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EVALUATION OF BIO-AGENTS FOR CONTROLLING FRUIT ROT/ ANTHRACNOSE OF BANANA CAUSED BY *COLLETOTRICHUM GLOEOSPORIOIDES IN-VITRO* CONDITION

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ABSTRACT
The effects of bio-agents on pathogenicity of *Colletotrichum gloeosporioides* and its potential to control fruit rot diseases of banana were determined. Addition of bio-agents reduced radial growth, spore production, spore germination and appressoria production of *C. gloeosporioides*, *in vitro* by dual culture technique method. Among three bio-agents *viz.*, *Trichoderma viride* (RAU, Pusa and New Delhi isolate), *Trichoderma harzianum* (Pusa and New Delhi isolate) and *Trichoderma virens* (RAU, Pusa isolate and New Delhi isolate), the Pusa isolate was significantly superior to New Delhi isolate. In case of Pusa isolate of *T. viride, T. harzianum* and *T. virens*, the radial growth of *C. gloeosporioides* were 19.7, 19.3 and 20.7 mm, respectively whereas New Delhi isolates *T. viride, T. harzianum* and *T. virens* produced 28.7 mm, 24.7 mm and 22.3mm radial growth, of the test fungus, respectively. *T. harzianum* (RAU, Pusa isolate) was found to be best to inhibit the radial growth (19.3mm) of *C. gloeosporioides*. It was closely followed by *T. viride* (19.7mm) of RAU, Pusa isolate. The cultural filtrate of these bioagents was less effective in inhibiting the conidial germination and length of germ tube of *C. gloeosporioides*.

INTRODUCTION

Fruit rot disease of banana cause varying degree of economic loss to harvested perishables produced all over the world, and control of fruit rot has been traditionally achieved by pre and post-harvest applications of fungicides (Eckert and Ogawa, 1988). However, producers have been compelled to seek alternative methods due to increased global demand for chemical-free fresh produce (Korsten, 2006), development of resistant strains of plant pathogens against currently used fungicides (Spotts and Cervanntes, 1986) and higher costs involved with synthetic fungicides. Among the currently available biological agents, non-fungicidal approaches, use of soft chemicals, natural chemicals, disinfectants, calcium applications, growth regulators, chemical elicitors to induce natural host defences, irradiation, hot water, modified atmosphere storage, special packaging and genetic manipulation of fruit have been practiced with varying degree of success (Coates and Johnson, 1997; Barkai-Golan,2001;Janisiewicz and Korsten, 2002; Korsten, 2006).

Bio-agents *Trichoderma harzianum, T. viride* and *T. virens* were used to control the fruit rot of banana. In addition, the ability of these bio-agents in controlling the anthracnose of papaya and banana (Sivakumar *et al.,* 2002) has been documented. Regulatory barriers for the use of these bio-agents are few, as most are classified as generally recognized as safe (GRAS). Moreover, bio-agents have been exempted from residue tolerance on all agricultural commodities. Treating produce with bio-agents is inexpensive and less sophisticated in comparison with other non-chemical alternatives such as biological control and heat treatment. Furthermore, the bio-agents is easily available and control measure can be implemented without much more professional expertise.

Use of biological antagonists has already been shown to be efficient methods for controlling post harvest diseases (Chalutz and Wilson, 1990; Smilanick and Denis-Arrue, 1992; Vinas *et al.,* 1998; Fan and Tilan, 2001; Sharma *et al.,* 2009).

We identified *Colletotrichum gloeosporioides* the causal organism of fruit rot of banana of several varieties. In these studies the fruit rot symptoms (i.e necrotic lesions with salmon-pink colour spores) developed at the ripe stage was defined as rotting which starts at the tip of the stem end of an individual banana finger and spread downwards. In present study, the potential of *Trichoderma* bio-agents was evaluated with the objectives of determining its effects on dual culture technique of C. *gloeosporioides*, the causal organism of fruit rot, anthracnose of banana.

Production has been seriously threatened by decreasing soil fertility and pests and diseases problems (Baker, 1940). The diseases are a major constrains of banana production both in field and also at post-harvest. Several postharvest diseases also affect the industry worldwide. Fruit rot and anthracnose diseases had been reported as being the most prominent.

Fruit rot of banana management by fungicides is very prominent but in recent years, growing countries are demanding for chemical-free fresh produce. Exploring new methods to reduce dependency on use of agrochemicals is a worldwide trend. There is the need to develop alternate postharvest treatments that are safe and acceptable to consumers. Therefore, to develop an alternative disease control programme of agrochemicals for the banana sector

(Rangaswami, 1988).

