Research Article

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Evaluation of native *Trichoderma* spp. against pathogens infecting small cardamom

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Abstract

A survey was carried out during 2008-2009 period in the high ranges of Idukki district for isolation and *in vitro* screening of native *Trichoderma* spp. against *Fusarium oxysporum* and *Colletotrichum gloeosporioides,* pathogens of small cardamom. All the 29 isolates were plated against the two selected plant pathogens in order to test their antagonistic potential and were found to be inhibitive to the growth of *F. oxysporum* and *C. gloeosporioides*. In dual cultures, out of the 29 isolates, on seventh day the isolates CT-4, CT-5, CT-10, CT-15, CT-22 and CT-23 showed above 85 per cent reduction on the growth of *Fusarium* and on tenth day the isolates CT-13, CT-18 and CT-23 showed above 34 per cent reduction on the growth of *Colletotrichum*. The biomass, spore production, the production potential of siderophore, indole acetic acid (IAA) and hydrogen cyanide (HCN) of these isolates were also assessed. Some of the isolates showed better HCN, IAA and siderophores production. This information can be employed for the exploitation of these biocontrol agents in the high ranges of Idukki district of Kerala for management of the two potential fungal pathogens of small cardamom.

Keywords: Biological control, cardamom, HCN, IAA, siderophore, *Trichoderma* spp.

Introduction

Biological control by antagonistic organism is a potential non-chemical means for crop protection against phytopathogenic fungi (Papavizas, 1985). It offers solutions to many serious problems of modern agriculture and is an essential component in the development of modern agriculture. *Trichoderma* sp. is an established eco-friendly biocontrol agent against a variety of plant pathogens. The antifungal abilities of *Trichoderma* have been known since 1930s. The major modes of action of *Trichoderma* spp. are (i) high competitive saprophytic ability, (ii) faster rhizosphere colonization, (iii) production of hydrolytic enzymes *viz.,* chitinase, glucanase, cellulose and (iv) production of toxins or antibiotics, volatile compounds, siderophores *etc.* The above mentioned factors, soil type, moisture, temperature and nutritional status of the soil have been found to influence the disease controlling efficacy of *Trichoderma spp.* (El-Katatny *et al*., 2000). *Trichoderma* have the ability to promote growth and induce resistance in plants (Harman, 2006). Small cardamom is affected by many fungal pathogens such as *Rhizoctonia solani*, *Pythium vexans*, *Phytophthora meadii* and *Fusarium oxysporum*. Among these fungal pathogens, *F. oxysporum*, a well-known wilt fungus is becoming a major pathogen of small cardamom. Root tip rot and leaf yellowing, rhizome rot, panicle wilt and pseudostem rot are the common symptoms caused by *F. oxysporum* in small cardamom. "Azhukal" disease caused by *P. meadii* and leaf blight caused by *Colletotrichum gloeosporioides* are also very common in cardamom plantations (Vijayan *et al*., 2009a). *Trichoderma* spp. are effective in control of soil/seed borne fungal diseases in several crop plants (Kubicek *et al*., 2001) including cardamom (Vijayan *et al*., 2008, Vijayan *et al*., 2009b). The investigation was also extended to evaluate the efficacy of native *Trichoderma*

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isolates against the two selected fungal pathogens of cardamom *viz., F. oxysporum* and *C. gloeosporioides* under *in vitro* conditions.

Materials and methods

Survey, isolation and identification

A survey was carried out during 2008-2009 in the high range forest soils of Idukki district for isolation and *in vitro* screening of native *Trichoderma* spp. against *F. oxysporum* and *C. gloeosporioides* pathogens of small cardamom. The soil samples were plated in *Trichoderma* specific medium (TSM) for isolating the *Trichoderma* spp. The isolates of *Trichoderma* cultures were identified based on spore characteristics and their morphological studies. All the fungal cultures were maintained on potato dextrose agar (PDA) medium in slants, with routine sub-culturing.

Dual culturing of *Trichoderma* against fungal pathogens

All the *Trichoderma* isolates were plated against the pathogens *F. oxysporum* and *C. gloeosporioides*, to test their antagonistic potential, on PDA at room temperature. The culture discs of pathogens (8 mm) were placed on PDA plate one cm away from the edge. After two days of pathogen inoculation, *Trichoderma* culture discs (8 mm) of same size were inoculated to the opposite side one cm away from the edge. Three replications were maintained for each isolate. The colony diameters of the pathogens as well as the antagonists were recorded up to 10 days and the percentage of growth reduction of pathogen was calculated.

