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# Efficacy of Different Bioagents on Mycelial Growth of Chickpea Wilt Complex Fungi *in Vitro*

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***Abstract* – Of the ten antagonists tested under *in vitro* condition, maximum inhibition in radial growth of *Sclerotium rolfsii* was observed with *Pseudomonas fluorescens* followed by *Trichoderma harzianum*, *T. viride* (Raipur isolate) and *Gliocladium virens* (Raipur isolate). More than 90 per cent inhibition of sclerotia production by *S. rolfsii* was also observed with these treatments. Maximum growth inhibition of *Rhizoctonia solani* was observed with *G. virens* (Parbhani isolate) followed by *P. fluorescens, G. virens* (Raipur isolate), *T. viride* (Raipur isolate) and *T. hamatum*, whereas 100 per cent inhibition of sclerotia production was observed with *P. fluorescens, G. virens* (Raipur isolate), *T. harzianum, T. hamatum* and *Aspergillus niger.* Inhibition of radial growth of *Fusarium oxysporum* f.sp. *ciceri* was maximum with *T. viride* (Raipur isolate), *T. hamatum*, *T. viride* (Parbhani isolate), *A. niger, T. harzianum, T. koningii, P. fluorescens*, *G. virens* (Parbhani and Raipur isolate) and *T. lignorum.***

***Keywords* – Bioagents, *Trichoderma*, *Pseudomonas Fluorescens*, Wilt, Chickpea, Root Rot, Collar Rot, *Gliocladium*.**

## I. INTRODUCTION

Chickpea is the most important pulse crop of India and accounts for approximately 75% of world's chickpea production. Chickpea wilt complex has been reported from 33 countries of the world causing 10-15% yield losses annually depending upon the environment condition. Chemical pesticides have its own limitations such as high cost, no availability, toxicity, development of resistant strains, environmental pollution and adverse effect on beneficial soil microflora and fauna has compounded the problem in India and all other countries producing chickpea. Biological control is a potential alternative to chemical fungicides. The best control method found is planting resistant varieties, although not all have been bred for every formae specialis of the pathogen. The biocontrol efficacy of antagonistic organism in managing the chickpea wilt causing fungi *viz., Fusarium oxysporum* f.sp. *ciceris, Rhizoctonia solani* and *S. rolfsii* under *in vitro* condition and observed efficiency of *Trichoderma* isolates, against *R. solani*, *S. rolfsii* and *F. oxysporum* f.*sp. ciceris* has great importance. Therefore, present study was undertaken with a view to manage chickpea wilt causing fungi by bio-agents for better utilization to increase productivity of chickpea.

## II. MATERIALS AND METHODS

Five species of *Trichoderma* (*viz.*, *T. viride, T. hamatum, T. lignorum, T. harzianum and T. koningii*), *Gliocladium virens, Aspergillus niger* and one bacterium, *Pseudomonas fluorescens* were sub-cultured from the stock culture of the department of Plant Pathology IGAU, Raipur. Also one isolate of each *T. viride* and *G. virens* obtained from Department of Plant Pathology, MAU, Parbhani, Maharashtra were used in the present study. These ten antagonists were evaluated for their antagonistic activity against the test fungi, *S. rolfsii, R. solani* and *F. oxysporum* f.sp. *ciceri*.

### *In vitro Testing of Antagonists by Dual Culture Technique*

Two isolate of *T. viride* (Raipur and Parbhani isolate), Two of *G. virens* (Raipur and Parbhani isolate), *T. hamatum, T. lignorum, T. koningii, T. harzianum, A. niger* and bacterium, *P. fluoresces* were screened for their

antagonistic activity in dual culture on Potato dextrose agar (PDA) in Petri plates. Twenty ml PDA medium was poured in each of the sterilized Petri plates. On solidification, 5 mm disc cut from the 7 days old culture of both the antagonist and the test fungus were inoculated separately on one half of the plate at the same time. For bacterial antagonist, sterilized blotter paper strip dipped in bacterial suspension and place on half medium and kept away. In control, antagonist was replaced with the test fungus. Each treatment was replicated thrice for every pathogen. All the plates were incubated at $25\pm2^0$C. Observations were made on the radial growth of the test fungus and antagonist when the fungus in control plate reached to rim of the plate. Number of sclerotia formed and their formation type also recorded after 15 days of incubation. The per cent growth inhibition of the test pathogen in presence of antagonist was calculated over control as bellow.