MATERIALS AND METHODS

C. gloeosporioides was isolated from the banana variety Fhia 3 (AABB), a highly susceptible variety to fruit rot, showing typical anthracnose symptoms. A pure culture of *C. gloeosporioides* with cinnamon colour colony morphology was obtained from a single cell culture and maintained in potato dextrose agar (PDA). The fungal culture was confirmed as *C. gloeosporioides* based on colony and spore characteristics as described by Photita *et al.* (2005). The bio-agents like *T. harzianum, T. viride and T. virens* were used to test efficacy against *C. gloeosporioides* by dual culture technique (Morton and Straube, 1955).

Screening of bio-agents by dual culture technique

Screening of bio-agents against the test fungus *C. gloeosporioides*, dual culture technique developed by Morton and Straube (1955) was adopted. Twenty ml sterilized molten PDA Medium was poured into sterilized Petri-plates of 90 mm diameter aseptically and allowed to solidity. Five mm discs of 7 days old culture of the test fungus and the bio-agent taken out with the help of sterilized cork borer were placed on PDA approximately 4 cm apart from each other. Three replications were maintained for each treatment. All the plates were then incubated in BOD incubator at a temperature of 28 ± 2°C. Observations on colony diameter of the test fungus were recorded after 7 days when the colony of the fungus was completely oven grown by the bio-agents.

Effect of culture filtrates of bio-agents on spore germination and length of germ tube of *C. gloeosporioides*

The culture filtrates of bio-agents were tested for their efficacy against spore germination and length of germ tube of *C. gloeosporioides*. The flasks containing 25 ml of potato-dextrose-broth medium were inoculated with bio-agents and incubated for 21 days. Thereafter, the whole fungal growth was homogenized in warning blender and filtered through filter paper. One drop of filtrate of liquid of these bio-agents were put on different glass slides in which spore from 7 days old cultures of *C. gloeosporioides* were mixed. The slides were kept in moist chamber at 28 ± 2°C. A control set was also run concurrently in which spores were mixed in sterilized distilled water. After 24 hours per cent spore germination and length of germ tubes were recorded under binocular microscope. All the experiment were replicated thrice, per cent inhibition over the control was calculated.

RESULTS AND DISCUSSION

Growth of *C. gloeosporioides* under dual culture, *Trichoderma* spp. obtained from different sources and *C. gloeosporioides* were cultured simultaneously in the same Petri-plate and incubated at 28 ± 2°C. Faster growth rate of bio-agent suppressed the growth of *C. gloeosporioides* which is evident from the data presented in Table 1.

All the bio-agents inhibited the radial growth of *C. gloeosporioides* significantly as compared to the control. *T. harzianum* and *T. viride* of RAU, Pusa isolate were found *at par* to each other in terms of suppression of radial growth of *C. gloeosporioides*. It is also evident from data that RAU, Pusa isolates significantly superior to New Delhi isolate. In case of Pusa isolate of *T. viride, T. harzianum* and *T. virens*, the radial growth of *C. gloeosporioides* were 19.7, 19.3 and 20.7 mm, respectively whereas New Delhi isolates *T. viride* and *T.*

Table 1. Effect of bio-agent against *Colletotrichum gloeosporioides* under dual culture

S. No.	Bio-agents	*Radial growth of *C. gloeosporioides* under dual culture after 7 days of inoculation on PDA medium (mm)	Per cent inhibition
1.	*Trichoderma viride* (RAU, Pusa isolate)	19.7	77.6(61.9)
2.	*Trichoderma viride* (New Delhi isolate)	28.7	67.7(55.4)
3.	*Trichoderma harzianum* (RAU, Pusa isolate)	19.3	78.9(62.5)
4.	*Trichoderma harzianum* (New Delhi isolate)	24.7	73.7(59.2)
5.	*Trichoderma virens* (RAU, Pusa isolate)	20.7	78.2(62.2)
6.	*Trichoderma virens* (New Delhi, isolate)	22.3	76.461.3
7.	Control	89.6	
	S. Em. ±	0.8	1.1
	CD at 5%	2.5	3.4

* Average of three replications.

