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# Screening of Biocontrol Agents Against *Rhizoctonia solani* Causing Web Blight Disease of Groundnut (*Arachis hypogaea* L.)

Sevugaperumal Ganesan<sup>1</sup> and Rajagobal Sekar<sup>2</sup>  
 Centre for Research & PG Department of Botany,  
 Thiagarajar College (Autonomous), Tamil Nadu,  
 India

## 1. Introduction

Soil borne plant pathogenic fungi cause heavy crop losses all over the world. With variable climate from region to region, most of the crops grown in India are susceptible to diseases caused by soil borne fungal pathogens. Among tropical, subtropical land crops groundnut (*Arachis hypogaea* L.) is an important annual oil seed crop, which provides vegetable oil as human food and oil cake meal as animal poultry feed. A large number of diseases attack groundnut in India (Ganesan and Sekar, 2004a).

Fungi cause majorities and several of them are yield reducers in certain region and seasons (Bowyer, 1999). Among the soil borne fungal diseases of groundnut, Web blight, caused by *Rhizoctonia solani* is the most common disease (Dubey, 2000).

Majority of work done on plant disease biocontrol relate to soil borne diseases using either bacteria or fungal antagonists (Montealegre *et al.*, 2003; Askar and Rashad, 2010; Pandya and Saraf, 2010). Among bacteria, *Pseudomonas* and *Bacillus* spp. are widely used. However, the use of antagonistic fungi, especially *Trichoderma* and *Gliocladium* spp. has been more extensive than their bacterial counterparts (Harman, 2000; Ganesan, 2004; Harman, 2006; Neha and Dawande, 2010). Bacteria isolated from the rhizosphere and belonging to a wide variety of genera have the potential to suppress diseases caused by a diversity of soil borne plant pathogens. But the information available on the antagonistic effect of rhizobacteria against *R. solani* is very scanty. *Rhizoctonia solani* Kuhn (teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk) is a widespread and an ecologically diverse soil-borne fungus, causing different types of diseases in many plant species. It causes root rot, stem rot, fruit and seed decay, damping-off, foliar blight, stem canker and crown rot in various crops (Guleria *et al.*, 2007).

Understanding the mechanism of action is important because it gives much useful information in determining the maintenance, enhancement and implementation of biological control. Biological control agents interact with phytopathogens directly or indirectly to reduce the population of pathogens or reduction in the ability of the pathogens to cause disease. In general, mechanisms implicated in antagonism towards the biological control of phytopathogenic fungi includes, A) Direct mechanism: parasitism, antibiosis, competition for nutrients or space, production of enzymes and inactivation of pathogen enzymes. B) Indirect mechanism: tolerance to stress through enhanced root and plant

development induced systemic resistance and solubilization and sequestration of inorganic nutrients (Liu, *et al.*, 1995; Chet, *et al.*, 1998; Altomare *et al.*, 1999; Harman, 2000; Viswanathan and Samiyappan, 2002; Ganesan *et al.*, 2003; Ganesan, 2004; Ganesan and Sekar, 2004a; 2004b; Gohel *et al.*, 2006; Harman, 2006; Pal and Gardener, 2006).

In the present work, isolation and identification of native antagonists against web blight causing pathogen (*Rhizoctonia solani*) and comparing their antagonism with biocontrol agents obtained from different type culture collection and study the mechanism of the antagonism against the pathogens were studied.

## 2. Materials and methods

### 2.1 Isolation of pathogen from groundnut field

Groundnut (*Arachis hypogaea* L.) plants showing web blight symptoms were collected from Pallapatty village crop field, Tamil Nadu, India (77°81' - 78° -2'E longitude and 9°5' - 10°5'N latitude). The pathogenicity of the isolated pathogen (*R. solani*) was tested as described by Singh and Thapliyal, (1998).

### 2.2 Isolation and maintenance of biocontrol agents

Isolation of bacterial biocontrol agents was made according to the method of Khot *et al.*, (1996). They were screened for their antagonistic activity against the pathogens by dual culture method, isolates which showed significant antagonistic activity was identified by using methods described in Bergey's Manual of Systematic Bacteriology (1984).

Isolation of fungal biocontrol agents was made from the pathogens infested field soil. Fungal species growing on the ungerminated sclerotia were isolated, screened for antagonistic activity by dual culture method, identified and maintained on Potato Dextrose Agar (PDA).

