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EFFECT OF THE METABOLITE OF *TRICHODERMA* SPP. ON THE GERMINATION OF SCLEROTIA AND GROWTH OF *SCLEROTIUM ROLFSII* SACC. AND PRODUCTION OF PECTOLYTIC AND CELLULOLYTIC ENZYMES

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The effect of the metabolite of three spp. of *Trichoderma* was observed on the germination of sclerotia and growth of *Sclerotium rolfsii,* isolated from tomato fruit, besides the production of pectolytic and cellulolytic enzymes. As a result, the metabolite of *Trichoderma* spp. prepared by incubating for 28 days at pH 7 and onward in potato dextrose medium completely checked the germination of sclerotia. As the incubation period of culture of *Trichoderma* spp. increased, the germination of sclerotia by metabolite decreased in the same proportion. The dry weight of mycelium and formation of sclerotia of *S. rolfsii* and production of pectolytic and cellulolytic enzymes were adversely effected by the metabolite of *Trichoderma* spp. with *T. harzianum* found to be most suppressive.

Keywords : Germination; Growth; Pectolytic and cellulolytic enzymes; *Sclerotium rolfsii; Trichoderma* spp.

Introduction

Vast literature is available on the biological control of pathogenic organisms[1-5]. Recently it has been in practice to control soilborne pathogens using pharmaceutical preparations of *Trichoderma* spp. Elad *et al*[3]. have reported the parasitism of *Trichoderma harzianum* on *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kuhn. *S. rolfsi* in groundnut field was controlled using molasses enriched soil granules as medium for *T. harzianum*[6]. The present paper deals with the effect of metabolite of *Trichoderma* spp. on the germination of sclerotia and growth of *Sclerotium rolfsii* Sacc. isolate of tomato fruit and production of pectolytic and cellulolytic enzymes.

Material and Methods

Sclerotium rolfsii Sacc. was isolated from ripe tomato fruit[7] and grown on potato dextrose agar (PDA) medium. Three species of *Trichoema* i.e. *T. harzianum, T. Koninghii* and *T. viride* were supplied by the then Head of the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi - 110012. *Trichoderma* spp. were grown on PDA slants at 28±1°C for for 7 days and stored at 8±1°C for future work.

Obtaining sclerotia of S. rolfsii : Sclerotia procured from tomato fruits were cultured on PDA at 25±1°C for 30 days when resulting sclerotia became black.

Preparation of metabolite of Trichoderma spp :- The metabolite was prepared in autoclaved liquid Czapek Dox (CD) and Potato dextrose (PD) medium taking them 100 ml in 300 ml conical flasks. The pH of the medium was adjusted to 4,5,6,7,8 and 9 using 0.1N HCl and 0.1 NaOH after autoclaving. A single bit of 4 mm diameter of seven days of culture of *Trichoderma* spp. was aseptically transferred to the media in flasks and incubated for 7,14,21 and 28 days at 28±1°C. For preparation of the metabolite, triplicate of flasks were maintained for each species of *Trichoderma,* each pH level and each incubation period. The metabolite was filtered aseptically through sterilized Whatman No. 1 filter paper in sterilized conical flasks. The filtrate was centrifuged at 10,000 rpm for 10 min.

Observation of the germination of sclerotia : Ten ml of the metabolite was taken in sterilized petri dishes of 10 cm diameter. Twenty sclerotia grown as above, were transferred to each petri dish with the help of sterilized forceps. Five replicates were set for each fungus, medium, pH and incubation period. The control was maintained of the medium only. The petri dishes containing sclerotia in metabolies were placed over distilled water in sealed desiccators to prevent evaporation. A period of seven days was spared for germination of sclerotia. Petri dishes were placed over black sheet of paper to correctly observe the white germling of sclerotia. The germinated sclerotia were counted considering the noted parameters and Mean was recorded.

Observation of growth of S. rolfsii : The growth of *S. rolfsii* was measured in terms of dry weight and formation of sclerotia. 100 ml of CD medium was maintained at pH5 after autoclaving. On cooling 2 ml of the metabolite prepared as above was added. Triplicate of the flasks was used for one

Table 1. Effect of metabolites of *Trichoderma* spp. prepared at different pH on incubation for varying periods on the germination of sclerotia of *S. rolfsii* growing in C.D. medium.

