Journal of Biological Control, 26 (2): 144-154, 2012

Research Article

Evaluation and selection of efficient isolates of *Trichoderma* species from diverse locations in India for biological control of anthracnose disease of grapes

INDU S. SAWANT, YOGITA R. RAJGURU, VARSHA P. SALUNKHE and PALLAVI N. WADKAR
National Research Centre for Grapes, Manjri Farm Post, Pune-Solapur Road, Pune 412 307, Maharashtra, India
Corresponding author E-mail: indulika18@yahoo.co.in

ABSTRACT: Thirty four isolates belonging to seven *Trichoderma* species from different geographical locations of India were screened *in vitro* for their antagonism to *Colletotri gloeosporioides* and the efficient isolates were further evaluated for their bio-efficacy *in vivo* and in field for control of anthracnose of grapes. In *in vitro* screening, all *Trichoderma* isolates overgrew *C. gloeosporioides* colony. Microscopic examination of hyphal interaction showed plasmolysis of hyphae of *C .gloeosporioides* by all *Trichoderma* isolates. The percent inhibition of radial growth of *C. gloeosporioides* by toxic volatile and non-volatile metabolites produced by different isolates of *Trichoderma* ranged from 40.8 to 63.6 and from nil to 65.9 respectively. In *in vivo* trial with 20 most antagonistic isolates, the lowest disease incidence was recorded in leaves treated with *T. harzianum* 5R obtained from Kodagu, followed by *T. hamatum* (NAIMCC- 1717) from Andaman and Nicobar islands and *T. asperellum* (NAIMCC 1769) from Pitchavaram. Survival studies on grape phylloplane with selected 11 isolates indicated that when applied at 5×10^6 spores per ml the population ranged from 4087.05 to 1185.42 cfu per cm^2 on day 5 which further declined by day 15. On foliar application of these eleven isolates in field, lowest PDI was recorded in *T. viride* (NAIMCC-1817) initially obtained from soil of western Ghats of Kerala. The other isolates which recorded low PDI values were *T. harzianum* (NAIMCC-1965) obtained from Darjeeling, *T. koningii* (NAIMCC-1938), *T. pseudokoningii* (NAIMCC-1775) obtained from Andaman & Nicobar islands and *T. harzianum* 5R obtained from Kodagu (Karnataka).

KEY WORDS: *Colletotrichum gloeosporioides, Trichoderma* spp. volatile metabolites, non-volatile metabolites, lysis, phylloplane

(Article chronicle: Received:22-9-2011 Revised:5-6-2012 Accepted: 14-6-2012)

INTRODUCTION

Anthracnose is an important disease of grapes in India, occurring on all green tender parts during warm, wet and humid weather. The main losses occur during the vegetative growth phase from April to October. The tender shoots may be completely burnt out with infection; while infection on the immature shoots reduces the cane fruitfulness, directly affecting the productivity. Infection during the fruiting phase may result in direct yield losses, if it rains during pre monsoon and post-monsoon period in Maharashtra (Sawant and Sawant, 2010).

Disease management is generally chemical intensive, but, there are reports of reduced efficacy of fungicides against anthracnose, especially carbendazim formulations under intensive use situations. The tolerant isolates are equally pathogenic and also exhibit cross tolerance to other fungicides and are also persistent in nature. Thus, there is a strong need to find alternate control methods which can be used for development of an effective IPM programme.

Though, *Elsinoe ampelina* (*Sphaceloma ampelinum*) is generally accepted as the pathogen of grape anthracnose, recent studies have shown that *Colletotrichum gloeosporioides* species complex has emerged as the dominant pathogen. Antagonism and parasitism of many *Colletotrichum* spp. by different species of *Trichoderma* and their subsequent use for biological control of anthracnose disease is reported in various crops (Shovan *et al*., 2008; Sobowale *et al*., 2010), including potential for control of *C. gloeosporioides* on brambutan (Sivakumar and Wilson, 2000) and grapes (Soytong *et al*., 2005).