The following biocontrol agents used in the present study were obtained from Indian Type Culture Collection (ITCC), New Delhi and Microbial Type Culture Collection (MTCC), Chandigarh. *Trichoderma harzianum* (ITCC-4572), *T. koningii* (MTCC-2385), *T. viride* (MTCC-800), *T. pseudokoningii* (MTCC-3011), *T. hamatum* (MTCC-2577), *T. reesii* (MTCC-798), *Bacillus megaterium* (MTCC-453), *B. pumilus* (MTCC-170) and *B. subtilis* (MTCC-121).

## 3. Antagonistic activity of biocontrol agents

### 3.1 Dual culture method

The antagonistic ability of the bacterial biocontrol agents was tested by dual culture technique. The antagonism between selected fungal antagonists and the pathogen *R. solani* was tested following the method suggested by Bell *et al.*, (1982). They were ranked according to modified Bell's ranking scale. R<sub>1</sub> = complete overgrowth, R<sub>2</sub> = 75% over growth, R<sub>3</sub> = 50% over growth, R<sub>4</sub> = locked at the point of contact, R<sub>5</sub> = pathogen over growing antagonist. The percentage of inhibition was calculated using the following formula: Percentage of inhibition =  $(A_1 - A_2) / A_1 \times 100$ , Where A<sub>1</sub> = Area covered by the pathogen in control, A<sub>2</sub> = Area covered by pathogen in dual culture.

## 4. Light microscopic and Scanning Electron Microscopic (SEM) observations

For light microscopic observations, the mycelium was aseptically removed from the site of interaction and mounted on microscopic slides using lactophenol cotton blue stain. Slides

were analyzed and photographed under photomicroscope (Nikon-20). For Scanning Electron Microscopic (SEM) studies, samples were mounted on the specimen stubs using fevicol adhesive. Small samples were mounted directly on Scotsch double adhesive tape, and were coated with gold to a thickness of 100 Å using Hitachi Vacuum Evaporator, Model HUS 5GB. Coated samples were analyzed in a Hitachi Scanning Electron Microscope model S-450 operated at 15kv, and photographed.

## 5. Volatile activity

To study the effect of volatile metabolites of biocontrol agents on *R. solani*, paired plate technique was followed (Dennis and Webster, 1971). Three replicates were placed for each treatment. Growth of the pathogen was recorded on the 3rd day and on 5th day after incubation and the percentage of inhibition was calculated using the formula, Percentage of inhibition =  $(A_1 - A_2) / A_1 \times 100$ .

Where  $A_1$  = Area covered by the pathogen in control,  $A_2$  = Area covered by pathogen in paired petriplate.

## 6. Non-volatile activity

Non-volatile activities of bacterial and fungal biocontrol agents were tested as described by Jariwala *et al.*, (1991) with slight modification. Loop full of bacterial biocontrol agents were inoculated in Nutrient broth and incubated in a shaker (120 rpm) for 48hrs at room temperature ( $28^\circ \pm 2^\circ$ ). After the incubation, cultures were centrifuged at 5000rpm for 15min. and the supernatant was used for antibiotic activity.

In the case of fungal antagonists, 1ml of spore suspension ( $1 \times 10^5$  cfu / ml) was inoculated in Potato Dextrose (PD) broth and incubated at room temperature for 1 week. The fungal mat and the spores were removed by filtration through double layer filter paper followed by centrifugation at 5000rpm for 15min. the supernatant was used for antibiotic activity. Culture filtrates were added to PD Agar medium at 25%, 50%, 75% and 100% concentration, the pH was adjusted to  $6.8 \pm 0.2$ . Then the medium was sterilized and poured in the sterile Petri plates.

Three day old actively growing *R. solani* cultures were removed from the edge of the colony using 4mm diameter cork borer and placed at the center of these culture medium and the plates were incubated at room temperature. Three replicates were maintained for each concentration. Plates containing PDA medium with pathogens alone served as control. Radial growth of the fungal colony was measured on 3rd day and 5th day after incubation. Percentage of inhibition was calculated using the formula described earlier.

## 7. Enzymatic activity

Two sets of Erlenmeyer flasks (250ml) containing 50 ml of czapek dox broth containing carboxy methyl cellulose (CMC) (inducer of  $\beta$ -1-4 endoglucanase) and minimal medium supplemented with chitin (inducer of chitinase), were inoculated with fungal and bacterial biocontrol agents respectively, Bacterial cultures were incubated on rotary shaker (120rpm) at ( $28^\circ \pm 2^\circ$ C) for 24hrs. Fungal cultures were incubated on rotary shaker (120rpm) at ( $28^\circ \pm 2^\circ$ C) for 1week. After incubation period, cultures were filtered using Whatman No.1 filter paper and filtrates were centrifuged at 5000rpm for 15 min. Supernatants were used as crude preparations for enzyme assays.

