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RESEARCH ARTICLE

In vitro evaluation of the different bioagents against *Rhizoctonia solani* in soybean

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ABSTRACT

Samples were collected and isolation was made from the root rot infected soybean plant which yielded a fungal species. That was identified as *Rhizoctonia solani* Kuhn. The infected plants showed browning and rotting of collar region and brown discoloration of infected tissues with easy uprooting and root decay. In the present investigation, it was observed that bio agent, *Trichoderma harzianum*, *Trichoderma viride, Trichoderma hamatum* and *Bacillus substalis* were effective in controlling the growth of the pathogen up to 82.15, 70.22, 67.97 and 55.98 per cent, respectively *in vitro*.

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INTRODUCTION

Soybean [*Glycine max* (L.) Merill] belongs to family fabaceae. It is originated from provinces of China and Manchuria and further spread to Korea, Taiwan, Japan, Australia and South Africa. It has become miracle crop of the 21st century due to its multifaced advantages. On the global scale soybean has come to the top of the list of oilseed crops and contributes over one third of the total supply of the vegetable oil over pool. The crop is suffered by many fungal diseases of which root rot caused by Rhizoctonia solani is the most destructive disease which occurs at pre-emergence or post-emergence stage of seedlings and causes significant losses in yield. It is soil borne disease and creates great problem in its management. Apart from soybean, *R. solani* is reported to cause sheath blight of rice, collar rot of passion fruit, banded sclerotial disease of maize, rhizoctonia leaf blight of sunflower and rhizoctonia rot of carrot, etc.

Now-a-days use of fungicides in plant protection is widely used because fungicides help to reduce disease incidence and thus, boost up the crop yield that meets the hunger of exploded population. However, fungicides are not a long term solution to maintain crop health. Applications of the synthetic chemicals have many ill effects on eco-system. Besides, their non-targeted effects and hazardous nature, petroleum based fungicides are more expensive and some loose their effectiveness because of development of resistant strains of pathogens. In this context, use of bio-agents and breeding for resistance, to control plant diseases is fully justified. During past several years, some noticeable success of disease control was achieved by using bio-agents, as they are economical and eco-friendly. These are the distinct possibilities for future and can be successfully exploited in the modern agriculture, especially within the framework of Integrated Disease Management System (IDMS).

MATERIALS AND METHODS

Soybean root rot samples were collected from experimental fields of College of Agriculture, Pune during *Kharif* season, 2009.

Bio-agents used for experiment :

- Trichoderma viride
- Trichoderma harzianum
- Trichoderma hamatum

– Bradyrhizobium japonicum
– Chaetomium globosum
– Pseudomonas fluorescense
– Bacillus subtilis

Isolation :

The affected portion of the stem and root of the samples were cut into small pieces, washed thoroughly in tap water to remove dirt. The pieces were then disinfected by 1:1000 mercuric chloride solutions for two minutes followed by serial washing in three changes of sterilized water to remove the traces of mercuric chloride. Three to four such pieces were then plated aseptically on sterilized Potato dextrose agar medium in each Petriplate. The Petri plates were then incubated at room temperature of 280 + 2^{0}C. Well isolated pure fungal colony, free from any contamination, was then transferred to agar slant by hyphal tip method.

By frequent sub-culturings, Rhizoctonia sp. was purified and agar slants showing pure fungal growth were maintained for further studies.

Inoculation (pathogenicity test) :

The pathogenecity test of the isolated pure organism obtained from damaged seedlings of soybean was conducted by soil inoculation method.

Pure culture of Rhizoctonia solani was multiplied on sterilized Corn meal sand medium. Corn meal sand medium was prepared by filling of Erlenmeyer flasks with 97 g of filter sand, 3 g of plain yellow corn meal and 30 ml of de-ionized water and was autoclaved at 1.05 kg/cm2 pressure at 121.50C temperature for 1 hour for three successive days. Mycelium suspension from one plate was transferred to one 250 ml flask. Inoculated flasks were placed in an incubator at 26^{0}C and shaken daily for 5 minutes. It was multiplied for 10 days and then uniformly mixed in the sterilized mixture of soil and compost (2:1 by volume) at the rate of 0.5 per cent v/v (Keinath, 1995).

Ten pots were sterilized with 5 per cent copper sulphate solution, out of that a set of five pots was filled with Rhizoctonia solani inoculated soil and remaining five pots were filled with sterilized soil served as control. The pots were then incubated for 15 days at room temperature, frequently stirred and watered, so that fungus could colonize in the soil. Twelve seeds of soybean (JS-335) were surface disinfected by dipping in 0.1 per cent mercuric chloride and were sown in each pot. Observations on germination and seedling mortality were recorded.

Re-isolation :

The pathogen were reisolated from the roots and stems of artificially inoculated plants showing typical symptoms. Cultures obtained were transferred on Potato dextrose agar slants for comparing with original cultures.

