SHORT COMMUNICATION

Factors affecting mycoparasitism of *Sclerotinia sclerotiorum* by *Trichoderma* spp.

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ABSTRACT: Studies on investigating the factors favoring mycoparasitism of *Sclerotinia sclerotiorum* by *Trichoderma* spp. indicated that both temperature and pH have a significant effect on mycoparasitism. *Trichoderma viride* at 15°C and *T. harzianum* (Th3) at 25°C were most effective in inhibiting mycelial growth of 86.90 percent and 78.96 percent respectively. In the case of pH, Th3 was inhibited up to 92 per cent growth of *S. sclerotiorum* at pH 5 while at pH 7 it was inhibited up to 79 per cent of growth of pathogen. In a combination of different temperature and pH, Th3 inhibited up to 81, 73 and 60 per cent growth at pH 5, 7 and 9 and Temperature at 15°C respectively. At 25°C Th3 inhibited 86, 82 and 55 per cent growth of pathogen at pH 5, 7 and 9 respectively. Th3 was very effective at temperature of 15°C, 25°C as well as pH of 5 and 7 against *S. sclerotiorum* by inhibiting their mycelial growth.

Keywords: Biocontrol agents, mycoparasitism, *Sclerotinia sclerotiorum*, *Trichoderma* spp.

White rot of mustard caused by *Sclerotinia sclerotiorum*, has become important disease in India as well as in world. It can cause up to 40 per cent yield losses leading to discouragement of among growers to cultivate the crop (Mukherjee, 2015). Eco friendly management is one among the best approach in different management practices, which is sound effective and safe to environment. *Trichoderma* species (Teleomorph: *Hypocrea*) is a filamentous ascomycetous fungus widely used as biocontrol agent (BCA) against many plant pathogens. It includes different economically important species viz., *T. harzianum*, *T. asperellum*, *T. viride*, *T. atroviride*, *T. virens* and *T. reesei* (Sharma et al., 2014). *Trichoderma* antagonize many phytopathogenic fungi, bacteria and invertebrates through mechanism of hyperparasitism, antibiosis, competition and induced systemic resistance. Mycoparasitism is the most considered and direct form of antagonism. It involves tropic growth of biocontrol agent towards the target organism (Arunachalam and Sharma, 2012). Mycoparasitism by *Trichoderma* is affected by many soil and environmental parameters, among these temperature and pH are the two most important. The optimum temperature and pH for growth differs among the *Trichoderma* species. Most *Trichoderma* strains are mesophilic and grow well at neutral to slightly acidic pH (Huang and Erickson, 2008). Understanding the factors favoring mycoparasitism of *Sclerotinia sclerotiorum* by *Trichoderma* spp. is essential for better management of white rot disease of mustard.

Pathogen cultures

Survey of major mustard growing areas was conducted

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during the Rabi crop season (2013-14) at post-flowering to pod formation stage of the crop, to record stem rot incidence and samples were collected from New Delhi, Rajasthan, Himachal Pradesh and Pantnagar. The fungal pathogens were isolated, identified and purified on PDA plates and were further grouped into four different isolates viz., SS26, SS41, SS43, SS40 on the basis of morphology, cultural characteristics and sclerotial bodies. Cultures were grown in potato dextrose agar plates and incubated at 28±2°C for 5-7 days for further studies.

Trichoderma cultures

Trichoderma spp. viz., *T. harzianum* (Th3) - ITCC-5593, *T. viride* - ITCC-8315, *T. atroviride* - ITCC-7445, *T. asperellum* - ITCC-8940 and *T. longibrachiatum* - ITCC-7444 from ITCC. *T. konongi* and *T. virens* from Biological Control Laboratory, Division of Plant Pathology, IARI, New Delhi and *T. harzianum* (Th5) was collected from IARI field and isolated were used for this study. Among these, three spp. viz., *T. harzianum* (Th3), *T. viride* and *T. harzianum* (Th5) were selected for this particular study because these three were found more effective in comparison to the others in previous study by Arunachalam and Sharma (2012). Cultures were grown in potato dextrose agar plates and incubated at 28±2°C for 5-7 days for dual plate assay.