### 7.1 Chitinase activity

Enzymatic hydrolysis of colloidal chitin was assayed following the release of free N-Acetylglucosamine (NAG) from colloidal chitin by spectrometric method (Ohtakara, 1988) and by clearing zone assay method (Frandsberg and Schnurer, 1998).

### 7.2 Clearing zone assay

The clearing zone assay was performed on chitin agar containing 0.15% chitin, 1.5% agar, and 0.02% Sodium azide ( $\text{Na N}_3$ ) in 50Mm potassium phosphate buffer (pH - 6.1). Crude enzymes samples (0.25ml) were added to wells (diameter 5-mm) made in the agar medium. The plates were incubated in a humidity chamber at  $28^\circ \pm 2^\circ\text{C}$  for 24hr. and the rate of clearing zones were measured.

### 7.3 Spectrometric method

Colloidal chitin was prepared from raw chitin, five grams of chitin powder was homogenized in 100ml of concentrated hydrochloric acid and left at  $20^\circ\text{C}$ . for 10 min. The suspension was poured into cold water under agitation and left to settle. The precipitate was washed with water and dried.

The reaction mixture containing 1 ml of 0.5% colloidal chitin, 2 ml of McIlvaines's buffer (equal volume of 0.2M disodium hydrogen phosphate and 0.1M of citric acid, pH 4.0) and 1ml of culture filtrate, was incubated for 20 min. at  $37^\circ\text{C}$  in a shaker (120rpm) and the reaction was stopped by boiling for 3 min. After centrifugation of this mixture (2000rpm for 30 min.) 1.5 ml of supernatant fluid was mixed with 2 ml of potassium ferricyanide reagent (0.05% potassium ferricyanide in 0.5 M sodium carbonate) and heated in boiling water bath for 15 min. The amount of N- acetyl glucosamine released was estimated by absorbance of reaction mixture at 420 nm. One unit of enzyme activity was defined as release of 1 micro mol N-acetylglucosamine (IU) / ml of culture filtrate / min.

### 7.4 $\beta$ - 1-4- endoglucanase activity

$\beta$ -1-4-endoglucanase attacks the 1-4- $\beta$ -glucosidic linkages of cellulose molecule randomly. Enzymatic hydrolysis of carboxy methylcellulose (CMC) was assayed by dinitro salicylic acid (DNS) method (Miller, 1959). The enzyme activity was expressed as release of 1 micromole glucose (IU) / ml of culture filtrate / min.

## 8. Result and discussion

Pathogen *Rhizoctonia solani* was isolated from web blight symptom showing infected groundnut plants and identified according to their colony characters and pathogenicity (Plate I). Fifteen bacterial biocontrol agents were isolated from soil. Among the isolates, 5 showed antagonistic activity against the pathogen. These isolates were identified as *Bacillus polymyxa*, *B. licheniformis*, *B. sphaericus*, *B. thuringiensis* and *Pseudomonas putida* based on different biochemical tests (Krieg, 1984; Sneath, 1984). These isolates were pure cultured and maintained on Nutrient Agar medium. Ten fungal antagonists were isolated and screened against *R. solani*, among these only 2 isolates showed significant reduction in the growth of the pathogen. They were identified as *Trichoderma longibrachiatum* and *T. virens* = *Gliocladium virens* (Plate II-3).

Several workers also found the successful control of *R. solani* in *invitro* condition using biocontrol agents (Dubey, 1998; Desai and Schlosser, 1999; Bunker and Mathur, 2001;

Ganesan and Sekar, 2004b; Bienkowski *et al.*, 2010). In the present work to understand the biocontrol of pathogens by antagonists, light and scanning electron microscopic observations, involvement of volatile and non-volatile metabolites and secretion of enzymes (chitinase and  $\beta$ -1-4-glucanase) have been made (Table 1, 2a & 2b, 3a & 3b, Fig. 1a & 1b, Plate I & II).

In dual culture method, after third day of incubation all the biocontrol agents inhibited the pathogen to varying degree. Among the bacterial biocontrol agents, Maximum level of growth reduction of *R. solani* was noted in *B. subtilis* (42.53%) followed by *B. polymyxa* (39.13%) and *B. licheniformis* (30.36%). Lowest level of inhibition was found with *B. pumilus* (11.84%). Percentage of inhibition varied with biocontrol agents used. Among the fungal biocontrol agents, 78.76% of inhibition was recorded with *T. virens* followed by *T. hamatum* (77.64%) and *T. harzianum* (72.24%). Minimum level of inhibition was noticed with *T. reesi* (33.06% (Table 1). Further incubation of plates showed reduction in number of sclerotial production by the pathogen, when compared with control. They were ranked according to modified Bell's ranking scale (Table 1).