In the present study, 34 isolates belonging to seven *Trichoderma* species from different geographical locations of India were screened *in vitro* for their antagonism to *C. gloeosporioides* and the efficient isolates were further evaluated for their bio-efficacy *in vivo* and in field for control of anthracnose of grapes.

MATERIALS AND METHODS

Fungal cultures and growth conditions

C. gloeosporioides isolate 20-P-6 isolated from naturally infected grape leaves was used for the study. Thirty four *Trichoderma* isolates belonging to seven species originally collected from diverse geographical locations were obtained from National Bureau of Agriculturally Important Microbials (NBAIM), Mau, India, except isolate *T. harzianum* 5R which was from the collection of the National Research Centre for Grapes. *C. gloeosporioides* was maintained on Czapek-Dox Agar (CDA) and *Trichoderma* isolates were maintained on PDA at 28 ± 0.5°C. A 5mm disc from five day old culture plates was used for all studies.

In vitro antagonism

The antagonism of the 34 *Trichoderma* isolates was studied on potato dextrose agar (PDA) by dual culture method (Dennis and Webster, 1971c). The 90mm PDA dishes were seeded with a disc of *C. gloeosporioides* and *Trichoderma* isolate placed near the edge and on opposite sides and incubated at 28 ± 0.1°C in a BOD incubator (Binder KB 400). Observations on days taken for contact of the two colonies and complete growth of *Trichoderma* over the *C. gloeosporioides* were recorded. Slides were prepared from the zone of interaction, stained with lactophenol cotton blue and observed in bright field at 400x in an Olympus BX 51 compound microscope for lysis of hyphae. Images were captured using digital camera system and processed in Microsoft Office 2010. The approximate percentage of the mycelium of *C. gloeosporioides* exhibiting lysis of protoplasm was noted for each field at 400x. Four observations were taken.

Production of toxic volatile metabolites

The production of toxic volatile metabolites was studied by the procedure described by Dennis and Webster (1971b). Petri dishes containing sterile media were inoculated centrally with either *C. gloeosporioides* or a *Trichoderma* isolate. The lids were discarded and the bottom of a *C. gloeosporioides* plate was inverted over a *Trichoderma* plate and the edges were sealed together with a transparent adhesive tape. An inoculated *C. gloeosporioides* plate inverted over an uninoculated PDA plate served as control. Three replications were maintained. Plates were incubated as indicated above. Radial growth was measured after 6 days of incubation and percent growth inhibition was calculated as :

$$\% \text{ inhibition} = \frac{X-Y}{X} \times 100$$

Where,

X = colony diameter of *C. gloeosporioides* in control plate

Y = colony diameter of *C. gloeosporioides* in test plate.

Production of toxic non-volatile metabolites

The effect of toxic extracellular non-volatile metabolites on radial growth of *C. gloeosporioides* was studied by the addition of cell free culture filtrates on agar medium (Dennis and Webster, 1971a). The *Trichoderma* isolates were grown in potato dextrose broth at 28 ± 0.1°C in a BOD incubator (Binder KB 400) for 15 days in dark without shaking (stationary phase culture). The culture was filtered through grade 42 Whatman cellulose filter paper. The filtrate was sterilized by passing through Whatman 13mm 0.2µ nylon syringe filter Puradisc. The sterilized filtrate was added to a sterilized pre-cooled PDA to give a final conc of 10% (v/v) before pouring in petri dishes. A disc of *C. gloeosporioides* was placed in the centre. Plates with unamended medium served as control. Three replications were maintained. Plates were incubated as above. Radial growth was measured after 6 days of incubation and percent growth inhibition was calculated as above.

