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Research Article

Efficacy of various fungicides against *in vitro* colony growth of *Tilletia indica* and *in vivo* control of karnal bunt disease of wheat

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Abstract

An *in vitro* evolution of ten fungicides against *Tilletia indica* (Mitra) Mundkur revealed that Dolomite and Shelter were the most *and* equally effective fungicides in inhibiting *in vitro* colony growth of the fungus and *in vivo* reduction of the percent infection of the grain of wheat by karnal bunt disease. The protective application (i.e. spray before inoculation) of Dolomite and Shelter were comparatively more effective in controlling wheat grain infection than their eradicative application (i.e. spray after inoculation). Crest, Agrohit, Altert plus and Antracol were comparatively less effective while Reconil-M, Aliette, Protocol prec-ombi and Thiomil were ineffective against the fungus and the Karnal bunt disease caused by it. It is therefore suggested that Dolomite and Selter should be used for seed treatment and for foliar application for Karnal bunt management in wheat under the agro ecological conditions of Punjab, Pakistan.

Introduction

Tilletia indica (*Neovossia indica* Mitra Mundkur) is the casual agent of Karnal bunt of wheat (*Triticum aestivum* L.). The disease was initially reported from India in 1931 (Mitra, 1931) and subsequently has been reported in Afghanistan, Iraq, Iran, Mexico, Nepal, Pakistan (Warham, 1986) and USA (Ykema *et al*., 1996). In early 50s, Karnal bunt disease was considered to be disease of minor importance and it was confined to hills of Pakistan. In 1971, the disease was reported from Sialkot, Gujranwala and Mardan districts during the crop years of 1966-71 (Hassan, 1971) and the frequency of disease ranged from traces to 2.0 percent. The disease remained endemic for considerable period of time in the Northern area of Pakistan and later it spread to south and was reported as far as Jhang, Khanewal and Muzaffargarh district of the Punjab (Bhatti and Ilyas, 1986). A little later the disease became wide spread throughout the Punjab and was prevalent in 23 districts with a frequency range of 0.32 to 3.50 per cent (Ilyas *et al*., 1989b). At present almost all commercial varieties of wheat under cultivation are susceptible to Karnal bunt and the disease incidence is aggravating with the passage of time (Shakoor et al., 2015). The disease reduces wheat yields and can cause a fishy, unpalatable odor and taste in wheat flour, reducing flour quality (Bonde *et al*., 1997; Sekhon *et al*., 1980; Singh and Bedi, 1985). Since karnal bunt affects the international trade of commercial wheat grain and movement of germplasm, the presence of the disease can cause economic loss to wheat exporting countries (Bonde *et al*., 1997). The yield and quality losses are generally minor; the most economic loss can be attributed to the quarantine status of the pathogen (Babadoost, 2000, Butler, 1990). For a country like Pakistan which now has entered into the list of exporters, the importance of disease cannot be ignored (Shakoor et al., 2014).

Control of Karnal bunt has now become a major concern in Pakistan due to scarcity or non- availability of resistance in commercial wheat varieties under cultivation. The gravity of the situation of the disease calls for evaluation of fungicitoxicants against the disease for its management. This paper reports on *in vitro* evaluation of various fungicides against the growth of Karnal bunt fungus and *in vivo* fungicidal control of the disease.

Materials and Methods

Preparation of Sporidial Culture of *Tilletia indica*

One year old bunted, wheat grain, infected with Teliospores of *T. indica*, of wheat cultivar AS-2002 obtained from Tareen Model Farm Lodhran, Punjab, Pakistan were used for sporidial and secondary sporidial culture of *T. indica* by the method of Torres *et al.* (1982). About 30-40 bunted grains were scratched with a dissecting needle to loosen the teliospores and were placed in 25 ml sterile distilled water and stirred for 5 to 10 minutes. Then the water suspension was filtered through a 120 mm mesh sieve, which retained the larger debris on to the sieve while spores and other small particles passed into the filtrate. The filtrate was centrifuge at about 3000 rpm for 1 minute, which sedimented the spores into a pellet, whereas lighter debris remained suspended. The water was decanted out and the teliospores in the tube were disinfected with a twenty times diluted solution of commercial bleach (5% chlorox) for one minute centrifugation. Teliospores were sedimented in a sort of pellet again, decanted out the chlorox and given two rinsing of sterile distilled water. The spores were again recovered by centrifugation and suspended in sterile water. One ml of spore suspension was poured and distributed evenly on each of several Petri-plates with 2 percent plain agar medium. The composition of plain agar medium was 20 gram agar dissolved in 1000 ml H_2O. The inoculated plates were incubated at 15-20^oC with a light/ dark regime of 14/10 hours. After about 10-12 days primary sporidia were obviously visibility formed as a result of teliospores germination on the surface of plain agar medium in the Petri-plates, having long thread like or star like structures. Using this primary sporidial culture, secondary sporidia were produced on PDA, for which about 100 ml autoclaved PDA was taken into 250 ml flask and the medium was solidified in the form of a slant. The slants were then inoculated with a primary sporidial culture by cutting culture blocks (3x3 mm size) and placing them on the upper edge of the slants. The slants in flasks were incubated at 18^0C. Within 3-4 days secondary sporidia were started to discharge forcibly from primary sporidia on PDA slants. The secondary sporidial culture thus obtained was consisted of brittle, crustaceous unibonate colonies with dendric margins.