S. No	Bacterial Biocontrol agents	Radial growth of <i>R. solani</i> (mm)*	% inhibition	Fungal Biocontrol agents	Radial growth of <i>R. solani</i> (mm)*	% inhibition
1	Control	74.53 ± 0.28	-	Control	74.70 ± 0.15	-
2	<i>B. subtilis</i>	42.83 ± 0.27	42.53	<i>T. harzianum</i>	20.73 ± 0.73	72.24 <sup>R1</sup>
3	<i>B. megaterium</i>	64.90 ± 1.21	12.92	<i>T. hamatum</i>	16.70 ± 0.24	77.64 <sup>R1</sup>
4	<i>B. pumilus</i>	65.70 ± 0.31	11.84	<i>T. viride</i>	38.63 ± 0.37	48.28 <sup>R2</sup>
5	<i>B. thuringiensis</i>	62.93 ± 0.40	15.56	<i>T. koningii</i>	26.93 ± 0.53	63.94 <sup>R3</sup>
6	<i>B. licheniformis</i>	51.90 ± 0.73	30.36	<i>T. pseukoningii</i>	53.73 ± 0.63	28.07 <sup>R4</sup>
7	<i>B. speriacus</i>	64.60 ± 0.34	13.32	<i>T. reesii</i>	50.00 ± 0.34	33.06 <sup>R5</sup>
8	<i>B. polymyxa</i>	45.36 ± 1.83	39.13	<i>T. longibrachiatum</i>	25.20 ± 0.57	66.26 <sup>R2</sup>
9	<i>P. putida</i>	65.33 ± 0.60	12.34	<i>T. virens</i>	15.86 ± 0.23	78.76 <sup>R1</sup>

\* = Each value is a mean of triplicate; ± = Std error; R<sub>1</sub>-R<sub>5</sub> = Bell's ranging

Table 1. Interaction of biocontrol agents against *R. solani* – Dual culture method

Light and scanning electron microscopic observations of antagonists and pathogenic organisms in dual culture indicate that the principle mechanism of fungal antagonism is due to mycoparasitism. Antagonistic hyphae coil the pathogenic hyphae. Pathogenic organism showed severe vacuolation followed by coagulation, shrinkage of cytoplasm and finally lysis of cells. In some cases the tip of the pathogenic hyphae showed bulbous and tapering end. Bacterial antagonists showed attachment with the pathogenic hyphae and lysis. Pathogenic mycelium on the clearing zone showed swelling of hyphal tips, cells were found to be bulbous, swollen with shrunken and granulated cytoplasm (Plate II).

Under Scanning Electron Microscope, the fungal antagonists showed coiling, and lysis of pathogens mycelium. With the bacterial biocontrol agents, the affected pathogenic mycelium showed attachment, lysis and disintegration (Plate II). During antibiosis, both volatile and nonvolatile secondary metabolities have been implicated in restricting the vegetative growth of pathogenic fungi. Volatile activity of *T. harzianum* showed 100% inhibition of *R. solani*, where as *T. viride* showed 93.44% of inhibition against *R. solani*. *B. speriacus* and *B. polymyxa* showed 87.30% and 94.08% of inhibition. *B. megaterium* and *B.*

*licheniformis* showed 95.7% and 94.41% of inhibition and *P. putida* showed 88.5% inhibition of *R. solani* mycelial growth (Table 2a & 2b). In general the inhibition percentage was higher on the 3<sup>rd</sup> day of incubation than on 5<sup>th</sup> day of incubation. No changes in the colony characteristics of fungal and bacterial antagonists were observed.

S. No	Biocontrol agents	Mycelial Length on 3 <sup>rd</sup> day (mm)*	% inhibition	Mycelial Length on 5 <sup>th</sup> day (mm)*	% inhibition
1	Control	30.96 ± 0.26	-	39.83 ± 0.15	-
2	<i>B. subtilis</i>	1.93 ± 0.23	93.76	3.53 ± 0.46	91.13
3	<i>B. megaterium</i>	1.33 ± 0.33	95.70	5.53 ± 0.41	86.11
4	<i>B. pumilus</i>	6.8 ± 0.20	78.03	14.10 ± 0.40	64.59
5	<i>B. thuringiensis</i>	11.66 ± 0.23	62.33	15.53 ± 0.27	61.00
6	<i>B. licheniformis</i>	1.73 ± 0.40	94.41	2.56 ± 0.83	93.57
7	<i>B. sphaericus</i>	3.93 ± 0.37	87.30	6.85 ± 0.45	82.80
8	<i>B. polymyxa</i>	1.83 ± 0.16	94.08	4.96 ± 0.18	87.54
9	<i>P. putida</i>	3.56 ± 0.23	88.50	8.56 ± 0.34	78.50

