Research Paper :

Influence of temperature and pH on antagonistic potential of *Trichoderma viride in vitro*

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SUMMARY

Effects of temperature and pH were determined on antagonistic potential of *Trichoerma viride* against *Sclerotium rolfsii* and *Rhizoctonia solani in vitro*. *T. viride* showed maximum antagonistic potential against *S. rolfsii* and *R. solani* at 25 to 30^{0}C which was indicated by greater colonization and growth of *T. viride* over *S. rolfsii* and *R. solani*. At 25 to 30^{0}C *T. viride* significantly checked the growth of *S. rolfsii* and *R. solani* and inhibited the growth of the pathogen and lost antagonistic potential at high temperature (35 to 45^{0}C). On the other hand, *S. rolfsii* and *R. solani* inhibited the growth of *T. viride* at high temperature (35 to 40^{0}C). Similarly, the most favourable pH for maximum antagonistic potential of *T. viride* against *S. rolfsii* and *R. solani* ranged between 5.5 to 6.5. *T. viride* showed maximum antagonistic potential against these two pathogens at 6.0 pH. Antagonistic potential of *T. viride* declined with decreasing in pH (below 4.5) as well as at high pH (above 7.5). This study revealed that 25 to 30^{0}C temperature and 5.5 to 6.0 pH were found to be optimal for antagonistic potential of *T. viride.* It can be concluded that many soils borne and seed borne fungal diseases can be controlled by using *Trichoderma* spp., especially in the *Kharif* and *Rabi* seasons when soil temperature ranges between 25 to 30^{0}C.

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Trichoderma species are known mycoparasites on several plant pathogens especially against soil-borne plant pathogens, (Papavizas, 1985). Köhl and Schlosser (1989) observed that only selected strains could tolerate extreme temperatures. Biocontrol agents may respond differentially to varied soil conditions. For example a soil-moisture-deficit beyond – 4.54 bars affected sporulation of *T. viride,* but not *T. harzianum* (Cole and Zvenyika, 1988). The antagonistic potential of *Trichoderma* spp. against *Fusarium udum* was not much altered by changing the environmental conditions. However, it was maximum at 35^{0}C ± 2 and pH 6.5 over a wide range of C/N ratios, (Spiegel *et al.,* 1991). The effectiveness of biocontrol agents depends on several parameters, that includes soil texture, water content, pH and crop history (Hagn *et al*., 2003; Berg *et al*., 2005); therefore their application should consider the environmental stress that could affect not only their survival in the soil, but also their ability to maintain their biocontrol capacity. A series of abiotic and biotic environmental parameters has an influence on the biocontrol efficacy of *Trichoderma*. Some important parameters to be considered are the effects of temperature, pH, water potential, the presence of pesticides, metal ions and antagonistic bacteria in the soil. The pH characteristics of the soil also belong to the most important environmental parameters affecting the activities of mycoparasitic *Trichoderma* strains. The agricultural importance of the genus is that some *Trichoderma* species possess good antagonistic abilities against plant pathogenic fungi, *e.g*. *Fusarium* (Sivan and Chet, 1986), *Pythium* (Naseby *et al.,* 2000) *Rhizoctonia* (Lewis and Papavizas, 1987*).* Studies are available on the effects of temperature on the spore germination and germ-tube growth (Magan, 1988), mycelial growth (Samuels, 1996), competitive saprophytic abilities (Naar and Kecskes, 1998) of *Trichoderma* strains. The optimum temperature for growth differs among the *Trichoderma* species (Samuels, 1996). One of the most important limitations of the use of *Trichoderma* strains as biofungicides is their low osmotolerance level. Biocontrol *Trichoderma* strains are applied in agricultural soils with certain pH-characteristics. Therefore, it is important to collect information about the effects of pH on mycelial growth and sporulation of *Trichoderma* strains with biocontrol potential. pH can also play a role in the regulation of extracellular enzyme

production, as it was demonstrated by Delgado *et al.* (2000). *Trichoderma* strains were able to grow in a wide range of pH from 2.0 – 6.0 with an optimum at 4.0. However, the mycelial growth of some of the examined plant pathogenic fungi had pH-optima at alkalic values.

