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Full Length Research Paper

Eco- friendly strategies for management of *Fusarium* wilt of *Pisum sativum L.*

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The efficacy of different microbes for their antagonistic ability was determined *in-vitro* against *Fusarium oxysporum* f. sp *pisi* by dual culture method. *T. viride* and *Pseudomonas fluorescens*-I exhibited maximum antagonistic activity *in vitro*. *In-vivo T. viride* and *Pseudomonas fluorescens* resulted in maximum reduction of seed rot root and wilt (*Fusarium oxysporum*) of pea. In addition, there was increased germination, mortality, shoot length, root length, fresh weight, vigour index and numbers of nodules. Among the microbes, *T. viride* performed better. Therefore, *T. viride* may be used for the reduction of root rot, wilt and mortality of pea.

Key words: *Fusarium* wilt, pea, bioagent, control.

INTRODUCTION

Pea (*Pisum sativum*) is one of the most important cash crops for farmers of Uttar Pradesh. It is attacked by several disease caused by fungi, bacteria and viruses. Among these diseases, wilt caused by *Fusarium oxysporum* schl. f. sp. *pisi* Synder and Hansen is the most destructive disease of the crop and occurs as an epiphyte almost every year. The disease is essentially soil borne and poses a greater problem in management by using fungicides which are uneconomical and their frequent and indiscriminate use often leads to atmospheric pollution and development of resistance in the pathogens. In this context, biological control is an alternative strategy of disease management which is eco-friendly. Several organisms have been successfully used as biocontrol agents, such as the species of *Trichoderma* (Raguchander et al., 1997), *Pseudomonas, Gliocladium Penicillium* (Castejón-Muñoz and Oyarzun, 1995), *Rhizobium* (Bradshaw et al., 1991), etc. Based on the review of literature, it appeared that biological seed treatment was feasible and attempts were made in the present study to select effective antagonists and biofertilizers which could be applied to pea seeds. These were evaluated by laboratory and field condition.

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MATERIALS AND METHODS

The soil samples were collected from pea growing areas of Allahabad (U.P.) in polythene bags. The samples were taken from the rhizosphere of healthy pea plant, pooled and then isolation of associated microbiota was made by dilution plate method (Waksman, 1916). The culture was identified on the basis of morphological characters documented in taxonomic key (Von Arx, 1978).

In vitro study

Antagonistic potential of the isolated soil microflora and those in laboratory collection against *F. oxysporum* was detected by dual culture technique on potato dextrose agar (PDA) medium with three replications for each isolates and control. They were incubated for 7 days at 24 ± 1°C.

In vivo study

This study was conducted in poly house of the department of Botany, University of Allahabad (2007 - 2008). Inoculum of *F. oxysporum* was prepared following the method of Dutt and Das 2002. Soil was mixed uniformly with cow dung (2:1). The dried soil was sterilized with formalin 40%; 5 ml formalin diluted with 20 ml of water for 4 kg soil at 0.1% weight of dry soil (Haque et al., 1995; Harnadez and Hill, 1983). The culture of *F. oxysporum* was then mixed with pot soil before seed showing (Chunje and Zhibiao, 1996; Gomez and Gomez, 1984).

The inoculum was applied only up to a depth of 6 cm in pot soils

Table 1. *In vitro* evaluations of antagonists against *F.oxysporum* f. sp. *Pisi.*

Antagonist	Inhibition (%)	Zone of inhibition (+) interaction (-)
T. viride	51.55	-
T. harzianum	34.55	-
Pseudomonas fluarescens-1	49.62	-
Pseudomonas fluarescens-2	50.08	+
Aspergillus niger	27.36	-
Gliocladium virens	26.39	-
Rhizobium strain-1	34.55	-
Rhizobium strain-2	32.28	-
CD 70.05	(1.059)	

Data represent the means of three replications.

that were 2 g. Culture of *F. oxysporum* was mixed with each pot soil. A total of 20 pots were inoculated without culture for three replications. Seed were counted, weight and packed in separate polyethylene bags as per treatment and replication. 2*Trichoderma* strains, 2 *Pseudomonas* strains 1*Gliocladium virens,* 1 *Aspergillus niger* and two biofertilizers (Rizobium-1 and Rizobium-2) were used for treating seed. Seeds were treated with selected antagonist strains and biofertilizers following the methods of earlier researchers (Das, 1988; Harnadez and Hiill, 1983; Hassain and Mohammed, 2002; Hassain, 2000).

