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Research Article

Antagonistic activity of endophytic *Trichoderma* against *Phytophthora* rot of black pepper (*Piper nigrum* L.)

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ABSTRACT: Endophytic isolates of *Trichoderma viride* and *T. pseudokoningii* from black pepper caused 64.4 and 65.6 per cent inhibition of mycelial growth of *Phytophthora capsici in vitro*. *In planta* screening showed that the isolate of *T. viride* was efficient in reducing *Phytophthora* rot incidence and enhancing plant growth characters. In pot and field experiments also this isolate resulted in the lowest infection by the pathogen.

KEY WORDS: Endophytes, *Trichoderma viride*, *T. pseudokoningii*, *Phytophthora capsici*, black pepper

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INTRODUCTION

Black pepper is one of the important export oriented spice crops of Kerala which holds a prominent place in the economy of the state. *Phytophthora* rot caused by *Phytophthora capsici* has become a major problem during rainy season for pepper cultivation resulting in huge crop loss. Even though many chemical control measures are recommended for the management of this disease, biocontrol is having due importance because of its cost effectiveness, ecofriendly nature and demand for organic products in the world market. Recently, endophytic microorganisms have received increased attention due to their ability to stimulate plant defense mechanism and survive in adverse ecological niches. Literature on the effect of endophytic fungi on plant pathogens is meagre, especially in India. Hence, an attempt was made in the present investigation to isolate and study the antagonistic effect of endophytic fungi from healthy black pepper on *P. capsici* causing *Phytophthora* rot disease.

MATERIALS AND METHODS

Plant samples were collected from healthy black pepper plants grown in the forest soils of Cherumkuzhy (Thrissur district) and Nilambur (Malappuram district) of Kerala. Endophytic *Trichoderma* was isolated from roots adopting the protocols suggested by Haiyan *et al*. (2005) and McInroy and Kloepper (1995) with slight modifications. Roots were cut into about one centimeter segments and surface sterilized by sequentially dipping in 1.05% sodium hypochlorite solution (2 min) and 70% ethanol (2 min), and rinsed with two changes of sterile water and final two changes in sterile 0.02 M phosphate buffer. From the final buffer wash 0.1 ml aliquot was transferred to *Trichoderma* selective medium (TSM) to serve as sterility check. Samples were triturated in 9.9 ml of buffer in a sterile pestle and mortar and 0.1 ml was plated on *Trichoderma* selective medium and pure cultures of these fungi were maintained for further studies.

Identification of *Trichoderma* isolates

Cultural and morphological characters of the selected *Trichoderma* isolates were studied and identified according to the key suggested by Rifai**.** The identities of the isolates were further confirmed by National Centre for Fungal Taxonomy, New Delhi (NCFT) and Indian Type Culture Collection, New Delhi (ITCC).

In vitro and *in planta* effect of *Trichoderma* isolates against *P. capsici*

In vitro antagonistic effect of endophytic *Trichoderma* isolates was tested against *P. capsici* by dual culture technique and per cent inhibition was calculated.

For *in planta* studies, cuttings of highly susceptible black pepper variety, Panniyur-1 were raised in polybags

using sterilized soil at three in each bag. Fifteen-day-old *Trichoderma* culture multiplied in potato dextrose broth was diluted to a concentration of 10^6 spores/ml and applied at the time of planting and 45 days after planting (DAP) @ 30 ml/bag. The inoculum was prepared by mixing 7-day-old *Phytophthora* culture in sterile sand oat medium (19:1) and incubated for two weeks. Challenge inoculation of the pathogen was done 30 DAP @10g/bag. Observations on number of plants infected and biometric characters like length of vine and number of leaves were recorded. Endophytic *Trichoderma* were reisolated from soil, root and peeled stem 135 days after last application.

Evaluation of endophytic** Trichoderma **against** P. capsici **under pot culture and field conditions

Trichoderma viride was further evaluated for its efficacy using rooted cuttings of Panniyur-1 and its efficacy was tested again in farmer's field. Observations on disease incidence and severity were recorded. 0–5 scale suggested by Vijayaraghavan (2003) was used for rating the severity. Coefficient of infection (CI) was calculated according to the formula suggested by Datar and Mayee (1981). (CI=PDI x PDS/ 100). Rhizospheric *Trichoderma* of black pepper and reference culture of *T. viride* were included as check in all studies. The table values were statistically analysed using DMRT.