The effect of low temperatures (Antal *et al.,* 2000), water activity and pH (Kredics *et al.,* 2000; Kredics *et al*., 2004; Begoude *et al.,* 2007), have been tested as stress factors that may affect wild or mutant biocontrol *Trichoderma* strains. Based on these previous studies an investigation was carried out to assess the optimum temperature and pH for maximum antagonistic potential of *T. viride* against *S. rolfsii* and *R. solani in vitro.*

MATERIALS AND METHODS

Isolation and maintenance of pathogens:

S. rolfsii and *R. solani* were isolated from infected soybean plants and rhizospheric soil samples. The diseased soybean plants and soil samples were collected from soybean growing areas of Latur, Nanded, and Solapur districts of Maharashtra state. Field survey was conducted during *Kharif* 2008 and 2009.

Isolation from infected plants :

A small bit of 5 mm diameter was carefully excised from infected plant parts, surface sterilized with 0.1% $HgCl_2$ and transferred onto potato dextrose agar (PDA) in Petri dishes. These plates were incubated for 5 days at 28^{0}C in BOD. After 5 days of incubation, pure culture of *S. rolfsii* and *R. solani* was obtained by hyphal tip culture and maintained on PDA slants at 4^0 C for further studies.

Isolation from soil:

Hundred mg soil samples was finely powdered and stirred in one ml sterile distilled water and serial dilutions were prepared and from 10^3 dilutions, 100 ìl was spread in Petri dishes containing on potato dextrose agar medium in three replicates. The plates were incubated at 28^{0}C for 4 days in BOD, and typical colonies of *S. rolfsii* and *R. solani* were isolated and sub-cultured on PDA medium. Twenty one isolates of *S. rolfsii and* 17 isolates of *R. solani* were obtained. Out of these isolates, the most virulent isolate of *S. rolfsii (*SR-07), and *R. solani* (RS-9*)* were used in the present study.

Isolation and maintenance of *Trichoderma* species:

Thirty seven isolates of *Trichoderma* spp. were isolated from soybean growing areas of Latur, Nanded, and Solapur districts of Maharashtra state. These isolates were identified as *T. viride, T. harzianum T. hamatum, T. koningii* and *T. reesei*. Most promising and antagonistic isolates of *T. viride* (TV-19) was used in the present study.

Effect of temperatures on antagonistic potential of *T. viride* against *S. rolfsii* and *R. solani:*

This was done by dual culture technique using 20 ml of potato dextrose agar medium in 90 mm culture plates. The agar medium in the culture plates is seeded with the potential antagonist and test pathogen (5 mm culture disks of five days old culture) opposite each other near the periphery of petriplates. The medium inoculated with the antagonists and pathogen alone served as control. These plates were incubated in BOD at 10, 15, 20, 25, 30, 35, 40, and 45^{0}C and 70% relative humidity. The plates incubated at 28^{0}C were served as control. Five replications were maintained at each temperature. After 72 hrs of inoculation observations were recorded on antagonism and inhibition zone by both antagonists and test pathogens.

Effect of pH on antagonistic potential of *T. viride* against *S. rolfsii* and *R. solani:*

Twenty ml of potato dextrose agar medium in 90 mm culture plates with different pH *viz*., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 was poured. Dual culture of the potential antagonists and test pathogens commonly is used. The agar medium in the culture plates is seeded with the potential antagonist and test pathogen (5 mm culture disks of five days old culture) opposite each other near the periphery of petriplates. The medium inoculated with the antagonists and pathogen alone served as control. These plates were incubated in BOD at 28^0 C and 70% relative humidity. Five replications were maintained at each pH. The plates incubated at 7.0 pH were served as control. After 72 hrs of inoculation observations were recorded on antagonism and inhibition zone by both antagonists and test pathogens.