Table 2: Effect of different bio-agents on spore germination and length of germ tube of *Colletotrichum gloeosporioides*

S. No.	Bio-agents	*Conidial germination % after 24 hrs.	Per cent inhibition	*Length of germ tube (µm) after 24 hrs.	Per cent inhibition
1.	*Trichoderma viride* (RAU, Pusa isolate)	80.3	13.3(21.3)	296.7	12.7(20.6)
2.	*Trichoderma viride* (New Delhi isolate)	85.0	8.0(16.1)	311.7	8.3(15.0)
3.	*Trichoderma harzianum* (RAU, Pusa isolate)	73.0	21.4(26.6)	265.7	21.8(27.7)
4.	*Trichoderma harzianum* (New Delhi isolate)	76.7	17.3(24.5)	279.3	18.0(25.0)
5.	*Trichoderma virens* (RAU, Pusa isolate)	87.7	5.5(13.1)	284.3	16.4(23.6)
6.	*Trichoderma virens* (New Delhi isolate)	88.3	4.6(10.1)	285.4	14.3(20.5)
7.	Control	92.7		340.6	
	S. Em. ±	1.1	1.8	7.4	3.0
	CD at 5%	3.5	5.6	23.0	9.5

* Average of three replications, ** Value given in parenthesis is after angular transformation.

harzianum produced 28.7 mm and 24.7 mm 22.3 mm radial growth, of the test fungus, respectively.

Effect of different bio-agents on spore germination and length of germ tubes of *C. gloeosporioides*

It is evident from the results that all the bio-agents were significantly superior to control in terms of conidial germination and length of germ tube. Conidial germination of *C. gloeosporioides* in culture filtrate of RAU, Pusa isolate of *T. viride, T. harzianum* and *T. virens* were found to be 80.3, 73.0 and 87.7 per cent respectively , whereas control exhibited 92.7 per cent. New Delhi isolate of *Trichoderma viride* and *T. harzianum* produced slightly higher percentage of conidial germination. In general RAU, Pusa isolates of bio-agents were found more effective than New Delhi isolates in terms of suppression of conidial germination and affecting the length of germ tube which was presented in Table 2.

Similar finding were observed by several scientist. Wisniewski and Wilson, (1992) reported that, though use of antagonistic micro-organisms and induction of resistance in harvested fruits have shown promise.

Several antagonistic micro-organisms have been found effective against post-harvest pathogens e.g. *Trichoderma* spp. reduce severity of a number of fruit rot (Pathak *et al.,* 1988). Majumdar and Pathak (1995) observed significant reduction in severity of guava fruit rots both in the pre and post inoculation treatments. Biological control of fruit rot and dieback of chillies using antagonistic micro-organisms and plant products has been attempted by Jeyalakshmi and Seetharaman (1998) and Jeyalakshmi *et al.* (1998).

Philip *et al.* (2000) studied the antagonistic potentiality of eight isolates of *Trichoderma* and an isolate of *Gliocladium virens* was tested *in vitro* against *C. gloeosporioides*. In general, *T. harzianum-1* and 2 and *T. viride-2* had shown high antagonism against *C. gloeosporioides* inhibiting their mycelial growth spore production and spore germination.

Findings of the present study elucidate the effects of Bio-agents on pathogenicity of *C. gloeosporioides* and development of fruit rot/anthracnose diseases of banana. *In vitro* effect of bio-agents against *C. gloeosporioides* by dual culture method were studied. A clear advancing green growth of *Trichoderma* spp. was observed over the colony of *C. gloeosporioides*. It was found that all the bio-agents inhibited the radial growth of test fungus significantly as compared to control. Pusa isolates of *T. harzianum* showed maximum inhibition (78.9) over the control as compared to *T. viride* (77.8) and *T. virens* (78.2), Mycoparasitism through physical contact by coiling and pathogen lysis in case of *Sclerotium* rolfsii by *T. harzianum* was observed by Upadhyaya and Mukhopadhyaya (1986). Disintegration of mycelia of test fungi may be due to the action of enzymes produced by *Trichoderma* spp. which would increase antagonism against plant pathogens. Studies on effect of bio-agents on spore germination and length of germ tube of *C. gloeosporioides* revealed that these bio-agents are not much effective for inhibiting the conidial germination and length of germ tube as compared to control. Philip *et al*. (2000) studied to antagonistic potentiality of *Trichoderma* spp. against *C. gloeosporioides* and found inhibition of the mycelial growth, spore production and spore germination of test fungus. Similar finding were observed by Wisniewski and Wilson, 1992; Korsten *et al*. (1994).

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