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Dose-response relationship in the microbial suppression of *Sclerotium rolfsii* by *Trichoderma pseudokoningii*, strain MTCC 3011

Sclerotium rolfsii is a devastating plant pathogen infecting over 500 species of plants[1]. It can infect seeds, seedlings, mature plants in the field, and cause diseases of fresh vegetables and rhizomes while in storage and during transit[2]. In recent years, the biological control as well as the management of this pathogen under field conditions[3], using its antagonistic fungi, *Trichoderma* spp., has emerged as a viable alternative to chemical fungicides. Recently, we have isolated a strain (MTCC 3011) of *T. pseudokoningii* from sclerotium of *S. rolfsii*[4]. This strain was found to be highly effective in suppressing the growth of *S. rolfsii* on ginger rhizome, and on several vegetables (beet, carrot, bitter gourd, elephant-foot yam, etc.) while in storage[4,5]. Under other species of *Trichoderma* (*T. viride*, *T. harzianum*, *T. virens*, *T. koningii*), *T. pseudokoningii* has been rarely reported in literature as a biocontrol agent[6]. Hence, there is a need for a thorough characterization of this species in order to use it as an effective biocontrol agent of plant diseases. In this paper, we report on the effect of the type of inoculum and inoculum density of *T. pseudokoningii* for its efficacy as a microbial-suppressive agent of *S. rolfsii*. We also propose the possible mechanism of biocontrol in this system on the basis of inferences from the present results as well as our previous observations.

The disease-control potential of *T. pseudokoningii* for *S. rolfsii* was studied using a bioassay described by us earlier[4]. Briefly; one side of sliced ginger

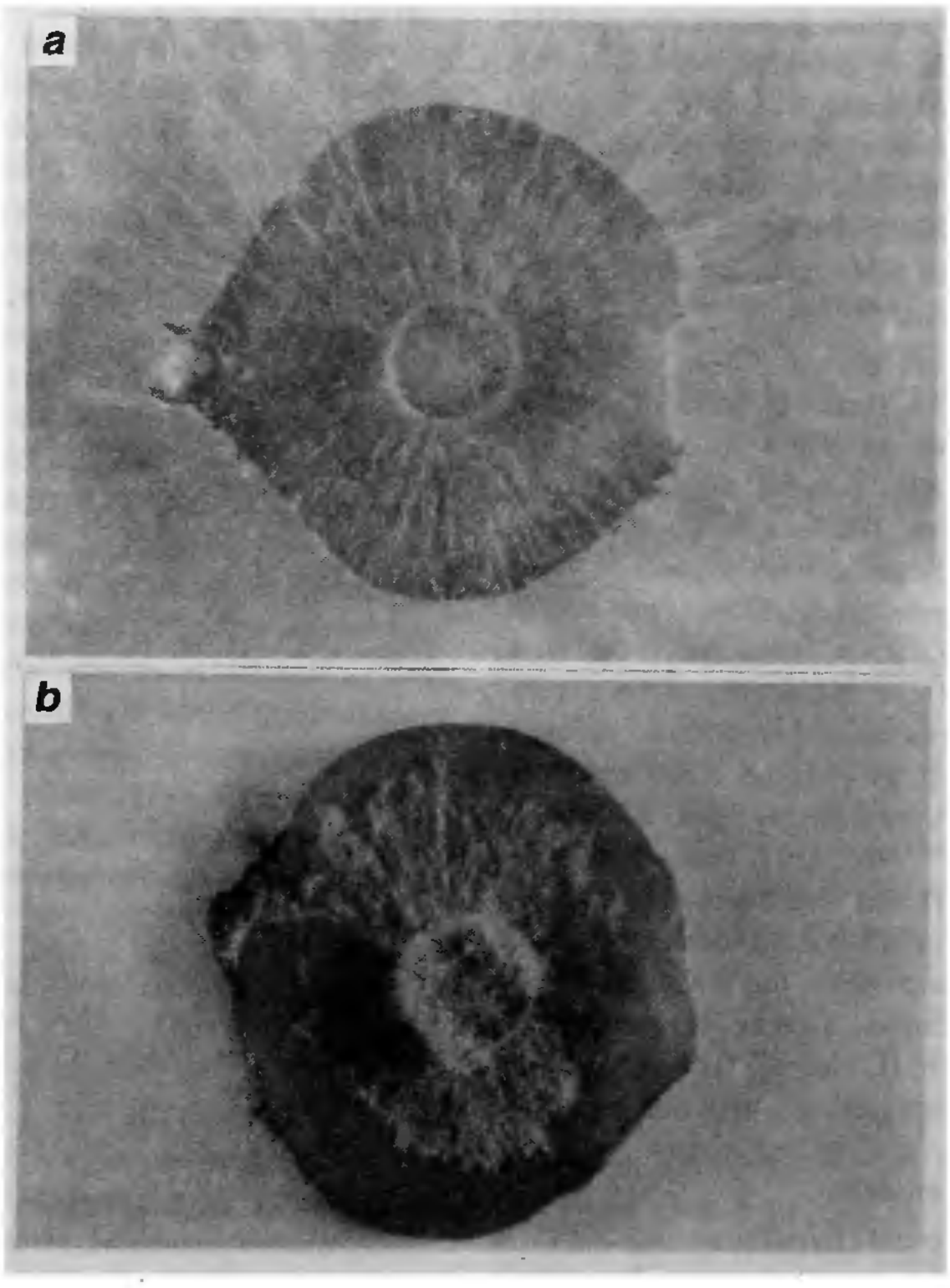

Figure 1. Overgrowth of *T. pseudokoningii* on *S. rolfsii* after 5 days of incubation. *a*, Growth of *S. rolfsii* on sliced ginger rhizome not inoculated with *T. pseudokoningii*; *b*, *S. rolfsii* growth being overgrown by *T. pseudokoningii* on sliced ginger rhizome inoculated with *T. pseudokoningii* (0.05 mg ml^{-1} mycelial suspension).

