J. Biol. Control, 22(2): 405-410, 2008

Eco-friendly strategies for management of *Sclerotinia* rot of french bean

PRANAB DUTTA, B. C. DAS and M. ISLAM*

Department of Plant Pathology, Assam Agricultural University, Jorhat 785013, Assam, India.
*Department of Life Sciences, Dibrugarh University, Dibrugarh 786001, Assam, India.
E-mail: pranabd1974@indiatimes.com

ABSTRACT: ***Sclerotinia*** **rot caused by** ***Sclerotinia sclerotiorum*** **is a serious menace to the cultivation of french bean (*Phaseolus vulgaris* L.) in Assam. *In vitro* efficacy of antagonist by dual culture method was tested against *S. sclerotiorum*. Out of all the antagonists *Pseudomonas fluorescens* was found best causing growth reduction of 64.93 per cent followed by *Bacillus subtilis* (62.86 %) and *Trichoderma harzianum* (59.08 %). Study on the mode of parasitism showed that the fungal antagonist initially caused coilling and then lysed the pathogen hyphae and the bacterial antagonist ceased pathogen growth after coming in contact with the pathogen. Effect of fungal antagonist *T. harzianum* and bacterial antagonist's *B. subtilis* and *P. fluorescens* as seed treatment against sclerotinia rot was evaluated under field conditions. Seed treatment with *T. harzianum* was found has most effective in improving seed germination (18.43 %), reducing 90.46 % infection and increasing yield (69.51%) over control plot. Efficacy of *B. subtilis* and *P. fluorescence* as seed treatment against the disease was next to *T. harzianum* treatment respectively.**

KEY WORDS: Antagonist, biological control, french bean, seed treatment, *Sclerotinia* rot,

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is one of the major pulse crops cultivated in India and around the world. White mold of french bean caused by *Sclerotinia sclerotiorum* (Lib) de Bary can infect all the stages of crop and has become important in recent times in India and elsewhere with high incidence and severe yield losses. The pathogen is known to infect about 400 plant species (Kolte, 1985) with no proven sources of resistance. In recent years an increasing consciousness about environmental pollution due to pesticides and development of fungicide resistant strains in plant pathogens has challenged plant pathologists to search for an ecofriendly tools for disease management. Biological control appears to be a promising ecofriendly strategy for managing various diseases in a range of crops. To be effective, biological disease control depends not only on suitable biocontrol organisms; but also on methods and strategies for introducing and maintaining the organism in crop. Considering these points in view, the present study was conducted to determine the possibility of the use of most effective fungal and bacterial antagonist as seed treatment for management of white mold of french bean.

MATERIALS AND METHODS

Fungi were isolated from rhizosphere soil samples of healthy french bean plants adjacent to or between two *Sclerotinia* rot affected plants in a field with 80 per cent incidence of the disease. Sclerotia of *S. sclerotiorum* lying in the affected field soil were also collected and made isolation of other fungi. Isolations were done in medium specific for each of the isolated dilution plate technique (Pramer and Schmidt, 1956).

***In vitro* study**

Antagonistic potential of the isolated soil microflora and those in laboratory collection, against *S. sclerotiorum* was detected by dual culture technique on potato dextrose agar (PDA) medium with five replications for each isolate and appropriate control. They were incubated for seven days at 20 ± 1° C.

Mode of parasitism of antagonists on *S. sclerotiorum* was studied by growing each of antagonists separately along with pathogen, one opposite to another on sterilized slides smeared with 1 per cent PDA. The slides were incubated at 20 ± 1° C for 48 h. The incubated slides were observed directly under compound microscope.

***In vivo* study**

In vitro efficacy of biological seed treatment on seed germination was studied by growing antagonist on PDA medium for 10 days for *T. harzianum* (@1 x 10^6 cfu/ml of water) and three days for bacterial antagonist (@1 x 10^9 cfu/ml of water at 27 ± 1 ° C and rolling the seeds of the cv Contender on the lawn of the fungal and bacterial growth. For comparison, dry seed treatment of carbendazim (@ 0.2% w/w) was applied before sowing. Part of the treated seeds was kept on moistened blotter at 23 ± 1° C in three replications for each treatment with appropriate untreated controls.

Field experiment with three most effective antagonists were laid out in randomized block design (RBD). The plot size was 1m x 2m with spacing of 30 cm x 10 cm.

