ISSN 0975 - 6299 Vol 2 / Issue 3 / Jul - Sept 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PATHOLOG

# IN VITRO EFFICACY OF VARIOUS FUNGAL AND BACTERIAL ANTAGONISTS AGAINST RHIZOCTONIA SOLANI, CAUSAL AGENT OF DAMPING OFF DISEASE IN CAPSICUN ANNUUM L.

**ABHINITI MALHOTRA*[1], TRIPTI AGARWAL[2] AND P. C. TRIVEDI[3]**

[1,2,3] Department of Botany, University of Rajasthan, Jaipur – 302055

**ABHINITI MALHOTRA**
Department of Botany, University of Rajasthan, Jaipur – 302055

## ABSTRACT

Chilli is an important vegetable and spice crop worldwide and one of the most important vegetables in India. The chief constituent of chilli, Capsaicin has antioxidant, antibacterial and anti-cancerous properties. *Rhizoctonia solani* that causes damping off disease of seedlings as well as root and stem rot in young transplants, is a major soil borne pathogen of chilli. In the present study, 13 isolates of biocontrol fungi and 4 bacterial strains were evaluated against *Rhizoctonia solani* using Dual culture technique. Microscopic observations of fungal growth in dual cultures revealed that growth inhibition of the pathogen occurred soon after contact with the antagonist due to the efficient coiling process followed by a substantial production of hydrolytic enzymes. The results show that among the fungal species *Gliocladium virens* and *T. harzianum* (T8) are the most effective isolates at 25°C and inhibit *R. solani* mycelial growth by 74.82% and 73.33% respectively. Among the bacterial strains maximum growth inhibition is caused by *P. fluorescens* P.f.1 (73.33%) followed by *P. fluorescens* P.f.2 (62.22%).

## KEYWORDS

*Capsicum annuum*, *Rhizoctonia solani*, *Trichoderma harzianum*, Dual culture.

## INTRODUCTION

Chilli is an important vegetable and spice crop throughout the world. Hot and sweet chilli pepper (*Capsicum annuum* L.) is one of the most important vegetables in India [1,2] and it is a basic ingredient of the Indian diet. Rajasthan is considered to be the seventh state in the production of red chilli. *Rhizoctonia solani, which* causes damping-off disease of seedlings as well as root and stem rot in young transplants, is a major soil-borne pathogen of chilli (*Capsicum annuum* L.). As Chilli is a vegetable crop, the use of chemicals for disease control is not advisable in view of its residual problems. Biocontrol of plant pathogens using antagonistic fungi and bacteria therefore plays a significant role.

Among the antagonistic fungi, *Trichoderma* species has been found as a promising biocontrol agent of *R. solani* in chilli[3].

## MATERIALS AND METHOD

Antagonist Fungal species were isolated from the rhizosphere of native fields of Jaipur and others were procured from Lucknow, Haridwar and Udaipur. Isolations were done in medium specific for fungi[4] and *Trichoderma*[5] by soil plate technique[6]. All cultures were maintained on PDA at 25 $\pm$ 2 $^0$ C.

Dual cultures[7] were set up with 10 *Trichoderma* spp. isolates to measure their effect on the mycelial growth of *R.solani*. Discs of 5mm were cut using sterilized cork borer from the periphery of 5 days old actively growing cultures and placed 3 cm apart on PDA plates, incubated for 7 days at 25 $\pm$ 2°C. Three petridishes were maintained for each treatment including control treatment devoid of biocontrol agent. Results are expressed as the mean of the percentage of inhibition of growth by Fokkema[8] formula (1973). % Inhibition

$$= \frac{100 \times R_1 - R_2}{R_1}$$

$R_1$ = radial growth of *R.solani* in control.
$R_2$ = radial growth of *R.solani* in dual inoculation.

## RESULT AND DISCUSSION

All the tested fungal and bacterial isolates caused significant inhibition in mycelial growth of *R.solani* in dual culture studies (table -1). Maximum growth inhibition was caused by *Gliocladium virens* (74.82%) followed by *T. harzianum* T8 isolate (73.33%), *T. viride* (67.42%) and T3 isolate of *T. harzianum* (67.40). Minimum inhibition was shown by *Paecilomyces lilacinus* (7.40%), while *Trichoderma hamatum* (47.40%) and *Trichoderma aureoviride* (52.59%) showed intermediate antagonism. *Gliocladium virens* and *Trichoderma viride* grew over the colony of *R.solani* just after 3-4 days of incubation. Later it covered the entire petriplate suppressing the growth of pathogen. On interaction with *Trichoderma harzianum,* isolates labeled (T2, T5, T6) growth were inhibited at the line of contact while some isolated showed visible inhibition zone. Among the biocontrol bacteria maximum growth inhibition was caused by *P. fluorescens* P.f.1 (73.33%) followed by *P.*

*fluorescens* P.f.2 (62.22%). All the data are statistically significant.

