00^{a}	0.00^{a}	0.00^{a}	100.00	34.20^{b}	48.60^{a}	41.40^{a}
Difenoconazole (3%)	1.25g	0.17^{b}	0.00^{a}	0.09^{a}	98.82	34.40^{a}	50.00^{a}	42.20^{a}
Flutriafol (25g L-1)	2.5 ml	0.00^{a}	5.29^{d}	2.65c	65.36	35.10^{a}	44.00c	39.55^{b}
Flutriafol (25g L-1)	1.25 ml	0.12^{a}	8.52^{e}	4.32^{d}	43.53	34.30^{a}	43.90c	39.10c
Carbendazim (50%WP)	2.5 g	0.09^{a}	0.03^{a}	0.06^{a}	99.22	34.97^{a}	50.90^{a}	42.94^{a}
Carbendazim (50%WP)	1.25 g	0.13^{a}	0.00^{a}	0.07^{a}	99.08	34.57^{a}	46.60^{b}	40.59^{b}
Control	-	3.05^{b}	12.24^{f}	7.65^{e}		32.50c	41.50^{d}	37.00^{d}
***CD (P≥0.05%)		0.984	1.663	1.32		0.81	2.85	1.83

* FSI = Flag smut incidence; **PDC= Per cent disease reduction over control; ***CD= Critical difference; Means followed by the same letter within a column are not significantly different according to Fisher's protected least significant difference test at (P≥0.05).

3 Results and Discussion

There is very little information available on the application of fungicides to contain FS of wheat. Therefore, in present study attempt has been made to illustrate the impact of fungicidal seed treatment for the management of FS. The triazole fungicides (Difenoconazole, Thiabendazole and Carboxin plus Thiram) have been reported as effective molecules as seed dressers for controlling Tilletia controversa responsible for dwarf bunt of wheat (Sitton *et al.,* 1993). Mechanistically, Difenoconazole targets ergosterol biosynthesis by inhibiting the fungal enzyme sterol-1-4-α-demethylase (Munkvold, 2009). Wang *et al.* (2002) highlighted the significant role of Difenoconazole as seed treatment in suppressing sharp eyespot (Rhizoctonia cerealis) incidence by 60-80% at seedling stage. Similar reports regarding the application of fungicides as seed dressing for the management of wheat pathogens have been published by several workers (Sundin *et al.,* 1999; Buechley and Shaner, 1996; Cook *et al.,* 2002). These research findings corroborate with present study, where significant and positive effect ($P \geq 0.05$) was found among all tested fungicides in their ability to suppress disease development and enhancing crop yield during both the cropping seasons (Table 1). Analysis of variance showed all fungicidal treatments had significantly less smut than untreated control ($P \geq 0.05$) in both the years. In 2016-17, FSI score for the untreated control was relatively more (12.24%) compared to 2014-15 (3.05 %). Pooled analysis indicated that the untreated control had the highest FSI (7.65%) compared to fungicidal seed treatments. Among fungicide treatments, the highest FSI score (4.32%) was recorded in case of Flutriafol (25g L^{-1}) treated seed with 1.25 ml kg^{-1} seed, although found better than untreated control (Table 1). Complete disease control (100%) was achieved with Difenoconazole seed treatment @ 2.5 g kg^{-1} seed. Similar level of wheat protection from Tilletia controversa by Difenoconazole seed treatment has been reported earlier (Sitton *et al.,* 1993). The other fungicide seed treatments showing at par and more than 99% PDC include Tebuconazole (5.36 %) @ 1.25 g kg^{-1} seed (99.61%), Carbendazim (50%WP) @ 2.5 g kg^{-1} seed (99.22 %) and Carbendazim (50 %WP) @ 1.25 g kg^{-1} seed (99.08 %). Results on the effectiveness of Tebuconazole for the management of smut diseases are in conformity with Goel *et al.* (2001) and Shekhawat and Majumdar (2013). Further, Singh and Singh (2011) revealed that seed dressing with Tebuconazole (0.333 g kg^{-1} seed) resulted in complete control of loose smut diseases and enhanced grain yield in wheat. They also highlighted ineffectiveness of Vitavax (2.5g kg^{-1} seed) to provide complete control of FS infection in wheat, as observed in present study. The powder based formulations [Difenoconazole (3 %), Tebaconazole (5.36 %) and Carbendazim (50 % WP)] at both concentrations provided significantly better disease protection (> 98.30 %) compared to the liquid fungicidal formulations [Flutriafol (25g L^{-1}), Carboxin (17.5%) + Thiram (17.5%) and Tebuconazole (5.36 %)] at both the tested concentrations (Table 1).

All the tested fungicides did not show any visible toxic effect on wheat. The fungicidal seed treatments significantly enhanced wheat grain yield ($P \geq 0.05$) in comparison to the untreated control (Table 1). In 2014-15 and 2016-17, the mean grain yield of the untreated control was 32.50 and 41.45 q ha^{-1}, respectively (Table 1). The application of Carbendazim 50% WP @ 2.5 g kg^{-1}, Difenoconazole (3%) @ 2.5 g kg^{-1} seed; Difenoconazole (3%) @ 1.25 g kg^{-1} seed, Tebuconazole (5.36%)@ 2.5 g kg^{-1} seed and 2.5 ml kg^{-1} seed led to a greater increase in the grain yield as compared to other seed treatment. Highest grain yield (42.94 qha^{-1}) was recorded from the plots where seed treated with Carbendazim 50% WP @ 2.5 g kg^{-1}, where as it was at par with Difenoconazole and Tebuconazole. However, Flutriafol (25g L^{-1}) was found less effective relative to other fungicides. Parallel to present findings, Smiley and Patterson (1995) reported wheat yield gain and profitability from Dividend (difenoconazole--1(2-[4-(4-chlorophenoxy)-2-chlorophenyl] 4-methyl-1,3-dioxolan-2-yl-methyl)1H-1,2,4 triazole) and Vitavax seed treatments. Loughman *et al.* (2005) reported that Tebuconazole (Folicur) was more effective than Flutriafol (Impact) in foliar disease reduction and yield gain, as observed in present study. Therefore, farmers should choose fungicides based on their efficacy.

The results of field trials conducted in 2014-15 and 2016-17 mentioned in the present study concluded that correct fungicide applications could greatly reduce FSI score and enhance yield gain in disease prone areas. Further, Difenoconazole (2.5 g kg^{-1} seed) is reported as a promising alternative for timely and effective management of FS of wheat. Therefore, the adoption of effective fungicides to combat *U. agropyri* as a short-term control strategy in absence of resistant cultivars could manage the Flag smut effectively.

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