In vivo bio-efficacy

The study was conducted on healthy detached leaves of grape cultivar Thompson Seedless. The 5th and 6th leaves from the top of growing shoots were detached and washed carefully under running tap water. The leaves were surface sterilized by immersing in 4% sodium hypochlorite solution for 1 min and rinsed thrice in sterile distilled water. The leaves were allowed to air dry and then sprayed with a suspension of *Trichoderma* containing 5 x 10^6 spore ml^{-1}. After 2 h, the leaves were spray inoculated with a suspension containing 1 x 10^6 ml^{-1} conidia of *C. gloeosporioides*. The fungal suspensions were prepared in sterile distilled water containing 1-2 drops of 0.05% Tween 80. The petiole of each leaf was dipped in approx. 25 ml sterile distilled water in a 50 ml beaker to maintain turgidity of the leaves. The beakers were arranged in a humid chamber at > 90% RH under natural daylight conditions at 25-28°C. Infection was apparent on the 3rd day and observations on disease were recorded as ratings on a 0-5 scale, where 0, no symptom; 1, 2, 3, 4, and 5 correspond to upto 10, 25, 50, 75 and >75% leaf area infected respectively. Three replications were maintained

and the trial was repeated twice. The percent disease index (PDI) was calculated as follows and the pooled data was used for analysis.

$$PDI = \frac{\text{Sum of total ratings}}{\text{No. of observations x maximum of scale}} \times 100$$

Survival of selected *Trichoderma* isolates on the grape phylloplane

Survival of selected 11 *Trichoderma* isolates was assessed on leaves of grape cv. Thompson Seedless grown in pots, which were maintained at 26 ± 4°C and 75 ± 25% RH under natural daylight conditions. The plants were sprayed with a suspension of *Trichoderma* containing 5 x 10^6 spores ml^{-1} to completely cover both adaxial and abaxial surfaces. Each isolate was replicated twice and 5 leaves were picked at zero day (3–4 h after application), 5th and 15th days after application to determine establishment and survival. Two 30mm discs from each leaf were cut, suspended in 100ml sterile distilled water with 1-2 drops of 0.05% Tween 80 and shaken at 120rpm for 30 min. The suspension was diluted and a 0.1ml of each dilution was spread on modified *Trichoderma* selective medium (Elad *et al*., 1981). The plates were incubated at 28 ± 0.1°C in a BOD incubator (Binder KB 400) for 5 days with intermittent light. Observations on number of colonies were recorded on the 5th day and total colony forming units (cfu) were calculated (cfu observed x dilution factor x 100) and converted to cfu's per cm^2 as follows, considering establishment on both the adaxial and abaxial surfaces.

$$CFU / cm^2 = \frac{\text{Total cfu in 100 ml}}{\text{Area of each disc } (\pi r^2) \text{ x no of discs x 2 (both surface)}}$$

Evaluation of selected *Trichoderma* isolates for the management of anthracnose in field

The field study was conducted on grape cultivar Thompson Seedless at the research farm of this Centre. Eleven isolates of *Trichoderma* selected on the basis of *in vitro* and *in vivo* studies were evaluated. The vines were sprayed with a suspension of *Trichoderma* containing 5 x 10^6 spores ml^{-1} to completely cover both adaxial and abaxial surfaces. Three applications were done at weekly intervals and disease was recorded as ratings on a 0-5 scale as above after seven days of each spray. The experiment was done in RBD with eight replications. Disease was observed on 10 leaves per replication.

Statistical analysis

The *in vitro* and *in vivo* data was analyzed by CRD, while the field data was analyzed by RBD. The percentage data was transformed before analysis. Means were compared by Duncan's Multiple Range Test. Correlation analysis was performed for the *Trichoderma* species and the percent lysis and inhibition data using 'correl' function of Microsoft Excel 2007.

RESULTS AND DISCUSSION

***In vitro* antagonism**

The *Trichoderma* species tested comprised of 12 isolates of *T. harzianum* from geographically different locations of West Bengal, Madhya Pradesh, Andaman and Nicobar islands, Kerala and Karnataka; 7 isolates of *T. viride* from West Bengal and Kerala; 4 isolates of *T. koningii* from Andaman and Nicobar islands and Uttar Pradesh; 4 isolates of *T. pseudokoningii* from Maharashtra and Andaman and Nicobar islands; 4 isolates of *T. hamatum* from Andaman and Nicobar islands; 2 isolates of *T. asperellum* from Tamil Nadu and Uttaranchal Pradesh and 1 isolate of *T. lectea* from Madhya Pradesh (Table 1). The isolates were either from the rhizosphere soil, non-rhizosphere soil or unspecified sites.

