J. Phytol. Res. 23(2) : 373-376, 2010

BIO- SCREENING OF RHIZOSPHERIC MYCOFLORA FOR THE CONTROL OF ANTHRACNOSE OF PEA CAUSED BY *COLLETOTRICHUM PISI*

RASHMI PANT
Department of Botany, Govt. P.G. College, Kota, India.

Antagonistic properties of rhizospheric mycoflora can be used as bio-control agent for many pathogens. In the present study rhizospheric mycoflora of pea plant were screened for their antagonistic properties against *Colletotrichum pisi* causal organism of anthracnose of pea. Both *in vitro* and *in vivo* experiments were conducted. Among the 16 microflora tested six showed good results. *Trichoderma viride* and *Gliocladium* spp. supported good plant growth along with disease protection. Treatments supplemented with *Rhizobium* fertilizer showed better results.

Keywords : Antagonism; *Colletotrichum pisi;* Pea plant; *Rhizobium;* Rhizospheric mycoflora.

Introduction

Pea occupies a very special position because of its importance both as a vegetable and as a pulse crop. A market survey of Kota district of Rajasthan revealed that about 17 to 30 percent of total crop was damaged due to anthracnose disease during 2008 - 09. Humid weather favours the disease. Chemically the disease is controlled by thiophanate methyl. However, the application of chemical should be reduced to preserve the agro eco system. Antagonistic properties of Rhizospheric mycoflora are good source of bio-control agents. Importance of bio-control agents against soil pathogen of tomato was studied by many workers[1,2]. Among the rhizospheric mycoflora plant growth-promoting rhizo-bacteria are of much importance[3]. They promote growth by controlling the pathogens and deleterious organisms[4]. Rhizospheric fungal flora also shows antagonistic properties against pathogens. Jeffries[5] mentioned the importance of biocontrol agents against diseases caused by *Colletotrichum.* There have been many reports of bio-control of *Colletotrichum* by rhizospheric fungi[6,7].

In the present study six rhizospheric fungi were studied for their antagonistic efficacy against the pathogen. Both *in vitro* and *in vivo* experiments were performed. In another set of experiment, culture of *Rhizobium* was added along with rhizospheric mycoflora. Addition of *Rhizobium* along with antagonistic rhizospheric fungi not only reduced the chances of disease but also enhanced plant growth.

Material and Method

Isolation of rhizospheric mycoflora-Rhizospheric mycoflora were isolated from the soil, 10-fold soil dilution were made. The cultures were developed and maintained on PDA. *Rhizobium* was obtained from Division of Microbiology, IARI, New Delhi. *Rhizobium* was mixed @ 25 gm / Kg.

Dual Culture to Test Antagonism-Dual culture plate technique[8] was used for testing antagonism against *C. pisi.* The pathogen was inoculated after one day of inoculating bio control agents. The diameter of colony was recorded and per cent inhibition was calculated by the formula given by Naik and Sen[9].

Pot Experiment - Two sets of experiment were performed, one with antagonistic fungi and another with antagonistic fungi + *Rhizobium* inoculants. In each set culture of pathogen *Colletotrichum pisi* was added @ 2 gm / kg .The pathogen was added four days before adding bio control agent. 10% of antagonistic fungi was mixed separately in each pot. Pots without antagonistic treatment were set as control. 10 seed per pot was sown next day.

In second set *Rhizobium* inoculants @ 25 gm / kg was mixed with antagonistic fungi. Pots with pathogen + *Rhizobium* and without antagonistic treatment were set as control. Seeds were pre-soaked in *Rhizobium* suspension for over night and 10 seeds per pot were sown next day.

Germination percentage was recorded for each pot. Seedlings from 1/3 pots of each set of experiment were harvested after 2 week. Washed and dried. Fresh weight were recorded they were then oven dried and dry wt. were recorded. Percentages of diseased and healthy

Table 1. Percentage inhibition of mycelial growth of pathogen in dual culture.

Sl. No.	Microbial antagonist	Inhibition of mycelial growth (percentage)
1.	*Aspergillus flavus*	51.62 (46.32)
2.	*Aspergillus niger*	62.46 (60.83)
3.	*Penicillium* spp.	54.70 (51.66)
4.	*Cladosporium* spp.	17.15 (22.43)
5.	*Gliocladium* spp.	65.10 (58.24)
6.	*Trichoderma viride*	67.02 (62.37)
		C.D. at 5% 2.75

Table 2. Effect of microbial antagonist on growth parameters and disease incidence in pea plants.