RESULTS AND DISCUSSION

The findings in present investigation indicated that *T. viride, S. rolfsii* and *R. solani* have different temperature optima for growth. *T. viride* showed 25-30^{0}C optimum temperature for the antagonist potential against S. *rolfsii,* and *R. solani*. In dual culture, *T. viride* overgrew *S. rolfsii* and *R. solani* at 25^{0}C to 30^{0}C, but at 35^0 C to 40^0 C, *S. rolfsii* and *R. solani* overgrew the colony of *T. viride*. This study indicates that *T. viride* was very effective in suppressing the growth of *S. rolfsii* and *R. solani* at 25 to 30^0 C temperature. Similarly the most favorable pH for maximum antagonistic potential of *T. viride* against *S. rolfsii* and *R. solani* ranged between 5.5 to 6.5. *T. viride* showed maximum antagonistic

potential against *S. rolfsii* and *R. solani* at 6.0 pH where *T. viride* grew very fast over the *S. rolfsii* and also check the growth. Antagonistic potential of *T. viride* declined with decreasing in pH (below 4.5) as well as at high pH (above 7.5). This study revealed that 25 to 30^{0}C temperature and 5.5 to 6.0 pH were found to be optimal for antagonistic potential of *T. viride* against *S. rolfsii* and *R. solani* (Table 1 and 2; Fig.1). The results of this study about the effects of temperature and pH on antagonistic potential of *Trichoderma* strains may be useful information for applicability of biocontrol strains in agricultural soils with 25 to 30^{0}C temperature and 5.5 to 6.5 pH. It can be concluded that many soils borne and seed borne fungal diseases can be controlled by using *Trichoderma* spp., especially in the *Kharif* and *Rabi* season when soil temperature ranges between 25 to 30^{0}C. Another important information can be reported from the present investigation that a *T. viride* check the formation

