

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.703.287>

## Bio-control of Root Rot of Brinjal Caused by *Rhizoctonia solani* Kuhn.

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### ABSTRACT

The present study revealed the biological control of root rot in brinjal caused by *Rhizoctonia solani* Kuhn. Two *Trichoderma* spp. and two bacteria namely *Bradyrhizobium japonicum* and *Bacillus thuringiensis* were screened against *Rhizoctonia solani* Kuhn. *In vitro* studies revealed that *T. harzianum* was found superior in restricting mycelial growth of *R. solani* followed by *T. viride*. *In vivo* assay the seed treatment with conidial suspension of bio-agent particularly *T. harizanium* ( $7.8 \times 10^6$  conidia ml<sup>-1</sup>) was found to be most effective in controlling root rot incidence followed by *T. viride*. In seedling treatment with conidial suspension only *T. harizanium* was found effective in reducing root rot incidence. Unautoclaved culture filtrate of *T. harizanium* was found effective even at lowest concentration of 10 % under *In vitro* studies. Only *B. japonicum* was found effective in arresting the mycelial growth under *in vitro* studies.

#### Keywords

Bio-control, *Rhizoctonia solani*, *Trichoderma* spp., *B. japonicum*, *B. thuringiensis*, Culture filtrate, Bio-control

#### Article Info

Accepted:  
20 February 2018  
Available Online:  
10 March 2018

### Introduction

Egg - plant (*Solanum melongena* L.) an important of the popular vegetable worldwide. It is affected by several diseases, which do not let the plants to grow and yield to a best of genetic potential. Among various pathogens, fungi constitute an important group as they inflict damage to crop plant at different stages. Among the fungal diseases, the root - rot caused by *Macrophomina* remains to be a challenging task in terms of management, since it is soil -borne in nature. It is distributed

worldwide and is prevalent in arid, sub - tropical and tropical climate, especially in the areas with low rainfall and high temperature. Various disease management methods have been implemented to combat and eradicate pathogenic fungi. These include cultural, regulatory, physical, chemical and biological methods. All these methods are effective only when employed well in advance as precautionary measure. Once a disease has appeared, these methods become impractical / ineffective. In that situation, chemical control offers a good choice to grower to control the

disease. Chemical pesticides have been in use since long and they provide quick, effective and economic management of plant diseases. However, in recent past, it has been realized that use of chemical in agriculture is not as beneficial as it was visualized. Chemical pose serious health hazards to an applicator as well as to a consumer of the treated material. In addition to target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in soil and contaminate the whole environment. Increasing awareness of humankind toward the ecosystem and environment has made a marked shift from synthetic materials to bio - products. Fungi constitute a major group of bio agents against various kinds of pests. A good number of fungi such as *Trichoderma*, *Gliocladium* can suppress the parasitism of *Fusarium* sp., *Rhizoctonia* sp., *Sclerotium* sp. The present investigation is, however, design in a way to investigate comparative efficacy of some species of a common *Trichoderma* against *Rhizoctonia solani* on Eggplant.

### **Materials and Methods**

To test the efficacy of two *Trichoderma* spp. and two bacteria viz. *Bradyrhizobium japonicum*, *Bacillus thuringensis* against target pathogen the cultures were received from department of plant pathology, college of agriculture Nagpur, and used in studies.

#### ***In vitro* assay of antagonists: Fungal antagonist**

This was done by zone of inhibition technique using potato dextrose agar after Woods (1957). Inoculum disc of 4mm. diameter was taken from 7 days old culture of *R. solani* and placed at four peripheral points on PDA plates. The fungal antagonist placed at centre. The experiment was set up in five replications and incubated at 27+1°C for 5 days. The zone of inhibition was measured after 120 hours.

Inhibition was determined with following formulae.

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition.

C = Fungal growth (colony diameter) in control.

T = Fungal growth in treatment.

#### ***In vitro* assay of culture filtrates of an antagonist against *Rhizoctonia solani***

For these studies the culture of *T. harzianum* and *T. viride* were grown on PDB medium in 250ml of Erlenmeyer flasks at 27+1°C for 20 days. The culture filtrate is filtered through absorbent cotton and double layered Whatman's filter paper No. 42. The cell free culture filtrate was used in the all studies. Half the quantity from complete filtrate was taken and sterilized in an autoclave at 10 lbs for 10 minutes. The fungitoxicity of culture filtrates of an antagonist was assayed by zone of inhibition with 'Paper Disk Method' given by Thornberry (1950). The concentrations of 10, 25, 50, 75 and 100 percent were used. Disc saturated with distilled sterile water served as a control.

#### **Measurement of fungitoxicity**

##### **Zone of inhibition**

The term 'Zone of Inhibition' used in this work refers to the distance between the center of disc and periphery of zone where the antifungal action on the growth of target fungi around the disc is visible in the form of hazy, almost clear and clear areas.