The antagonist produces enzymes which causes lysis of cell wall components of the pathogenic fungi. According to observations of Agarwal[9] (2002), the antagonists *viz. Gliocladium virens* and *Trichoderma harzianum* causes lysis of cell wall, vacuolation in cytoplasm and fragmentation of the fungal hyphae, thereby restricting the growth of the pathogen.

Microscopic examination at the point of contact of two fungi revealed that the overgrowing mycelium of the antagonist penetrated the mycelium of the pathogen and the tip of the hyphae of the pathogen swelled and curled making it ineffective.

These observations indicate that pathogens lose their viability after colonization with antagonists[10,11]. Shalini[12] *et. al*. (2007) studied the effect of 17 strains of *Trichoderma* against *R.solani in vitro*.
All the strains including *T.harzianum, T.viride and T.aureoviride that are* tested inhibited the growth of *R. solani* because the isolates coiled around the hyphae of *Rhizoctonia solani* and formed appresoria and hook-like structures.

The light microscopic observation on dual culture assay showed that the hyphae of all *Trichoderma* isolates could grow parallel to the hypae of *Rhizoctonia solani*. The growth inhibition of *Rhizoctonia solani* in presence of *Trichoderma* spp. could be attributed to all the three modes of antagonism *in vitro* viz. competition, antibiosis and mycoparasitism.

In the present study *Gliocladium virens* grew over the colony of *R.solani* within just 3 days of inoculation. This overgrowth may be due to its fast growing nature, rapid sporulation, secretion of gliotoxin[13] and cell wall lytic enzymes such as chitinase, endochitinase and β-1,3-glucanase[14]. Howell and Stipanovic[15] (1983) isolated a new antibiotic, diketopiperazine from the cultural filtrate of *G.virens* and gave a trivial name "gliovirin".

In the present study *T. viride* was found superior to many *T.harzianum* isolates. *T. viride* was also reported by Amin[16] *et al*. (2010) as an important antagonist with the highest percent inhibition against soil borne pathogens of different vegetables viz., *Rhizoctonia solani* , *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* under *in vitro* conditions. Lin[17] *et al*. (1994) isolated "Tricholin", a ribosome-inactivating protein from the culture broth of *Trichoderma viride* and is shown to exert fungicidal effects on *Rhizoctonia solani* through a multi-hit kinetic interaction.

*Pseudomonads* protects the plant from pathogens by activating defense genes encoding chitinase, β–1,3 glucanase and peroxidase[18]. Battu[19] *et al.* (2009) isolated Twenty *P .fluorescens* strains from rhizosphere soil samples collected from rice seedlings grown in Andhra Pradesh and Tamil Nadu. Among 20 strains, *P. fluorescens* P.f 05 was found significantly inhibiting the mycelial growth of rice pathogens *Magnaporthe grisea* and *Rhizoctonia solani*. To further characterize the production of antifungal metabolites by strain P.f.05, several growth media were used. Among the media tested, King's B medium at 120 rpm with pH of 7.0 and 40% of dissolved oxygen incubated at 28 C yielded maximum amount of secondary metabolites. Among them, four secondary metabolites were identified through thin layer chromatography. Of this four, one particular metabolite was found to be a significantly higher inhibitior of the mycelial growth of two rice pathogens when compared to other three metabolites. Of interest, this metabolite was further characterized by HPLC, NMR and Mass Spectroscopy and identified as 2, 4-diacetyl-phloroglucinol (DAPG).

Thus, *G. virens* was the most effective in causing significant suppression of growth and sclerotia formation of *R. solani in vitro* through production of volatile and non-volatile antibiotics followed by *Trichoderma harzianum* and *P. fluorescens* P.f.1.

The biocontrol agents appeared quite prospective for further exploration for practical and economic control of root rot of chilli in conventional fields.

