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Biological control of Karnal bunt of wheat and its evaluation at a hot spot location in North-West India

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ABSTRACT

Biological control of Karnal bunt of wheat (c.o. *Tilletia indica*) was tried under artificially inoculated conditions at Karnal and under natural field conditions on large scale at a hot spot location, Dhaulakuon for two crop seasons. Foliar spray of *Trichoderma* spp. at the critical growth stage (Zadoks' growth stage of 41-49) proved very effective in controlling the Karnal bunt disease. Per cent disease control provided by *T. viride* (str. Ecoderma) provided disease control level at par with *T. harzianum* (str. Th1) and the recommended chemical propiconazole (Tilt 25EC) during 2002-03 crop season. In the earlier crop season also these treatments were very effective. It emerged that the efficacy of spray of the bioagent fungus depends upon the time of spray, i.e., the critical crop growth stage. Biological control proved more effective during low-disease year than the high disease year. Biological control can provide an effective alternative to the chemical control method during low-KB years.

Key words: Biological Control, Karnal bunt, Wheat, *Tilletia indica* , *Triticum aestivum*, *Trichoderma*, propiconazole

Karnal bunt, caused by *Tilletia indica* Mitra is one of the most important diseases of wheat (*Triticum aestivum*) in the north-west India. Apart from damaging the grain/seed, it causes impediments in global trade. Various approaches have been adopted for the management of this disease, including the use of fungicides (Smilanick *et al.* 1998; Zang and Morgado 1998; Sharma *et al.* 1994; Aujla *et al.* 1989, Singh and Prasad 1980; Singh *et al.* 1985; Singh *et al.* 1989; Sharma *et al.* 2005). Though the use of resistant varieties is the most viable and economical approach but truly resistant varieties are not available. Because of the importance of this disease in seed production, stringent seed certification standards have been fixed (Sharma and Agarwal 1996). Use of Chemicals is the most effective method for the control of this disease, but the continued dependence on chemical pesticides leads to health and environment hazards and related issues. Biological control offers an option or alternative to the chemical control. Agarwal *et al.* (1995) found that teliospore germination of *T.indica* is significantly reduced by species of *Trichoderma* and *Gliocladium*. However, no systematic information is available on the use of bioagent fungi for the control of this disease under field conditions. The present studies were thus conducted to evaluate the efficacy of biological control measures for the management of Karnal bunt disease under field conditions.

MATERIAL AND METHODS

The experiment was conducted in two phases. Under the first phase, it was conducted under artificially inoculated conditions at experimental farm in Karnal (Haryana) during 1996-97 and 1997-98 crop season, using a susceptible variety WL -711. Subsequently, in the second phase, a large scale field trial was conducted at the disease hot-spot location, Dhaulakuon in Paonta Valley of Himachal Pradesh under natural field conditions, using variety WH 542 during 1998-99 and was further repeated during 2002-03 crop season.

The bioagents, were raised and multiplied on sterilized sorghum grain under aseptic conditions. For this, sorghum grains were soaked overnight in distilled water. Fifty grams of grain were soaked in each of the 200 ml conical flasks and sterilized through autoclaving at 15 pounds pressure for 20 minutes. These flasks were then inoculated by the above mentioned bioagent fungi to raise their pure cultures. These flasks were incubated at 25 ± 1°C. Inoculated flasks were shaken at regular intervals to avoid the clumping of grains. After 8-10 days of incubation, the cultures were taken for making the suspension of the fungal cultures in sterilized water. Concentration of the suspension was made to 10^6 cfu (colony forming units) and the crop was sprayed by using suspension @ 500 l/ha. The spray was done at the early earhead emergence stage, i.e. the crop growth stage 41-49 on Zadoks scale (Zadoks *et al.* 1974), which is the most vulnerable stage of the crop for infection of *T. indica* (Nagarajan, *et al.* 1997). Sprays were done in the afternoons so that the dew occurring late in the evening and during the night, is able to help in establishment of the bioagent fungus. In artificial inoculations, the KB pathogen, *T. indica* was syringe inoculated at the vulnerable stage, 48 hours after the bioagent spray. Pathogen was inoculated as per technique followed for screening of germplasm against Karnal bunt (Gill *et al.* 1993 Sharma *et al.* 2002). To ensure the prevalence of congenial microclimate for both the pathogen and the bicontrol fungi, dense mist was created for 2-3 hours over the crop canopy with the help of perfo sprayers for the first five days after inoculations with *T. indica* to simulate the KB epidemic creating conditions (Nagarajan *et al.* 1997).

