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EVALUATION OF DIFENOCONAZOLE 3% WS AS SEED TREATMENT AGAINST KARNAL BUNT IN WHEAT

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ABSTRACT

The present study was undertaken to evaluate the efficacy of difenoconazole 3% WS against Karnal bunt along with some recommended fungicide. Based on the data of two crop seasons it can be concluded that the spraying of propiconazole 25% EC @ 500ml/ha at anthesis was most effective fungicide both in terms of managing the Karnal bunt of wheat and improving the yield. Among the seed treatments, seed treatment with thiram @ 3.00g/kg seed was found most effective in improving the field emergence. However, the test fungicide difenoconazole 3% WS had no beneficial effects on field emergence, disease management and yield as compare to check. Nevertheless, the test fungicide difenoconazole 3% WS had no phytotoxic effects on wheat plants during both the crop seasons even at the highest dose tested.

INTRODUCTION

Karnal bunt of wheat caused by *Tilletia (Neovossia) indica* (Mitra) Mundkur, was first reported in 1931 Karnal, India. For many years it was a minor disease found only in Northwestern India. During 1969-70, it was unusually widespread in Northwest India and since 1974-75; it has been distributed throughout Northern India from West Bengal to the western border. Until the early 1970s, the distribution of *T. indica* was restricted to Asia and included most of northwestern India (Mundkur and Thirumalachar, 1952; Rush *et al.*, 2005). In 1972, it was reported in Sonora, Mexico and by 1983, it was being intercepted in railroad cars entering the United States from Mexico (Boratynski *et al.*, 1985). In 1996, *T. indica* it was reported in Arizona (Ykema *et al.*, 1996), resulting in the U.S. Department of Agriculture declaring an "extraordinary emergency" and a national survey for the presence of *T. indica*. Currently, the distribution of *T. indica* includes small areas in California, Arizona, and Texas in the United States, Afghanistan, India, Iran, Iraq, Mexico, Nepal, Syria and the Northern Cape Province of South Africa (Rong *et al.*, 2004, Rush *et al.*, 2005). The incidence of Karnal bunt varies considerably from year to year due to its dependence on favorable weather during heading (Bedi *et al.*, 1949, Fuentes-Davila, 1996, Gill *et al.*, 1993, Rush *et al.*, 2005, Singh, 1994, Singh *et al.*, 1996). It does not cause any economic yield loss, but poses impediments in world trade, since; the pathogen is on the quarantine list of several countries (Nagarajan *et al.*, 1997; Nagarajan and Sharma, 2001). At present, it is reported from several countries in the Asian and North American continents including India, Pakistan, Afghanistan, Syria, Iran, Iraq, Mexico, USA and South Africa (Nagarajan *et al.*, 1997; Nagarajan and Sharma, 2001; Sharma *et al.*, 1998; Singh, 1997a). Most of the countries, from where it is not reported, are afraid of introducing it, sometimes based on valid reasons, but mostly due to trade and political reasons (Sharma *et al.*, 2007). The disease has historically caused minor overall yield and quality losses in countries where it occurs (Brennan *et al.*, 1990, Rush *et al.*, 2005, Singh, 1994). Significant yield or quality losses are typically localized, occurring in highly susceptible cultivars grown in fields with high inoculum density during seasons with unusually favorable weather. However, losses could be high in certain weather conditions. In 1996, Iran lost more than 100,000 ha of wheat crop due to this disease. The epidemic happened when an outbreak occurred in - arid southeast of the country (Torabi *et al.*, 1996).

Resistance to Karnal bunt has been reported in several Indian wheats (Swati *et al.*, 2014) but most cultivars are susceptible. Application of foliar fungicides has achieved significant control of Karnal bunt. Two or more applications of propiconazole at or after spike emergence reduced the incidence of infected seeds by 95% (Aujla *et al.*, 1989, Singh *et al.*, 1993, Smilanick *et al.*, 1987a). An unfortunate consequence of applying systemic chemicals to wheat at late growth stage is the high level of residue in the grain at harvest. The systemic fungicide concentration needed for activity against *T. indica* may result in excessive residue and pose a regulatory issue.