Period of incubation (in days)	*Trichoderma* spp.	pH of the medium					
		4	5	6	7	8	9
7	*T. harzianum*	14.0±1.22	17.0±3.74	17.0±3.74	11.0±1.58	10.0±1.58	9.0±2.9
	T. koninghii	20.0±1.87	15.0±2.23	15.0±1.22	15.0±2.23	12.0±1.22	10.0±1.58
	T. viride	32.0±1.22	26.0±1.87	23.0±1.22	19.0±3.31	10.0±1.58	-
	Control	61.0±1.87	51.0±3.99	48.0±4.63	48.0±3.31	47.0±3.74	40.0±3.53
14	*T. harzianum*	13.0±1.99	12.0±1.22	-	-	-	-
	T. koninghii	14.0±1.87	7.0±1.22	-	-	-	-
	T. viride	12.0±1.22	11.0±0.99	-	-	-	-
	Control	60.0±3.53	50.0±4.89	47.0±2.45	48.0±2.73	48.0±3.74	39.0±5.82
14	*T. harzianum*	7.0±1.22	5.0±0.00	-	-	-	-
	T. koninghii	7.0±1.22	5.0±0.00	-	-	-	-
	T. viride	9.0±0.99	6.0±0.91	-	-	-	-
	Control	60.0±3.53	50.0±5.82	46.0±2.99	47.0±3.16	46.0±3.16	40.0±2.45
28	*T. harzianum*	5.0±0.00	4.0±0.99	-	-	-	-
	T. koninghii	5.0±0.91	4.0±0.99	-	-	-	-
	T. viride	9.0±1.87	6.0±0.99	-	-	-	-
	Control	61.0±2.45	51.0±2.45	47.0±1.99	48.0±3.74	48.0±2.91	41.0±2.45

- = No germination of sclerotia, t value of the germination of sclerotia at pH 4 and 5=0.43 (NS)

Table 2. Effect of metabolites of *Trichoderma* spp. prepared at different pH on incubation for varying periods on the germination of sclerotia of *S. rolfsii* growing in PD medium.

Period of incubation (in days)	*Trichoderma* spp.	pH of the medium					
		4	5	6	7	8	9
7	*T. harzianum*	11.0±0.99	13.0±1.99	7.0±1.22	-	-	-
	T. koninghii	17.0±1.99	16.0±1.87	7.0±1.87	-	-	-
	T. viride	18.0±1.22	16.0±1.87	9.0±1.87	-	-	-
	Control	57.0±1.99	55.0±3.53	42.0±1.99	31.0±0.99	25.0±0.99	19.0±1.22
14	*T. harzianum*	15.0±2.23	13.0±1.99	-	-	-	-
	T. koninghii	12.0±1.22	13.0±1.99	-	-	-	-
	T. viride	17.0±1.99	17.0±1.99	-	-	-	-
	Control	58.0±1.22	56.0±2.45	41.0±3.74	32.0±2.45	24.0±2.99	20.0±2.23
21	*T. harzianum*	13.0±1.22	12.0±1.22	-	-	-	-
	T. koninghii	9.0±0.99	7.0±1.22	-	-	-	-
	T. viride	11.0±1.87	9.0±0.99	-	-	-	-
	Control	59.0±3.99	56.0±3.99	41.0±3.31	32.0±3.74	25.0±3.66	19.0±3.31
28	*T. harzianum*	10.0±1.58	9.0±1.87	-	-	-	-
	T. koninghii	9.0±1.87	8.0±1.22	-	-	-	-
	T. viride	11.0±0.99	8.1±1.22	-	-	-	-
	Control	58.0±0.99	55.0±1.99	42.0±2.55	32.0±3.39	25.0±2.91	20.0±3.53

- = No germination of sclerotia, t value of the germination of sclerotia at pH 4 and 5 =0.43 (NS)

sp of *Trichoderma* and the control. Two sclerotia were added to each flask and incubated for 15 days at 28±1°C. The resulting mycelial mat was filtered over pre-weighed and predried. Whatman No. 1 filter paper and dried at 60±1°C for 24 hr and finally desiccating over fused $CaCl_2$ in sealed desiccators for next 72 hr and the final weight was taken. Dry weight of the mycelium was recorded deducting the weight of filter paper from the final weight.

Twenty ml of CDA medium was sterilized in large culture tubes. On cooling and before solidifying 2 ml of the metabolites prepared as above was added separately of each species of *Trichoderma*. The tubes were shaken to mix the metabolite and the medium and poured in the petri dishes of 10 cm diameter. Triplicate of petri dishes were maintaned for 30 day at 28±1°C. The resulting sclerotia were counted and mean of triplicate was recorded and CD value was calculated.

