Research Paper :

In vitro evaluation of the different botanical extracts against *Rhizoctonia solani* infesting soybean

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SUMMARY

Samples were collected and isolation was made from the root rot infected soybean plant which yielded the pathogen, which 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. It was observed that the botanical extracts of *Allium sativum*, *Zingiber officinale* and *Eucalyptus* sp. inhibited the mycelium of the test fungus to the extent of 100 per cent followed by *Azadirachta indica*, *Ocimum sanctum*, *Hibiscus rosa-sinesis* and *Pongamia pinnata.*

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Soybean [*Glycine max* (L.) Merill.] belongs to family Leguminaceae. 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 most destructive and occurs at pre-emergence or post-emergence stage of seedlings and causes significant losses in yield. It is soil borne disease and creates great problems 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.

During the ancient time noticeable successes in plant disease management were achieved. Plant originated medicines for tree dressings, fumigation and seed treatment against different diseases were suggested in the past (Nene and Thaplyal, 1993). Later with the advent of chemistry, many chemicals were introduced in plant disease control. As they showed quicker results, people started using them on large scale. Therefore, the use of 'Natural plant products' remained neglected.

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 plant extracts, to control plant diseases is fully justified. During past several years, some noticeable success of disease control was achieved by using plant products, as they are economical and eco-friendly. There are the distinct possibilities for future and can be successfully exploited in the modern agriculture, especially within the framework of Integrated Disease Management System.

MATERIALS AND METHODS

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

Plants and their parts used for extraction

Botanical name	Common name	Plant part used
Curcuma longa	Turmeric	Rhizomes
Zingiber officinale	Ginger	Rhizome
Allium sativum	Garlic	Cloves
Azadirachta indica	Neem	Leaves
Eucalyptus sp.	Nilgiri	Leaves
Hibiscus rosa-sinesis	China rose	Leaves
Lantena camera	Ghaneri (wild)	Leaves
Ocimum sanctum	Tulsi	Leaves
Vinca rosea	Sadaphuli	Leaves
Pongamia pinnata	Karanj	Seed

Isolation:

The affected portion of the stem and root of the samples were cut in to 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 Petriplates were then incubated at room temperature of $28^0 \pm 2^0$C. Well isolated pure fungal colony, free from any contamination, was then transferred to agar slant by hyphal tip method.

By frequent sub-culturing of pathogen, *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 infected 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/ cm^2 pressure at 121.5^0C 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 26^0C and shaken daily for 5 minutes. It was multiplied for 10 days (Rollins *et al.,* 1999) and then uniformly mixed in the sterilized mixture of soil and compost (2:1 by volume) at the rate of 0.5 % 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 fungus was 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 of Mycology and also from the literature. The fungus was identified up to species level 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.

Extraction of anti-fungal plant extracts:

The crude extracts were obtained as per the method described by Bambode and Shukla (1973). In this method, 10 g of the plant part were weighed and thoroughly washed. The plant material was then crushed in the mortar with the help of pestle by adding 10 ml sterilized distilled water. After that, the crushed material was strained through double layered muslin cloth and filter paper (Whatman No.1) and the filtrate obtained was used in the experiment for further studies.

Effect of selected botanical extracts on mycelia inhibition of *Rhizoctonia solani* in *vitro*:

The effect of different plant extracts on mycelial

growth by poisoned food test was carried out (Nene and Thapliyal, 1993). In this test, all the plant extracts obtained were tried against the fungus. The plant extract (whole) was supplemented with sterilized PDA in 1:2 proportions and the 'poisoned' medium was poured in Petridishes. A mycelial disc of the test fungus, 0.5 cm in diameter, was cut from the periphery of a 7 days old culture of fungus and aseptically inoculated into the medium. Each set was replicated thrice. The first control was run side by side using only distilled sterilized water and the second one with using thiram 0.25%. The Petriplates were incubated at room temperature (28 ± 1^0C). Observations on mycelial inhibition were recorded when the mycelial growth in first control set touched the edges of the Petriplate. Before analyzing the data, percentages were converted into arc sin 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% level of significance were worked out as per Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Symptomatology:

Browning and rotting of tissue at collar region of young seedling were noticed in infected plants while at later 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 in to browning and rotting of tissues. *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 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 reisolation:

Isolation:

Isolations made from the infected roots showing typical symptoms of rotting which 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 the 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 in to cent per cent mortality. Pre-emergence and post-emergence mortality observed in soil inoculation were 33.33 per cent and 66.66 per cent, respectively.