RESULTS AND DISCUSSION

Isolation of endophytic fungi from roots of black pepper yielded typical colonies of two types of *Trichoderma* spp. (Isolate No. BPT-8 and BPT-9) on *Trichoderma* selective medium. These isolates showed very fast growth, good sporulation and yellowish brown pigmentation on potato dextrose agar (PDA) medium. Based on cultural and morphological characters, endophytic *Trichoderma* (BPT-8) isolated from Cherumkuzhy area of Thrissur District was identified as *T. viride* (NCFT 1302.07) and BPT-9 from Nilambur (Malappuram Dist.) was identified as *T. pseudokoningii* (ITCC-6437.06).

In vitro studies on the antagonistic activity revealed that both isolates (BPT–8 and BPT–9) were equally effective showing 64.4 and 65.6 per cent inhibition in the growth of *P. capsici*. Among the two endophytes screened *in planta*, *T. viride* was found to be the best as it showed good plant growth characters and no disease incidence as compared to the other endophytic *T. pseudokoningi* (Table 1). Reisolation from soil, root and stem resulted in 264, 144 and 124 colonies of *T. viride* and 192, 82 and 196 colonies of *T. pseudokoningii* at 10^{-2} dilution indicating the endophytic nature of these *Trichoderma* isolates in black pepper and also their ability to survive in soil. *T. viride* isolate was again tested for its efficacy under pot culture condition using rooted cuttings and also under field condition. In both pot culture and field experiments, the treatment with *Trichoderma* spp. were better than the control (Table 2). Among the three *Trichoderma* isolates tested, the lowest infection was recorded in the case of endophytic *T. viride*. A significant difference in coefficient of infection was noticed in *T. viride* treatments compared to control in under both pot and field experiments.

Many workers have reported the antagonistic ability of endophytic *Trichoderma* against different pathogens. Sobowale *et al*. (2007) observed the efficacy of endophytic *T. pseudokoningii* and *T. harzianum* against *Fusarium verticilloides* in maize and also stated that re-isolation of any of the *Trichoderma* species from different points within maize stem other than point of inoculation would be suggestive of their endophytic ability**.** Mejia *et al.* (2008) reported endophytic *Trichoderma* spp. as biocontrol agent against *Moniliophthora roreri* in cacao. Bailey *et al.* (2008) also noticed antagonistic activity of endophytic *T. harzianum, T. hamatum* and *T. asperellum* against *Moniliophthora roreri* of *Theobroma cacao.* Samuels *et al*. (2000) reported endophytic *T. stromaticum* against cacao witches' broom pathogen. However, this is the first report from India on endophytic *Trichoderma*

Table 1. *In planta* screening of selected *Trichoderma* spp. on *Phytophthora* disease incidence and biometric characters of black pepper

Trichoderma isolate	*Per cent disease incidence	Biometric character	
		No. of leaves	Length of vine (cm)
T. viride (BPT-8)	0	5.80 (5.667^{ab})	15.60 (15.516^{ab})
T. pseudokoningii (BPT-9)	3.3	4.80 (4.667b^{c})	12.10 (11.567^{ab})
T. harzianum (Rhizosphere isolate)	0	7.60 (7.500^{a})	20.20 (19.950^{a})
T. viride (Reference culture from KAU)	0	3.20 (3.333b^{c})	10.00 (8.016^{b})
Control	20	3.40 (3.167^{c})	8.40 (7.226^{b})

* Values are means of six replications. Figures in parentheses are square root transformed values

Table 2. Evaluation of endophytic *Trichoderma* against *P. capsici* under pot and field conditions

Treatments	Pot culture experiment			Field Experiment		
	Per cent disease disease	Per cent disease severity	Coefficient of infection	Per cent disease incidence	Per cent disease severity	Coefficient of infection
T. viride (endophyte)	47.36* (47.360^{b})	17.60 (17.346^{c})	8.34 (8.34^{b})	16.667 (2.260^{c})	16.667 (2.2601^{c})	2.72 (2.260^{b})
T. harzianum (rhizosphere)	47.61 (47.774^{b})	24.00 (23.996^{b})	11.43 (11.43^{b})	33.333 (3.813^{b})	20.000 (3.064^{b})	6.67 (3.064^{b})
T. viride (reference culture)	50.0 (50.126^{b})	24.80 (24.796^{b})	12.40 (12.40^{b})	50.000 (5.366^{b})	46.667 (5.190^{ab})	23.33 (5.191^{b})
Control	77.77 (77.714^{a})	37.6 (37.586^{a})	29.24 (29.24^{a})	83.333 (8.472^{a})	80.000 (8.296^{a})	66.66 (8.297^{a})

* Values are means of six replications. Figures in parentheses are square root transformed values

viride and *T. pseudokoningii* from black pepper against *P. capsici.* The present studies reveal the possibility of exploitation of endophytic *Trichoderma* spp. for the better management of disease as endophytes are well protected and show better ability to survive. Moreover, direct involvement of endophytes will also increase resistance against *Phytophthora* rot.

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