During 1996-97 crop season, the trial was laid out in plots of 7.5m² (10 rows of 3m length each, with row to row spacing of 25 cm) in an unreplicated manner, with following treatments: *Trichoderma viride* (Str. SS), *T. harzianum* (Str. Th-1), *Gliocladium virens*(Str. Gv-1), *T. viride* Str-Funginil, and chemical fungicide propiconazole (Tilt 25EC @0.1%) as

sprays in comparison to a control plot which was inoculated with *T. indica* and was sprayed with plain water only.

In each of the plots, five earheads were syringe inoculated with *T. indica* whereas, the bioagent sprays were given on plot basis. Per cent incidence of KB was recorded after harvest in the five inoculated earheads by counting the number of infected and healthy grains. During 1997-98 crop season, this trial was repeated. It was sown in Randomized Block Design (RBD) with four replications. Strain 'SS' of *T. viride* tested during 1996-97 crop season did not give any encouraging results, hence, it was replaced by another strain of *T. viride*, namely TV-1 based on its faster growth and profuse sporulation under *in vitro* conditions. Five earheads from each plot were syringe inoculated with *T.indica*, 48 hours after the sprays of the bioagent fungi. Disease incidence was recorded after harvest by calculating the percent infected grains in these ear heads.

In the second phase, this trial was conducted at Dhaulakuon in Paonta Valley of Himachal Pradesh. This trial was conducted during 1989-99 and then repeated during 2002-2003 crop season. Dhaulakuon is an established hot spot location for the Karnal bunt disease (Sharma *et al.* 2001). Trial was laid out on a large seed multiplication farm in RBD with five replications and the plot size was kept as 200m^2. The trial was conducted under natural field conditions. Four treatments, *viz. T. harzianum* (str. Th 1), *T. viride* (Str. TV-1), *T. viride* (str. 212) and propiconazole (Tilt 25EC @0.1) sprays were evaluated during 1998-99 while *T. viride* (str. Ecoderma), replaced *T. viride* -212 and an additional treatment of *Gliocladium virens* was tried during 2002-2003. *T. viride* str. Ecoderma, being a commercially available product in the form of water dispersible powder, was used @ 4 gm/litre of water. All other treatments remained the same. During both the years of study, one treatment with spray of plain water was kept as control for comparison. Data on disease incidence was calculated by counting the number of diseases and healthy grains in the grain samples as follows:

Disease incidence (D.I.) = IG/T.G. X 100, where I. = infected grains and T.G. total no. of grains in the sample (i.e. 2000). Per cent disease control was calculated by comparing the disease incidence in the individual treatments with that in the untreated control.

RESULTS AND DISCUSSION

The trial conducted at Karnal under artificially inoculated conditions in the field showed that strain 'SS' of *T.viride* did not prove effective during 1996-97 and hence this was not included during the next crop season. The level of disease control was only 36.3 per cent with *T. viride* str 212 (during 1997-98) and 37.84 per cent with *G. virens* during 1996-97 (Table 1). The field trial conducted during 1998-99 crop season under natural field conditions at the hot spot location, confirmed, the findings of 1996-97 and 1997-98 studies conducted at Karnal. The disease incidence was reduced from 5.86 per cent in control to 2.68 and 2.74 per cent under *T. harzianum* str. Th-1 and *T. viride* str. Tv-1 sprayed plots, meaning thereby 54.3 and 53.2 per cent control respectively, over the untreated plot, in comparison to 88.7 per cent control in propiconazole (Tilt 25EC) sprayed plots which had 0.66% incidence of KB (Table 2).