All the *Trichoderma* species and isolates made contact with the *C. gloeosporioides* colony within 2-4 days and overgrew it within 4-5 days of contact and 4 isolates viz. *T. harzianum* (NAIMCC-1741), *T. viride* (NAIMCC-1823), *T. pseudokoningii* (NAIMCC-1775) and *T. hamatum* (NAIMCC-1719) which took more than 5 days to overgrow (Table 1). The *C. gloeosporioides* hyphae exhibited typical lysis of the protoplasm (Figure 1a, 1b, 1c, 1d, 1e and 1f); the estimated values for percent lysis ranged from 30.00 in *T. viride* (NAIMCC-1952) to 82.5 in *T. viride* (NAIMCC-11817) (Table 1). Thus, all tested isolates irrespective of their geographical location exhibited antagonism to the *C. gloeosporioides* isolate of grapes. *Trichoderma* species are well known producers of extracellular lytic enzymes *viz.* chitinases, beta-1, 3-glucanases, proteases etc. (Verma *et al.,* 2007) which cause lysis of the protoplasm as well as the cell wall and is a major mechanism of antagonism.

Production of toxic volatile and non-volatile metabolites

All the *Trichoderma* species and isolates produced non volatile metabolites which inhibited the radial growth of *C. gloeosporioides* by 40.8 per cent (*T. harzianum,* NAIMCC-1756) to 63.6 per cent (*T. hamatum,* NAIMCC-

Table 1. Details of *Trichoderma* isolates studied and their antagonism to *Colletotrichum gloeosporioides*

NAIMCC Acc. No.	Species	Source of isolate	Time taken to contact (days)	Days to grow completely	Per cent degradation of protoplasm	Inhibition of radial growth by	
						volatile metabolites (%)	non-volatile metabolites (%)
NAIMCC-1727	*T. harzianum*	Coimbatore – sugarcane, rhizosphere	3	4	65.0 (53.7)	53.6 (47.1)	33.7 (35.4)
NAIMCC-1741	*T. harzianum*	Indore – soyabean	4	> 5	35.0 (36.2)	58.2 (49.7)	24.1 (29.4)
NAIMCC-1742	*T. harzianum*	Andaman & Nicobar islands – pumpkin, rhizosphere	3	5	42.5 (40.6)	50.5 (45.3)	24.9 (29.9)
NAIMCC-1744	*T. harzianum*	Thiruvananthapuram	3	5	77.5 (62.1)	54.0 (47.3)	11. (19.1)
NAIMCC-1745	*T. harzianum*	Thiruvananthapuram	3	4	37.5 (37.7)	51.1 (45.6)	04.4 (11.9)
NAIMCC-1746	*T. harzianum*	Thiruvananthapuram	3	4	62.5 (52.2)	55.1 (47.9)	03.7 (10.9)
NAIMCC-1756	*T. harzianum*	Kerala – soil of western ghats	3	5	57.5 (49.3)	40.8 (39.7)	20.0 (26.5)
NAIMCC-1961	*T. harzianum*	Malda – rhizosphere	3	5	72.5 (58.4)	56.0 (48.4)	43.3 (41.1)
NAIMCC-1962	*T. harzianum*	Darjeeling – Sukna forest soil,	3	5	42.6 (40.6)	45.9 (42.6)	43.7 (41.4)
NAIMCC-1965	*T. harzianum*	Darjeeling – acacia, rhizosphere	3	5	72.5 (58.6)	57.9 (49.5)	45.6 (42.4)
NAIMCC-1967	*T. harzianum*	Darjeeling – acacia, rhizosphere	3	4	62.5 (52.2)	50.0 (45.0)	26.3 (30.8)
–	*T. harzianum* 5R	Kodagu – citrus, rhizosphere	4	5	67.5 (55.2)	53.0 (46.8)	10.0 (18.2)
NAIMCC-1812	*T. viride*	Coimbatore – sugarcane, soil	3	> 5	50.0 (45.0)	62.5 (52.2)	03.7 (09.1)
NAIMCC-1817	*T. viride*	Western Ghats of Kerala – soil	3	5	82.5 (65.8)	63.0 (52.5)	43.0 (40.9)

