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IN-VITRO EVALUATION OF FUNGAL ANTAGONISTS AND PLANT EXTRACTS AGAINST FRENCH BEAN ROOT ROT CAUSED BY *RHIZOCTONIA SOLANI* KHUN.

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Abstract

Study were conducted by using fungal bio-agents and plant extracts against French bean root rot caused by *Rhizoctonia solani* Khun under *in-vitro* condition. In this experiment, the antagonistic activity of six fungal bio-agents were used to know the effect of fungal antagonists on the production of sclerotia of *Rhizoctonia solani* were tested. The sclerotial production of *Rhizoctonia* was drastically reduced in the presence of *T. viride* – 52 (IIHR), *T. viride* - 56 (IIHR) and *T. viride* - 27 (IIHR) recording 2.97, 2.37 and 2.08 sclerotia per microscopic field. Whereas, least number of sclerotia 0.48 produced in *T. harzianum* – 55 (NBAII). Significant reduction of sclerotial growth of *R. solani* by *T. viride, T. harizianum* was examined under *in vitro* trails. Interaction of the pathogen and antagonist on PDA medium were observed and also recorded mycelia growth on PDA medium visible contact of the both *R. solani* and fungal antagonists were observed after two days of inoculation. In *T.viride*-56 occupy the full growth (90mm) on the fungus after ten days of inoculation. The efficacy of four plant extracts *viz.,* Neem, pongamia, subabul gliricidia were evaluated and observed the radial growth of the mycelium. Neem leaf extract found maximum inhibition (90mm) of the fungus at 5, 10 and 15 per cent concentration followed by pongamia leaf extract seven days after inoculation. Among the leaf extract, neem and pongamia leaf extracts were found to be effective against root rot pathogen, *R. solani*.

Key words : Bio-agents, *Trichoderma viride*, *Rhizoctonia solani* Neem Ponamia and *in-vitro*.

Introduction

French bean (*Phaseolus vulgaris* L.), belongs to the family Fabaceae which is also known as snap bean, kidney bean and garden bean. It is an important protein source for many developing countries Markhart (1985). It is consumed as green pod vegetable and as well as dry seeds. French bean is domesticated in Mexico, Peru and Colombia about 8000 years ago Schoonhoven and Voysest (1991). It is widely cultivated in tropics, sub tropics and temperate regions. In India and most of the tropical Asia, it is a major vegetable crop, where indigenous pulses are also preferred (Duke, 1981). French bean is consumed as immature tender fruits, green grains as vegetables and dry grain (Rajamah). The nutritive value of 100g of green pod contains 1.7g protein, 0.1g fat, 4.5g carbohydrate, 1.8g fibre and is also rich in minerals and vitamins. It has some medicinal properties in control of diabetes, cardiac problems and natural cure for bladder burn. It has both carminative and reparative properties against constipation and diarrhoea respectively (Duke, 1981).

The statistics with respect to this crop is very deficient owing to the small area of production and short duration (Anonymous, 2002). However, as per the FAO estimates, the French bean is grown in the world in an area of 0.83 m ha with annual production 5.64 mt with productivity of 6.76t/ha. India, annually, French bean is grown in an area of 0.15 m ha with annual production of 0.42mt and productivity of 2.8 t/ha (FAO, 2007). In Karnataka, it is grown an area of 15,699 ha with productivity of 1,67,856 tonnes (Anonymous, 2004). French bean affected by many fungal, bacterial, viral and nematode diseases. Among the fungal diseases, root rot is the one of the most destructive disease especially in high rain fall coupled with high soil moisture, relative humidity and soil

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temperature (23-25ºC) favoured for the development of disease. The disease was first reported in 1994 from Solan, Himachala Pradesh (Sachin Upmanyu *et al*., 2003). The web blight and root rot severity varies between 7.50 and 56.0 and 20.51 and 44.06 per cent in bush and pole-type French bean varieties, respectively.