In vitro evaluation

Antagonistic potential of *Trichoderma* spp. was studied by dual culture assay against *S. sclerotiorum*. The pathogen inoculated PDA plates alone served as a control (Dennis and Webster, 1971). Three replications

of the each culture plates were incubated at different temperature level i.e. 15°C, 25°C and 35°C for measuring colony growth both in control and dual culture plates. The plates were evaluated based on the grading method (Bell *et al.*, 1982). The radial growth of the pathogen was measured after full growth and the percent inhibition was calculated as follows: $PI = (C - T) \times 100/C$, where PI is the percent inhibition of mycelial growth; C is the radial growth of pathogen in control plates (cm) and T is the radial growth of pathogen in dual culture (cm). To study the effect of pH on mycoparasitism, PDA medium was prepared and autoclaved. pH of media was adjusted to 5, 7 and 9. On the media, the pathogen and selected species of *Trichoderma* were dual cultured and kept at 25°C. Three replications for each *S. sclerotiorum* isolates with each *Trichoderma* spp. were kept. These dual cultured plates were kept for seven days and observed for growth of pathogen. The radial growth of pathogen in treatment and control were measured and percentage mycelium growth inhibition was calculated by using previously mentioned formula. To study the effect of combination of temperature and pH, media of different pH (5, 7 and 9) were prepared and the pathogen and selected species of *Trichoderma* were dual cultured and kept at different temperature level of 15°C, 25°C and 35°C. The radial growth of pathogen in treatment and control were measured and percentage mycelium growth inhibition was calculated by using previously mentioned formula.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using OPSTAT software (www.hau.ernet.in/about/opstat.php). Differences between treatment mean values were determined following least significant difference (LSD) test at $P < 0.05$.

Effect of different temperature level on mycoparasitic activity

Temperature has a direct effect on growth of *Trichoderma*, *S. sclerotiorum* and mycoparasitism activity of *Trichoderma*. The results indicated that at different temperature level, *Trichoderma* mycoparasitize *S. sclerotiorum* in different manner. At 15°C temperature all the three species of *Trichoderma* inhibited all the isolates of *S. sclerotiorum*. *T. viride*, Th3 and Th5 inhibited 86.90, 80.98 and 72.57 per cent growth of SS26 respectively and indicate that *T. viride* was better than other two *T. harzianum* isolates. At 25°C temperature both the pathogen and *Trichoderma* grew very well and rapidly. At this temperature *T. viride*, Th3 and Th5 inhibited 68.96, 75.97 and 66.18 per cent growth of *S. sclerotiorum* isolate SS26 respectively. At 35°C growth of all *Trichoderma* spp. was good but most of isolates of *S. sclerotiorum* were unable to grow. At this temperature per cent inhibition of *S. sclerotiorum* was observed to be from 83.33 per cent to 90 per cent. The very poor growth of *S. sclerotiorum* at 35°C was due to effect of high temperature. The results on effect of different temperature level on mycoparasitism of *S. sclerotiorum* by *Trichoderma* spp. indicate that at temperature 15°C and 25°C *T. viride* and Th3 were significantly efficient (Fig. 1).

Effect of different pH level on mycoparasitic activity

Results on effect of different pH levels on mycoparasitism of *S. sclerotiorum* by *Trichoderma* shows that at pH 5 all the *Trichoderma* spp. inhibited all the isolates of *S. sclerotiorum* ranging from 80.85 to 92.91 per cent. At this pH all the *Trichoderma* spp. grew very well but isolates of *S. sclerotiorum* were unable to grow effectively. *T. viride*, Th3 and Th5 inhibited 86.73, 92.12 and 84.29 per cent growth of *S. sclerotiorum* isolate SS26 respectively. At pH 7 both *Trichoderma* and *S. sclerotiorum* grow very well. *T. viride*, Th3 and Th5 inhibited 69.71, 75.53 and 66.40 per cent growth of *S. sclerotiorum* isolate SS26 respectively. At pH 9 isolates of *S. sclerotiorum* grew very well but *Trichoderma* spp. grew slowly and growth inhibition of *S. sclerotiorum* isolates by *Trichoderma* spp. recorded between range of 30.73 to 43.59 per cent (Fig. 2). At pH 9 *T. viride*, Th3 and Th5 inhibited 37.92, 42.63 and 35.15 per cent growth of *S. sclerotiorum* isolate SS26 respectively.

Effect of combination of temperature and pH on mycoparasitic activity

Results shows that at different combination of temperature and pH levels *Trichoderma* inhibited growth of *S. sclerotiorum* isolates differently. At 15°C temperature *Trichoderma* spp. reacted differently at different pH against *S. sclerotiorum*. At pH 5, 7 and 9 *T. harzianum* (Th3) inhibited 81.30, 73.24 and 59.54 per cent growth of the isolates of *S. sclerotiorum* (Table 1). So at 15°C temperature Th3 was efficient in controlling all the isolates of *S. sclerotiorum* at different pH levels. At 25°C Th3 was good in controlling *S. sclerotiorum* at different pH levels.