**TABLE - 1**
**In vitro antagonism of fungal and bacterial strains against Rhizoctonia solani.**

| S.No. | TEST MYCOFLORA | CONTROL (mm) | INTERACTION (growth of pathogen) (mm) | % GROWTH INHIBITION OF *R.solani* | GRADE |
|---|---|---|---|---|---|
| 1. | *Trichoderma harzianum($T_1$)* | 45 | 15.33 | 65.93 | $R_1$ |
| 2. | *Trichoderma harzianum($T_2$)* | 45 | 31.33 | 30.37 | $R_4$ |
| 3. | *Trichoderma harzianum($T_3$)* | 45 | 14.66 | 67.42 | $R_1$ |
| 4. | *Trichoderma harzianum($T_4$)* | 45 | 25.33 | 43.71 | $R_3$ |
| 5. | *Trichoderma harzianum($T_5$)* | 45 | 30.33 | 32.60 | $R_4$ |
| 6. | *Trichoderma harzianum($T_6$)* | 45 | 24.66 | 45.20 | $R_4$ |
| 7. | *Trichoderma harzianum ($T_7$)* | 45 | 18.33 | 59.26 | $R_2$ |
| 8. | *Trichoderma harzianum ($T_8$)* | 45 | 12.00 | 73.33 | $R_1$ |
| 9. | *Trichoderma viride* | 45 | 14.66 | 67.42 | $R_1$ |
| 10. | *Trichoderma aureoviride* | 45 | 21.33 | 52.60 | $R_2$ |
| 11. | *Trichoderma hamatum* | 45 | 23.66 | 47.42 | $R_2$ |
| 12. | *Gliocladium virens* | 45 | 11.33 | 74.82 | $R_1$ |
| 13 | *Paecilomyces lilacinus* | 45 | 41.66 | 7.42 | $R_5$ |
| 14. | *P. fluorescens* P.f1 | 45 | 12 | 73.33 | $R_1$ |
| 15. | *P. fluorescens* P.f2 | 45 | 17 | 62.22 | $R_2$ |
| 16. | *Pseudomonas species* | 45 | 41.66 | 7.42 | $R_5$ |
| 17. | *Bacillus subtilis* | 45 | 35 | 22.22 | $R_4$ |
| | C.D. AT 5% | | 1.96 | 4.36 | |

## REFERENCES


1. Bose T.K., and Son M.G., "Chilli and *Capsicum*". In vegetable crops of India. Calcutta: Nayaprakashan, 343-378, (1986).
2. Chadha K.L. and Ramphal, Vegetable production in India. *Indian Horticulture*, 33: 4-7, (1989).
3. Bunker R.N. and Mathur K., Antagonism of local biocontrol agents to *Rhizoctonia solani* inciting dry root rot of chilli. *J. Mycol. Pl. Path.,* 31: 50–52, (2001).
4. Martin James P., Use of acid, rose Bengal and streptomycin in the plate for estimating soil fungi. *Soil Science*, 69: 215-232, (1950).
5. Elad Y., Chet I., V. Boyle and Henis Y., Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* scanning electron microscopy and fluorescent microscopy. *Phytopathology*, 73: 85-88, (1983).
6. Parkinson D., New methods for qualitative and quantitative study of fungi in the rhizosphere. *Pedologie*., 7: 146-154, (1957).
7. Wood R.K.S., The control of diseases of lettuce by use of antagonistic organism. The control of *Botrytis cinerea*. *Pres. Annl. Appl. Biol.,* 38 :203, (1951).
8. Fokkema N.J., The role of saprophytic fungi in antagonism against *Drechslera sorokiniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. *Physiol. Pl Pathol*., 3 :195-205, (1973).
9. Agarwal S.C., Sharma S. and Prasad K.V.Y., Efficacy of biological components in wilt management of chickpea. *Indian J. Pulses, Res.,* 15 (2):177-178, (2002).
10. Elad Y., Chet I. and Katan J., *Trichoderma harzianum* a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology*, 70 :119-121, (1990).
11. Gokulapalan, C., and Nair, M.C., 1984, "Antagonism of a few fungi and bacteria against *Rhizoctonia solani. Indian J Microbiol.,* 24:57-58, (1984).
12. Shalini S. and Kotasthane A. S.,Parasitism of *Rhizoctonia solani* by strains of *Trichoderma* spp. *Electronic Journal of Environmental, Agricultural and Food chemistry*., 6 (8):2272-2281, (2007).
13. Maheshwari D.K., Dubey R.C. and Sharma V.K., Biological effects of *Trichoderma virens* on *Macrophomina phaseolina* causing charcoal rot of peanut. *Indian.J.Mircobiol.,* 41:251-256, (2001).
14. Di Pietro A., Lorito M., Hayes C., Broadway K. and Harman G.E., Endochitinase from *Gliocladium virens*. Isolation, characterization, synergistic antifungal activity in combination with gliotoxin. *Phytopathology*., 83:308–313, (1993).
15. Howell C.R. and Stipanovic R.D.,Gliovirin, a new antibiotic from *Gliocladium virens* and its role in the biological control of *Pythium ultimum*. *Can. J. Microbiol*., 29:321-324, (1983).
16. Amin F., Razdan V.K., Mohiddin F.A., Bhat K.A. and Banday S., Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. *Journal of Phytology*., 2(10):38–41, (2010).
17. Lin A., Lee T.M. and Rern J.C., Tricholin, a new antifungal agent from *Trichoderma viride*, and its action in biological control of *Rhizoctonia solani*. *J. Antibiot.*(Tokyo). 47(7):799-805, (1994).
18. Chen C., Belanger R.R., Benhamou N.A. and Paulitz T.C., Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR). *Physio. Mol. Pl. Pathol.,* 56:13-23, (2000).
19. Battu P.R., Reddy M.S. and Kumar K.V., Characterization of antifungal metabolites of *Pseudomonas fluorescens* and their effect on mycelial growth of *Magnaporthe grisea* and *Rhizoctonia solani*. *International Journal of Pharm Tech Research.,* 1(4):1490-1493, (2009).