Rhizoctonia solani Khun; causing root rot disease is a soil inhabitant, polyphagus and a facultative parasite. It is known to cause disease in many crops including rice, barley, urd bean soybean, potato etc. (Sachin Upmanyu *et al*., 2003). This pathogen causes a variety of symptoms like crown rot, sheath blight, web blight, root rot, etc., on different hosts. French bean is predominantly grown by poor and marginal farmers. Root rot causes 40 per cent yield loss. As the disease is minor and sporadic in nature, extensive studies have not been carried out, but it is on the increase in the recent past particularly under high soil moisture coupled with high humidity (Abram Mathew and Gupta, 1996).

Materials and Methods

The present experiment was conducted on effect of fungal antagonists and plant extracts against French bean root rot caused by *Rhizoctonia solani* at department of Plant Pathology, GKVK, Bangalore, Karnataka. The material used and methods followed in conducting the experiment were described below.

Mechanism of inhibition of the pathogen by the antagonists in dual culture

To know the antagonistic activity of six fungal bio-agents *viz.*,*Trichoderma viride*-56(IIHR), *Trichoderma viride*-56(IIHR 27), *Trichoderma viride*-52(IIHR), *Trichoderma viride*-14(NBAII), *Trichoderma viride*-16 (GKVK and *Trichoderma harzianum* (NBAII)) against *Rhizoctonia solani* were conducted under *in vitro* condition. Mechanism of inhibition was studied by dual culture method growing of test fungus and bio-agents on a solidified PDA in Petri dish. Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. Both bio-agents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus. The plates were incubated at 27±1°C and observations were made on the hyphal interactions. After both the fungi came in with each other, the contact zone was cut using a sharp blade and taken out. It was washed gently with water mounted under lacto phenol over a clean glass slide and observations were made under (10X) microscope. Viable population of Sclerotina of *Rhizoctonia solani* in the presence of bio-agents were recorded.

In-vitro evaluation of fungal antagonists against *Rhizoctonia solani*

Six isolates are tested for their antagonistic activity against *Rhizoctonia solani* by duel culture plate method on PDA medium and observation recorded the mycelia growth of fungus up to ten days. For each treatment four replications are maintained.

In vitro evaluation of plant extracts against *Rhizoctonia solani*

Four locally available plant extracts are evaluated against *Rhizoctonia solani* following the procedure given by Gerard Ezhilan *et al*. (1994) with slight modification (Table.1). Fresh plant leaves are washed with tap water and sterilized water. It was then processed with sterile distilled water at the rate of 1 ml g^{-1} of tissues (1:1 v/w) with the pestle and mortar and filtered through fine cloth. This also formed the standard plant extract solution (100%). The extract of different plant leaves are incorporated and sterilized at 1.1 kg cm-2 for 20 minutes. To study the anti-fungal activity of plant extract, the poisoned food technique was followed. Five, ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of sterilized molten potato dextrose agar medium respectively. So as to get 5, 10 and 15 per cent concentrations. The medium was thoroughly shaken for uniform mixing of the extract and then after adding the botanicals again the media was sterilized. Twenty ml of medium was poured into each of the 90 mm sterilized Petri plates. Each plate was inoculated with 5 mm mycelial discs of taken from the periphery of fungal culture and incubated at 27±1^{0}C till the growth of colony touched the periphery in the control plate. The disc was placed upside down in the centre of the Petriplate, so that the mycelium was in direct contact with the medium poisoned with the requisite plant extract at required concentration. Three replications were maintained for each treatment. Suitable control plates were maintained where in culture discs were inoculated into the center of potato dextrose agar plates without plant extracts. Mean colony diameter in each case was recorded by taking the diameter the colony in two directions. Radial growth of the fungus was measured after 3 and 7 days of inoculation.

