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Full Length Research Paper

Evaluation of antagonistic fungi against charcoal rot of sunflower caused by *Macrophomina phaseolina* (Tassi) Goid.

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***In vitro*, sensitivity of *Macrophomina phaseolina* (Tassi) Goid determined through inhibition zone technique to various antagonistic fungi viz., *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viride*, *Trichoderma harzianum* and *Penicillium capsulatum* amended into PDA medium. All the antagonists reduced the colony growth of *M. Phaseolina* significantly compared to the control *A. flavus* was proved to be the most effective (66.00%) in reducing the colony growth of *M. Phaseolina* followed by *A. niger* (55.55%), *T. viride* (51.11%), *T. harzianum* (26.67%) and *P. capsulatum* (11.11%) respectively over control. *A. flavus* due to it antifungal metabolites activities was the most effective while *P. capsulatum* was the least effective. Seeds of four varieties treated with the culture of *A. flavus*, *A. niger*, *T. viride* and *P. capsulatum* and there combinations were sown in pots having infested soil (*M. phaseolina*). Results showed reduction in disease incidence of charcoal rot on sunflower cultivar G-66 with antagonist, *A. flavus* (100%) followed by *A. niger* (64.86%) *P. capsulatum* (63.79%) and *T. viride* (31.89%) over control. Decrease in disease incidence over control was 100% where seed was treated with combination of *A. niger* and *A. flavus* while *A. niger* and *T. viride* combination was least effective on G-66 (30.80%). All antagonists reduced the disease incidence on G-66 (Highly resistant), HRBS-1, (Resistant), G-72 (Moderately susceptible) and G-51 (susceptible) but were most effective on highly resistant variety (G-66) while least effective on susceptible variety (G-51).**

Key words: Sunflowers, *Macrophomina phaseolina*, antagonistic fungi.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) belongs to the family Asteraceae, which is an important oil seed crop. It is grown as spring and autumn crop in Punjab, Sindh and N.W.F.P. The crop in Pakistan is cultivated on an area of 537000 acres with an annual seed production of 656000 tons (Anonymous, 2006). Germany, Italy, France and Israel are leading countries in sunflower seed production. Average yield of Germany, Italy, France, Israel and Pakistan is 2476, 2228, 2334, 2417 and 1241 kg/acre respectively. Average yield of sunflower seed in Pakistan is much less than other world leading countries in sunflower production. Low yield of sunflower in Pakistan can be attributed to several biotic and abiotic constraints (Khan, 2007). Mirza and Beg (1983) conducted the first survey of the sunflower crop in the central and northern areas of Pakistan and reported up to 90% yield losses due to *Macrophomna phaseolina* (Tassi) Goid, the cause of charcoal rot of sunflower in Pakistan. Charcoal rot of sunflower was reported for the first time in 1984 from Faisalabad (Mirza, 1984) and later from other areas of Punjab, Sindh and N.W.F.P as serious threat to sunflower (Steven et al., 1987).

Charcoal rot followed by head rot and leaf spots, is the most serious disease in Pakistan (Ahmad et al., 1991). Various disease management methods have been implemented to combat and eradicate pathogenic fungi. These include cultural, regulatory, physical, chemical and biological methods. All these methods are effective only

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Table 1. Seeds variety and status.

Name of variety	Status
G-66	Highly resistant
HRBS-1	Resistant
G-72	Moderately susceptible
G-51	Susceptible

when employed well in advance as precautionary measure (Sharma, 1996; Kata, 2000). Once a disease has appeared; these methods became impractical/ineffective. In that situation, chemical control offers a good choice to growers to control the disease. Chemical pesticides have been in use since long and they provide quick, effective and economic management of diseases. However in recent past, it has been realized that use of chemicals in agriculture is not as beneficial as it was visualized. Chemicals pose serious health hazards to an applicator as well as to consumer of the treated material. In addition to the target organism, pesticides also kill various beneficial organisms. Their toxic form persists in soil and contaminates the whole environment (Hayes and Laws, 1991). Increasing awareness of human kind toward the ecosystem and environment has made a marked shift from systemic materials to bio-products. Fungi constitute a major group of bio agents against various kinds of pests. A good number of fungi such as *Trichoderma*, *Gliocladium* can suppress the parasitism of *Fusarium* sp., *Rhizoctonia* sp., *Sclerotium* sp (Rajappan and Ramaraj, 1999; Lifshitz et al., 1986). The present investigation is, however, design in a way to investigate comparative efficacy of some antagonistic fungi against charcoal rot of sunflower caused by *M. phaseolina in vitro* and in pots.