Sl. No.	Microbial antagonist	Percentage of seed germination	Fresh /dry wt.	Length of radicle	Disease incidence (percentage)
1.	*Aspergillus flavus*	59.02	0.270 / 0.16	6.23	40.00
2.	*Aspergillus niger*	68.85	0.271 / 0.16	6.27	36.66
3.	*Penicillium spp.*	46.78	0.268 / 0.15	6.17	43.34
4.	*Gliocladium spp.*	72.36	0.302 / 0.15	6.39	33.34
5.	*Trichoderma viride*	76.91	0.340 / 0.16	7.15	23.34
6.	Control	43.55	0.187 / 0.11	5.93	60.00
	C.D. at 5 %	2.02		0.23	3.2

Table 3. Effect of fungal antagonist + *Rhizobium* on growth parameters and disease incidence in pea plant.

Sl. No.	Microbial antagonist +*Rhizobium*	Percentage of seed germination	Fresh /dry wt.	Length of radicle	Disease incidence (percentage)
1.	*Aspergillus flavus*	73.59	0.287 /0.19	6.58	30.00
2.	*Aspergillus niger*	77.39	0.288 / 0.19	6.61	23.34
3.	*Penicillium* spp.	61.05	0.334 / 0.20	6.40	36.66
4.	*Gliocladium* spp.	82.44	0.360 / 0.21	6.84	16.67
5.	*Trichoderma viride*	83.81	0.365 / 0.21	7.32	10.00
6.	*Rhizobium*	63.16	0.287 / 0.19	6.63	33.34
	C.D. at 5%	3.20		0.25	2.7

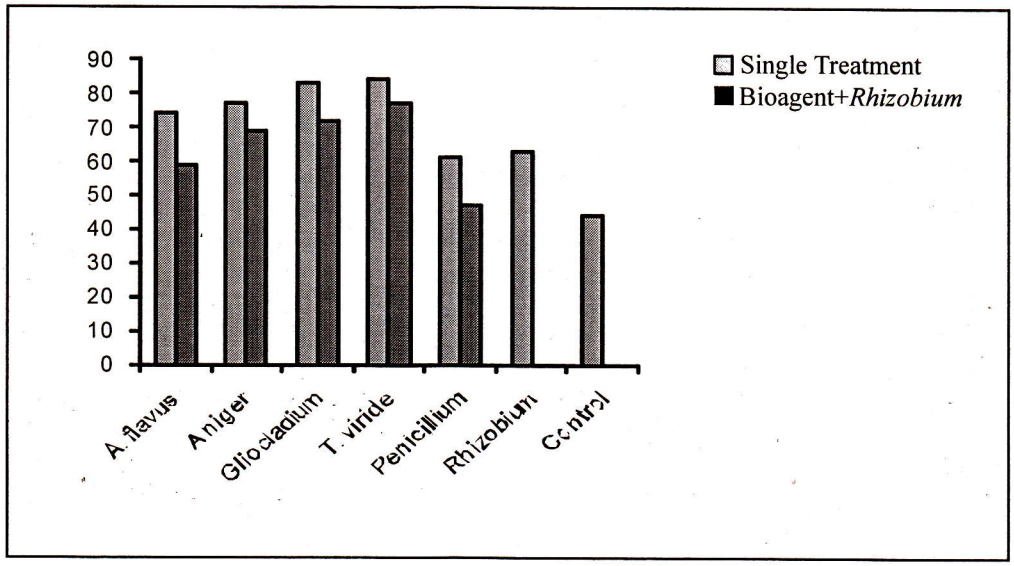

Fig.1. Percentage of seed germination.