$$\text{Growth inhibition (\%)} = \frac{\text{Growth of test fungus in control plate} - \text{Growth of test fungus in presence of antagonist}}{\text{Growth of test fungus in control plate}} \times 100$$

## III. Results and Discussion

### *Dual Culture Technique (Under Laboratory Condition)*

The antagonistic activity of *Trichoderma* sp., *Aspergillus niger*, *Gliocladium virens* and *Pseudomonas fluorescens* isolated from native soil as well as *G. virens* and *T. viride* from Parbhani was studied under *in vitro* condition against *Sclerotium rolfsii* Sacc., *Rhizoctonia solani* Kuhn and *Fusarium oxysporum* f.sp. *ciceri* (Padwick) Snyder and Hansen by dual culture technique and the data presented in Table 1, Table 2 and Table 3, respectively.

It is evident from Table 1 that, maximum growth inhibition of *S. rolfsii* was observed with *P. fluorescens* (71.85%) followed by *T. harzianum* (65.56%), *T. viride* (R) (65.19%) and *G. virens* (R) (61.48%), it was minimum with *G. virens* (P) (31.11%) followed by *T. koningii* (34.81%), *T. viride* (43.33%) and *T. lignorum* (44.81%) (Plate 1a). Significant difference was observed within the treatment. Maximum inhibition of sclerotia production was observed with *T. harzianum* (99.46%) followed by *T. viride* (R) (95.80%), *G. virens* (R) (94.54%), *P. fluorescens* (93.65%) and *A. niger* (89.62%), it was minimum with *G. virens* (P) (49.37%). All the antagonists were effective in inhibiting the mycelial growth (31.11-71.85%) and sclerotial production (49.37 - 99.46%) over control. The *Pseudomonas fluorescens* as an important antagonist inhibiting the growth of *S. rolfsii* was also reported by several workers (Hebber *et al.*, 1991; Rangeshwaran and Prasad, 2000; Maheshwari *et al.*, 2002; Rangeshwaran *et al.*, 2002; Revathy and Muthusamy, 2003).

Data presented in Table 2 showed that 54.81 to 90.74 per cent inhibition of mycelial growth and 73.68 to 100 per cent inhibition of sclerotial production of *R. solani* were observed with all antagonists tested (Plate 1b). Maximum growth inhibition was observed with *G. virens* (P) (90.74%) followed by *P. fluorescens* (85.93%), *G. virens* (R) (64.44%), *T. viride* (R) (61.48%) and *T. hamatum* (59.63%), whereas 100 per cent inhibition of sclerotia production was observed with *P. fluorescens, G. virens* (R), *T. harzianum, T. hamatum* and *A. niger*. The *G. virens* showed inhibitory effect under *in vitro* condition against *R. solani* was also reported by Chung and Chung (1998), Prasad *et al.* (1999), Mukherjee and Tripathi (2000), Prasad and Rangeshwaran (2001) and Zeid *et al.* (2002).

Data (Table 3) revealed that all the antagonists were found to be significantly effective over control in inhibi-

-ting the mycelial growth of *F. oxysporum* f.sp. *ciceri*. These antagonists inhibited 62.22 to 87.03 per cent mycelial growth of the fungus. Maximum growth inhibition was observed with *T. viride* (R) (87.03%), *T. hamatum* (85.92%), *T. viride* (P) (84.81%), *A. niger* (82.95%), *T. harzianum* (81.11%), *T. koningii* (72.92%), *P. fluorescens* (70.36%), *G. virens* (P) (67.77%), *G. virens* (R) (62.22%) and *T. lignorum* (62.22%) (Plate 1c). The *T. viride* gave superior control against *F. oxysporum* by dual culture technique was also reported by several workers (Ushamalini *et al.*, 1997; Gupta *et al.*, 2003; Rao and Kulkarni, 2003; Sangle and Bambawale, 2004).