### **In vitro assay of antagonist: Bacteria**

This was done by dual culture technique (Laha *et al.*, 1992) using PDA. Mycelial disc of 6mm diameter from 5 days old lawn of *Rhizoctonia solani* was placed in center of PDA plates. Simultaneously cultures of *Bradyrhizobium japonicum* and *Bacillus thuringiensis* were streaked on both the sides about 2cm away from the test pathogen. The agar plate inoculated with fungus served as control. Five replications were kept for treatment. The plates were incubated at 27+2°C and observations were recorded for 4 days. The diameter of fungal colony with and without bacterial streaking was measured for determining interaction effect.

### **In vivo assay of antagonist: Seed treatment**

To study the effect of bio-agent seed treatment on seed germination and seedling mortality, the seeds of brinjal were washed with sterile water for four to five times, dried in shade and then soaked in conidial suspension of *T. harzianum* ( $7.8 \times 10^6$  Spores  $\text{ml}^{-1}$ ) and *T. viridi* ( $3.3 \times 10^7$  spores  $\text{ml}^{-1}$ ) for an hour and these seeds were used for sowing in pot culture experiment. The experiment was arranged in five replications. For each replication 30 seeds were used (30 seeds /pot). The observations regarding number of seeds germinated and number of seedlings rotted were recorded and data is present in Table 3 and 3a.

### **Seedling treatment**

The seeds of brinjal were washed with sterile water for four to five times, dried in shade and used for sowing. After one month, seedlings were removed from pot without damaging root system and treated with conidial suspension of *T. harzianum* ( $7.8 \times 10^6$  conidia /ml) and *T. viride* ( $3.3 \times 10^7$  conidia /ml) by root dip

method and treated seedlings were planted in pot containing sick soil. Five seedlings per pot were planted and experiment was arranged in five replications. The observations recorded till 30<sup>th</sup> days after planting. The data regarding percent root rot incidence was recorded and presented in table 4 and 4a.

### **Results and Discussion**

The data collected from in vitro assay of fungal antagonist was statistically analyzed by using Completely Randomised Design (CRD) and it was found that the *T. harzianum* was significantly superior in restricting the growth of pathogen followed by *T. viride*.

Similar results were obtained by Lorito *et al.*, (1993), Dilip Monga, (1993), Benhamou and Chet (1993), Haran *et al.*, (1996), Table 1.

From the Table 2, it is revealed that only unautoclaved culture filtrate of *T. harzianum* was found to be effective and formed the zone of inhibition even at lowest concentration of 10 percent whereas zone of inhibition was not formed by culture filtrate of *T. viride* autoclaved and unautoclaved. The zone of inhibition formed in case of *T. harzianum* is directly proportional to the concentrations of culture filtrate.

As the concentration of culture filtrate increases the zone of inhibition also increases as compared to control. The zone of inhibition obtained at 10, 25, 50, 75, and 100 percent concentrations were 27.33, 33.33, 40.66, 47.83, and 57.66 mm. respectively.

Several fungi produces antagonistic substances i.e. antibiotics in their substrate and have been found effective in controlling plant diseases *in vitro*. Similar results were obtained by Agrawal *et al.*, (1975) and Pandey (1985).

**Table.1** *In vitro* assay of antagonists (*Trichoderma harzianum* and *Trichoderma viride* against *Rhizoctonia solani*)

Sr. No.	Treatments	Growth of <i>Rhizoctonia solani</i> colony diameter (mm)					Mean	% Inhibition
		R-I	R-II	R-III	R-IV	R-V		
1	<i>T. harzianum</i>	14.5	12.75	11.35	13.25	13.20	13.01	69.41
2	<i>T. viride</i>	40.40	39.30	38.20	39.10	40.20	39.44	7.28
3	Control	39.05	43.00	43.75	44.50	42.43	42.54	-

Result: 'F' test:- Significant, SE:- 0.688, C.D. at 5%:- 1.50

**Table.2** *In vitro* assay of culture filtrate of an antagonist against *Rhizoctonia solani*

Sr. No.	Treatment Concentrations in percent	Zone of inhibition in mm against <i>Rhizoctonia solani</i>			
		<i>Trichoderma harzianum</i>		<i>Trichoderma viride</i>	
		Autoclaved	Unautoclaved	Autoclaved	Unautoclaved
1	100	-	57.66	-	-
2	75	-	47.83	-	-
3	50	-	40.66	-	-
4	25	-	33.33	-	-
5	10	-	27.33	-	-
6	Control	-	0.00	-	-

**Table.3** Effect of seed treatment with bio-agent *T. harzianum* and *T. viride* on seed germination and seedling mortality