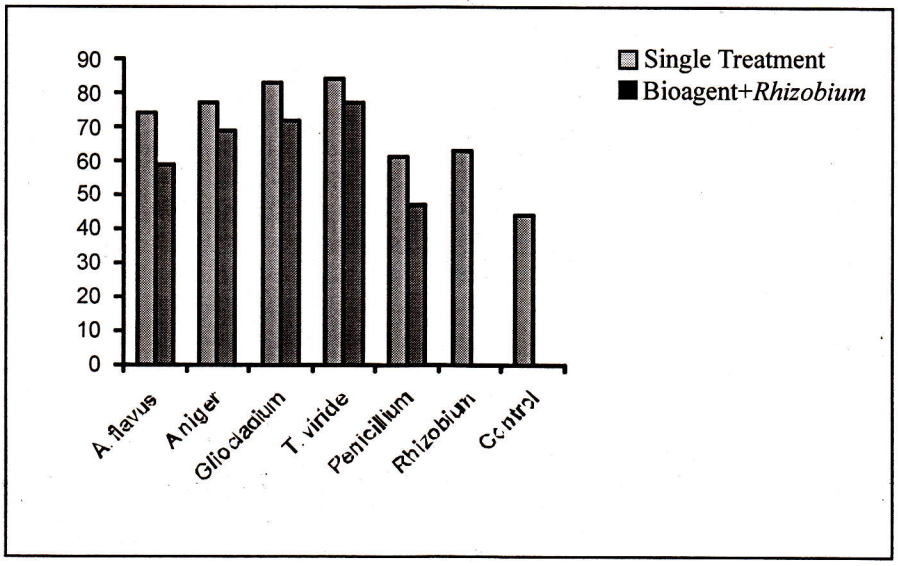

Fig.2. Disease incidence in pea plant.

plant were recorded after third month.

Results and Discussion

The results of *in vitro* experiment showed that all the antagonist well control the mycelial growth of *Colletotrichum pisi.* In each case, inhibition was more than 50% except for *Cladosporium* spp. (Table 1).

Cladosporium showed the least control (17.15) while *Trichoderma viride* showed maximum control (67.02) closely followed by *Gliocladium* spp. (65.10). *A. niger* showed better control (62.46) as compared to *A. flavus* (51.62). As *Cladosporium* spp. did not show significant reduction in the growth of pathogen only the remaining five antagonist were taken for pot experiments.

Pot experiment with antagonist treatment showed that all supplement favour seed germination over control. Germination was maximum in case of *Trichoderma viride* (76%), closely followed by *Gliocladium* spp. (72%).Treatments with *Penicillium* spp. did not show much improvement over control. Fresh and dry weight of seedling and length of radicle also showed improvement in each treatment. Results were better in treatments with *T. viride* and *Gliocladium* (Table 2).

Antagonistic treatment reduced the disease incidence up to 20 - 40 percent as compared to control. Number of diseased plants were reduced up to half in case of *A. niger* and *Gliocladium*. In pots containing *Trichoderma viride,* only seven out of thirty plants showed diseased symptoms. Diseased incidence fell from 60 % in control to 23 % in *T. viride* treated plants.

In next set of experiment with additional supplement of *Rhizobium* culture all the growth parameter showed better results. Germination of seeds showed 20 to 45% increase, with maximum in *Rhizobium* + *Trichoderma* treatment closely followed by *Rhizobium* + *Gliocladium.* There was a sharp decrease in disease incidence in each case. Only 10% plant showed disease symptoms in *Rhizobium* + *Trichoderma* treatment. (Table 3).The results showed that antagonistic rhizospheric mycofloras are quite effective as a bio-control agent. They multiply fast in native soil[10] and compete with pathogen. *T. viride* and *Gliocladium* spp. multiply at a faster rate and inhibit the growth of *Colletotrichum pisi* upto 65%. Hjelijord and Tronsmo[11] also mentions *Trichoderma* and *Gliocladium* as strong bio control agents against soil pathogens. *Aspergillus niger* showed good inhibition. Potential of *Aspergillus* as bio control agent against soil pathogen is very high[12]. It appeared from the results that all these antagonist not only reduce the chances of disease but also promote growth. In case of rhizospheric microflora of tomato reduction in disease severity along with improved health was also reported[13,14].

Rhizobium bacteria are an important member of the rhizospheric microflora. They promote growth by controlling the pathogen and deleterious organism. Increasing *Rhizobium* population in the rhizosphere showed a marked increase in seed germination and other growth parameter. In addition, it also showed a sharp decline in disease incidence. The results suggest that a suitable rhizospheric antagonistic microflora can work as a bio-control agent and may enhance plant growth.

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