Experimental plots were made sick by soil inoculating (@ 500 g/m^2) the inoculam of *S. sclerotiorum* in sclerotial and mycelial form obtained from 4% maize meal sand medium incubating at 28 ± 1°C for 21 days as per the method described by Dutta and Das (2002).

Antagonists were grown on PDA medium. After attaining full growth at a temperature of 27± 1°C culture was scrapped and suspended in sterilie distilled water. Concentration of fungal antagonist and bacterial antagonist 1x 10^6 spore / ml and 1 x 10^9 cfu / ml respectively was maintained. To this suspension 2 per cent (w/v) carboxy methylcellulose was added. Surface sterilized seeds with 70 percent ethanol were soaked in the suspension for 4 hr at 28±1° C on horizontal shaker. Seed soaked in sterile water was kept as control. Percentage seed germination, percentage disease incidence (PDI) and shoot length (cm/plant) was recorded at 30, 60 and 90 days after seed germination. Recording of above ground shoot length (cm) of ten plants at random across each plot and calculating their average as the mean shoot length for the plot measured as shoot length. Seed yield was recorded at final harvest. Dividing the difference between the data in the control and that in treatment by that in the control and then multiplying the resulting quotient with 100 calculated percentage increase or reduction due to any treatment. The data was statistically analyzed as par the design using ANOVA.

RESULTS AND DISCUSSION

All the tested microorganisms caused significant ($P< 0.05$) inhibition in mycelial growth of *S. sclerotiorum* in dual culture studies (Table 1). The highest reduction (64.93 %) was caused by *P. fluorescens* followed by *B. subtilis* and *T. harzianum* on PDA. Mycelial growth reduction recorded in case of *T. viride* and *T. koningii* was statistically at par with each other. Antagonism of *S. sclerotiorum* by *B. subtilis* (Loeffler *et al.*, 1986), *P. fluorescens* (Das *et al.*, 2001), *G. virens*, *Trichoderma* spp (Sharma *et al.*, 1992, 1999; Das *et*

al., 2001) *A. terreus* (Das *et al., 2001*) have been reported earlier. Growth inhibition caused by antagonists in the present study might be due to production of antibiotics by *P. flourescens* (O' Sullivan and O' Gara, 1992), *B. subtilis* (Turner and Backman, 1991) *Trichoderma* sp. (Chet *et al.*, 1981) as reported earlier.

Study on mode of parasitism showed that mycelial growth of *S. sclerotiorum* seemed to stop growing when came it in contact with *P. fluorescens* and *B. subtilis* and thereby results in complete restriction of growth of *S. sclerotiorum* (Table 2) and thereby severe vacuolation followed by coagulation and shrinkage of cytoplasm was observed after 7 days of incubation. *Aspergillus terreus* and *Gliocladium virens* also coiled around the pathogen hyphae but *A. terreus* caused lyses of *S. sclerotiorum* hyphae at the branching point and at the tip. Such observation was of mycoparasitism was also earlier made for *B. subtilis* (Loeffler *et al.*, 1986), *T. harzianum* (Mukhopadhyay, 1994), *A. terreus* (Roy and Sayre, 1984).

Among the different antagonists used as seed treatment *T. harzianum* caused 100% seed germination followed by *P. flourescens* (95.55%) and *B. subtilis* (93.33%) (Table 3). While in the field experiment, seed treatment with carbendazim (0.2%) provided significantly higher seed germination (95.55%) compared to control, followed by *T. harzianum* (88.88%) and *P. flourescens* (88.66%) without any significant difference between the later two antagonists, explaining the importance of any good seed treatment (Table 4). These observations were in the same line with those recorded for seed germination. Earlier *T. harzianum* has also been found to provide a better initial plant stand in case of soybean (Dutta and Das, 1999). Similar observation of increase seed germination of onion has been reported earlier (Rajendra and Ranganathan, 1996). Thus seed treatments with biological agent helped to prevent gaps in plant stand as compared to control and may have effected a reduction in stress caused by the soil borne diseases.

All the antagonists showed as a better protection by causing significant reduction in incidence of *Sclerotinia* rot (Table 4). Seed treatment with *T. harzianum* was the best among all the days of observation. Success of *Trichoderma* in cheking *Sclerotinia* rot as seed treatment in chickpea (Sharma *et al.*, 1999) has been reported earlier. Disease suppresion by seed treatment with *P. flourescens* (Rosales and Mew, 1982) and *B. subtilis* (Das, *et al.*, 1999) have been reported earlier. They also reported that lower disease incidence was due to ability of the bacteria to colonize roots as well as migration and establishment on plant surface as they grew. Similar mechanism might be involved in the enhancement of antagonistic activity in the present study. Seed treatment with carbendazim caused significant reduction on disease incidence at all the days of observation.