Application of chemicals to seed is safest, cheapest and could be effective means of controlling most seed-borne pathogens and is most widely followed disease control practice used in all crops (Nene and Thapliyal, 1979 and Sharvelle, 1979). Hexachlorobenzene and cyano (methylmercuric) guanidine have been reported to prevent the germination of teliospores of *T. indica* in tests in Mexico (Joshi *et al.*, 1983). However, in India, only thiram 75 % WS has been registered with the CIB as seed dressing fungicide against Karnal bunt. The chemical is in use for the more than 3 decades. Continuous use of fungicides for long period of time may pose threat of development of resistance in pathogens. Thus products may become less effective - or even useless for controlling resistant pathogens and pests. Identification of new molecules that are effective against target pathogen and rotating them with other available fungicides can be one of the methods to manage the pesticide resistance. With this objective the present study was undertaken to evaluate the efficacy of Difenoconazole 3% WS against Karnal bunt. The test fungicide has been registered in India for the management of loose of wheat, however, not for Karnal bunt. Therefore, present investigation was carried out to test the efficacy of difenoconazole 3% WS against Karnal bunt of wheat.

MATERIALS AND METHODS

The experiment was conducted at Norman E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar to test the efficacy of difenocanazole 3% WS as seed treatment against Karnal bunt in wheat during *Rabi* 2014-15 and 2015-16 crop seasons. The experiment was laid out in a Randomized Block Design (RBD) with three replications and seven treatments. Seeds were pretreated with required quantity of chemicals as per the treatment schedule (Table 1) using slurry method (Sharma *et al.*, 2015). Treated seeds were further dried under shade before sowing in the field. However, spraying of propiconazole 25% EC @ 500ml/ ha was done at anthesis stage. Variety UP 262 was sown on 03.12.2014 and 08.12.2015 having 9 rows of 5 meters length at 23 cms row to row distance. The crop was raised as per the recommended agronomic practices. The details of the treatments are mentioned in Table 1.

The observations on Karnal bunt were recorded after threshing. A working sample of 2000 seeds was obtained from each replication and was visually examined for Karnal bunt infection. Karnal bunt incidence was calculated using the following formula (Fuentes-Davila and Rajaram 1994, Sharma *et al.*, 2007, Kumar, 2018):

Per cent Karnal bunt incidence = (No. of infected seeds/2000) × 100

Per cent increase in yield and avoidable yield loss (AYL) due to Karnal bunt were calculated separately using grain yield data from the management trials using following formula:

$AYL = [(Yp - Yu)/Yp] \times 100$

Where, Yp= Yield under protected condition and Yu = Yield under unprotected condition (Kumar, 2011, 2013, and Nagaraja *et al.*, 2012).

Evaluation of phytotoxicity of difenoconazole 3% WS in wheat

The test fungicide difenoconazole 3% WS was applied as seed treatment at the concentration of 2.00 and 4.00 ml/kg seed and compared with untreated control. Variety UP 262 was used for evaluation of phytotoxicity. Ten plants were randomly selected and phytotoxicity symptoms (leaf chlorosis, leaf tip burning, leaf necrosis, leaf epinasty, leaf hyponasty, vein clearing, wilting and rosetting) were recorded at 1, 3, 5, 7, and 10 days after field emergence as per CIB guidelines using a rating scale of 0-10 (Muthukumar and Udhayakumar, 2015, Kumar, 2018).

RESULTS AND DISCUSSION

Effect of Difenoconazole 3% WS on field emergence of wheat

Effect of difenoconazole 3% WS on field emergence of wheat was recorded on variety UP 262. The data presented in table 2 revealed that during 2014-15, the field emergence of wheat ranged from 87.80 to 92.90 per cent being maximum (92.90 per cent) from the seeds treated with thiram 75% WS @ 3.00 g/ kg seed. No treatment except seed treatment with thiram 75% WS @ 3.00 g/ kg seed was significantly superior over untreated control.

Similar results were obtained during the year 2015-16, the field emergence of wheat ranged from 88.26 to 93.78 per cent. Maximum field emergence (93.78 per cent) was recorded from the seeds treated with thiram 75% WS @ 3.00g/ kg seed. In 2015-16 also, no treatment except seed treatment with thiram 75% WS @ 3.00 g/ kg seed was significantly superior over untreated control.