For seed coating, selected antagonistic strains containing 2×10^7 cfu/ml spore suspension seed treatment as liquid formulation was used. The seeds were dipped in the broth for 15 min. In the case of biofertilizers, seed were initially moistened with molasses at 50 g per kg seed. Then seeds were thoroughly mixed with biofertilizers (50 g/kg seed). Each biofertilizer contained 3×10^9 *rhizobium* cells/mg. In the control, seeds were not treated with selective antagonist strains or biofertilizers. The inoculants coated seed were placed in a cool and dry place under shade for drying. Treated seeds and non treated seeds for control were sown in previous prepared soil in pots. 5 seeds were sown in each pot. Observation was made every day in the morning on root rot and wilt infected plants. Interculture operations were done to maintain normal hygienic condition of crop growth. No plant protecting chemicals (insecticides and fungicides) were used for control of pests and diseases of crop. Data were recorded for germination seed rot, root rot, wilt plant, stand seedling vigour, number of nodules per plant, number of green pods per plant and plant height for vigour index. Data were recorded for germination up to the15th day of sowing. 5 plants were randomly selected, uprooted carefully from pot and washed in tap water. Then, root length and shoot length were recorded. Vigour index was calculated by the formula of Baki and Anderson (1973) as shown below:

Vigour index = Mean shoot length + mean root length) × Germination (%)

The data were subjected to statistical analysis for mean values and test of significance.

RESULTS AND DISCUSSION

The data (Table 1) indicate that all the antagonists inhibited the mycelial growth of *F. oxysporum* f. sp. *Pisi. T. viride* (51.55), *P. fluorescens*-1 (49.62) and *P. fluorescens* -2 (50.08) showed maximum inhibition and were statistically different. These were significantly higher than other antagonists such as *rhizobium*-1 (34.55) and *rhizobium* -2 (32.28). However *T. hamatum* (35.55), *Gliocladium virens* and *Aspergillus niger* (27.36) were found least effective. The zone of inhibition was observed only in *Pseudomonas fluorescens-2* whereas, other treatment revealed zone of interaction. The inhibitory effect of *Trichoderma* spp. against *F. oxysporum* f. sp *pisi* was probably due to hyperparasitism, mycoparasitism, competition for space and nutritional sources and antagonistic chemicals produced and released into the environment. *Trichoderma* spp. has been reported to produce antibiotic compound (Trichodermin), extracellular enzymes (chitinase, cellulose), unsaturated monobasic acid and peptides (Alamethicine) that either damage plant pathogens or enhance their population in maintaining favorable balance as a portion of the biota (Mukherji and Garg, 1988). *Trichoderma* spp. has also been employed in biological control of disease of vegetable crops caused by soil borne plant pathogens (Baker and Cook, 1974; Chet, 1987; Chet and Baker, 1981; Elad et al., 1980).

In vivo evaluation

Germination seed rot, wilt and plant stand were significantly affected by antagonist and biofertilizers (Table 2). The highest germination (93.25) were recorded when seeds were treated with *T. viride* which was statistically similar to *P. fluorescens*-1 (90.75). The lowest seed germination (72.33) was recorded in the control. The highest seed rot (27.65) was observed in control, while the lowest (6.75%) in *T. viride* followed by *P. fluorescens-1* (9.25%). The highest wilt (48.65%) was observed in control and the lowest wilt (8.49%) in *T. viride* which was statistically similar to *P. fluorescens*-1 (1.56). Maximum plant stand (91.51%) was recorded in *T. viride* which was statistically similar to *P. fluorescens*-1 (88.44). The lowest plant stand (51.35) was recorded in untreated control. Shoot length, root length, fresh weight,

Table 2. Effect of biocontrol agents on seed germination, seed rot and wilt of Pea in pot condition.