Seed treatment with *T. harzianum* provided the highest yield (69.51 q ha^{-1}) followed by *B. subtilis* and *P. fluorescens* (Table 4). Lowest yield (41.00 q ha^{-1}) was recorded in control plot where *S. sclerotiorum* was applied alone. Increased shoot length with higher yield observed in this study might be associated with reduction in *Sclerotinia* rot incidence. Moreover, earlier report also shown increased germination with higher yield when *T. harzianum* (Chet, 1987; Dutta, and Das, 1999), *B. subtilis* (Das, *et, al.*, 1999) was used as seed treatment for disease management. Thus the study demonstrated the potential of the natural disease control tools for managing *Sclerotinia* rot of french bean.

ACKNOWLEDGEMENT

The authors are grateful to the Director of Research (Agri) and Heads, Department of Plant Pathology, AAU, Jorhat, Assam and Life Sciences, Dibrugarh University, Dibrugarh, Assam and Directorate of Research (Agri), AAU, Jorhat for providing the required facilities.

REFERENCES

Chet, I. 1987. *Trichoderma* application, mode of action and potential as a biological agent of soil borne plant pathogenic fungi. In: *Innovative Approaches to Plant*

Table 1. Efficacy of antagonists on mycelial growth of *Sclerotinia sclerotiorum*.

Treatment	Mycelial Growth (mm)*	(%) reduction in mycelial growth
Fungal Antagonists		
T. harzianum	22.75	59.08
T. viride	27.64	50.29
T. koningii	25.25	5459
A. terreus	30.75	44.69
G. virens	26.75	51.89
Bacterial antagonists		
B. subtillis	20.65	62.86
P. flourescens	19.50	64.93
Control	55.60	0.00
CD (P<0.05)	3.88	-

* After 5 days of inoculation, Data are mean of five replication

Table2. *In vitro* screening of microorganisms for antagonistic activity towards *Sclerotinia sclerotiorum*

Microorganism/ Antagonist	Antagonism in dual culture	Mode of parasitism (Visual) on pathogen hyphae
Fungal Antagonists		
T. harzianum	+	Initially coiling, formation of loops among pathogen hyphae, later on shrinkage of cytoplasm causing lysis of pathogen hyphae.
T. viride	++	Sporulation in and around pathogen hyphae, coiling, lysis.
T. koningii	++	Coiling with thick rope like appearance, lysis
A. terreus	++	Adhering of spores around hyphae, disintegration of protoplasm
G. virens	++	Distribution of spore around hyphae and disintegration of protoplasm, air bubling within the pathogen hyphae
Bacterial antagonists		
B. subtillis	++	Lysis near the cotact zone, disintegration and Breaking of cytoplsmic continuty inside the mycelium
P. flourescens	++	

Table 3. Effect of biological seed treatment on seed germination *in vitro*

Treatment	Seed germination (%)	% Increase of seed germination
T. harzianum	100.00 (90.00)	18.43
B.subtilis	93.33(13.50)	10.53
P.fluorescens	95.55(77.82)	13.16
Carbendazim (0.2% w/w)	85.55(67.66)	1.31
Control	84.44(66.76)	-
CD (P<0.05)	4.21	

*Mean of 5 replications 5 days after sowing treatment at 25 ±1°CFigure in the parentheses are arc sine transformed value.

Table 4. Effect of biological and chemical seed treatment on disease incidence and yield (q / ha) of french bean

Treatment	Disease Incidence*						Yield *	
	30 DAG	% Reduction over control	60 DAG	% Reduction over control	90 DAG	% Reduction over control	q ha $^{-1}$	% Increase over control
Tricho derma harzianum	0.45 (3.85)	97.14 (80.26)	3.50 (10.78)	95.07 (77.17)	8.69 (17.14)	90.46 (72.01)	69.50	69.51
Bacillus subtilis	1.11 (6.05)	92.93 (74.58)	14.81 (22.63)	79.14 (62.82)	33.00 (35.06)	63.78 (52.99)	58.00	41.46
Pseudo monas flourer scens	0.00 (4.05)	90.07 (71.63)	21.90 (27.30)	69.15 (56.26)	40.78 (39.69)	55.24 (48.01)	54.50	32.93
Carben dazim (0.2%)	0.00 (4.05)	100.00 (90.00)	2.50 (9.09)	88.03 (65.76)	7.27 (24.56)	81.04 (64.19)	55.500	34.15
Control	15.71 (23.55)	-	70.99 (57.41)	-	91.10 (72.64)	-	41.00	-
CD (P<0.05)	1.07		4.44		7.61		3.21	-

*Data are mean of four replications, Figure in the parentheses are arc sine transformed value, DAG: days after germination.