NAIMCC Acc. No.	Species	Source of isolate	Time taken to contact (days)	Days to grow completely	Per cent degradation of protoplasm	Inhibition of radial growth by	
						volatile metabolites (%)	non-volatile metabolites (%)
NAIMCC-1822	*T. viride*	Kerala – soil of western ghats	2	4	32.5 (34.5)	53.4 (47.0)	48.9 (44.3)
NAIMCC-1823	*T. viride*	Kerala – soil of western ghats	3	5	70.0 (56.9)	49.3 (44.6)	48.5 (44.1)
NAIMCC-1951	*T. viride*	Darjeeling – Mahananda reserve forest	3	4	42.5 (40.7)	46.5 (43.0)	59.6 (50.5)
NAIMCC-1952	*T. viride*	Darjeeling – Sukna forest soil	3	4	30.0 (33.0)	53.6 (47.1)	45.6 (42.4)
NAIMCC-1956	*T. viride*	Darjeeling – tea, rhizosphere	3	5	37.5 (37.7)	54.3 (47.5)	0.00 (0.00)
NAIMCC-1760	*T. koningii*	Andaman & Nicobar islands – tomato, rhizosphere	3	5	50.0 (45.0)	47.4 (43.5)	25.6 (30.3)
NAIMCC-1761	*T. koningii*	Maunath Bhanjan – soil	3	4	40.0 (39.1)	48.6 (44.2)	43.3 (41.1)
NAIMCC-1762	*T. koningii*	Odisha – fish pond sediment	2	3	70.0 (56.9)	53.1 (46.8)	45.2 (42.2)
NAIMCC-1938	*T. koningii*	Andaman & Nicobar islands – clove, rhizosphere	2	5	75.0 (60.1)	57.5 (49.3)	33.0 (35.0)
NAIMCC-1774	*T. pseudokoningii*	Coimbatore – sugarcane, rhizosphere	3	5	57.5 (49.4)	41.6 (40.1)	48.1 (43.9)
NAIMCC-1775	*T. pseudokoningii*	Andaman & Nicobar islands – cowpea, rhizosphere	4	> 5	72.5 (58.6)	47.8 (43.7) (43.7)	41.5 (40.1)
NAIMCC-1776	*T. pseudokoningii*	Andaman & Nicobar islands – cucumber, rhizosphere	3	4	37.5 (37.7)	50.9 (45.5)	65.9 (54.3)
NAIMCC-1777	*T. pseudokoningii*	Pune – onion, rhizosphere	2	5	45.0 (42.1)	44.7 (41.9)	27.8 (31.7)
NAIMCC-1716	*T. hamatum*	Andaman & Nicobar islands – ivy gourd, rhizosphere	2	5	62.5 (52.2)	59.3 (50.4)	39.6 (39.0)

NAIMCC Acc. No.	Species	Source of isolate	Time taken to contact (days)	Days to grow completely	Per cent degradation of protoplasm	Inhibition of radial growth by	
						volatile metabolites (%)	non-volatile metabolites (%)
NAIMCC-1717	*T. hamatum*	Andaman & Nicobar islands – bottle gourd, rhizosphere	3	4	52.5 (46.4)	62.2 (52.0)	08.5 (16.8)
NAIMCC-1719	*T. hamatum*	Andaman & Nicobar islands – lady's finger, rhizosphere	3	> 5	42.5 (40.6)	50.1 (45.0)	00.0 (00.0)
NAIMCC-1720	*T. hamatum*	Andaman & Nicobar islands – brinjal, rhizosphere	2	4	40.0 (39.1)	63.6 (52.9)	25.2 (30.0)
NAIMCC-1769	*T. asperellum*	Pitchavaram – soil	3	5	55.0 (47.9)	52.5 (46.4)	16.7 (24.1)
NAIMCC-1937	*T. asperellum*	Uttaranchal – soil	3	4	47.5 (43.5)	53.0 (46.7)	08.5 (16.8)
NAIMCC-1763	*T. lectea*	Indore – soyabean	3	4	60.0 (50.7)	49.3 (44.6)	53.7 (47.1)
–	Control		–	–	00.0 (0.00)	00.0 (0.00)	00.0 (0.00)
CD (0.05)	–	–	6.4	06.0	4.8		

Figures in parentheses are arcsine transformed values.