Morphological studies and identification :

The fungus isolated from infected seedlings of soybean was critically examined under the compound microscope. The fungus was studied for its morphological characters by referring standard books in Mycology and also from the literature. The fungus was identified up to species levels on the basis of its morphological characters and with the help of "Fungus identification services" at 'Agharkar Research Institute' (Maharashtra Association for the Cultivation of Sciences), Pune (M.S.).

Symptomatology :

Diseased seedlings of soybean were carefully uprooted and symptoms developed on the seedlings, stem and underground parts were studied.

Effect of bio-agents against *Rhizoctonia solani in vitro* :

The antagonistic effects of different bio-agents *viz., Trichoderma viride, T. harzianum, T. hamatum, Chaetomium globosum*, *Bacillus subtilis* and *Pseudomonas fluorescence* against *Rhizoctonia solani* were studied by adopting dual culture technique (Thibhuvanamala *et al.,* 1999). Bio-agents were obtained from Biological Nitrogen Fixing Scheme, College of Agriculture, Pune and Agharkar Research Institute, Pune.

The antagonistic effects were carried out in Petri plates poured with sterilized PDA. The fungal cultures *viz., Trichoderma viride*, *T. harzianum, T. hamatum* and *Chaetomium globosum* were grown separately on PDA plates for 7 days. A 5 mm disc of the antagonistic fungus was cut from the 7 days old culture with the help of sterilized cork borer and placed at one end of the Petri plates aseptically, which were already poured with PDA. In the opposite end, a similar disc of the test fungus was placed. The discs were placed in such a manner that both the test fungus and bio-agent would get an equal opportunity for their growth.

In case of bacterial antagonist, it was streaked for 1cm length instead of placing a culture disc. The petri plates were incubated at room temperature (28 + 10^{0}C). The experiment was conducted with three replications. The first control plate contained only the test fungus and without bioagent and second control plate contained Thiram 0.25%. The observations were recorded when the mycelial growth in the first control plates reached the edges of petri plates. Per cent inhibition of growth was calculated by the following formula (Arora and Upadhyay, 1978) :

$$\text{Per cent growth inhibition} = \frac{\text{Colony diameter in Control plate} - \text{Colony diameter in Interaction plate}}{\text{Colony diameter in control plate}} \times 100$$

Treatment details :

Effect of bio-agents against *Rhizoctonia solani in vitro*	
Sr. No.	Treatments
T_1	*Trichoderma viride*
T_2	*Trichoderma harzianum*
T_3	*Trichoderma hamatum*
T_4	*Bradyrhizobium japonicum*
T_5	*Chaetomium globosum*
T_6	*Pseudomonas fluorescence*
T_7	*Bacillus subtilis*
T_8	Control (No treatment)
T_9	Control (with Thiram @ 0.25%)

Before analyzing the data, percentages were converted into arcsin values. The data were subjected to statistical analysis by following the standard method for analysis of variance. The standard errors for treatment mean and critical difference at 5 per cent level of significance were worked out as per Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions habe been presented under following sub heads:

Symptomatology :

Browning and rotting of tissue at collar region of young seedling were noticed in infected plants while at latter stage plant showed brown discolouration at ground level. Affected plant gradually turned yellow with water-soaked lesions at basal stem. The affected plants were easily pulled out. The white mycelial growths of *R. Solani* with black mustard seed like sclerotial bodies were observed on severely affected roots. The secondary roots were found decayed while tap roots remained unaffected. The infected seedlings showed higher levels of pre and post-emergence root rot symptoms and seed rot with greatly reduced plant stand. These results are in conformity with the earlier description of many research workers. Similar results were obtained by Celetti *et al.* (1990) who described *Rhizoctonia solani* infecting the collar region of soybean resulting into browning and rotting of tissues. The *Rhizoctonia solani* infection to tap roots was noticed by Nelson *et al.* (1996). In present study, brown discoloration at ground level and death of rotten plants was found similar to the observations recorded by Singh *et al.* (1973). The symptoms observed in the present investigations are similar to those described by Dorrance *et al.* (2003) who observed seed and root rot with greatly reduced plant stand.

Isolation, pathogenecity and re-isolation :

Isolation :

Isolations made from the infected roots showing typical symptoms of rotting yielded the fungal culture of *Rhizoctonia solani*. The growth of fungus from the infected tissue was distinctly visible after four days in Petriplate containing Potato dextrose agar medium. The pure culture of fungus was obtained by hyphal tip method on Potato dextrose agar medium, later on it was transferred and maintained on Potato dextrose agar slants for further studies.

Pathogenecity :

The pathogenecity test of *Rhizoctonia solani* was conducted by soil inoculation method. Observations regarding seed germination, mortality percentage, numbers of days required for rotting were recorded and results are presented in Table 1. The symptoms expressed were found to be similar to that of naturally infected plants. In soil inoculation method, the typical rotting symptoms were drying of plants after 20 days and it resulted into cent per cent mortality. Pre-emergence and post-emergence mortality observed in soil inoculation was 33.33 per cent and 66.66, respectively.