Siderophore production

For siderophore production, potato dextrose broth (PDB) was inoculated with 10^8 *Trichoderma* spores mL^{-1} and incubated in a rotary shaker (150 rpm) at 28 ^{0}C for 10 days. Culture filtrates were obtained by filtering through Whatman No. 42 filter paper. One mL of this culture filtrate mixed with an equal volume of 2 per cent ferric chloride solution. The presence of red colour indicated the presence of siderophore and the absorbance was measured at 440 nm using UV-Spectrophotometer (Neilands, 1981).

Production of hydrogen cyanide (HCN)

All the *Trichoderma* isolates were inoculated on PDB for quantitative determination of the production of HCN by these isolates. A strip of sterilized filter paper saturated with 0.5% picric acid in 2% (w/v) sodium carbonate were exposed to the inoculated broth. The mouth of flasks were then tightly sealed with parafilm and incubated at 30 ^{o}C for 7 days. A change in color of the filter paper from yellow to light brown indicated the production of HCN (Bakker and Schippers, 1987). The picrate paper was immersed in 10 mL of water for about 30 minutes with occasional gentle shaking along with blank picrate paper. The absorbance was measured at 510 nm of the picrate solution using UV-Spectrophotometer. The total cyanogens content in ppm was calculated by the equation (Bradbury *et al.,* 1999).

Total cyanogen content (ppm) = 396 x absorbance.

Indole acetic acid production

Indole acetic acid (IAA) production was quantitatively measured by modified Tang and Bonner Method (Goron and Weber 1950). The isolates were inoculated in PDB and incubated at 28 $^{\circ}$C for 10 days in a rotary shaker at 150 rpm. The culture filtrates were collected by centrifuging at 5000 rpm for 20 minutes. One mL of this culture filtrate was mixed with 4 mL of Fe-H_2SO_4 reagent and incubated for 15 minutes and the absorbance was read at 530 nm using UV-Spectrophotometer.

Broth culture studies

The isolates were tested for spore production and mycelial biomass in broth cultures. The cultures were inoculated in PDB and incubated in shaker at 28 ^{o}C (150 rpm) for 10 days. After 10 days, spore production was observed in all isolates, thereafter mycelial mat was removed by filtration and the biomass was recorded.

Results and discussion

From the survey conducted during 2008-2009, 23 *Trichoderma* spp. were isolated from various locations of Idukki district and compared with six different *Trichoderma* isolates obtained from ICRI, Myladumpara (Table 1). All the twenty nine isolates of *Trichoderma* spp. were plated in dual culture

Table 1. *Trichoderma* isolates used for the study

Isolate code	Place of collection
CT-1	Kajanappara -1
CT-2	Kajanappara -2
CT-3	Mathikettan
CT-4	Mali
CT-5	Senapathi -1
CT-6	Senapathi -2
CT-7	Kattapana
CT-8	Myladumpara -3
CT-9	Kadamakuzhi -1
CT-10	Vandenmedu
CT-11	Kadamakuzhi -2
CT-12	Rajakumari
CT-13	Kattapana -1
CT-14	Kattapana -2
CT-15	Elappara -1
CT-16	Anakkara
CT-17	Elappara -2
CT-18	Vattapara
CT-19	Peermedu
CT-20	Pethotti
CT-21	Anavilasm
CT-22	Chakkupallam
CT-23	Kajanappara -3
VT-1	Cumbumettu
VT-2	Myladumpara -2
VT-3	Kizhavikulam
VT-4	Ranni
VT-5	Namari
T-12	Myladumpara -1

against two common cardamom fungal pathogens *viz., F. oxysporum* and *C. gloeosporioides* to test their antagonistic potential. The isolates inhibited the mycelial growth of *F. oxysporum* from 43.2 to 98.2 per cent and the inhibition of *C. gloeosporioides* ranged from 2.6 to 41.2 %. On seventh day the isolates CT-4, CT-5, CT-10, CT-15, CT-22 and CT-23 showed above 85.1 per cent reduction on the growth of *Fusarium* and on tenth day the isolates CT-13, CT-18 and CT-23 showed above 34.1 per cent reduction on the growth of *Colletotrichum* (Table 2). The reason for the inhibition may be competition for food and space, production of antibiotics, cell wall degrading