As mentioned earlier, the field trial was repeated during 2002-03 crop season to confirm the findings under field conditions (Table 2). The results showed that the level of disease during this crop season was in general low, because it was a low disease year in the region (Sharma *et al.* 2003). The disease incidence in untreated control was 2.0 per cent. The level of disease under *T. viride* str. Ecoderma was the lowest among the bioagent treatments (0.08%) and was similar to the sprays of propiconazole. The other treatments showed a disease control level of 89.0, 87.5 and 62.5 per cent under *T. harzianum* str. Th-1, *T. viride* str. Tv-1 and *G. virens* Str. Gv-1. These studies confirmed the findings of 1998-99 crop season at the 'hot spot' location and the findings under the controlled conditions during 1996-97 and 1997-98. The level of disease control was higher during 2002-2003 crop season as compared to 1998-99 crop seasons under natural

Table 1 Biological control of KB under artificially inoculated conditions in the experimental field.

Treatment	KB incidence %		Percent Disease control	
	1996-97	1997-98	1996-97	1997-98
T. viride str. 'SS'	13.10	-	11.49	
T. harzianum str. Th-1.	8.20	5.80	44.50	74.03
T. viride str.Funginil	8.90	-	39.86	
Gliocladium virens str. GV-1	9.20	-	37.84	
Propiconazole (Tilt 25EC @ 0.1%)	1.30	3.84	91.22	82.80
T. viride str. TV-1.	-	7.80	-	65.07
*T. viride str.*212	-	14.23		36.27
Control (untreated)	14.80	22.33	-	-
C.D. at 5%	-	8.87		

Table 2 Performance of biological control in a large farm at Dhaulakuan, a hot spot location for Karnal bunt.

Treatment	1998-99		2002-2003		Average	
	D.I.*	P.D.C.*	D.I.	P.D.C.	D.I.	P.D.C.
T.harzianum str. Th-1.	2.68	54.3	0.22	89.0	1.45	63.6
T.viride str. TV-1.	2.74	53.2	0.25	87.5	1.49	62.1
T.viride str.212	3.04	48.1	-	-	-	-
Propiconazole (Tilt 25EC@0.1%)	0.66	88.7	0.08	96.0	0.37	90.6
T.viride str. *Ecoderma*	-	-	0.08	96.0		
Gliocladium vriens str. GV-1	-	-	0.75	62.5		
Control (untreated)	5.86	-	2.00	-	3.93	
C.D. at 5%	2.19		0.24			

*D.I.: Disease Incidence; P.D.C.: Per cent Disease Control

field conditions. It seems that the efficacy of the bioagent fungi is improved under low disease conditions. Similar observations have been made earlier in case of biological and integrated management of loose smut of wheat (Sharma and Kumar 1997).

Antagonistic fungi, especially *Trichoderma* and *Gliocladium* spp. have been used extensively for the control of various foliar, seedling and soil-borne diseases. (Mukhopadhyay 1987, Mukhopadhyay and Mukherjee 1998). Seed-borne diseases of wheat have caught attention only in the recent years for management through the use of bioagents (Sharma and Kumar 1997; Sharma 2000). Preliminary studies (DWR 1996; Aggarwal *et al.* 1995) could indicate the possibility of using bioagents for the management of Karnal bunt of wheat under *in vitro* or controlled conditions, however, their utility under field conditions was not evaluated. The present studies have clearly shown the usefulness of the bioagents against this important disease of wheat on large scale field trial at the hot spot location. Biological control of KB has been found to be very encouraging through the present studies. The results of the large scale trial show that this technology can be successful at farmers' field level and holds promise for future. Use of biological control agents has big scope under organically produced wheat since, it involves the control of disease through non-chemical means.

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