Table 1: Pathogenecity test for *Rhizoctonia solani* on soybean in glasshouse

Sr. No.	Particulars	Sick soil	Control
1.	No. of seeds sown	12	12
2.	No. of seeds germinated	8	12
3.	No. of non- germinated seeds	4	0
4.	No. of plants root rotted	8	0
5.	No. of days required for root rotting after inoculation	20	-
6.	Per cent mortality		
a	Pre-emergence per cent	33.33	0
b	Post-emergence per cent	66.66	0
c	Total mortality per cent	100.00	0

Re-isolation :

The fungus was re-isolated from roots of artificially infected plants. The reisolated fungal culture was compared with respective original culture and found identical to the original culture in all respects which was used for further studies. For the confirmation of the Koch's postulates of the isolated organism, *Rhizoctonia solani* Kuhn. was found pathogenic to soybean and it was in the conformity with the work done by Naik and Ui (1981) and Anderson *et al.* (1988). *Rhizoctonia solani* isolated from roots and stems of soybean, proved pathogenic to the soybean crop as reported by Nelson *et al.* (1996). The diseased samples with typical symptoms were collected during survey from commonly grown varieties of soybean. The diseased samples invariably yielded species

Table 2 : Effect of bio-agents against *Rhizoctonia solani in vitro*

Treatment No.	Bio agent used	Mean colony diameter (mm)	*Per cent inhibition of mycelium
T_1	*Trichoderma viride*	25.32	70.22 (56.93)
T_2	*Trichoderma harzianum*	15.18	82.15 (65.02)
T_3	*Trichoderma hamatum*	27.23	67.97 (55.54)
T_4	*Bradyrhizobium japonicum*	48.17	43.33 41.16)
T_5	*Chaetomium globosum*	49.47	41.80 (40.28)
T_6	*Pseudomonas fluorescence*	48.38	43.09 (41.02)
T_7	*Bacillus subtilis*	37.42	55.98 (48.44)
T_8	Control (No treatment)	85.00	0 (0.00)
T_9	Control with *thiram*	0.00	100 (90.00)
	S.E.±		0.64
	C.D. at 5%		1.92

symptoms to that of original culture used for inoculation.

Morphological studies :

Morphological characters of the test fungus were recorded from seven days old culture grown on Potato dextrose agar medium. Mean length, breath, septation of mycelial observations were measured with the help of ocular micrometer. The mycelium of *Rhizoctonia solani* Kuhn. when observed under microscope gave septae, hyaline and branched appearance. Hyphal branching was at the right angle to parent hypha. The mycelial strand was found varied in length while width measured about 5 µm and length was 45 to 120 µm.

Seven days old fungus culture of pathogen grown on Potato dextrose agar was found feathery white in colour in the beginning and turned brown to dark blackish with advanced age of culture. Surface of colonies were light brown and dense. Mycelium was branched, septate and hyaline. Hyphal branching was right angle to parent hypae. Mycelium was variable in length and width. Dark brown sclerotia were 162 µm in diameter. These results are in agreement with Singh *et al.* (1973) who reported that width of hyphae from 5 to 8 µm. Prasad *et al.* (1987) also reported dark brown sclerotia of 0.32-2.32 mm in diameter. On the basis of the morphological characters, culture was identified as *Rhizoctonia solani* Kuhn. The identification was confirmed by referring to the Mycologist, Agharkar Research Institute, Pune.

Sclerotial formation :

The sclerotia of *Rhizoctonia solani* Kuhn. were brownish to dark brown in colour with round to regular shape. The sclerotia formation started after 6 days of inoculation. The variation in size and shape of sclerotia was also observed. The sclerotia measured about 90 to 210 µm in diameter with average 162 µm.

Identification of the fungus :

Identification of fungus was done after a critical study. Based on the morphological characters, the fungus was tentatively identified as *Rhizoctonia* sp. as per the standard description and figures given by Barnett and Hunter (1972). Further, the species identification was also confirmed by referring the culture to the Mycologist, Agharkar Research Institute, Pune as *Rhizoctonia solani* Khun.

Effect of bio-agents against *Rhizoctonia solani in vitro* :

The results of the present investigation are recorded in Table 2. Treatment differences were statistically significant. Antagonists *Trichoderma harzianum*, *Trichoderma viride, Trichoderma hamatum* and *bacillus substalis* were effective in controlling the growth of the pathogen by 82.15, 70.22, 67.97 and 55.98 per cent, respectively. On the other hand, *Bradyrhizobium japonicum, Pseudomonas fluorescence* and *Chaetomium globosum* gave minimum per cent inhibition of the pathogen *i.e.* 43.33, 43.09 and 41.80, respectively and *Bradyrhizobium japonicum*, *Pseudomonas fluorescence* were found at par with each other. The observations are in agreement with the reports of Elad *et al.* (1980), Harman *et al.* (1980), Shah-da Liu and Baker (1980), Venkatasubhaiah *et al.* (1989) and Deshmukh (1990).

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