Results and Discussion

The six fungal antagonistic microorganisms *viz.*, *Trichoderma viride*-56 (IIHR), *Trichoderma viride*-27 (IIHR), *Trichoderma viride*-52 (IIHR), *Trichoderma viride*-14(IIHR), *Trichoderma viride*-16 (GKVK) and *Trichoderma harzianum*(NBAII),were evaluated against *R. solani* by dual culture technique for their antagonistic effect under *in-vitro* conditions as explained

Table 1 : List of botanicals evaluated against *Rhizoctonia solani* using poison food technique.

S. no.	Botanical name	Common name	Family	Part used
1.	*Azadirachtaindica*	Neem	Meliaceae	Leaves
2.	*Pongamia pinnata*	Hongae tree	Leguminaceae	Leaves
3.	*Leucaenaleuco cephala*	Subabul	Fabaceae	Leaves
4.	*Gliricidiama culata*	Akven	Fabaceae	Leaves

Table 2 : Effect of fungal antagonists on the production of sclerotia of *Rhizoctonia solani.*

S. no.	Bioagent	Sclerotia per microscopic field*
1	*Trichodermaviride* - 56 (IIHR)	2.38
2	*Trichodermaviride* - 27 (IIHR)	2.08
3	*Trichodermaviride* –52 (IIHR)	2.97
4	*Trichodermaviride* – 14 (IIHR)	1.09
5	*Trichodermaviride* -16 (GKVK)	1.93
6	*Trichodermaharzianum* – 55 (NBAII)	0.48
	Mean*	**1.82**
	S. Em±	**0.096**
	CD@1%	**0.27**

**Mean of 4 observations per microscopic field.

against *Rhizoctonia solani* reveals that, sclerotial production of *Rhizoctonia* was drastically reduced in the presence of *T. viride* –52 (IIHR), *T. viride* - 56 (IIHR) and *T.viride* - 27 (IIHR) recording 2.97, 2.37 and 2.08 sclerotia per microscopic field (table 2). Whereas, least number of sclerotia 0.48 produced in *T. harzianum* – 55 (NBAII) (table 2). Das *et al.* (1996) also observed that, the sclerotial production of *Rhizoctonia* was drastically reduced in the presence of *T. viride and T. harizianum* under *in vitro* trails.

In-vitro evaluation of fungal antagonists against *Rhizoctonia solani*

Interaction of the pathogen and antagonist on PDA medium were observed up to ten days after inoculation. The observation on mycelia growth on PDA medium was recorded. Visible contact of the both *R. solani* and fungal antagonists were observed two days after inoculation. Visible inhibition of test fungus in TV56, TV16 and TH55 observed six days after inoculation. Overlapping and TV56 occupy the full growth (90mm) on the medium after ten days of inoculation (table 3). Upadhyay and Bharat Rai (1983) the coiling of hyphae was observed in *R. solani* by *T.virens*. The interaction between *T. harzianum* and *R. solani* was studied by Benhamou and Chet (1993) and reported that coiling of *T. harzianum* hyphae around *R.solani* was an early event preceding hyphal damage; the contact between the two fungi was mediated by a fine extra cellular matrix originating from cells of *R.solani*. Of the five fungal bio-control agents tested *in vitro* against *R. solani*, *T. harzianum* was the most effective in causing significant suppression (60%) of both growth and sclerotia formation and followed by *T.virens* (50%). Further, *T. harzianum* against *R. solani* was found to effective was reported by Bunker and Mathur (2001).

Table 3 : Interaction of pathogen and antagonists on PDA media.

S. no.	Treatments	Observation on mycelia growth-days in PDA									
		1	2	3	4	5	6	7	8	9	10
1	RS+TV56	+	+	+	++	++	+++	+++	+++	+++	+++
2	RS+TV27	+	+	+	+	++	++	++	++	+++	+++
3	RS+TV52	+	+	+	+	++	++	++	++	+++	+++
4	RS+TV14	+	+	+	+	+	+	+	++	++	+++
5	RS+TV16	+	+	+	++	++	+++	+++	+++	+++	+++
6	RS+ Th55	+	+	+	++	++	+++	+++	+++	+++	++++

Rs = *Rhizoctonia solani*
Tv = *Trichodermaviride*
Th = *Trichodermaharzianum*
++++ = Overlapping and T. h on *R.solani*
+ = Visible growth of both the organism
++ = Visible contact
+++ = Visible inhibition of test fungus

in 'Material and Methods'.