Disease Control). Chet, I (Ed). Wiley Interscience Publication, New York, pp 137-160

Chet, T., Harman, G. E. and Baker, R. 1981. *Trichoderma harzianum* its hyphal interaction with *Rhizoctonia solani* and *Pythium sp. Microbial Ecology*, **7**; 29-38.

Das, B. C., Dutta, P. and Begum K. H. 2001. *In vitro* efficacy of some indegenious biocontrol agents against *Sclerotinia sclerotiuorum* (Lib) de Bary causing collar rot of vegetables crop. *National Seminar on Water and land Management Including Cad for Socio Economic Upliftment of NE Region* NERIWALM.22-23rd Nov.2001, at Gwuahati.p136-138.

Das, B. C., Devi, G., and Dutta, P. 1999. *Bacillus subtilis*: A bioagent for management of sheath blight of rice. *Indian Journal of Plant. Pathology*, **17** (1&2): 75-77.

Dutta, P. and Das, B. C. 1999. Effect of seed pelleting and soil application of *Trichoderma harzianum* in the management of stem rot of soybean. *Journal of Mycology and Plant pathology*, **29** (3): 317-322.

Dutta, P. and Das, B.C. 2002. Management of collar rot of tomato by *Trichoderma* spp. and chemicals. *Indian Phytopathology*, **55** (2): 235-237

Kolte, S. J. 1985. *Disease of Annual Edible oilseeds.* Vol. II. *Rape seed- Mustard and Sesame Disease* CRC Press Inc. Boca Raton, Florida 135 pp.

Loeffler, W., Tshan, J. S. M., Vani Honakam, N., Keagler, M., Knopp, E., Hsid, T. F. and Wu, T. G. 1986. Antifungal effects of bacilysin and fengymycin from *Bacillus subtilis* 29-3, a comparision with activities of other *Bacillus* antibiotics. *Journal of Phytopathology* **115**: 201-203.

Mukhopadhyay, A. N. 1994. Biocontrol of soil borne fungal plant pathogens current status and future prospects and potential limitations *Indian Phytopathology*, **47**: 119-126.

O, Sullivan, D. J. and O, Gara, F. 1992. Traits of fluorescent *Pseudomonas* spp. involved in supression of plant root pathogens. *Microbiological Review*, **56:** 662-676.

Pramer, D and Schmidt, E.L. 1956. Experimental soil microbiology. Butler Publ. Co. Minnepolis, p. 107.

Rajendra, K and Ranganathan, K. 1996. Biological control of onion basal rot (*Fusarium oxysporum* fsp. Cepae) by combined application of fungal and bacterial antagonists. *Journal of Biological Control*, 97-102.

Rosales, A. M and Mew, T. W. 1982. Antagonistic effect of soiul microorganism on rice sheath blight. *International. Rice Research Newsletter*, **7**: 12-13.

Roy, A. K. and Sayre, R. M. 1984. Electron microscopical studies of *Trichoderma harzianum* and *Trichoderma viride* on *Rhizoctonia solani* f. sp. Sasakii *Indian. Phytopathology*, **37**:710-712.

Sharma, B. K., Singh, B. M. and Sugha, S. K. 1992. Fungal antagonists of *Sclerotinia sclerotiorum* (Lib) de Bary *Mysore Journal of Agricultural Science*, **26:** 271-275.

Sharma, S. K., Verma, B. R. and Sharma, B. K. 1999. Biocontrol of *Sclerotinia sclerotiorum* causing stem rot of chickpea. *Indian Phyotpathology*, **52**: 44-46.

Turner, J. T. and Backman, R. A. 1991. Factors relating to peanut yield increase after seed treatment with *Bacillus subtilius*. *Plant Disease*, **75**: 347-353.

(Received: 151.04.2008; Revised: 29.05.2008; Accepted: 05.08.2008)