In vitro Evaluation of Fungicides against the Growth of *T. indica*

In vitro evaluation of various fungicides at 40, 60, 80 and 100 g /ml against the colony growth of *T. indica* was carried by inhibition zone technique (Khan and Ilays, 2007). A weighed quantity of each of 10 fungicides (Table 1) was amended into the distilled sterilized water to obtain each of the 40, 60, 80 and 100 g/gm (ppm) aqueous concentration. Twenty ml of sterilized i.e. autoclaved potato dextrose agar medium (dextrose 20 g, starch 20 g, agar agar 200 g dissolved in water to make the volume 1000 ml) was poured in several 90 mm diameter autoclaved Petri-plates and allowed to solidify. A secondary sporidial culture of *T. indica* grown on a PDA slant in a 250 ml conical flask, was scratched with a sterilized scalpel, removed and stirred thoroughly in 200 ml distilled sterilized water taken in a 1000 ml sterilized beaker. This sporidial suspension was filtered aseptically through sterilized muslin cloth and the suspension was diluted further to get about 10,000 sporidia/ml of water. One ml of this sporidial suspension was transferred to each of 90 mm diameter PDA plates and spread uniformly on the surface of PDA with a sterilized bended (L-shaped) glass rod. With the help of sterilized 6 mm diameter cork borer, a well as made in the centre of each PDA plate with sporidial suspension spread on its surface. The wells of the four PDA Ptri-plates were filled with aqueous solution of each of four concentrations of the each test fungicide. The four PDA plates with wells were filled with distilled sterilized water which served as control. The Petri-plates were labeled with the fungicides and its dosage rate. All the Petri-plates with wells either with a fungicide solution or distillated sterilized water were put at 5^oC in a refrigerator for 24 hours to allow the diffusion of fungicide solution water into the medium of the Petri- plates. These Petri-plates were then incubated at 20^oC. After 5 days of incubation the diameter of inhibition zone of *T. indica* colony around the well for each of the concentration of the test fungicides was measured.

Table 1: Fungicides evaluated against *in vitro* colony growth of *Tilletia indica* and *in vivo* control of karnal bunt disease of wheat grains by foliar spray

Common name	Chemical name & % composition	Formulation	Dose Rate	Manufacturer
Protocol Precombi	Thiophanate Methyl(52.50%) + Diethofeen carb (12.5%)	65 W.P.	500g/ 100 lit water	**Agrolet Co.**
Agrohit	Diamethomorph(6%) + Mancozeb (44%)	50 W.P.	350g/ acre	**Agrolet Co.**
Dolomite	**Metalaxyl (15%) + Mancozeb (65%)**	**58 W.P.**	**250g/ acre**	**M/S Pak. China Chemicals**

Alert plus	**Fosetyl aluminium(40%)** + **Mancozeb (30%)**	**70 W.P.**	**300-400g/ acre**	**Agrolet Co.**
Crest	Carbendazim	50 W.P.	100g/ acre	**M/S Pak. China Chemicals**
Antracol	Propineb (61.25%) + Provali Carb (5.5%)	75 W.P.	250g/ 100 lit water	**Agrolet Co.**
Shelter	Mancozeb (80%)	80 W.P.	600g/ acre	**M/S Pak. China Chemicals**
Thiomil	Thiophaneti methyle (70%)	70 W.P.	200g/ acre	**M/S Pak. China Chemicals**
Reconil-M	Chlorothalonil (30%) w/w + Mancozeb (40%) w/w + other ingredients (30%) w/w	70 W.P.	330g/ acre or 150g/ 100 lit water	**M/S Pak. China Chemicals**
Aliette	**Aluninium ethyl phosphate**	**80 W.P.**	**2.5g/ lit water**	**Rhoune-poulenc-Agrochimie**

Table 2: Mean inhibition zones of colony of *Tilletia indica* by various fungicides at 4 dosages rates amended in PDA medium.

