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BIOLOGICAL CONTROL OF CHARCOAL ROT OF SUNFLOWER

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ABSTRACT

In *in vitro* biological control of charcoal rot disease of sunflower (caused by *M. phaseolina*), *Trichoderma harzianum*, Arachniotus sp. and Penicillium sp. provided 93, 100 and 50 per cent disease control respectively. *Trichoderma harzianum* significantly promoted vigour and dry stem weight where as Arachniotus sp. increased vigour, dry stem weight, plant yield and dry root weight over the non-infected healthy plants. Penicillium sp. did not affect vigour but significantly decreased plant yield and dry stem weight.

In *in vivo* control *Trichoderma harzianum*, Arachniotus sp. and Penicillium sp. provided 85, 91 and 60 per cent disease control. *Trichoderma harzianum* and Arachniotus sp. increased plant vigour and head size of the sunflower. The three antagonists also significantly reduced extent of linear stem infection of the diseased plants.

INTRODUCTION

In Pakistan sunflower (*Helianthus annuus* L.) is known to suffer from root and stem rot disorder called charcoal rot disease (Anonymous, 1980). The disease is caused by a soil borne pathogen, *Macrophomina phaseolina* (Ilyas *et al.*, 1981). Symptoms of the disease (wilting, flagging, defoliation and loss of plant vigour) become prominent just prior to crop maturity and the disease causes 20-50 per cent yield losses. (Acimovic, 1962; Ilyas *et al.*, 1981). Commercial cultivars lack resistance against *M. phaseolina* (Anonymous, 1980) and chemical control of the pathogen is too expansive to be practicable (Watanabe *et al.*, 1970 Ilyas *et al.*, 1975). The biological control involving the use of antagonistic microorganisms appears to be feasible and an economical control measure (Dhingra, 1973; Ghaffar, 1982). This paper reports on the *in vitro* and *in vivo* control of charcoal rot of sunflower by antagonistic soil fungi.

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MATERIAL AND METHODS

Preparation of inoculum of pathogen and isolates of antagonistic fungi

A highly pathogenic isolate of *M. phaseolina* used in this study was recovered from an infected sunflower stem, suffering from root and stem rot disease symptoms. After growth at 30°C, stock cultures on PDA were held at 4°C until used. Isolates of *Trichoderma harzianum*, Arachniotus sp. and Penicillium sp. antagonistic to the growth of *M. phaseolina* and inhibiting respectively 47, 61 and 43 per cent mycelial growth of *M. phaseolina in vitro* (when the inocculum of either of the antagonists and *M. phaseolina* were placed 60-65 mm apart on Richard agar medium), were used for the biological control studies, and were maintained on Richard agar medium at 4°C, after their growth at 25°C.

In vitro control of root and stem rot disease

The earthen pots of 45 x 30 cm size were filled with equal amount of steam sterilized soil amended with 1% wheat straw by weight. Before amending soil with wheat straw, culture of each antagonist from one Richard agar medium plate (alongwith the medium) was thoroughly hand mixed with wheat straw of each pot. Then equal amount of *M. phaseolina* inoculum (obtained from a 7-day old petri dish culture) was added in the form of small PDA blocks, containing mycelium and sclerotia of the pathogen, placed at 10 and 20 cm below the soil surface in the pots. The pots without antagonist but with *M. phaseolina* and without antagonist and without *M. phaseolina* served as infested and uninfested control, respectively. Ten sunflower seeds were planted in each pot and the pots were watered frequently with sterilized water. The treatments were replicated three times. Fifteen days after plant emergence, 5 vigorously growing seedlings were left in each pot, the other were uprooted. At maturity, data were recorded on per cent plant infection, plant height, yield/plant, dry stem and dry root weight. Data were analyzed statistically and differences between treatments were determined by Duncan's Multiple Range Test at 5 per cent level of significance.