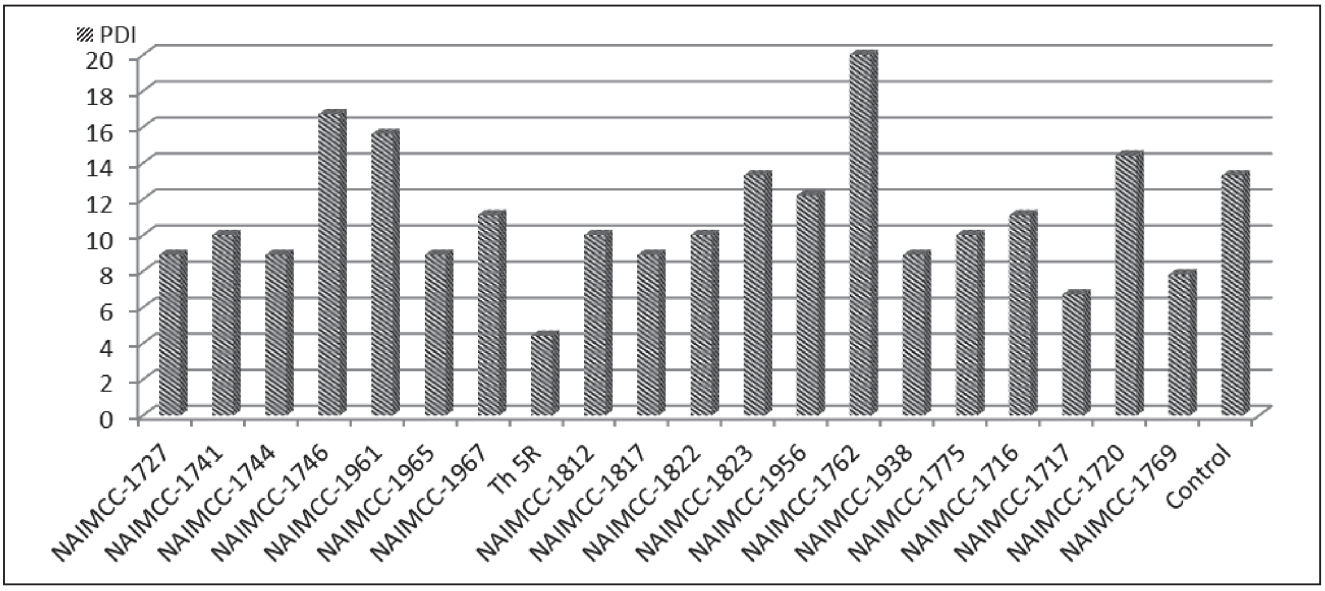

Fig. 1 PDI of anthracnose on grape leaves treated with different *Trichoderma* isolates

1720). However, the isolates differed widely in the production of non-volatile metabolites. Isolate *T. pseudo-koningii* (NAIMCC-1776) gave maximum inhibition of 65.9 per cent but there were five isolates which did not inhibit at all or caused negligible inhibition of less than five percent (Table 1). Eziashi (2006) and Rini and Sulochana (2007) also found that certain isolates of *Trichoderma* did not produce toxic non-volatile metabolites. Tronsmo (1978) had shown that production of metabolites by *Trichoderma* is temperature dependent; volatile metabolites are produced at higher temperatures and non-volatile metabolites at lower temperatures. The high incubation temperature of 28°C in the present study might have favored production of volatile rather than the non-volatile metabolites. Though the inhibitory effect of volatile and non-volatile metabolites of *Trichoderma* species against many plant pathogens is well documented, there are only a few reports on their effect on *Colletotrichum* species (Ajith and Lakshmidevi, 2010; Faheem *et al.,* 2010) who showed the inhibitory effect of volatile metabolites produced by isolates of *T. virens, T. harzianum, T. viride, T. reesei* and *T. saturni-sporum* against *C. capsici.*