Observing the effect of the metabolite of Trichoderma spp on the production of pectolytic and cellulolytic enzymes by S. rolfsii : One hundred ml of CD medium supplemented with 2% apple pectin was adjusted to pH 6.5. Two sclerotia of *S. rolfsii* were inoculated. These were divided into two sets. In one set of the culture, 2 ml of the metabolite prepared from 28 days old culture of *Trichoderma* spp. was added while another set served the control in which 2 ml of the CD medium only was added. The culture was incubated at 28±1°C for 15 days. The culture filtrate was filtered and centrifuged as noted earlier for getting the metabolite. This filtrate serving as enzyme preparation (EP), was stored at 2°C. The EP prepared as above, was used as enzyme source for assaying following enzymes secreted by *S. rolfsii*. Protopectinase (PP) activity was assayed by potato disc method[8]. Polygalacturonase (PG) and polymethyl galacturonase (PMH) activities were measured[9]. Pectin methylesterase (PME) was assayed by continuous titration method using standard alkali to neutralise pectic acid[10]. C1 of cellulolytic enzyme activity was estimated by column clearing technique[11] using cellulose powder as substrate. Cx activity was assayed[12] using carboxymethyl cellulose as substrate.

Results and Discussion

The metabolite prepared by incubating the culture in CD medium for 28 days proved the most toxic followed by 21 and 14 days at all the pH (Table 1) except that prepared by incubating the culture for 7 days, the effect of which is not appreciating. The metabolite prepared at pH6 and above proved to be the most toxic suppressing the germination of sclerotia completely. Doubtlessly, the increasing pH as evinced by the control also adversely effected the germination of sclerotia but the difference in the number of germinated sclerotia is not at all comparable with the effect of metabolite. The results on PD medium is not appreciably different (Table 2). The metabolite at pH6 and onward was found to suppress the germination of sclerotia when the culture was incubated for 14, 21 and 28 days. The effect of pH on germination of sclerotia was similar to that observed in CD medium. There appeared no difference in the effect of the three spp of *Trichoderma*. The dry weight of mycelium and the number of sclerotia of *S. rolfsii* were significantly reduced due to *Trichoderma* spp (Table 3). *T. harzianum* was observed most effective followed in succession by *T. koninghii* and *T. viride*. The activity of pectolytic and cellulolytic enzymes (Table 4) was suppressed by the metabolites and the sequence of effectiveness was noted as above for the growth and formation of sclerotia of *S. rolfsii*.

Several authors[13-15] have reported various pH optima for different isolates of *S. rolfsii*. pH between 2 to 8 has been found better for mycelial growth and sclerotium formation[16]. It is sure that the prolongation of incubation period and maintenance of pH beyond 6 for the growth of *Trichoderma* spp. raise the magnitude of toxicity of metabolite that suppresses the germination of sclerotia which can be contemplate for the manufacture of biocontrol agents of *S. rolfsii* isolate of tomato. The suppression of growth of mycelium and production of sclerotia of *S. rolfsii* under the influence of the metabolite of *Trichoderma* spp clearly point out that some sort of biochemicals are secreted out by *Trichoderma* spp that disturb the growth and sclerotium formation. Doubtlessly the parastitic nature

Table 3. Dry weight of the mycelium of *S. rolfsii* and the number of sclerotia formed due to the metabolite of three spp of *Trichoderma*

Trichoderma spp.	Dry weight (in mg)	Number of sclerotia
T. harzianum	329.00±5.11	150.33±4.33
T. koninghii	393.66±4.69	175.60±5.72
T. viride	493.00±3.49	228.66±9.46
Control	732.00±11.51	337.00±7.08

C.D. for dry weight of mycelium = 26.26
C.D. for the number of sclerotia = 18.45

Table 4. Effect of the metabolites of *Trichoderma* spp. on the production of pectolytic and cellulolytic enzymes by *Sclerotium rolfsii*.

Trichoderma spp.	Pectolytic enzymes (%)				Cellulolytic enzymes (%)	
	PP	DP	PG	PME*	Cx	Ci**
T. harzianum	30.00	55.90	53.00	0.59	51.12	16.80
T. koninghii	33.25	60.00	59.27	0.65	56.33	17.50
T. viride	38.40	67.75	64.87	0.70	60.55	18.10
Control	39.50	67.25	70.16	0.90	64.72	18.70

* 0.1 N NaOH used to neutralized pectic acid released in course of the reaction.
** Length of the cleared column in mm.

of *T. harzianum* on *S. rolfsii* has been proved[3,17] and recently it has been reported that *T. harzianum* has no antibiotic activity but it is a mycoparasite producing β-1-3 glucanase and chitinase enzymes[18]. The antagonistic activity of *Trichoderma* spp. becomes evident if observed the germination of sclerotia, growth of mycelium and formation of sclerotia. Besides these, attenuated pectolytic and cellulolytic enzyme activity compels to realise that meagre amount of hydrolytic product would be released by the activity of noted enzymes secreted insufficiently by *S. rolfsii* under the influence of *Trichoderma* spp. and consequently less nutrition would be available to *S. rolfsii* creating a situation of starvation[19]. Moreover, per cent rotting of tomato fruit due to *S. rolfsii* has been found greatly reduced due to the noted spp of *Trichoderma*[20].

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