Antagonist biofertilizers	Germination (%)	Seed rot (%)	Wilt (%)	Plant stant (%)
T. viride	93.25	6.75	8.49	91.51
T. harzianum	79.90	20.10	25.34	74.66
Pseudomonas fluorescens-1	90.75	9.25	11.56	88.44
Pseudomonas fluorescens-2	81.39	18.61	24.76	75.24
Gliocladium virens	83.95	16.05	17.34	82.66
Rhizobium-2	87.69	12.31	13.86	86.14
Rhizobium-1	82.88	17.12	20.01	79.99
Aspergillus niger	86.11	13.89	15.70	84.30
Control	72.33	27.67	48.65	51.35
CD (P>0.01)	5.084	5.084	4.081	4.084

Data represent the means of three replications.

Table 3. Effect of seed treatment with antagonist and biofertilizer on plant (growth of pea in *Fusarium oxysporum* f.sp. *pisi* infested in pot).

Antagonist and biofertilizer	Shoot length plant (cm)	Root length plant (cm)	Fresh Wt (g)	Nodules/ plant	Seedling vigour	Mortality (%)
T. viride	20.5	31.3	4.1	72.7	5030.3	3.3
T. harzianum	17.4	22.1	2.6	21.3	3422.6	16.7
Pseudomonas fluorescens-1	20.0	28.1	4.0	56.0	4328.1	6.7
Pseudomonas fluorescens-2	16.4	20.7	2.6	32.7	2637.5	26.7
Gliocladium virens	18.8	23.1	2.5	28.0	3763.8	10.0
Rhizobium -1	15.8	26.2	4.1	75.7	4206.0	10.0
Rhizobium -2	16.1	23.4	2.5	31.3	3427.8	16.7
Aspergillus niger	18.2	24.7	3.2	12.7	3570.7	16.7
Control	10.5	7.5	1.2	8.0	718.0	73.3
CD (P=0.05)	2.9	4.0	1.1	18.9	910.4	10.6

number of nodules per plant, seedling vigour and mortality percentages were significantly influenced by the antagonist and biofertilizers (Table 3). The highest shoot length (20.5 cm) was noted in the seed treated with *T. viride* which was statistically similar to *P. fluorescens*-1(20.0 cm) and followed by *G. virens* (18.8), *A. niger* (18.2), *T. harzianum* (17.4) and *P. fluorescens-2* (16.4). *Rhizobium* S-2 (16.1) and *Rhizobium S-1 (*15.8) untreated control produced the lowest shoot length (10.5 cm). Though the treatments did not show any significant effect on root length it ranged from (7.50 cm) control to (31.3 cm). In all treatment the highest fresh weight per plant was 4.1 g in *T. viride* which was statistically similar to *Rhizobium* S-1 (4.1 g), *Pseudomonas fluorescens* (4.0 g), *A. niger* (3.2 g), *T. harzianum* (2.6 g) and *G. virens* (2.5 g). Higher vigour index of seedling (5030.3) was obtained from *T. viride* and the lowest (718.8) was in untreated control. Maximum number of nodules per plant (757) was observed in *Rhizobium S*-1 which was statistically similar to *T. viride* (72.2) and the lowest (8.0) in untreated control. The lowest mortality (3.3%) was observed in *T. viride* and the highest mortality percentage was observed in the untreated control (73.3%) Table 3. Chakrobarty and Chakraborty (1989) reported that bacterization of seed with *Rhizobium leguminosorum* biovar viceae was highly effective in reducing the severity of food rot in Pea caused by *Fusarium solani* f. sp *pisi,* while Perveen and Ghaffar (1991) reported that *Rhizobium meliloti* gave complete control of *Fusarium oxysporum* on 30 days and 120 days old tomato plant with higher seed germination as compared with untreated control. *Pseudomonas* has been reported to improve plant growth parameter and suppress the attack of different pathogens and diseases caused by them leading ultimately to improved crop yield (kloepper et al., 1986; Davision, 1988; Jeon et al., 2003). Effectiveness of *T. koningi, T. viride, Gliocladium catenulatum* and *G. raseum* against pea root rot complex was demonstrated by Lacicowa and Pjeta (1994).

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