Fig. 1. Percent inhibition activity of *Trichoderma* spp. at different temperature against SS26 isolate of *S. sclerotiorum*

Fig. 2. Percent inhibition activity of *Trichoderma* spp. at different pH against SS26 isolate of *S. sclerotiorum*

Table 1. Combined effect of temperature and pH on mycoparasitic activity of *T. harzianum* (Th3) against SS26 isolate of *S. sclerotiorum*

Temperature	Inhibition of mycelia growth (%)		
	pH Levels		
	5	7	9
15°C	81.3 (64.3)	73.2(58.8)	59.5 (50.4)
25°C	86.3 (68.3)	81.5 (64.5)	54.9 (47.8)
35°C	90.0 (71.6)	83.9 (66.3)	65.0 (53.7)
Factors	C.D.	SE(d)	
Temperature(A)	1.61	0.76	
pH(B)	1.61	0.76	
Factor(A X B)	2.80	1.32	

*Value in parenthesis representing arc sin transformed value

At pH 5, 7 and 9 Th3 inhibited 86.34, 81.56 and 54.96 per cent growth of the isolates of *S. sclerotiorum*. At 35°C the growth of *S. sclerotiorum* was not so effective but results of growth inhibition varied at different pH levels. At pH 5 Th3 inhibited 90.04 per cent growth of *S. sclerotiorum*. At pH 7 and 9 it's inhibited 83.97 and 65.02 per cent growth of *S. sclerotiorum*, respectively.

White rot caused by *S. sclerotiorum* is an important disease of mustard crop. Biological methods of disease management are ecologically and economically safe. The optimum temperature for growth differs among the *Trichoderma* species. Most *Trichoderma* strains are mesophilic. *T. viride* was the most abundant species in early spring and autumn in a spruce forest soil. Abiotic and biotic environmental parameters may have negative influence on the biocontrol efficacy of *Trichoderma* strains (Bandyopadhyay *et al.*, 2003; Kredics *et al.*, 2004). *T. asperellum*, TaDOR673 was highly tolerant to heat shock of 52°C with a mean spore count (log c.f.u/ml) of 4.33 (Poosapati *et al.*, 2014). *T. harzianum* isolates grew well at temperature 20°C, pH 5 and molasses agar medium (Meena *et al.*, 2014). As compare to these, results of present study also showed that *T. harzianum* (Th3) grew very well at 15°C and 25°C and inhibited the *S. sclerotiorum* very effectively which is also a low temperature loving pathogen. *T. viride* also showed very good results at 15°C and 25°C. At 35°C pathogen growth was not observed in dual culture plates as well as in control. The absence of growth of pathogen at 35°C was mainly due to the effect of temperature and it is not due to mycoparasitic activity of *Trichoderma*.

Both *Trichoderma* spp. and *S. sclerotiorum* are influenced by different levels of pH. Mycoparasitic activity of *Trichoderma* changes at different level of pH. *Trichoderma* strains were able to grow at 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 alkali values (Kredics *et al.*, 2004). Jackson *et al.* (1991) have found that optimum biomass production of three *Trichoderma* isolates occurred at pH ranges between 4.6 and 6.8. Similarly, our studies also showed that at all the three pH (5, 7 and 9) *T. harzianum* (Th3) grew very well and inhibited the *S. sclerotiorum*. At pH 5 and 7, *T. viride* also

showed good results by inhibiting pathogen up to 86.73 per cent and 69.71 per cent, respectively. At pH 9 both *T. harzianum* (Th3) and *T. viride* showed pathogen inhibition upto 43.59 and 40.04 per cent which is not considered to be effective as biocontrol agents.

The outcome of the study on combination of temperature and pH levels showed that at 15°C, *T. harzianum* (Th3) was most effective at all the three pH levels. It parasitizes the pathogen very effectively at pH 5 and 7 but at pH 9 it inhibited the pathogen up to 59.54 per cent. Temperature of 25°C favored the growth of both pathogen and *T. harzianum* (Th3) at pH 5 and 7 but at pH 9 the growth was suppressed. At 35°C growth of *Sclerotinia* isolates was suppressed at pH 5 and 7 but at pH 9 the pathogen grew very well in the control plates but in dual culture plates it showed that pathogenic growth was inhibited up to 65% by *T. harzianum* (Th3).

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