In vivo control of root and stem rot disease

The effect of each of the three antagonists on the *in vivo* control of the disease was studied in a field where sunflower crop was not planted for the last ten years. The treatments were planted with sunflower crop in subplots of

1.8 x 6.0 meter size. Before sowing the seed beds were throughly ploughed and soil of each treatment was mixed with wheat straw contaminated either with *M. phaseolina* or with antagonist or both or with non-contaminated wheat straw (uninfested control) at the rate of 100 kg straw/acre. The calculated amount of wheat straw for each subplot (treatment) was hand mixed thoroughly with inoculum of *M. phaseolina* and the antagonist, each taken from 7-day old culture plates, at the rate of 50 petri plates/acre of either of *M. phaseolina* or the antagonist. The contaminated or uncontaminated straw was broad casted on the surface of the soil of each subplot and mixed into the soil with the help of a small hand plough. The distance between treatments (subplots) was 60 cm and between replications 90 cm, whereas plant to plant distance was 30 cm. The treatments were replicated four times.

At maturity, the data were recorded on per cent plant infection, extent of linear infection in diseased stem, plant and head size. The data were analyzed statistically and differences between treatments were determined by Duncan's Multiple Range Test at 5 per cent level of significance.

RESULTS AND DISCUSSION

In vitro evaluation of the effect of three antagonists revealed that *Trichoderma harzianum*, Arachniotus sp. and Penicillium sp. significantly reduced the incidence of root and stem rot disease over the infested control (Table 1). The use of *Trichoderma harzianum* and Arachniotus sp. provided 93 and 100 per cent disease control, although there was no significant difference between the extent of disease control by the two antagonists, whereas with Penicillium sp. the extent of disease control was 50 per cent of the infested control. Arachniotus sp. applied to the seed have been reported to control pre-and post-emergence damping-off of Urid beans caused by *Rhizoctonia bataticola* (Dhingra, 1973). *M. Phaseolina* infection significantly reduced plant height (31.5%) over the non infected healthy plants Trichoderma harzianum significantly increased plant height (16.3%) and dry stem weight (10.9%) over non-infected healthy plants, whereas Arachniotus sp. not only increased plant height (29.3%) and dry stem weight (21.8%) but also increased seed yield per plant (7.8%) and dry root weight (13%) over the noninfected healthy plants.

Akhtar (1982) postulated that antagonists (*Trichoderma harzianum* and Arachniotus sp.) besides releasing antifungal metabolites, produce growth hormonic substances which increase the vigour and yield of the crop plants. Though Penicillium sp. had no effect on the *in vitro* increase of plant height, yet there was a significant decrease in seed yield/plant (11.7%) and dry stem weight (10%) over the healthy noninfected plants This was, probably, on account of the fact that antifungal metabolites released by Penicillium sp. may be toxic to host plants.

In *in vivo* evaluation, the three antagonists not only provided significant disease control but also reduced the extent of linear infection of diseased stem over the infected control (Table 2). In doing so *Trichoderma harzianum* and Arachniotus sp. were statistically equally more effective than Penicillium sp. Ten per cent disease incidence with the extent of linear infection statistically equal to that of infested control, was found in noninfected control. This was probably due to naturally occurring soil born inoculum of the pathogen in the field soil. Although there was no significant effect of the antagonists on the height of diseased plant, each of the three antagonists significantly increased height of healthy plants. *Trichoderma harzianum* and Arachniotus sp. were statistically equally more effective than Penicillium sp. in stimulating an increase in height of the healthy plants (Table 2). This again may be on account of growth hormonic substances produced by the antagonists. There was no *in vitro* effects of Penicillium sp. on plant height (Table 1). This was probably on account of the fact that antifungal metabolites (Penicillins, etc.) released by Penicillium sp. were also toxic to the sunflower plants growing *in vitro* in the absence of other soil microorganisms. Under *in vivo* conditions, the antifungal metabolites were, perhaps, disintegrated by soil microorganisms and the hormonic substances released by the Penicillium sp. caused a significant increase in plant height. Contrary to plant height both *Trichoderma harzianum* and Arachniotus sp. caused a significant increase in the diameter of the flower heads of both the diseased and healthy plants. Penicillium sp. neither affected the head size of the diseased nor that of the healthy plants (Table 2). Effect of the three antagonists on yield could not be evaluated on account of birds damage to crop near maturity.