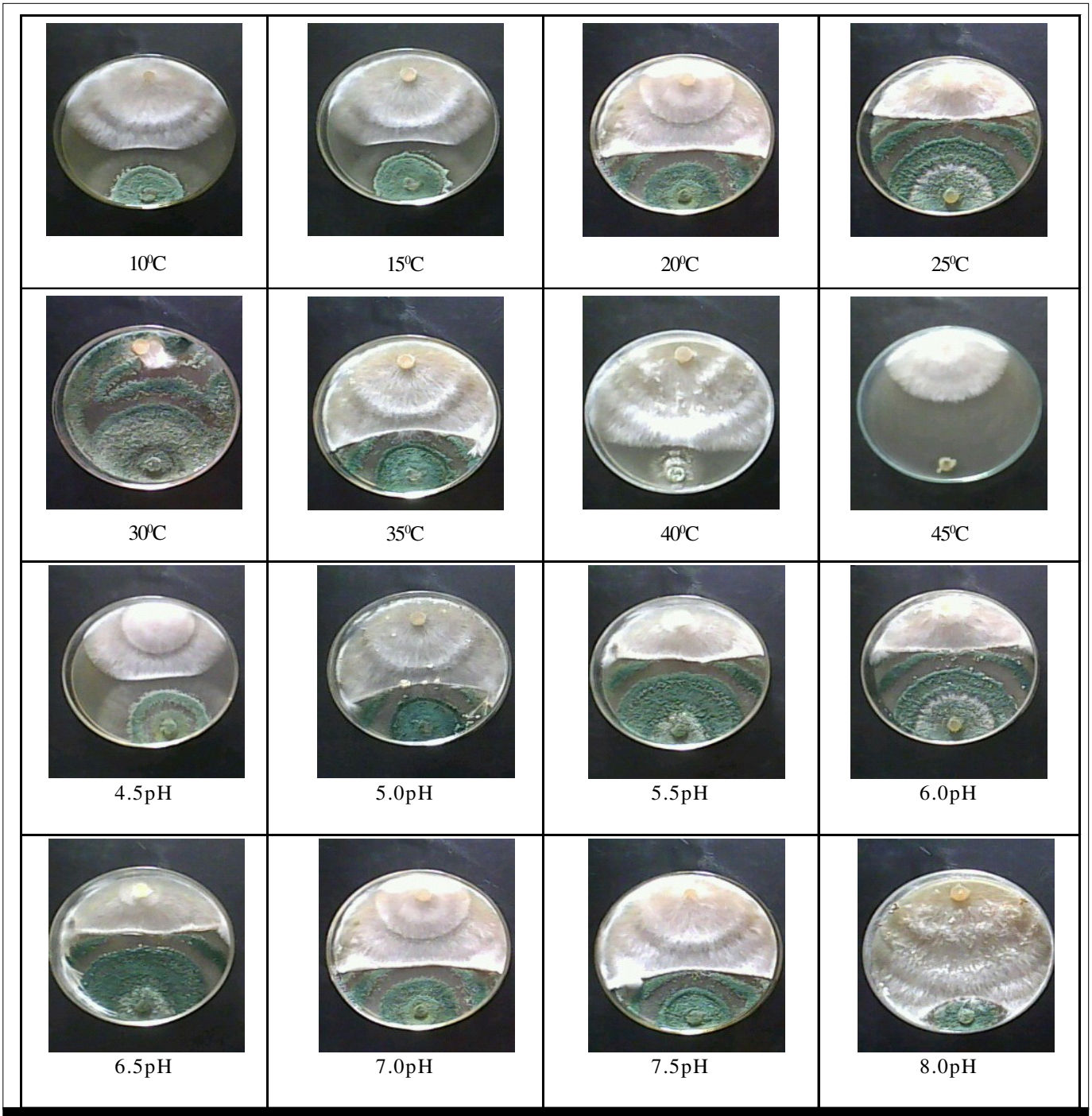

Fig. 1 : Inhibition of *S. rolfsii* and *R. solani* by *T. viride* at different temperature and pH

Table 1 : Percentage inhibition of *S. rolfsii* and *R. solani* by *T. viride* at different temperatures

Temperature (ºC)	Percentage inhibition by *T. viride**	
	S. rolfsii	*R. solani*
10	17.7	21.74
15	20.7	27.59
20	37.1	41.93
25	76.4	77.68
30	80.0	82.33
35	28.5	25.58
40	11.0	13.51
45	0.0	0.00
C.D. (P=0.05)	0.53	0.62
C.V. %	5.89	6.57

*Mean of five replications

Table 2 : Percentage inhibition of *S. rolfsii* and *R. solani* by *T. viride* at different pH

pH	Percentage inhibition by *T. viride**	
	S. rolfsii	*R. solani*
4.0	32.9	33.9
4.5	37.7	40.7
5.0	46.7	45.1
5.5	72.0	76.7
6.0	77.6	83.0
6.5	59.6	54.1
7.0	57.5	51.6
7.5	31.5	31.2
8.0	19.7	20.0
C.D. (P=0.05)	0.53	0.56
C.V. %	4.49	4.74

*Mean of five replications

of sclerotia by *S. rolfsii*.

Sankar and Jeyarajan (1996) reported that temperature of 20-30°C was optimum for the storage of the formulation at which even after 75 days and the product contained 206-271x10^6 cfu/g. In general, *Trichoderma* spp., are favored by acidic soil conditions (Chet and Baker, 1981; Papavizas, 1985). These observations confirm results obtained by Kredics *et al*. (2000; 2003) and Lupo *et al*. (2002), who classified *Trichoderma* spp. as a mesophilic organism. These results also agree with those obtained by Kucuk and Kivanc (2005) where the recovery of different *Trichoderma* strains correlated well with temperature. Development of *Trichoderma* strains was better when incubated at 24^{0}C than at 15^{0}C (Knudsen and Bin, 1990). The production and activity of antibiotics is decreasing with the increase of soil pH (Howell, 1998).

Conclusion:

– *T. viride* was more effective in reducing the growth of against *S. rolfsii* and *R. solani* at 25 to 30^{0}C.

– This study revealed that 5.5 to 6.0 pH were found to be optimal for antagonistic potential of *T. viride* against *S. rolfsii* and *R. solani.*

– Soil borne and seed borne fungal diseases can be controlled by using *Trichoderma* spp., especially in the *Kharif* and *Rabi* season when soil temperature ranges between 25 to 30^{0}C.

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