Mechanism of inhibition of the pathogen by the antagonists in dual culture

Impact of dual culture on population of bio agents

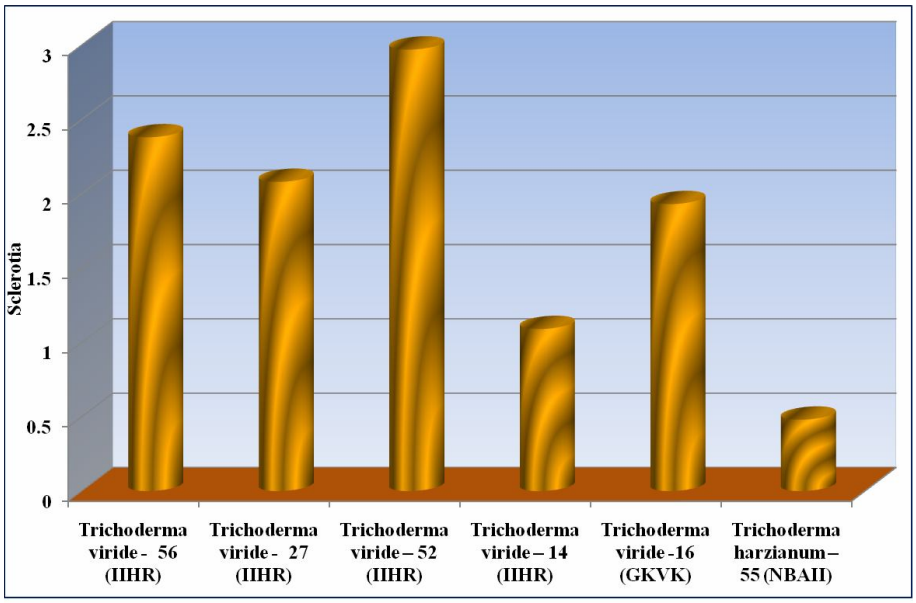

Fig. 1 : Effect of fungal antagonists on the production of sclerotia of *Rhizoctonia solani*.