\* = Each value is a mean of triplicate ± = Std error

Table 2a. Volatile activity of bacterial biocontrol agents against *R. solani*

S. No	Biocontrol agents	Mycelial Length on 3 <sup>rd</sup> day (mm)*	% inhibition	Mycelial Length on 5 <sup>th</sup> day (mm)*	% inhibition
1	Control	31.10 ± 0.75	-	43.66 ± 0.17	-
2	<i>T. harzianum</i>	-	100	0	100
3	<i>T. hamatum</i>	7.00 ± 0.40	77.49	10.66 ± 0.17	75.58
4	<i>T. viride</i>	-	100	2.86 ± 0.24	93.44
5	<i>T. koningii</i>	3.13 ± 0.27	89.93	10.93 ± 0.23	74.96
6	<i>T. pseudokoningii</i>	19.46 ± 0.60	37.42	32.96 ± 0.32	24.50
7	<i>T. reesii</i>	24.90 ± 0.45	12.91	39.10 ± 0.46	10.44
8	<i>T. longibrachiatum</i>	1.00 ± 0.00	96.78	4.46 ± 0.29	89.78
9	<i>T. virens</i>	2.56 ± 0.21	91.76	5.06 ± 0.17	88.41

\* = Each value is a mean of triplicate ± = Std error

Table 2b. Volatile activity of fungal biocontrol agents against *R. solani*

These results indicate that antagonistic organisms produce volatile compounds having antibiotic activity. Non-volatile activity of the antagonistic organisms against pathogens revealed significant reduction of pathogenic growth. The maximum level of inhibition of *R. solani* (100%) was obtained from *T. harzianum* at 100% conc. of culture filtrates. Among the bacterial antagonists *B. subtilis* produced 90.31% of inhibition of *R. solani* mycelium at 100% concentration of culture filtrates. However, decreased concentrations were less inhibitory to the growth of *R. solani* (Fig. 1a & 1b).

Isolates of *Trichoderma* excrete some growth inhibitory substances. Of these, alkyl pyrons, isonitriles, polyketides, peptaibols diketopiperazines, sesquiterpenes, and steroids have frequently been associated with biocontrol activity (Howell, 1998; Sivasithamparam and Ghisalberti, 1998). Production of heat labile antifungal substances by *Bacillus* to control different fungal pathogens was reported by several workers (Cubeta *et al.*, 1985; Podile *et al.*, 1987; Dileepkumar *et al.*, 1988). Cyanides were also considered as volatile metabolites produced by bacterial biocontrol agents (Laha *et al.*, 1996; Rangheshwaran and Prasad, 2000).

Biocontrol agents are known to produce various enzymes like  $\beta$ -1-3 glucanase, cellulase ( $\beta$ , 1-4,glucanase), chitinase and proteases, which are involved in the antagonistic activity against phytopathogenic fungi (Frandsen and Schnurer, 1998; Singh *et al.*, 1999; Berg *et al.*, 2000; Ramamoorthy and Samiyappan, 2001). In the present study *T. harzianum* and *T. koningii* showed maximum chitin utilization in clearing zone assay. In the case of bacterial antagonists, except *B. pumilus* all the other antagonists showed high level of chitin degradation (Table 3a & 3b).

S. No	Biocontrol agents	Clearing zone assay of Chitinase	Chitinase activity IU/ml	$\beta$ -1-4 Glucanase activity IU/ml
1	<i>B. subtilis</i>	+++	14.10	58.80
2	<i>B. megaterium</i>	+++	11.47	58.00
3	<i>B. pumilus</i>	++	9.73	70.80
4	<i>B. thuringiensis</i>	+++	19.81	71.60
5	<i>B. licheniformis</i>	+++	12.98	58.80
6	<i>B. sphaericus</i>	+++	16.27	30.00
7	<i>B. polymyxa</i>	+++	17.29	87.20
8	<i>P. putida</i>	+++	15.06	71.20