Fungicides	Mean Inhibition zone (mm) at 4 dosage rates			
	40 ppm	**60 ppm**	**80 ppm**	100 ppm
Crest	2.52 H*	3.40 EF	3.75 E	**3.77 E**
Dolomite	3.50 EF	5.70 C	7.75 A	**7.77 A**
Agrohit	0.25 L	0.50 L	0.5 0 L	**0.5 L**
Alert plus	0.00 L	0.00 L	2.00 J	**3.1 FG**
Shelter	3.35 EF	4.30 D	6.45 B	**7.75 A**
Reconil	0.00 L	1.00 K	1.00 K	**1.00 K**
Aliette	0.00 L	0.00 L	0.00 L	**0.00 L**
Antracol	2.25 I J	2.8 0 GH	3.50 EF	**3.5 EF**
Protocol pre-combi	0.00 L	0.00 L	0.00 L	**0.00 L**
Thiomil	0.25 L	0.25 L	0.5 L	**0.5 L**
Water (control)	**0.00 L**	**0.00 L**	**0.00 L**	**0.00 L**

* Value having the same letters do not differ at 5% level of significant.

In Vivo Control of Karnal Bunt Disease of Wheat by Protective and Curative (eradicative) spray of fungicides

A. **Protective Spray**

Wheat grains of highly susceptible cultivar AS-2002 were sown in sub-plots of 1.53 x 0.92 meter in size, with three replications, with sub-plots to sub plot distance 60 cm, repeat to repeat distance 90 cm, row to row distance in sub-plot 30 cm and plant to plant distance in a row 15 cm. At boot stage, the sub plots were sprayed with each of ten fungicides at their recommended dosage rates (Table 1). The sub plots sprayed with tap water served as control. Each treatment was replicated four times. The layout of the experiment was in RCBD. After 48 hours of fungicidal spray, each of the twenty heads in each subplot was boot inoculated with 3 ml of sporidial suspension of *Telletia indica* (10000 spores/ml). The sub-plots inoculated with distilled sterilized water served as control. Inoculated plants were tagged and labeled. The field was irrigated to lower the temperature. Agronomic practices were homogenously applied in all the replications. At maturity plants were harvested and hand threshed. The data of inoculated heads were recorded on percent grain infection. The data were analyzed statistically by subjecting it to Randomized Complete Block Design (RCBD) and Duncan Multiple Range Test (DMRT) to visualize the difference between the effects of various fungicidal spray treatments.

Table 3: Percent decrease in wheat kernels infection by *Tilletia indica* by protective and eradicative spray of various fungicides

Fungicides	Spray of fungicides before Inoculation		Spray of fungicides after inoculation	
	Mean percent kernel infection	**Percent decrease in kernel infection over**	**Mean percent kernel infection**	Percent decrease in kernel infection
Crest	22.45 EFG*	33.79	28.81 BC	**7.30**
Dolomite	12.73 H	62.45	19.01 FG	**38.84**
Agrohit	22.16 EFG	34.65	28.64 BC	**7.82**
Alert plus	27.49 CD	18.93	28.89 BC	**7.04**
Shelter	12.24 H	63.90	18.50 G	**40.48**
Reconil	33.01 AB	2.71	26.41 CDE	**14.98**
Aliette	30.86 ABC	8.99	30.97 ABC	**0.35**
Antracol	19.94 FG	41.19	23.59 DEF	**24.07**
Protocol pre- combi	33.39 AB	1.53	30.10 ABC	**3.15**
Thiomil	32.43 AB	4.36	28.87 BC	**7.08**
Control	**33.91 A**	**0.00**	**0.00 ABC**	**0.00**

*Values having the same letters do not differ at 5% level of significance

B **Curative Spray Evaluation**

In another experiment the variety (AS-2002) was sown in various sub-plots and the experiment was designed similar to that of protective application of fungicides. When crop reached to boot leaf stage each of the twenty heads in each replication were boot inoculated with 3 ml of sporidial suspension of *Tilletia indica* (10000 spores/ml). Distal sterilized water inoculated sub- plots served as control. After 48 hours of artificial inoculation, each of the test fungicides was sprayed at their recommended dosage rates (Table 1.) following RCBD design. Inoculated plants were tagged and labeled and field was irrigated. At maturity, the inoculated heads were harvested, hand thread and % seed infections for each spray treatment were determined. The data were analyzed statistically by subjecting it to RCBD and DMRT to visualize the difference between various fungicidal spray treatments.