MATERIALS AND METHODS

In order to determine the comparative activity of different test antagonistic fungi against the pathogen (*M. phaseolina*) both the pathogen and the test antagonistic microbe were grown opposite to each other on PDA in the same Petri dish. Petri dishes containing 20 ml of PDA were inoculated in the centre at the opposite to *M. phaseolina*. Cultures of *M. phaseolina* and tested antagonistic fungi, *A. niger*, *A. flavus*, *P. capsulatum* and *T. harzianum* were obtained from the culture bank of the Department of Plant Pathology, University of Agriculture, Faisalabad. The lukewarm PDA was poured in 9 cm Petri plates. After solidification 1 cm disc of antagonistic microbes were placed in the center of Petri dish. 1 cm disc of the 5 day old culture of *M. phaseolina* were taken through sterilized cork borer and placed opposite to the test antagonist on PDA near the periphery of Petri dish. The treatments were arranged as,

T0 = Control (untreated)
T1 = *Aspergillus niger V. tieghen*.
T2 = *Aspergillus flavus*
T3 = *Penicillium capsulatum*
T4 = *Trichoderma harzianum*
T5 = *Trichoderma viride*

All the Petri dishes were placed in an incubator at 30°C. The experiment was conducted in completely randomized design (CRD) with three replications and there were nine Petri dishes in each treatment. The data recorded on the basis of inhibition zone produced in Petri dishes after 24, 48 and 72 h and were subjected to analysis of variance.

Evaluation of various antagonists and their combinations on the incidence of charcoal rot of sunflower (in pots)

The sterilization of the soil was done by autoclaving it in small earthen pots, twice at 20 psi for 40 min. After sterilization, the soil was left as such for 5 - 7 days before making any treatment. Nine days old cultures of *M. phaseolina* were added at the rate of 1 and 1/2 Petri dish of 9 cm diameter in the form of small agar blocks. These agar blocks placed in the soil in three layers at 4, 8, and 12 cm depth in pots. Three pots were kept under each treatment. After the addition of inoculums to the soil, the optimum soil moisture (40 - 50% water holding capacity) was maintained throughout the course of the experiment. The pots were incubated for 5 - 7 days. Surface disinfected seeds were soaked in the culture of antagonistic fungi prepared by dissolving ¼ Petri dish of the culture in 10 ml distilled water + 2 drops of gum Arabic for 60 min. The air dried seeds coated with antagonistic fungi of the following varieties were stored at room temperature (Table 1). Seeds of these varieties treated with the following antagonists under the following treatments.

T0 = Untreated control
T1 = *A. niger*
T2 = *A. flavus*
T3 = *T. viride*
T4 = *P. capsulatum*

Seeds of these selected varieties were also treated in combination of these antagonists also. As shown in Table 2.

Experiment was performed in Glass House at the University of Agriculture Faisalabad 7 Feb, 2007 for the evaluation of efficiency of antagonistic fungi as seed treatment. Seeds of four sunflower cultivars (G-66, HRBS-1, G-72, and G-51) with different levels of resistance (Highly resistant, Resistant, Moderately susceptible and susceptible) were treated with antagonistic fungi (*A. niger*, *A. flavus, T. viride* and *P. capsulatum*) sown in pots having infested soil (*M. phaseolina*) in complete randomized block design with three replications.

The seeds of these varieties treated with antagonistic fungi were sown in ` soil in earthen pot (24 x 20 cm size) with three replications for each treatment. Surface disinfected seed sown in pots with sick soil treated as control. Seeds of the above mentioned varieties were also treated with fungicide for test comparison. Pots kept in glass house at 30°C and drenched with sterilized tap water. Germinated plants were thinned. Disease incidence was calculated at maturity for each test line by using formula;

Disease incidence = (Number of infected plants/Total number of plants) x 100

RESULTS AND DISCUSSION

In vitro evaluation of antagonistic fungi against *M. phaseolina*

A. niger, *A. flavus*, *T. viride*, *T. harzianum* and *P.*

Table 2. Seeds of selected varieties in combination with antagonists.