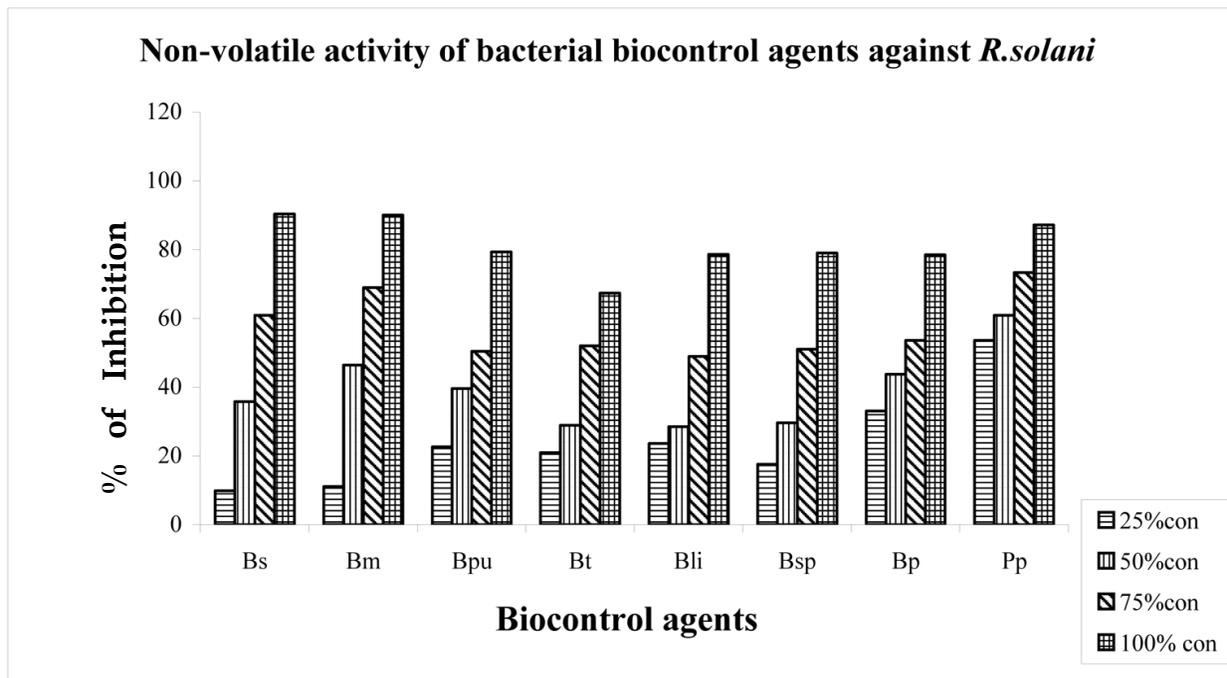
+++ = Higher level of clearing zone ++ = Moderate level