Table 1. Effect of pre-treatment with *T. pseudokoningii* on the mycelial growth of *S. rolfsii* on sliced ginger rhizomes

Type of inocula of *T. pseudokoningii*	Concentration ml^{-1}	Linear growth* of *S. rolfsii* (mm) ± SE			
		24 h	48 h	72 h	96 h
Mycelial suspension	50 mg	0	0	0	0
	5 mg	2.0 ± 0.6	**	**	**
	0.5 mg	3.3 ± 0.3	4.0 ± 0	**	**
	0.05 mg	7.7 ± 0.3	10.0 ± 1.2	10.0 ± 1.2	**
Conidial suspension	10^8	0	0	0	0
	10^7	2.3 ± 0.3	2.3 ± 0.3	**	**
	10^6	4.7 ± 0.9	4.7 ± 0.9	4.7 ± 0.9	**
	10^5	7.3 ± 0.9	7.3 ± 0.9	7.3 ± 0.9	**
	10^4	8.3 ± 0.7	9.7 ± 0.3	9.7 ± 0.3	**
	10^3	10.3 ± 0.3	19.7 ± 0.3	54.3 ± 1.2	65.0 ± 2.9
Control	–	11.7 ± 0.9	23.3 ± 2.8	52.7 ± 1.5	69.0 ± 4.6

*Mean of 3 replicates; **Growth of *S. rolfsii* overgrown by *T. pseudokoningii*.

rhizome was dipped in either a conidial inocula (harvested from 7-day-old plate culture) or mycelial inocula (prepared by blending in sterile water, blotted-dry mycelia that was harvested from 4-day-old shake culture) of *T. pseudokoningii*. The excess suspension was drained off, and the rhizome slices, treated side upwards, were placed on 3 layers of filter papers in petri dishes, 9 cm in diameter. The filter papers were moistened with 3 ml sterile water. The slice was then inoculated centrally with mycelial discs, 7 mm in diameter, of *S. rolfsii* cut from the margin of a 3-day-old culture. The plates were incubated at 24–28°C in polyethylene bags lined with moist tissue papers. The linear growth, from the edge of the inoculum disc of *S. rolfsii* was recorded daily.

The data on the biocontrol potential of *T. pseudokoningii* for *S. rolfsii* indicated that both the conidia and mycelia were effective in suppressing the mycelial growth of *S. rolfsii* on ginger rhizomes (Table 1). 50 mg ml^{-1} of mycelial suspension and 10^8 ml^{-1} of conidial suspension completely suppressed the growth of *S. rolfsii*. The ability to suppress *S. rolfsii*, however declined with the reduced inoculum level of *T. pseudokoningii*; 10^3 conidia ml^{-1} was ineffective. At the other concentrations though *S. rolfsii* showed growth initially, however, it failed to grow with time (unlike in the control, where *S. rolfsii* continued to grow (Figure 1 *a*)), and finally was overgrown by the *T. pseudokoningii*, as revealed by the profuse green growth of the antagonist on the pathogen (Figure 1 *b*). Microscopic examination of such growth revealed mycoparasitic coiling of *S. rolfsii* hyphae by *T. pseudokoningii*, and complete lysis. Plating of these growths on media amended with benomyl (to selectively suppress the growth of *T. pseudokoningii*) resulted in no fresh growth of *S. rolfsii*, suggesting killing of *S. rolfsii* by *T. pseudokoningii*.

One of the most significant factors in any effective biocontrol programme is the understanding of the mechanism of biocontrol. For example, in the present study, such information will help in further improving the performance of the antagonist. *S. rolfsii* being a menace mostly of the seeds/standing crops, a good amount of work has been carried out on the mechanism of biocontrol when *Trichoderma* spp. are applied to soil or seeds. In soil-borne infections, both mycelia and sclerotia (resting structures) play an important role in causing infection. Biological control of *S. rolfsii* in soil has been postulated to be due to the parasitism of sclerotia[7]. However, under the post-harvest conditions, the pathogen spreads in the form of mycelia only. Therefore, a post-harvest system forms a unique model to study the ability of the antagonist to suppress the mycelial growth of the pathogen exclusively. Mycelial growth of *S. rolfsii* can effectively be inhibited either through mycoparasitism/enzymatic lysis or antibiosis. Our earlier studies indicated that antibiosis does not play an important role in inhibiting *S. rolfsii* in a post-harvest system[8]. The present study has clearly established the role of mycoparasitism leading to hyphal lysis (presumably through the production of lytic enzymes like chitinases and β-1,3-glucanase) in biological suppression of *S. rolfsii* mycelial growth (Figure 1). The method described here (ginger slice bioassay, at lower dose of the antagonist) could be used as a model of *S. rolfsii in situ*, which would be more reliable than the widely-used dual inoculation technique in culture medium.

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