J. Indian bot. Soc. ISSN - 0019 - 4468 Vol. 93 (1 & 2) 2014 : 111-113

# ANTIMYCOTIC ACTIVITIES OF SOME RHIZOSPHERE FUNGI AGAINST *MYROTHECIUM RORIDUM* CAUSING LEAF SPOT OF COTTON

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A study was undertaken to investigate the antimycotic activities of some rhizosphere fungi of cotton against *Myrothecium roridum*. A total of five species viz. *Aspergillus flavus, A.niger,Trichoderma viride,T.harzianum* and *P.oxalicum* were tested for antimycotic activity against *Myrothecium roridum* by dual culture method in *in vitro*. There was little difference among the rhizosphere fungi in causing inhibition of the pathogen after three days of inoculation in dual culture, but significant difference was observed on 5$^{th}$ days of incubation. Fast growing antagonist was found to be most promising than slow growing antagonist. Among the five antagonists tested *in vitro* against *Myrothecium roridum* , *T.harzianum* was found to be most effective as compared to others.

**Key words:** Antimycotic, cotton, *Myrothecium* ,rhizosphere, *Trichoderma, Penicillium.*

The constant use of fungitoxic chemicals adds to the environmental pollution, consequently, efforts are under way for finding alternatives to chemical fungicides. Microorganisms which establish in the rhizosphere are ideal candidates for use as biocontrol agent, since rhizosphere provides the front line defense for root against attack by pathogens (Montensions *et al.*2002).The control of plant disease by chemicals has been subject of public concern and security due to the possible harmful effect on the environment and their undesirable effect on the non-target microorganisms (Gerlagh *et al.* 1999).

Biological management is the control of microorganisms by another, which reduces the inoculums density of disease producing activities of pathogen by one or more organisms. Application of biological agents for reduction of diseases is an integral component of cultivation. Weindling (1932) started the first work on *Trichoderma* and discovered that *T.lignorum* controls the growth of number of soil borne fungi. *Trichoderma* and their strains or isolates are known to differ in their potential to control the disease (Venkat Kota, 2006).*Trichoderma* are ubiquitous, soil borne,green-spored ascomycetes with multiple attributes in biocontrol,enzyme production and novel antibiotics (Siameto *et al.* 2010).

Biocontrol is one of the advanced technique utilized in agricultural field, which is found to be more selective and with no side effects. It is cheap, Biological agents are self propagating and self perpetuating there is no risk of resistance and are non-hazardous. The biocontrol fungi have a potential to restrict other fungi and arrest of pathogen achieved by way of antibiosis, mycoparasitism and competition.

The main aim of the present study was to evaluate the antimycotic properties of some rhizosphere fungi against *Myrothecium roridum* causing leaf spot cotton.

## MATERIALS AND METHODS

### a) Isolation of Pathogen :

Infected leaves of cotton were collected from various cotton fields in Amravati region. *Myrothecium roridum* was isolated from diseased leaves of various cotton varieties and maintained on (Potato Dextros Agar) PDA slants. PDA have been selected to maintain pure cultures of isolates in the research laboratory. The isolates viz. *Myrothecium roridum, Aspergillus flavus ,A.niger,Trichoderma harzianum,T.viride and Penicillium oxalicum* were identified from available literature. Pathogenecity test was confirmed in the laboratory on potted cotton plants by Koch's postulate method.

### b) Isolation of Antagonists :

Antagonistic fungi were collected from the rhizosphere of cotton plants during the rainy season by serial dilution method (Waksman,1927). Only five fungi were selected for antagonistic study viz. *Asper-*

Received on October 27,2012 Accepted on December 19, 2013

*gillus flavus, A.niger, Trichoderma harzianum, T.viride* and *Penicillium oxalicum*.

**c) Antagonistic Study :**

The dual culture method was adopted to observe the antimycotic effect of different isolates of the test antagonists. Test antagonists were screened against the pathogen *Myrothecium roridum* in dual culture on potato dextrose agar medium in petriplates. Autoclaved PDA medium was poured into petriplates and allowed to solidify. 5 mm disc of pathogen was kept in centre of petriplate while antagonists were inoculated 4 cm apart from it on PDA. In control only disc of the pathogen was inoculated. The petriplates were inoculated at $27.2^0$ C. The radial mycelial growth of colony in diameter were measured on $3^{rd}$,$5^{th}$ and $7^{th}$ days after incubation. Mycelial diameter of each treatment was compared with control plates. The percent inhibition of mycelial growth was calculated by using formula-

$$\text{Per cent inhibition} = \frac{TFC - TFTr}{TFC} \times 100$$

Where, TFC= Test fungus in control
TFTr= Test fungus in treatment.