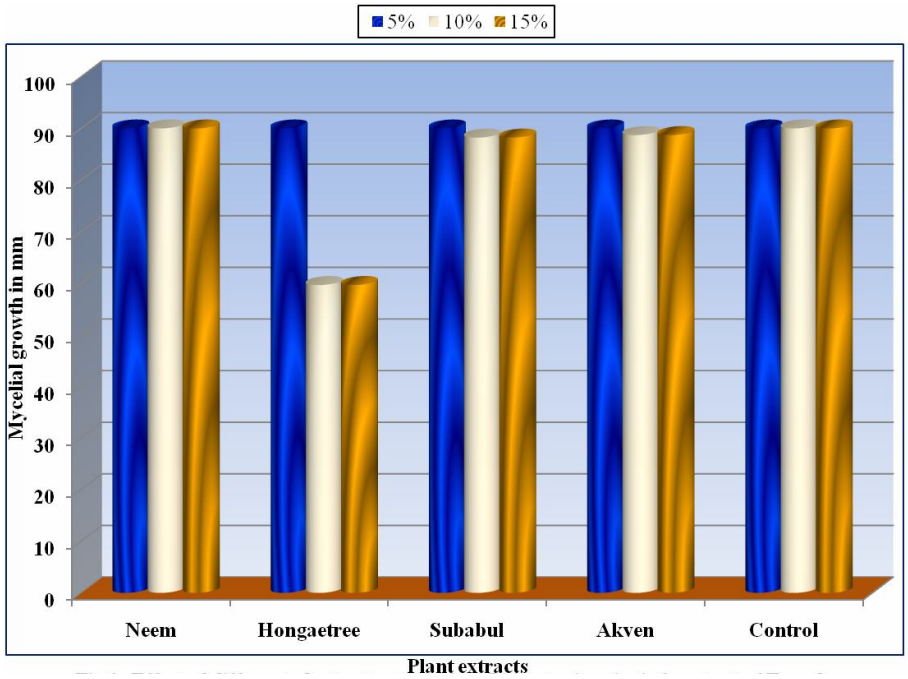

Fig. 2 : Effect of different plant extracts against *Rhizoctonia solani* of root rot of French.

In vitro studies of botanicals

The efficacy of four plant extracts was evaluated at different concentrations by 'Poisoned food technique' as described in 'Material and Methods'. The radial growth of the mycelium was recorded in 3 and 7 days after inoculation. At 3 days after inoculation, neem leaf extract recorded maximum radial growth of the fungus, the radial growth was 46.83, 44.50 and 41.23mm at 5, 10 and 15 per cent respectively followed by Pongamia leaf extract. In pongamia leaf extract, the growth was 39.36, 34.16 and 88.65mm at 5, 10 and 15 per cent, respectively. In case of Subabul showed least radial growth of the mycelium were recorded. In this treatment recorded fungal growth upto 36.20, 30.45 and 28.81mm at 5, 10 and 15 per cent respectively (table 4, fig. 2). At 7 days after inoculation,in case of neem,the maximum radial growth of the fungus were recorded upto 90.00mm at 5, 10 and 15 per cent followed by pongamia leaf extract. The radial growth in this extract was 90.00, 88.71 and 88.71mm at 5, 10 and 15 per cent concentration respectively. In case of Neem leaf extract, least radial growth was recorded. The radial growth in these extract

Table 4 : Effect of different plant extracts against *R.solani.*

S. no.	Plant extracts	Radial growth of the mycelium (mm) 3 days			Mean*	Radial growth of the mycelium (mm) 7days			Mean*
		5 per cent	10 per cent	15 per cent		5 per cent	10 per cent	15 per cent	
1	Neem	37.38	31.68	28.66	32.57	90.00	90.00	90.00	90.00
2	Pongamia	39.36	34.16	21.70	31.74	90.00	59.66	59.66	66.70
3	Subabul	36.20	30.45	28.81	31.82	90.00	88.23	88.23	87.81
4	Gliricidia	46.83	44.50	41.23	44.18	90.00	88.71	88.71	84.65
	Control	67.33	66.66	66.00	66.66	90.00	90.00	90.00	90.00
Mean		45.42	41.49	37.28		90.00	83.32	78.18	
			B	**C**	**B× C**		**B**	**C**	**B× C**
S. Em±			**0.51**	**1.47**	**1.14**		**0.39**	**1.14**	**0.88**
CD @ 1%			**0.65**	**1.90**	**3.29**		**0.51**	**0.72**	**2.56**

*Mean of three replications.

was 90.00, 59.96 and 59.96mm at 5, 10 and 15 per cent respectively. However, the pongamia leaf extract found to be effective against this pathogen (table 4, fig. 2).

The maximum inhibition of the fungus was recorded at 5, 10 and 15 per cent followed by pongamia leaf extract. Among the leaf extract, neem and pongamia leaf extracts were found to be effective against root rot pathogen, *R. solani*. The inhibition effect against *R. solani* is may be due to production of fungistatic properties (Shivapuri *et al*., 1997). Reddy *et al*. (2002) tested the efficacy of plant products on mycelia growth and sclerotial production of *R. solani*. Biswas and Roychoudhury (2003) evaluated the relative efficacy of six botanical formulations against the sheath blight disease of rice and found Neemazal and Achook performed best in reducing the disease severity of *R. solani*.

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