Table 2. *In vitro* inhibition of mycelial growth of *F. oxysporum* and *C. gloeosporioides* and production of secondary metabolites by *Trichoderma* isolates

Trichoderma isolate	per cent inhibition in *F. oxysporum* on 7th day	per cent inhibition in *C. gloeosporioides* on 10th day	Secondary metabolites production		
			HCN (ppm)	IAA (µg mL^{-1})	Siderophore (OD)
CT-1	62.6	21.2	180.6	2.5	0.2
CT-2	70.1	2.6	21.9	-	0.0
CT-3	50.3	30.2	81.2	5.6	0.2
CT-4	89.9	11.1	41.2	-	0.5
CT-5	89.2	9.3	35.2	53.7	0.5
CT-6	69.9	28.1	3.9	57.5	0.7
CT-7	62.6	23.1	48.7	52.5	0.9
CT-8	55.9	26.3	0.8	-	0.1
CT-9	74.8	12.9	55.0	-	0.3
CT-10	90.9	14.4	18.6	44.0	0.6
CT-11	62.8	19.8	41.2	12.5	0.2
CT-12	54.9	14.4	38.8	-	0.5
CT-13	57.3	34.5	3.9	-	0.2
CT-14	72.6	14.9	39.9	-	0.6
CT-15	85.2	14.4	22.6	8.8	0.5
CT-16	53.6	30.9	41.6	-	0.5
CT-17	50.5	13.8	47.1	-	0.3
CT-18	47.6	35.2	2.8	16.3	0.7
CT-19	57.2	18.6	3.6	5.5	0.2
CT-20	87.4	10.8	16.5	13.0	0.5
CT-21	71.7	9.5	76.4	5.6	0.6
CT-22	85.3	11.1	15.8	-	0.3
CT-23	98.2	41.2	64.6	3.8	0.6
VT-1	77.2	10.0	63.8	40.8	0.9
VT-2	67.7	21.5	13.4	13.0	0.4
VT-3	43.2	30.7	20.9	15.0	0.5
VT-4	72.9	29.0	59.8	7.5	0.5
VT-5	54.9	23.6	15.8	2.5	0.6
T-12	96.9	12.5	65.7	17.3	0.4
CD (1%)	1.1	0.3	6.1	-	-

(-) Not detected

enzymes and toxins. The production and the secretion of the fungal cell wall degrading enzymes and compounds affecting the integrity of fungal membrane and cell walls are considered as the key steps in the antagonistic ability of *Trichoderma* (Elad *et al.,* 1982; Lorito *et al*., 1994; Chet *et al*., 1998 and Kredics *et al*., 2000).

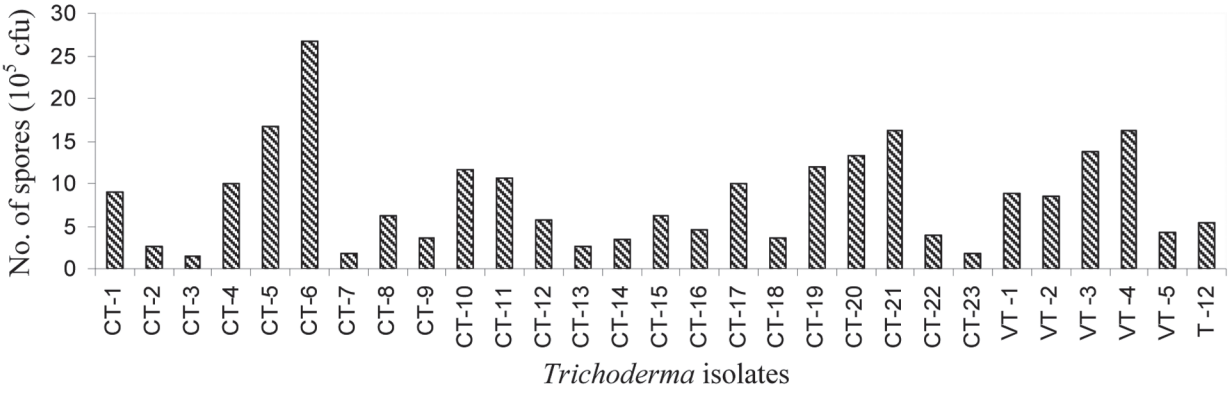

Fig 1. Variability in spore production (cfu x 10^5 dilution) by *Trichoderma* isolates