Re-isolation:

The fungus was reisolated from roots of artificially infected plants. The re-isolated 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

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**		
	Pre-emergence per cent	33.33	0
	Post-emergence per cent	66.66	0
	Total mortality per cent	100.00	0

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 of *Rhizoctonia solani*. The culture reproduced similar 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 and other 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. The result in respect of morphology and sporulation of the fungal pathogen, *Rhizoctonia solani* Kuhn. produced feathery white colored mycelium which turned brown to dark brown with advance in age. Mycelium was branched, septate and hyaline. Hyphal branching was at 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. The sclerotia in present study were dark brown coloured and 0.5 to 1.5 µm in diameter whereas, Lakshamanan and Nair (1985) reported sclerotia 1.3 - 1.8 mm x 1.14-1.5 mm and width of hyphae 6.25-8.93 µm. Prasad *et al*. (1987) also reported dark brown sclerotia of 0.32-2.32 mm in diameter. On the basis of morphological characters of the respective fungus, 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 selected botanical extracts on mycelial inhibition of *Rhizoctonia solani in vitro:*

Effect of crude extracts of the entire test plants on mycelia inhibition of the test fungus was studied separately and data were analyzed statistically (Table 2). The cent

Table 2 : Effect of selected botanical extracts on mycelial inhibition of *Rhizoctonia solani in vitro*

Treatment No.	Plant extracts used	Mean colony diameter (mm)	*Per cent inhibition of mycelium (Average of three replication)
T_1	*Curcuma longa* L.	28.28	65.52 (54.06)
T_2	*Zingiber officinale* L.	0.00	100 (90.00)
T_3	*Allium sativum* L.	0.00	100 (90.00)
T_4	*Azardirachta indica*	10.72	86.93 (68.83)
T_5	*Eucalyptus* sp. Labill.	0.00	100 (90.00)
T_6	*Hibiscus rosa-sinesis*	14.59	82.21 (65.07)
T_7	*Lantena camera* L.	38.63	52.90 (46.66)
T_8	*Ocimum sanctum* L.	11.98	85.40 (67.55)
T_9	*Vinca rosea* L.	81.68	0.40 (3.65)
T_{10}	*Pongamia pinnata (L.)* Pierre	19.52	76.20 (60.81)
T_{11}	Control (No treatment)	82	0 (0.00)
T_{12}	Control (With *thiram* @ 0.25%)	0.00	100 (90.00)
	S.E.±		0.60
	C.D. (P=0.05)		1.75

per cent mycelial growth of the fungus was inhibited by the extracts of *Allium sativum*, *Zingiber officinale* and *Eucalyptus* sp. *in vitro*. These treatments were statistically superior to all other treatments, and they were at par among themselves. The next treatments in the order of per cent mycelial growth inhibition were observed in *Azardirachta indica* (86.93), *Ocimum sanctum* (85.40), *Hibiscus rosa-sinesis* (82.21) and *Pongamia pinnata* (76.20) followed by *Curcuma longa* (65.52). The results of the remaining treatments were not found promising in inhibition of mycelium of the test fungus. The crude extract of *Allium sativum*, *Eucalyptus* sp. and *Zingiber officinale* inhibited cent per cent mycelial growth of the *Rhizoctonia solani*. The results are in agreement with those obtained by Bang (1995), Sunderraj *et al*. (1996), Ezhilan *et al*. (1994) and Kurucheve *et al*. (1997).

Extracts of *Azardirachta indica* and *Ocimum sanctum* inhibited the mycelia growth of *R. solani* to the extent of 86.93 and 85.40 per cent, respectively. Similar results were obtained by Tewari *et al*. (1991), Narain and Satapatty (1977) and Shivpuri *et al*. (1997). From the above results, it was found that the crude extract of *Allium sativum*, *Eucalyptus* sp. and *Zingiber officinale* proved to be the most effective followed by those of *Azardirachta indica* and *Ocimum sanctum* against *R. solani*. The performance of the remaining plant extracts was poor. The significant treatment differences indicated that the inhibitory activity of the active principle in the different plant extracts differed from plant to plant.

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