Increase in germination and maintenance of healthy root system through seed treatment is an important criterion for wheat cultivation. The wheat seed when sown in the field are affected by large number of soil borne fungi causing seed and seedling rot at various stages of plant growth, which, directly influence the yield of crop. It is reported that wheat seed treated with Vitavax-200 (carboxin + thiram) was found to be a technology that resulted in a consistent 10% yield increase for wheat cultivation, in Bangladesh (Meisner *et. al*, 1993, Badaruddin *et. al*, 2002). Seed treatment with Carboxin + thiram has been reported to improve emergence of seedlings under field condtions (Purchase *et al.*, 1992). However, in present study no significant effect of Carboxin + thiram on field emergence was recorded. In the present study only seed treatment with thiram significantly improved the field emergence of seedlings. Search for the literature revealed that nobody has reported such type of result, therefore it should be considered as first record.

Evaluation of Difenoconazole 3% WS against Karnal bunt (*Tilletia indica*) of wheat

The experimental data presented in table 3 revealed that the incidence of Karnal bunt during 2014-15 crop season ranged from 0.03 to 0.22 per cent. It is evident from the results that among the various treatments spraying of propiconazole 25% EC @ 500ml/ha was found to be the best in managing the Karnal bunt disease, however, rest of the treatments were significantly ineffective.

Similar results were obtained during 2015-16 crop season. The Karnal bunt incidence ranged from 0.02 to 0.23 per cent being highest in untreated control and lowest with spraying of propiconazole 25% EC @ 500ml/ha. Like 2014-15, spraying of propiconazole 25% EC @ 500ml/ha was found to be the best in reducing the disease incidence. However, rests of the treatments were ineffective in reducing the incidence of Karnal bunt.

Pooled analysis of the data of two crop seasons indicated that the spraying of propiconazole 25% EC @ 500ml/ha at anthesis provided 89.13 per cent disease control over untreated control.

This indicated that none of the fungicidal seed treatment is effective in managing the Karnal bunt disease under field conditions. This may be because the disease is seed and soil-borne however the infection occurs by air-borne inoculum at the time of flowering (Mitra, 1931, Mundakar, 1943). Only the allantoid types of secondary sporidia are the real incitant of Karnal bunt in nature (Dhaliwal and Singh, 1988). Warham *et al.* (1989) and Gill *et al.* (1993) have reported chemical seed treatments to be ineffective in killing the teliospores of *T. indica* on seeds of wheat, with the exception of mercurial compounds which are banned in most of the countries including India. Even if fungicides are effective, the effect of seed treatment alone with fungicides may not persist until anthesis, the most vulnerable stage for seed infection by *T. indica* (Dhaliwal and Singh, 1988). However, foliar sprays of propiconazole, tebuconazole, hexaconzole, thifluzamide, diniconazole etc. were shown to be effective against natural infection in India (Aujla *et al.*, 1989, Singh *et al.*, 1989, 1992, 1993, 1998, Gill *et al.*, 1993, Smilanick *et al.*, 1987b). The test fungicide needs to be evaluated through spray trials rather

Table 1: Details of the fungicides used as seed treatment and spray against Karnal bunt of wheat

Chemical	Chemical name	Trade name	Formulation	Dosage/kg seed g. a.i./ kg seed	Forml. (ml/g/ kg seed)	Source
Untreated control	-	-	-	-	-	-
Difenoconazole	1-[[2-[2-chloro-4-(4-chlorophenoxy)	Dividend	3% WS	0.03	1.00	Syngenta India
Difenoconazole	phenyl]-4-methyl-1,3-dioxolan-2-yl]			0.06	2.00	Ltd.
Difenoconazole	methyl]-1,2,4-triazole			0.09	3.00	
Difenoconazole				0.12	4.00	
Thiram	dimethylcarbamothioylsulfanyl N, N-dimethylcarbamodithioate	Thiram	75% WS	2.25	3.00	National Pesticides & Co. Maharashtra, India
Carboxin 37.5% + Thiram 37.5%	5,6-dihydro-2-methyl-1,4-oxathiine-3-carboxanilide + Dimethyl carbamothioylsulfanyl N,N-dimethyl carbamodithioate	Vitavax Power	70% WS	2.25	3.00	Dhanuka Agritech Ltd., India
Propiconazole	1-[[2-(2,4-dichlorophenyl)-4-propyl-1, 3-dioxolan-2-yl]methyl]-1,2,4-triazole	Tilt	25% EC	125	500ml/ha	Syngenta India Ltd.