Sr. No.	Seed treated with	No. of seeds sown	No. of seeds germinated	Percent seed germination	No. of seedlings rotted	Percent rotting
1	<i>T. harzianum</i>	150	127	84.66	15	11.81
2	<i>T. viride</i>	150	90	60.00	27	30.00
3	Control	150	85	56.66	35	41.17

**Table.3a** Effect of seed treatment with bio-agent on seedling mortality (Statistical analysis)

Sr. No.	Treatment	Percent seedling mortality					Treatment mean
		R-I	R-II	R-III	R-IV	R-V	
1	<i>T. harzianum</i>	10.00	13.33	10.00	10.00	6.66	9.998
		(18.44)	(21.39)	(18.44)	(18.44)	(15.00)	(18.342)
2	<i>T. viride</i>	16.66	13.33	26.66	13.33	20.00	17.996
		(24.12)	(21.39)	(31.11)	(21.39)	(26.56)	(24.914)
3	Control	23.33	20.00	26.66	16.66	30.00	23.330
		(28.86)	(26.56)	(31.11)	(24.12)	(33.21)	(28.770)

(Figures in parenthesis are arcsine values)

Result: 'F' test:- Significant, SE:- 1.52, CD at 5%:- 3.33)

**Table.4** Effect of seedling treatment with bio agent on root rot incidence of brinjal *in vivo*

Sr. No.	Treatment	No. of seedlings planted	No. of seedlings rotted	% incidence of root rot	% decrease in root rot incidence over control
1	<i>T. harzianum</i>	25	7	28	52
2	<i>T. viride</i>	25	18	74	6
3	Control	25	20	80	-

**Table.4a.** Effect of seedling treatment with bio agent on root rot incidence of brinjal *in vivo*

Sr. No.	Treatment	Percent seedling mortality					Treatment mean
		R-I	R-II	R-III	R-IV	R-V	
1	<i>T. harzianum</i>	40.00 (39.23)	40.00 (39.23)	0.00 (0.00)	20.00 (26.56)	40.00 (39.23)	32.00 (28.85)
2	<i>T. viride</i>	80.00 (63.44)	60.00 (50.77)	80.00 (63.44)	60.00 (50.77)	80.00 (63.44)	72.00 (58.37)
3	Control	80.00 (63.44)	100.00 (90.00)	60.00 (50.77)	80.00 (63.44)	80.00 (63.44)	80.00 (66.21)

(Figures in parenthesis are arcsine values)

Result: 'F' test significant, SE- 6.02, CD at 5 %:- 13.13

From the Table 3 and 3a, it is observed that when brinjal seeds were treated with spore suspension of *T. harzianum* and *T. viride* the percent seed germination was increased by 84.66 percent and 60 percent respectively as compared to control *i.e.* 56.66 percent. Data regarding seedling mortality was analyzed and it is observed that seed treatment with spore suspension of *T. harzianum* was found significantly superior in controlling seedling mortality of brinjal followed by *T. viride*.

Table 4 and 4a, revealed that the percent root rot incidence in case of *T. harzianum* was 28 percent where as 74 percent in case of *T. viride* as against 80 percent in control. The percent decrease in root rot incidence over control in regard to *T. harzianum* and *T. viride* was 52 and 6 percent respectively. From the statistical analysis it is clear that seedlings treatment with spore suspension of *T. harzianum* was found significantly superior in controlling root rot incidence. Similar results were reported by Marshall (1982), Lifshitz *et al.*, (1985), Beagle, Ristaino and Papavizas (1985), Sivan *et al.*, (1987), Harman *et al.*, (1989).

#### Effect of antagonistic bacteria on the growth of fungus

In the present investigation, *B. japonicum* and *B. thuringensis* were used *in vitro* by dual culture method and it was found that only *B. japonicum* arrested radial mycelial growth of *R. solani* from both sides of streaking. Similar results were recorded by Balasundaram *et al.*, (1988), Blum *et al.*, (1991), Gangopadhyay and Grover (1994), Gnanamanikam *et al.*, (1995).

Finally it is concluded from the present investigation, that some bio agent produces antagonistic substance *i.e.* antibiotics in their substrate and have been found effective in controlling plant disease *in vitro*. *T. harzianum* is a promising bio control agent for the management of root rot of brinjal followed by *T. viride*. The *Bradyrhizobium japonicum* arrested the growth of *R. solani* in *in vitro* studies.

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#### How to cite this article:

Shah, M.J., A.N. Buldeo and Bandgar, M.S. 2018. Bio-control of Root Rot of Brinjal Caused by *Rhizoctonia solani* Kuhn. *Int.J.Curr.Microbiol.App.Sci.* 7(03): 2469-2474.  
doi: <https://doi.org/10.20546/ijcmas.2018.703.287>