Table 1. *In vitro effect of antagonistic soil saprophytes on disease incidence, plant height, seed yield/plant, dry stem and dry root weight of sunflower*

Treatments	Disease incidence (%)	Plant height (cm)	Seed yield (gm)	Dry stem weight (gm)	Dry root weight (gm)
Trichoderma harzianum + *M. phaseolina*	7.0 c*	107 b	10.0 b	12 2 b	5.9 ab
Arachinotus sp. + *M. phaseolina*	0.0 c	119 a	11.0 a	13 4 a	6.1 a
Penicillium sp. + *M phaseolina*	50.0 b	95 c	9.0 c	9.9 d	5.3 b
No antagonist + *M phaseolina* (infested control)	100.0 a	63 d	0.0 d	10.0 d	4.2 c
No antagonist + No *M. phaseolina* (Noninfested control)	0.0 c	92 c	10.2 b	11.0 c	5.4 b

* Figures in the same column with the same letter do not differ significantly at 5% level of significance.

Table 2. *In vivo effect of antagonistic saprophytic fungi on per cent mortality by Macrophomina phaseolina, extent of linear infection in diseased stem, plant height and head size of sunflower plants*

Treatments	Disease incidence (%)	Extent of linear infection of diseased stem above soil level (cm)	Plant height (cm)		Head size (cm)	
			Healthy	Diseased	Healthy	Diseased
Trichoderma harzianum + *M. phaseolina*	15 c*	25 c	199 a	141	16.0 a	9 8 a
Arachniotus sp. + *M. phaseolina*	9 c	24 c	203 a	144	15 8 a	10.2 a
Penicillium sp. + *M.phaseolina*	40 b	49 b	183 b	134	14.9 b	7.1 b
No antagonist + *M.phaseolina* (infested control)	68 a	111 a	143 c	138	14.8 b	7.0 b
No antagonist + No *M. Phaseolina* (Non-infested control)	10. c	104 a	147 c	132 NS	14.4 b	7.5 b

* Figures in the same column with the same letter do not differ significantly at 5% level of significance.
NS = Non-significant.

REFERENCES

Acimovic, M. 1962. *Macrophomina phaseolina* as a causal agent of sunflower wilt in Vogrodine. Tast. Bilja, 70 : 125-138.

Akhta, C.M. 1982. Biological control of Rhizoctonia root rot of cotton and Fusarium wilt of linseed. Five year research report. Ayub Agri. Res. Inst., Faisalabad. P-63.

Anonymous. 1980. Survey of diseases of oilseed crops. Ann. Rept. Plant Path. Section. Oilseed Res. Inst., Faisalabad.

Anonymous. 1980. Screening of promising lines of sunflower against charcoal rot of sunflower. Ann. Rept. Plant. Path. Section. Ayub Agri. Res. Inst., Faisalabad.

Dhingra, O.D. 1973. Biological control of *Rhizoctonia bataticola* on Urid beans. Phytopath. Z. 76 : 23-29.

Ghaffar, A. 1982. Studies on the integrated control of sclerotial fungi. Fourth Ann. Res. Rept., Deptt. of Bot., Univ. of Karachi. P-41.

Ilyas, M.B. 1975, The response of *M. phaseolina in vitro* and its sclerotia in soil to various soil fungicides. Ph. D. Thesis. Univ. of Illinois, U.S.A.

Ilyas, M.B., M.A. Randhawa and M.A.R. Bhatti 1981. Biology, host parasite relationship and control of *M. phaseolina*. Tech. Bull. No. 1. Soc. Adv. Agri. Sci., Pakistan, Unvi. Agri., Faisalabad. P-22

Watanabe, T., R.S. Smith, and W.C. Snyder. 1970. Populations of *Macrophomina phaseoli* in soil as affected by fumigation and cropping. Phytopath. 60 : 1717-1719.