Table 2: Effect of Difenoconazole 3% WS on field emergence of wheat

Treatment	Dose g. a.i./kg seed	Product dose ml/g per kg seed	Field emergence (%) 2014-15	2015-16	Pooled
T1 Untreated control	0.03	1.00	87.80	88.26	88.03
T2 Difenoconazole 3% WS	0.06	2.00	88.00	88.42	88.21
T3 Difenoconazole 3% WS	0.09	3.00	88.03	89.55	88.79
T4 Difenoconazole 3% WS	0.12	4.00	89.16	89.58	89.37
T5 Difenoconazole 3% WS	2.25	3.00	89.96	90.35	90.16
T6 Thiram 75% WS	2.25	3.00	92.90	93.78	93.34
T7 Carboxin 37.5 + thiram 37.5 % WS	125	500ml/ha	92.80	93.29	93.05
T8 Propiconazole 25% EC	0.03	1.00	87.88	88.39	88.14
CD at 5%			5.04	5.07	

Table 3: Effect of Difenoconazole 3% WS against Karnal bunt of wheat

Treatment	Dose g. a.i./kg seed	Product dose ml/g per kg seed	Karnal bunt incidence (%) 2014-15	2015-16	Pooled mean	Disease control (%)
T1 Untreated control	-	-	0.22	0.23	0.23	-
T2 Difenoconazole 3% WS	0.03	1.00	0.18	0.18	0.18	20.29
T3 Difenoconazole 3% WS	0.06	2.00	0.15	0.18	0.17	27.54
T4 Difenoconazole 3% WS	0.09	3.00	0.12	0.18	0.15	34.78
T5 Difenoconazole 3% WS	0.12	4.00	0.12	0.10	0.11	52.90
T6 Thiram 75% WS	2.25	3.00	0.12	0.17	0.14	38.41
T7 Carboxin 37.5 + thiram 37.5 % WS	2.25	3.00	0.15	0.10	0.13	45.65
T8 Propiconazole 25% EC	125	500ml/ha	0.03	0.02	0.03	89.13
CD at 5%			0.13	0.16		

Table 4: Effect of Difenoconazole 3% WS on grain yield of wheat

Treatment	Yield (kg/plot)			Increase in yield (%)	AYL (%)
	2014-15	2015-16	Pooled mean		
T1 Untreated control	4.57	4.47	4.52	-	-
T2 Difenoconazole 3% WS	4.67	4.63	4.65	2.88	2.80
T3 Difenoconazole 3% WS	4.97	4.77	4.77	5.46	5.24
T4 Difenoconazole 3% WS	4.77	4.82	4.84	7.12	6.61
T5 Difenoconazole 3% WS	4.87	4.88	4.93	8.96	8.32
T6 Thiram 75% WS	4.80	4.75	4.78	5.64	5.44
T7 Carboxin 37.5 + thiram 37.5 % WS	5.10	5.07	5.08	12.46	11.02
T8 Propiconazole 25% EC	5.13	5.11	5.12	13.35	11.72
CD at 5%	0.55	0.60			

Table 5: Evaluation of phytotoxicity of Difenoconazole 3% WS on wheat during 2014-15 and 2015-16 (pooled mean)

Days of observation after field emergence	Treatment	Phytotoxicity symptoms Chlorosis	Tip burning	Necrosis	Epinasty	Hyponasty	Vein clearing	Wilting	Rosetting
1st day	Difenoconazole 3% WS @ 2.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Difenoconazole 3% WS @ 4.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Untreated control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3rd day	Difenoconazole 3% WS @ 2.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Difenoconazole 3% WS @ 4.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Untreated control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5th day	Difenoconazole 3% WS @ 2.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Difenoconazole 3% WS @ 4.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Untreated control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7th day	Difenoconazole 3% WS @ 2.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Difenoconazole 3% WS @ 4.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Untreated control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10th day	Difenoconazole 3% WS @ 2.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Difenoconazole 3% WS @ 4.00 ml/kg seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Untreated control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS

than seed treatment alone.