T5	T1 + T2	*Aspergillus niger* + *Aspergillus flavus*
T6	T1 + T3	*Aspergillus niger* + *Trichoderma viride*
T7	T1 + T4	*Aspergillus niger* + *Penicillium capsulatum*
T8	T2 + T3	*Aspergillus flavus* + *Trichoderma viridae*
T9	T2 + T4	*Aspergillus flavus* + *Penicillium capsulatum.*
T10	T3 + T4	*Trichoderma viridae* + *Penicillium capsulatum*
T11		Seeds treated with Vitavax @ 2 gm/kg of seed

[Vitavax (Carboxin) is systemic fungicide used as seed treatment against damping-off diseases caused by Rhizotonia].

Table 3. Analysis of variance for the effect of antagonistic fungi on growth of *M. phaseolina*.

SOV	DF	SS	MS	F. Cal	P-ratio
Antagonists (A)	5	20.907	4.181	4.115	0.0000**
Hours (B)	2	19.893	9.947	9.790	0.0000**
A X B	10	10.160	1.016	6.639	0.0000**
Error	54	8.264	0.153		
Total	71	59.224			

** Highly significant at P<0.05.

Table 4. Inhibition zone (diameter in cm) produced by antagonistic fungi.

S/N	Micro organism	24 h	48 h	72 h	Means hours
1	*Aspergillus niger*	0.30de	0.80cd	2.50a	1.20a
2	*Aspergillus flavus*	0.40de	1.30b	2.70a	1.46a
3	*Pencillium capsulatum*	0.10e	0.30de	0.50de	0.30c
4	*Trichoderma harzianum*	0.30de	0.70cd	1.20bc	0.73b
5	*Trichoderma viride*	0.050de	1.10bc	2.30a	1.30a
6	Control	0.00e 0.26c	0.00e 0.70b	0.00e 1.73a	0.00d

capsulatum significantly (P<0.05) inhibited the growth of *M. phaseolina* on PDA after incubation for 72 h at 30°C. It was noted that *A. flavus* was the most inhibitors species, followed by *A. niger, T. viride*, *T. harzianum*, and *P. capsulatum*. Inhibition of *M. phaseolina* by *A. niger*, *A. flavus* and *T. viride* did not differ significantly. *P. capsulatum* was the least effective antagonist against the pathogen, where as *T. harzianum* showed an intermediate action Tables 3, 4 and Figures 1, 2.

These results agreed with previous reports that soil micro-organisms including *Arachniotus* sp., *A. aculeatus*, *A. niger*, *A. flavus* and *T. viride* effectively inhibited growth of *M. phaseolina in vitro* (Michener and Snell, 1949; Cruz and Hubble 1975; Phillips, 1986). Sheikh 1981 showed that *A. flavipes* had marked inhibitory effects on *M. phaseolina* in agar culture. Zaki (1988) also concluded that *A. niger*, *A. flavus*, *T. harzianum*, *T. viride* and *Penicillium* sp., produce fungistatic metabolites which reduce the growth of *M. phaseolina*. *In vitro* efficacy of *T. harzianum* was tested against the eggplant root-rot pathogen, *M. phaseolina* and found that *T. harzianum* produced maximum inhibition zone of 18.20% (Hesamedin, 2008).

Management of charcoal rots disease of sunflower by seed treatment with antagonistic micro organisms in pot experiment

The experiment was performed in the University Experimental Glass House in Faisalabad on 7 February, 2007 to evaluate the efficiency of antagonistic fungi as

Table 5. Analysis of variance for the management of charcoal rots disease of sunflower by seed treatment with antagonistic micro organisms in pot experiment.

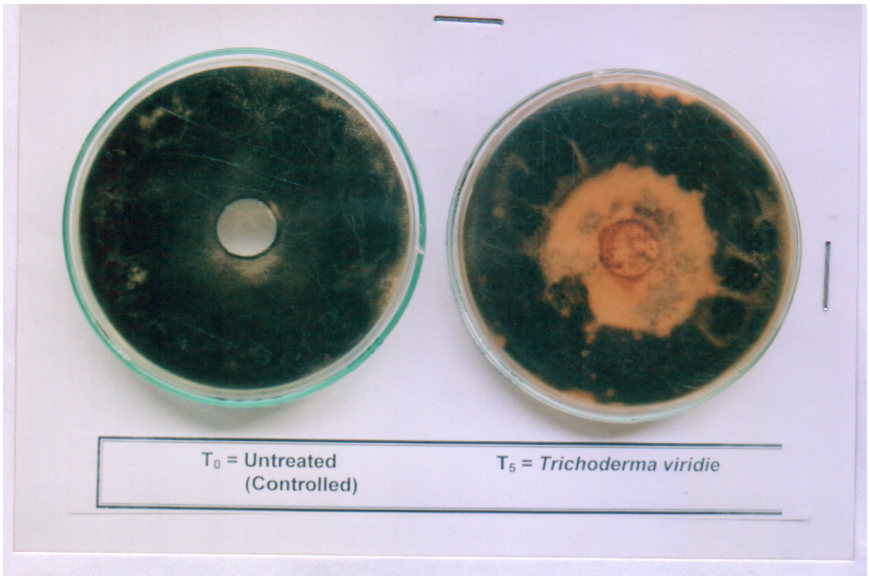
Source of variance	DF	SS	MS	F Value
Treatments (A)	11	43403.808	3616.957	585.933 **
Varieties (B)	3	87169.179	29056.393	4706.93 **
Interaction (A x B)	36	15637.987	4.4.389	70.37 **
Error	104	642.000	6.173	
Total	154	146852.974		