Table 3a. Enzymatic activity of bacterial biocontrol agents

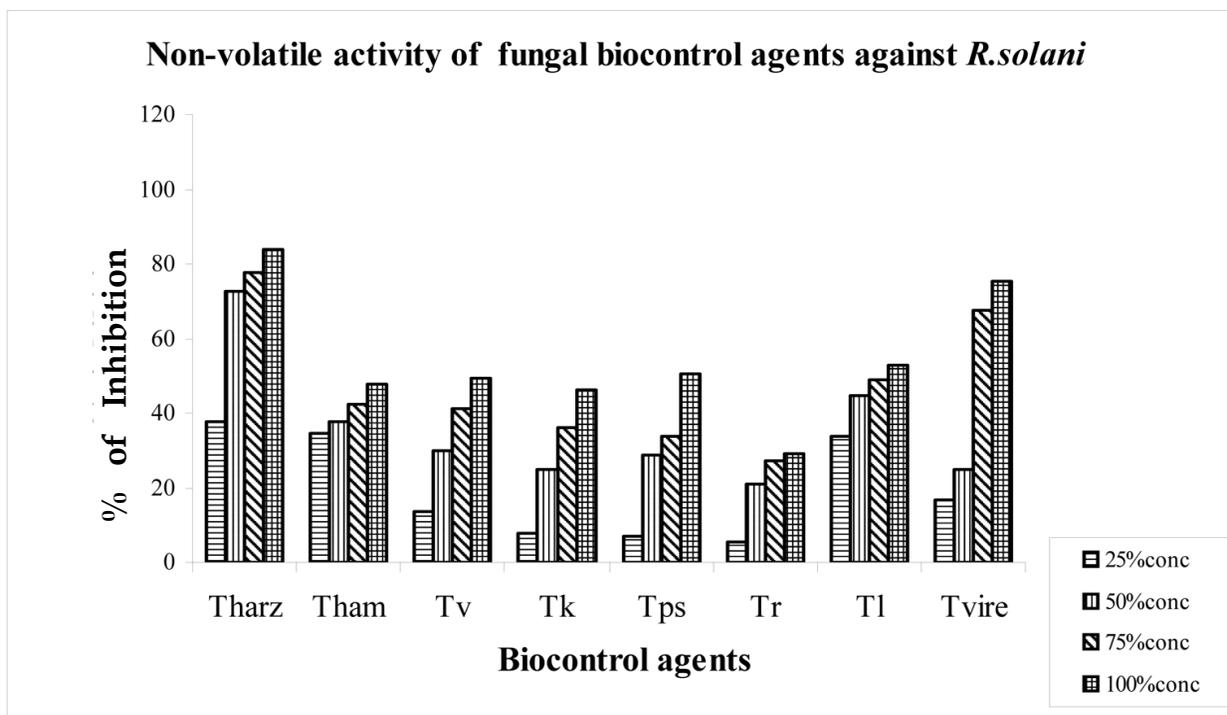
S. No.	Biocontrol Agents	Clearing zone assay of Chitinase	Chitinase Activity IU/ml	$\beta$ -1-4Glucanase Activity IU/ml
1.	<i>T. harzianum</i>	+++	5.51	7.60
2.	<i>T. hamatum</i>	++	2.32	4.80
3.	<i>T. viride</i>	-	0.12	33.20
4.	<i>T. koningii</i>	+++	4.00	60.40
5.	<i>T. pseudokoningii</i>	+	3.80	34.40
6.	<i>T. reesii</i>	-	0.33	46.80
7.	<i>T. longibrachiatum</i>	++	0.85	6.40
8.	<i>T. virens</i>	++	2.50	36.80

+++ = Higher level of clearing zone; ++ = Moderate level; + = Low level  
- = No clearing zone

Table 3b. Enzymatic activity of fungal biocontrol agents



(a)



(b)

Fig. 1.

Maximum level of chitinase enzyme production was observed in *B. thuringiensis* (19.81 IU/ml). Among the fungal antagonists *T. harzianum* showed maximum activity (3.80 IU/ml). In the CMC amended medium maximum level of  $\beta$ -1.4-endoglucanase activity was observed in *B. polymyxa* (87.2 IU /ml). Among the fungal antagonist maximum level of enzyme activity was observed in *T. koningii* (60.41 IU /ml) (Table 3a & 3b). The levels of chitinase and glucanase increase dramatically as soon as a pathogen attack occurs (Ferraris *et al.*, 1987). Both these enzymes are responsible for disrupting the fungal cell wall and/or prevention of hyphal growth (Vaidya *et al.*, 2001; Gohel *et al.*, 2004). There was great concern regarding screening techniques for biocontrol strains of *Trichoderma*; the first and quickest ones were screens for antibiotic production and/or mycoparasitism in petridish assays (Harman, 2006).



Plate I. Growth characters and pathogenicity of *Rhizoctonia solani*

1. Groundnut plants showing web blight disease
2. White hyaline mycelium of *R. solani* (on 3<sup>rd</sup> day of incubation) (Left) with irregular dark brown sclerotia on 7<sup>th</sup> day of incubation (Right)
3. Light microscopic picture of *R. solani* mycelium (400X)
4. Web blight symptom on groundnut plant in pot culture condition (left), healthy plants (Right)