Results and Discussion

An *in vitro* evaluation of ten fungicides (Table 1), amended into PDA, against colony growth of *T. indica* revealed that the effectiveness of the fungicides in reducing colony growth depended on kind of fungicide and its dosage rate evaluated. However, the efficacy of fungicides for inhibiting colony growth of *T. indica* increased with an increase in their dosage rates (Table 2).Dolomite and Shelter at their 40µg/ ml and 100µg/ ml dosage rates were the most and statistically equally effective fungicide in inhibiting the colony growth of *Tilletia indica* as these fungicides produced 7.77 and 7.75 mm diameter inhibition zones of the fungus. Shelter however at 100 g/ml dosage rate displayed the same and statistically equal effectiveness as Dolomite at 80 g/ml. Crest, Antracol Alert plus at 100 g/ml dosage rate were less effective than Dolomite and Shelter as these fungicides produced 3.77, 3.50, 3.10 mm diameter inhibition zones respectively. However, there was no statistically difference between the effectiveness of Crest and Antracoll and between that of Antracol and Alert plus. Alert plus and Antracol at 100 mg/ml dosage rate displayed the same effectiveness as Dolomite and Shelter displayed at 40 mg/ml. Reconil, Agrohit and Thiomil were the least effective fungicides against *T. indica*, while Aliette and Protocol pre-combi were ineffective in inhibiting the growth of the fungus.

The *in vivo* evaluation of effectiveness of the 10 test fungicides, as protective spray (i.e. spray before inoculation) and as eradicative spray (i.e. spray after inoculation) revealed that the protective sprays were more effective than eradicative sprays in controlling Karmal bunt disease of field grown wheat plants. The protective spray of Dolomite and Shelter, though statistically equally effective were the most effective and caused 62.45 and 63.90 percent reduction in occurrence of Karnal bunt disease respectively, while the eradicative spray of Dolomite and Shelter reduced 38.84 and 40.48 percent disease respectively on wheat grain and there was no statistically difference between the effectiveness of both fungicides. The protective sprays of Crest, Agrohit, Alert plus and Antracol were intermediate in their effectiveness and respectively caused 33.79, 34.65, 18.93 and 41.19 percent reduction in Karnal bunt infection of wheat grains. However, there was not statically difference between the effectiveness of Crest, Agrohit and Antracol. Antracol being comparatively less affective caused 24.07 percent decrease in kernel infection by its curative spray. The eradicative sprays of Crest, Agrohit and Alert plus were ineffective in controlling wheat kernel infection. The protective as well as predicative sprays of Reconil M. Alliette, Protcol pre-combi and Thiomil were ineffective in the control of wheat kernel infection by *T. indica*.

Karnal bunt disease of wheat has assumed on alarming situation in the Punjab, Pakistan during the previous two to three decades (Anon, 2005) and has been reported to cause, depending upon the cultivar affected, up to 30 percent grain losses (Anon, 1986; Ilyas *et al*., 1989). This calls for control of disease either by the use of host resistance or through the use of chemotherapy. Since resistance to karnal bunt disease in available commercial wheat cultivars is absent (Anon., 2005), the chemotherapeutic control of the disease can be achieved by foliar application of fungicides at proper plant stage growth (Singh and Prasad, 1980; Singh *et al*., 1985; Ilays *et al*., 1989a). Foliar spray of fungicides may protect the plants from infections or eradicate the established infection (Vyas, 1984). The studies of this paper revealed that Dolomite and Shelter fungicides were not only the most and equally effective fungicides in inhibiting the in vitro colony growth of *T. indica* but these fungicides were also the most and equally effective for *in vivo* control of the karnal bunt infection of wheat grains. However, the protective applications of these fungicides were comparatively more effective in controlling wheat grain infection than their eradicative applications. Under the present situation of scarcity of resistance to Karnal bunt disease the control of the disease through chemotherapy is not uncommon (Singh and Prasad, 1980; Singh *et al*., 1985; Ilyas *et al*., 1989a; Krishna and Singh, 1982). Krishna and Singh (1982) reported that Bavistine (Derosal-60) and Bayleton, when sprayed at boot leaf growth stage prior to inoculation (protective spray) controlled the karnal bunt disease. Singh and prasad (1980) found that a single spray of Benomyl or Bevistine or Dithane M-45 at boot leaf growth stage was effective against Karnal bunt disease. Singh *et al*. (1985) also reported that spray of either Mancozeb or Carbendazim can prevent karnal bunt disease of the wheat plants. Ilays *et al*. (1989a) reported that Spoltes, Tilt or Baycor were the most effective fungicides that significantly reduced the grain infection, the least effective spray were that of Bayleton, Topas C-50 and Dithane M-45 while that of Brassicol (PCNB) Bayfidan and Bavistine were intermediate in their effectiveness for reducing karnal burnt disease over the unsprayed control.