Table 6. Effect of antagonistic fungi on the incidence of charcoal rot on sunflower varieties in pot culture.

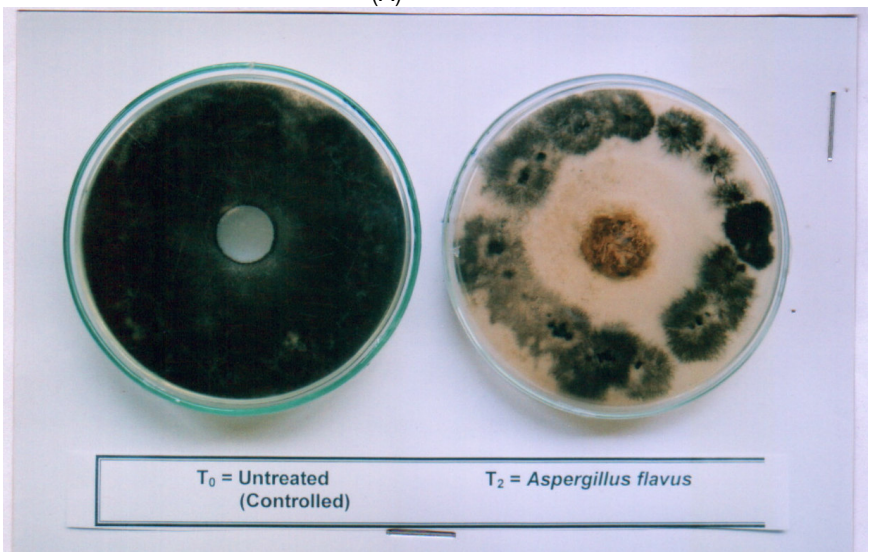
Treatments		G-66		HRBS1		G-72		G-51	
		D.I	% decrease over control	D.I	% decrease over control	D.I	% decrease over control	D.I	% decrease over control
T0	Control (untreated)	61.67	-	81.37b	-	90.17a	-	90.27 a	-
T1	*A. niger*	21.67 e	64.86	22.67 e	72.13	62.67 c	30.00	85.27 a	5.54
T2	A. flavus	0.00 f	100.00	22.67 e	72.13	42.33 d	53.05	82.33 c	8.79
T3	*T. viride*	42.00 d	31.89	43.00 d	47.15	62.67 c	30.49	90.23 a	0.04
T4	*P. capsulatum*	22.33 e	63.79	61.67 c	24.21	41.67 d	53.78	90.23 a	0.04
T5	T1 + T2 = *A. niger* + *A. flavus*	0.00 f	100.00	22.67 e	72.13	22.33 e	75.23	82.67 b	8.42
T6	T1 + T3 = *A. niger* + *T. viride*	42.67 d	30.80	21.67 e	73.36	42.67 d	52.67	90.17 a	0.11
T7	T1 + T4 = *A. niger* + *P. capsulatum*	42.33 d	31.36	42.33 d	47.98	63.00 c	30.13	90.20 a	0.05
T8	T2 + T3 = *A. flavus* + *T. viride*	21.67 e	64.86	22.67 e	72.13	42.00 d	53.42	82.33 b	8.79
T9	T2 + T4 = *A. flavus* + *P. capsulatum*	42.33 d	31.36	63.00 c	22.57	62.00 c	31.24	90.10 a	0.18
T10	T3 + T4 = *T. viride* + *P. capsulatum*	42.33 d	31.36	80.67 b	0.86	82.00 b	9.06	90.23 a	0.04
T11	Seed Treatment with fungicide (vitavex)	0.00 f	100	42.33 d	47.98	42.00 d	53.42	62.00 c	31.31