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Table 1. Mycelial and sclerotial inhibition of *Sclerotium rolfsii* by different antagonists (Dual culture technique).

| Antagonistic isolate | *Growth (mm) 5 DAI | | **Per cent growth inhibition | *Number of sclerotia formed (15 DAI) | **Per cent inhibition of sclerotia production |
|---|---|---|---|---|---|
| | *Sclerotium rolfsii* | Antagonist | | | |
| ***T. viride* (R)** | 31.34 | 58.67 | 65.19 (53.84) | 15.67 | 95.80 (78.24) |
| ***T. viride* (P)** | 51.00 | 39.00 | 43.33 (41.15) | 48.33 | 87.03 (68.89) |
| ***T. koningii*** | 58.67 | 31.34 | 34.81 (36.14) | 54.00 | 85.51 (67.63) |
| ***T. lignorum*** | 49.67 | 38.34 | 44.81 (42.01) | 41.67 | 88.82 (70.53) |
| ***T. harzianum*** | 31.00 | 59.00 | 65.56 (54.04) | 02.00 | 99.46 (86.02) |
| ***T. hamatum*** | 43.34 | 48.00 | 51.85 (46.04) | 69.00 | 81.48 (64.54) |

| | | | | | |
|---|---|---|---|---|---|
| ***G. virens* (P)** | 62.00 | 70.00 | 31.11 (33.88) | 188.67 | 49.37 (44.64) |
| ***G. virens* (R)** | 34.67 | 55.34 | 61.48 (51.62) | 20.33 | 94.54 (76.49) |
| ***A. niger*** | 44.00 | 46.00 | 51.11 (45.62) | 38.67 | 89.62 (71.20) |
| ***P. fluorescens*** | 25.34 | 74.67 | 71.85 (57.74) | 23.67 | 93.65 (75.39) |
| **Control** | 90.00 | - | - | 372.67 | |
| **SEm ±** | | | **0.4342** | | **0.6788** |
| **CD (5%)** | | | **1.28** | | **2.00** |

**Figures in parenthesis are Arcsine transformed values; DAI – Days after Inoculation;

* Average of three replication; R – Raipur isolates; P – Parbhani isolates.

Table 2. Mycelial and sclerotial inhibition of *Rhizoctonia solani* by different antagonists (Dual culture technique).

| **Antagonistic isolate** | ***Growth (mm) 5 DAI** | | ****Per cent growth inhibition** | ***Number of sclerotia formed (15 DAI)** | ****Per cent inhibition of sclerotia production** |
|---|---|---|---|---|---|
| | ***Rhizoctonia solani*** | **Antagonist** | | | |
| ***T. viride* (R)** | 34.67 | 55.34 | 61.48 (51.62) | 1.33 | 89.47 (71.10) |
| ***T. viride* (P)** | 38.34 | 51.67 | 57.41 (49.24) | 3.33 | 73.68 (59.12) |
| ***T. koningii*** | 39.00 | 51.00 | 56.67 (48.81) | 2.33 | 81.58 (65.73) |
| ***T. lignorum*** | 40.00 | 50.00 | 55.56 (48.17) | 1.33 | 83.47 (71.39) |
| ***T. harzianum*** | 38.34 | 51.67 | 57.41 (49.24) | 0.00 | 100.00 (89.39) |
| ***T. hamatum*** | 36.34 | 43.67 | 59.63 (50.53) | 0.00 | 100.00 (89.39) |
| ***G. virens* (P)** | 8.34 | 81.67 | 90.74 (72.26) | 2.67 | 78.95 (62.59) |
| ***G. virens* (R)** | 32.00 | 82.00 | 64.44 (53.38) | 0.00 | 100.00 (89.39) |
| ***A. niger*** | 40.67 | 49.34 | 54.81 (47.74) | 0.00 | 100.00 (89.39) |
| ***P. fluorescens*** | 12.67 | 77.34 | 85.93 (67.95) | 0.00 | 100.00 (89.39) |
| **Control** | 90.00 | - | - | 12.67 | |
| **SEm ±** | | | **0.3814** | | **1.7211** |
| **CD (5%)** | | | **1.13** | | **5.08** |

**Figures in parenthesis are Arcsine transformed values; DAI – Days after Inoculation;

* Average of three replication; R – Raipur isolates; P – Parbhani isolates.

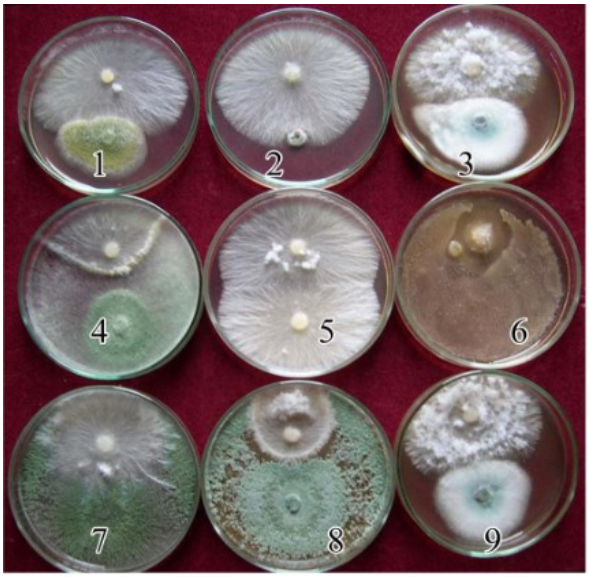
Table 3. Effect of different antagonists on radial growth (mm) of *Fusarium oxysporum* f.sp. *ciceri* by dual culture technique.