The isolates showed variability in siderophore production. Siderophore production varied from 0.03 to 0.99 (OD) (Table 2). The isolate CT-7 from Elappara region showed maximum siderophore production whereas the isolate CT-2 from Kajanappara region showed very low production of siderophore in PDB. Siderophores are the iron chelating compounds having low molecular weight. The productions of siderophores create an iron deficient environment which is unfavorable for the plant pathogens to exist. Microbial siderophore may stimulate plant growth directly by increasing the availability of iron in the soil surrounding the roots or indirectly by competitively inhibiting the growth of plant pathogens with less efficient iron uptake system (Marek- Kozaczuk *et al*., 1996).

The isolates showed variability in HCN production and it ranged from 0.8 to 180.6 ppm. The isolate CT-1 produced maximum HCN content (180.6 ppm) followed by CT- 3 (81.2 ppm) and the isolate CT-8 produced very small quantity of HCN (0.8 ppm) followed by CT-18 (2.8 ppm) (Table 2). Production of HCN may help to inhibit the growth of pathogens by volatile compounds. In addition to competition for limited carbon sources in the rhizosphere, antagonism can be mainly attributed to the production of antibiotics, siderophores and cyanides (Kloepper *et al*., 1980).

Out of twenty nine isolates, nineteen produced detectable amount of IAA in PDB. Among these isolates the isolate CT- 6 showed maximum IAA production (57.5 µg mL^{-1}), the isolates CT-1 and VT-5 produced less amount of IAA (2.5 µg mL^{-1}) and the IAA production were not detected in isolates CT-2, CT-4, CT-7, CT-8, CT-12, CT-13, CT-14, CT-16, CT-17 and CT-22 (Table 2). The microbes produce IAA that may stimulate plant cell proliferation or elongation (Glick *et al*., 1998).

In broth culture studies the isolates showed variability in sporulation and biomass production.

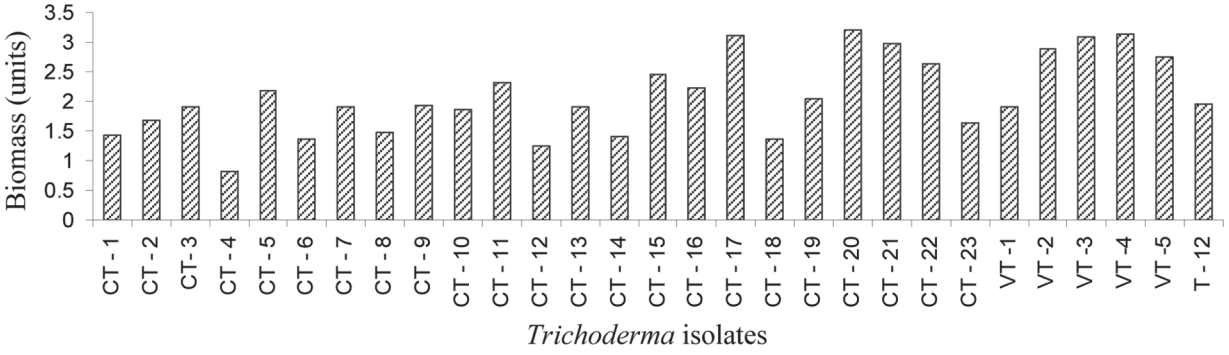

Fig 2. Variability in biomass production by Trichoderma isolates on PDB at 28^0C for 10 days

Some of the isolates did not sporulate well when grown on PDB even after ten days of incubation (Fig. 1 and 2).

The antifungal abilities of *Trichoderma* spp. was due to mycoparasitism (Weindling, 1932) and due to the effect of volatile and non volatile metabolites produced by these antagonists (Howell, 1998). Biological control by antagonistic microorganisms is a potential, non-chemical, eco-friendly and sustainable approach for managing plant diseases. Many fungi including *Trichoderma* have the potential to reduce the disease through biocontrol mechanisms which include antibiosis, mycoparasitism and competition for nutrients and space (Harman, 2000).

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