LSD value = 3.484

seed treatments. Seeds of four sunflower cultivars (G-66, HRBS-1, G-72, and G-51) with different levels of resistance (highly resistant, resistant, moderately susceptible and susceptible) were treated with *A. niger*, *A. flavus*, *T. viride* and *P. capsulatum* before sowing in pots containing soil inoculated with *M. phaseolina* in a complete randomized design with three replicates pot experiment. Disease incidence was significantly reduced as compared to controls in which seed was not coated with antagonists. Analysis of variance for the effect of antagonists on the incidence of charcoal rot indicated a significant effect for treatments, varieties and the possible interactions (Table 5). Disease incidence of *M. phaseolina* infection on sunflower seed line G-66 was reduced with *A. flavus*, followed by *A. niger*, *P. capsulatum* and *T. viride* (Table 6). Disease was fully controlled by the combination of *A. niger* and *A. flavus*, whereas few control occurred in treatment T6 (*A. niger* + *T. viride*).



(A)



(B)

Figure 1. Effect of antagonistic fungi on *M. phaseolina*.

The highest decreases in disease incidence occurred with Treatments T2, T6, T5 and T8 on cultivars G-66, HRBS-1, G-72 and G-51 respectively, whereas minimum reduction occurred with Treatments T10, T10, T10 and T3 on cultivars G-66, HRBS-1, G-72 and G-51, respectively (Table 6). Treatment with *A. flavus*, and the *A. flavus* + *A. niger* combination gave control equal to that treatment with the fungicide vitavax. These results strengthen the opinion that control with fungicides can be replaced by biological control because *A. flavus*, *A. niger* and *P. capsulatum* significantly protected the roots and stem of sunflower plants from infection by *M. phaseolina*.

The present study, therefore, supported the view that microbial antagonists may be more effective when applied to seeds than when applied to soil because of the proximity to the infection court (Kommedahl and Windels, 1981). These results agreed with previous findings that the application of *Arachniotus* and *Aspergillus* spp controlled *M. phaseolina* infections on *Vigna mungo* L. and *Arachis hypogaea* (Dhingra and Khare, 1973; Jackson, 1965).

The present study therefore provided strong evidence that seed treatments could be used in crop production for the prevention o f disease caused by seed-borne and

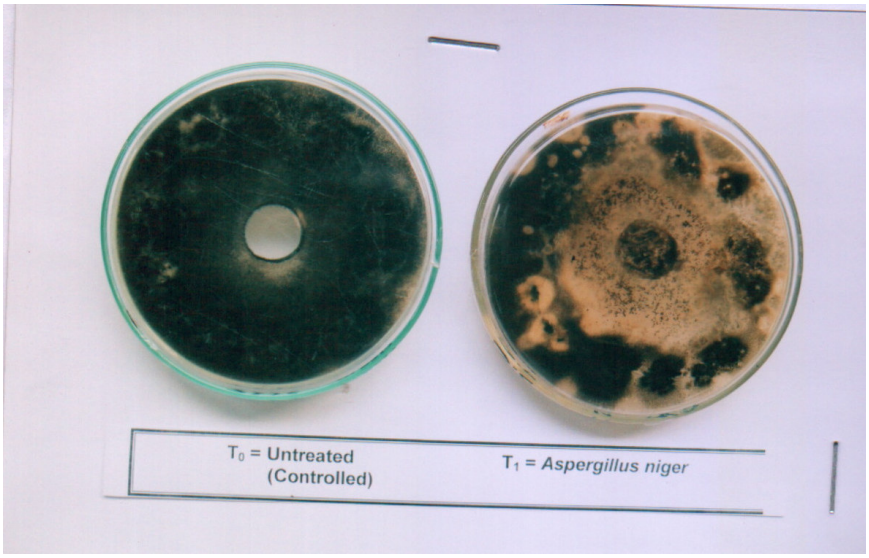


Figure 2. Effect of antagonistic fungi on *M. phaseolina*.

soil- borne pathogens, since it appears to be both effective and feasible. Delivery of antagonists directly to soil requires a large amount of material. Seed treatment is therefore, an alternative method for introducing biological control agents into the soil- plant environment. Antagonists applied to seeds not only have the potential for protecting the seed but being the initial colonizer of the emerging, root give a protective effect against root infecting pathogens (Chang and Kommedahl, 1968; Henis and Chet, 1975; Windels, 1981).

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