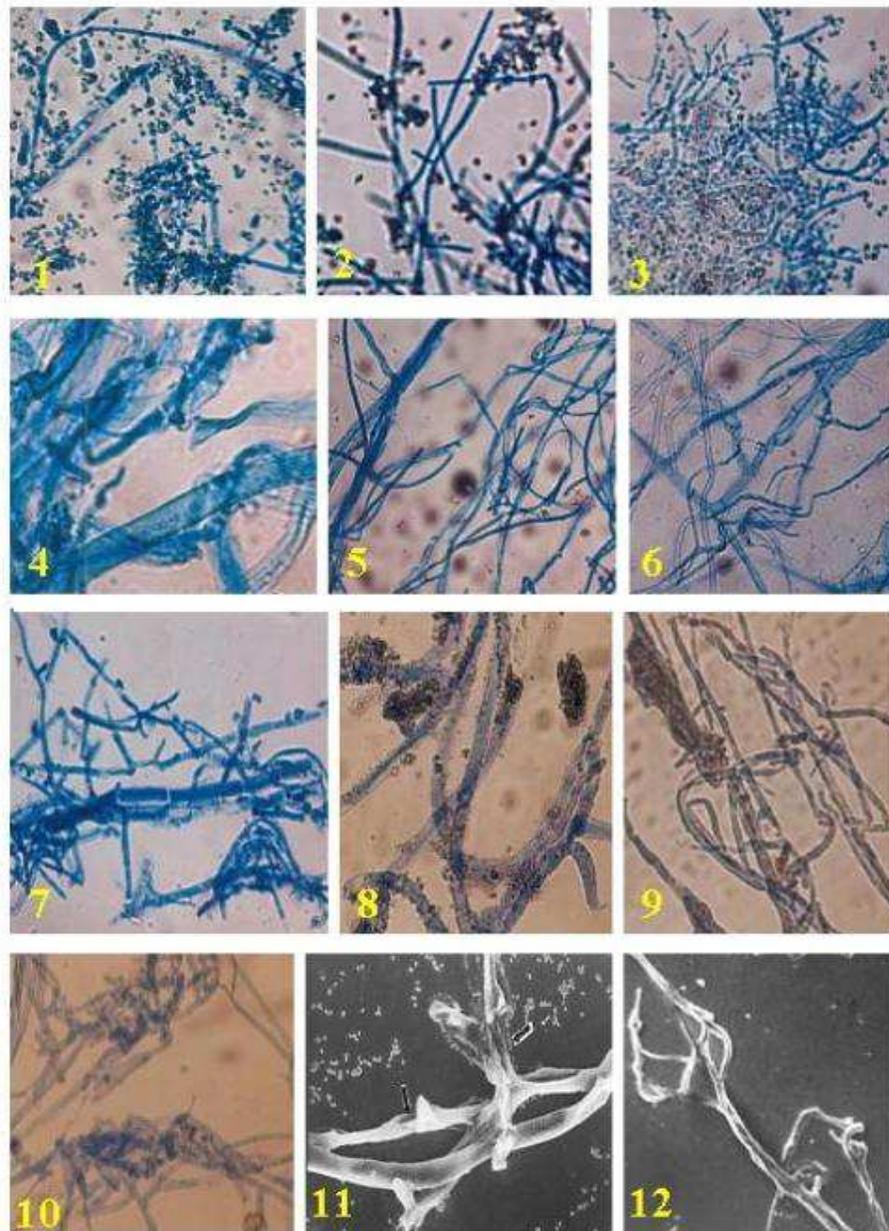


Plate II. Interaction between biocontrol agents and pathogens

Light Microscopic observation

1. Sporulated mycelium of *Trichoderma harzianum* (200X)
2. Sporulated mycelium of *T. hamatum* (200X)
3. Sporulated mycelium of *T. virens* (200X)
4. Coiling of *T. harzianum* hyphae on *R. solani* mycelium (400X)
- 5 & 6. Coiling and intermingling of *T. hamatum* mycelium with *R. solani* mycelium (200X).
7. Lysis and defragment of *R. solani* mycelium due to *T. virens* (200X)
8. Light micrographic picture of interaction between *B. polymyxa* and *R. solani*- showing attachment of *B. polymyxa* cells on *R. solani* mycelium (200X)
9. Interaction between *B. megaterium* and *R. solani*- clumping of *R. solani* mycelium (200X)
10. Interaction between *Pseudomonas putida* and *R. solani* showing defragmented mycelium

of *R. solani* (200 X)

#### Scanning Electron Microscopic observations

11. Interaction between *T. harzianum* and *R. solani*- showing coiling and lysis of its host mycelium (800 X)

12. Interaction between *B. polymyxa* and *R. solani* showing abnormal structure of host mycelium (120 X).

### 9. Conclusion

The work indicates that both fungal and bacterial biocontrol agents were effective against *R. solani*. These biocontrol agents inhibit the pathogen by antibiosis (volatile or non-volatile) or by enzyme production or by parasitism or by combinations of these methods.

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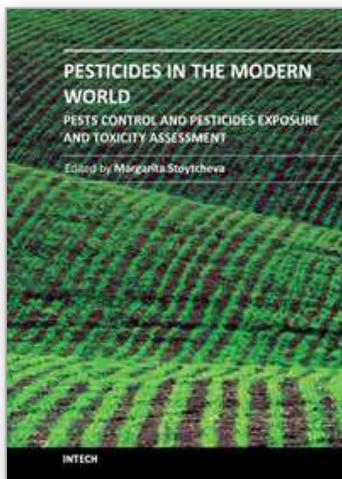
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University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
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Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
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