## RESULTS AND DISCUSSION

The data from Table 1 showed that *Trichoderma harzianum* was more effective which control the radial mycelial growth of the pathogen *Myrothecium roridum* upto 58.33 percent on $3^{rd}$ day of incubation followed by *Trichoderma viride* (38.88%). *Aspergillus flavus* and *Penicillium oxalicum* showed less than 31 percent growth inhibition. *Aspergillus niger* was found to be least effective (16.16%) on $3^{rd}$ day of incubation. *Trichoderma harzianum* showed 100 per cent growth inhibition on $5^{th}$ day of incubation followed by *Aspergillus flavus*(97.22%), *Penicillium oxalicum*(95.45%), *Trichoderma viride*(93.18%) and *Aspergillus niger* (84.09%). But as the days progressed in incubation, all the five antagonistic fungi showed 100 per cent growth inhibition upto $7^{th}$ day of incubation. The radial mycelia growth of pathogenic fungi *Myrothecium roridum* was inhibited more effectively since $3^{rd}$ day of incubation by *Trichoderma harzianum*.

From the present investigation it is clear that *Trichoderma harzianum* showed maximum growth inhibition right from the beginning as compared to other four tested antagonistic fungi. Upadhyay and Mukhopadhyay (1986) noted that *Trichoderma harzianum* produces extracellular enzymes $\beta(5,3)$ gluconase and chitinase which are capable of degrading cell walls of pathogenic fungi. Sonawane and Pawar (2001) reported that *Trichoderma harzianum* was highly effective in controlling the vegetative growth of the pathogen.

Arumugam *et al*.(2003) studied the antagonistic effect of *Trichoderma harzianum,* and isolated the enzyme

**Table 1** : Effect of different antagonists on radial mycelial growth of *Myrothecium roridum.*

| Sr. No. | Antagonistic fungi | Radial mycelial growth (mm)* | | | % growth inhibition | | |
|---|---|---|---|---|---|---|---|
| | | 3 DAI | 5 DAI | 7 DAI | 3 DAI | 5 DAI | 7 DAI |
| 1 | *Aspergillus flavus* | 25.00 | 1.00 | 0.00 | 30.55 | 97.72 | 100.00 |
| 2 | *Aspergillus niger* | 30.00 | 7.00 | 0.00 | 16.66 | 84.09 | 100.00 |
| 3 | *Trichoderma viride* | 22.00 | 3.00 | 0.00 | 38.88 | 93.18 | 100.00 |
| 4 | *Trichoderma harzianum* | 15.00 | 0.00 | 0.00 | 58.33 | 100.00 | 100.00 |
| 5 | *Penicillium oxalicum* | 25.00 | 2.00 | 0.00 | 30.55 | 95.45 | 100.00 |
| 6 | Control | 36.00 | 44.00 | 51.00 | - | - | - |

*****Mean of three replicates. **DAI** - Days After Incubation.

chitosanase. They pointed that the enzyme chitosanase inhibited the conidial germination of *Myrothecium verrucaria* .Therefore it is clear that enzyme secreted by the antagonist shows potential antimycotic property which inhibits the growth of pathogen. Similar findings have been observed by Brozova (2004) and Gupta (2004).

Chindambaram *et al*.(2004) also found that bioagents like *Trichoderma* were significantly effective for controlling cotton diseases.

The present study indicates that management of diseases by potential biocontrol agent like *Trichoderma harzianum* is effective against *Myrothecium roridum.* It is eco-friendly, non-hazardous and safe to environment.

The author is grateful to Dr. B.T Raut, Ex- Professor and Head, Department of Plant Pathology, Dr. P.D.K.V., Akola; Dr.D.M.Sawant (Associate Dean) College of Agriculture, Pune and Dr.A.U.Pachkhede, Professor and Head, Department of Botany, B. B .Science College, Amravati for constant inspiration, encouragement and healthy support.

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