References

Anonymous 1986. Annual Research Report. Wheat Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan.

Anonymous 2005. Second annual progress report of the ALP project, Identification of sources of resistance to Karnal bunt disease of wheat, Crop Disease Research Program, Institute of Plant and Environment Protection, NARC Islamabad. Pp. 1-29.

Babadoost M. 2000. Comment on the zero tolerance quarantine of Karnal bunt of wheat. Plant Dis., 84:711-712.

Bhatti MAR, and Ilays MB. 1986. Wheat diseases in Pakistan. In: Problems and progress of wheat pathology in South Asia. Malhotra Pub. House, New Delhi, India.

Bonde MR, Peterson GL, Shaad NW and Smilanki JL. 1997. Karnal bunt of wheat. Plant Dis. 81:1370-1377.

Butler L. 1990. Karnal bunt, quarantine and the international shipment of CIMMYT wheat seed. Proc. Bein. Workshop Smut Fungi, 7th International Congress, Mexico, p-22.

Hassan SF. 1971. Final technical report of fundamental studies on rusts and smuts of small grains in Pakistan. Cereal Disease Research Institute, PARC, Islamabad, P.100-110.

Ilays MB, Iftikhar K, and Arshad M. 1989a. Chemical control of Karnal bunt of wheat. Pak. J. Phytopath., 1(1-2):20-25.

Ilyas MB, Majeed K and K. Iftikhar K. 1989b. Survey of karnal bunt disease of wheat in the Punjab, Pakistan. Pak. J. Phtopath., 1(1-2):48-51.

Khan MA and Ilyas MB. 2007. Chemotherapy of plant diseases (Lab. manual). Department of Plant Pathology, Univ. Agri., Faisalabad. Pakistan.

Krishna A, and Singh RA. 1982. Evaluation of fungicides for the control of Karnal bunt of wheat. Pesticides, 16(8):7.

Mitra M. 1931. A new bunt of wheat in India. Ann. Appl. Biol., 18:178-179.

Sekhon KS, Saxena AK, Randhawa SK and Gill KS. 1980. Effect of Karnal bunt disease on quality characteristics of wheat. Bull. Grain Tech., 18(3):208-212.

Shakoor MA, Ahmad M, Ghazali HZ, Ahmad S, Balouch MA, Anjum R, and Hussain M. 2014. Chemotherapy of Karnal bunt of wheat: A Review, Int. J. Adv. Res. Biol. Sci., 1(9):163-188.

Shakoor MA, Ahmad M, Shahid MR, Hussain M, Yousaf MA, Ahsan M, Shah J, and Malghani AL. 2015. Screening of advanced lines and commercial varieties of wheat against karnal bunt. Int. J. Adv. Res. Biol. Sci., 2(5):17-22.

Singh A, and Prasad R. 1980. Control of Karnal bunt of wheat by a spray of fungicide. Indian J. Mycol and Plant Pathology, 10(Abstr).

Singh DV, Srivastava KD, Joshi LM and Verma BR. 1985. Evaluation of some fungicides for the control of Karnal bunt disease of wheat. Indian Phytopath., 38:571-73.

Singh PP and Bedi PS. 1985. Effect of Karnal bunt infection on gluten characteristics and protein fraction of wheat grain. Ann. Appl. Biol. 1(2):223-225.

Torres E, Bekel G and Prescort JM. 1982. Karnal bunt.-CIMMYT Report on Wheat Improvement. PP. 68-70.

Vyas SC. 1984. Systematic fungicides, Tata McGraw Hill Publishing Co. Ltd., New Delhi, pp. 360.

Warham EJ. 1986. Karnal bunt disease on wheat. Literature Review, Trop. Pest Manage. 32:229-242.

Ykema RE, Floyed JP, Palm ME and Peterson JL. 1996. First report of Karnal bunt of wheat in United States. Plant Dis., 80:1207.