| **Antagonistic isolate** | ***Growth (mm) 8 DAI** | | ****Per cent growth inhibition** |
|---|---|---|---|
| | ***Fusarium oxysporum* f.sp. *ciceri*** | **Antagonist** | |
| ***T. viride* (R)** | 11.67 | 78.34 | 87.03 (68.88) |
| ***T. viride* (P)** | 13.67 | 76.34 | 84.81 (67.04) |
| ***T. koningii*** | 24.34 | 65.67 | 72.92 (58.64) |
| ***T. lignorum*** | 34.00 | 56.00 | 62.22 (52.06) |

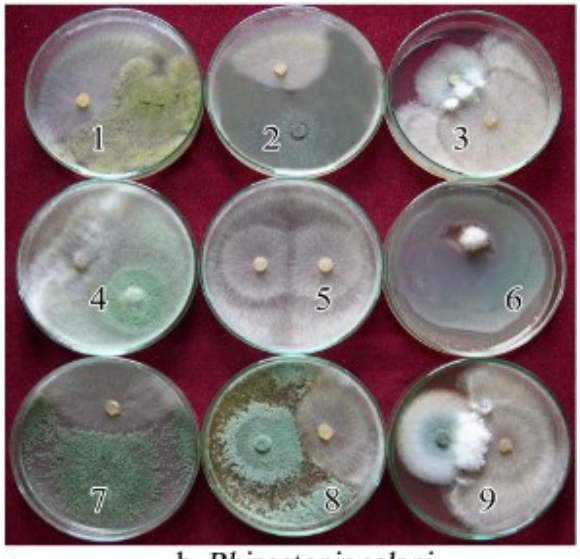
| | | | |
|---|---|---|---|
| ***T. harzianum*** | 17.00 | 73.00 | 81.11 (64.23) |
| ***T. hamatum*** | 12.67 | 77.34 | 85.92 (67.95) |
| ***G. virens* (P)** | 29.00 | 61.00 | 67.77 (55.40) |
| ***G. virens* (R)** | 34.00 | 56.00 | 62.22 (52.05) |
| ***A. niger*** | 15.34 | 74.67 | 82.95 (65.59) |
| ***P. fluorescens*** | 26.67 | 75.34 | 70.36 (56.99) |
| **Control** | 90.00 | - | - |
| **SEm ±** | | | **0.4683** |
| **CD (5%)** | | | **1.38** |

**Figures in parenthesis are Arcsine transformed values; DAI – Days after Inoculation;

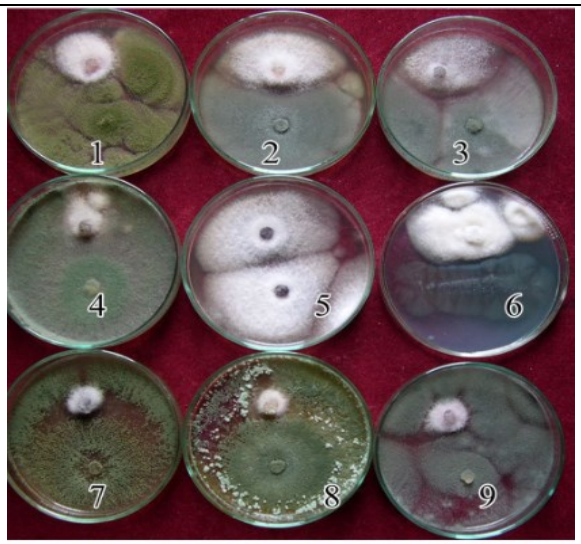
* Average of three replication; R – Raipur isolates; P – Parbhani isolates.




a. *Sclerotium rolfsii*




b. *Rhizoctonia solani*




c. *Fusarium oxysporum* f.sp. *ciceri*

Plate 1. Mycelial inhibition of wilt complex fungi by different antagonists.