Effect of Difenoconazole 3% WS on grain yield of wheat

Wheat variety UP 262 was planted to evaluate the effect of seed treatment with difenoconazole 3% WS, thiram 75% WS and carboxin 37.5 + thiram 37.5% WS and spraying of propiconazole 25% EC on grain yield (table 4). During the crop season 2014-15, the yield varied from 4.57 to 5.13 kg per plot, being lowest in untreated control and highest in treatment wherein spraying of propiconazole 25 % EC @ 500ml/ha at anthesis was given. It is evident from the results (table 4) that only the treatment i,e. spraying of propiconazole 25 % EC @ 500ml/ha at anthesis was significantly superior over untreated control. However, rests of the treatments were significantly at par with untreated control.

In 2015-16 crop seasons, the yield ranged from 4.47 to 5.11 kg per plot and only the treatment wherein spraying of propiconazole 25 % EC @ 500ml/ha was given at anthesis had significantly higher yield as compared to untreated control. Rests of the treatments were at par with untreated control.

Per cent increase and avoidable yield loss in yield were calculated on the basis of pooled mean of two crop seasons (2014-15 and 2015-16). It indicated that the treatment wherein spraying of propiconazole 25 % EC @ 500ml/ha was given at anthesis recorded maximum 13.35 per cent increase and 11.72 per cent avoidable yield loss over untreated control.

Present findings are in accordance with the work of Duczek and Jones-Flory (1994) who reported that applications of propiconazole on spring wheat at various growth stages at Outlook, Saskatchewan showed that the optimal time to spray was between the extension of the flag leaf growth stage to the medium milk growth stage. The maximum yield increase was about l0% on the soft white spring wheat, Fielder, compared to a 3% yield increase on the hard red spring wheat, Katepwa. Significant effect of seed treatment with Vitavax-200 (carboxin + thiram) on yield of wheat has also been reported (Meisner *et. al*, 1993, Badaruddin *et. al*, 2002) in Bangladesh. However, in our study no significant effect of carboxin + thiram on grain yield of wheat has been observed.

Evaluation of Phytotoxicity of Difenoconazole 3% WS in wheat.

Difenoconazole 3% WS was evaluated at 2 doses i.e. 2.0 and 4.0 ml/kg seed at 1, 3, 5, 7 and 10th days after field emergence for phytotoxicity symptoms (Table 5). Plants were observed for symptoms of phytotoxicity viz., leaf chlorosis, leaf tip burning, leaf necrosis, leaf epinasty, leaf hyponasty, vein clearing, wilting and rosetting. No phytotoxic symptoms were noticed even at the highest tested dose of 4.00 ml /kg seed of Difenoconazole 3% WS during both the crop seasons (2014-15 and 2015-16).

Our results are in agreement with the work of Sharma *et al.*, (2012) who conducted multi-location field trials at DWR, Karnal, PAU, Ludhiana, GBPUA&T, Pantnagar and CCS HAU, Hisar for testing the efficacy of difenoconazole for management of loose smut of wheat, it's effect on germination and phytotoxixicty on crop. Based on the multi-location data of two years, they have reported that the seed treatment with Dividend 3 WS-FS @ 2 to 3 g/Kg seed can be recommended (difenoconazole) for controlling the loose smut of wheat. No adverse effect of difenoconazole on germination and phytotoxicity was observed.

Based on the data of two crop seasons it can be concluded that the spraying of propiconazole 25% EC @ 500ml/ha at anthesis was most effective both in terms of managing the Karnal bunt of wheat and improving the yield. Among the seed treatments, seed treatment with thiram @ 3.00g/kg seed was found most effective in improving the field emergence. However, the test fungicide difenoconazole 3% WS had no beneficial effects on field emergence, disease management and yield as compare to check. Nevertheless, the test fungicide difenoconazole 3% WS had no phytotoxic effects on wheat plants during both the crop seasons even at the highest dose tested.

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