There was no correlation between the *Trichoderma* species and the percent lysis of mycelium ($r^2 = 0.104$), and the percent inhibition of growth by volatile ($r^2 = 0.024$) or non-volatile ($r^2 = 0.055$) metabolites, indicating that antagonism against *C. gloeosporioides* is not a property of any particular species of *Trichoderma,* but rather the property of a particular isolate.

***In vivo* bio-efficacy**

In the *in vivo* study, the PDI value in control was 13.3, while in the twelve *Trichoderma* treatments it ranged from 4.4 to 20.0. Among the isolates, the lowest disease was recorded in *T. harzianum* 5R (4.4 PDI) followed by *T. hamatum* (NAIMCC-1717) and *T. asperellum* (NAIMCC-1769) which recorded PDI values of 6.7 and 7.8 respectively (Fig. 1). The PDI values in these isolates were significantly less than that recorded in control. In an earlier study too foliar application of a spore suspension of *T. harzianum* was found to inhibit the development of *C. gloeosporioides* induced lesions on guava leaves (Pandey *et al.,* 1993).

Five other isolates *viz. T. koningii* (NAIMCC-1938), *T. viride* (NAIMCC-1817) and *T. harzianum* (NAIMCC-1727, 1744 & 1965) also recorded PDI values on par with *T. harzianum* 5R, but these PDIs were also on par with that of control. In the other isolates, though the PDI values appeared to be higher than control, they were on par. Only one isolate *viz. T. koningii* (NAIMCC-1762) recorded PDI value significantly higher than control. This was surprising as this isolate had exhibited high *in vitro* antagonism. Such anomalous behavior was also reported by Anees *et al*. (2010) who found that the *Trichoderma* isolate T40 which was highly inhibitory *in vitro* actually enhanced disease intensity *in vivo.* Similar observations were also made by Asran-Amal *et al*. (2005) where an antagonistic *Trichoderma* isolate actually reduced plant growth in the presence of pathogen.

Survival of selected *Trichoderma* isolates on the grape phylloplane

The first eleven isolates with least PDI values were selected for their establishment on the grape phylloplane. When applied at 5×10^6, the initial counts were about 1×10^5 per cm^2 (on both adaxial and abaxial leaf surfaces). After five days of application, the cfu counts ranged from 4087.05 (*T. harzianum* 5R) to 1185.42 (NAIMCC-1775) per cm^2 showing a reduction in their population (Fig. 2). In a study on the survival of 4 species of *Trichoderma viz. T. harzianum, T. hamatum, T. atroviride* and *T. longibrachiatum* on strawberry leaves, it was found that the numbers declined rapidly in the first three days, but, the decline was less rapid during subsequent period (Freeman *et al.,* 2004), till no colonies could be detected on day 14. However, in the present study, colonies could be detected even after 15 days of application. The cfu ranged from 56.62 (NAIMCC-1717) to 290.16 (NAIMCC-1744) cfu per cm^2 (Figure 2). But, as the cfu counts were low, the *Trichoderma* applications in the field bio-efficacy trial were scheduled at weekly intervals.

Evaluation of selected *Trichoderma* isolates for the management of anthracnose in field

In field, the average RH value during the period of study ranged from 88.7 ± 8.7%. The PDI value was least (11.00) in *T. harzianum* 5R treated vines, followed by *T. viride* (NAIMCC-1817) and *T. harzianum* (NAIMCC-1727) which recorded PDI values of 13.50 and 13.76 respectively (Table 2). The PDI in these three treatments were on par and significantly less than that recorded in control (18.00). In the two subsequent observations, the PDI of anthracnose increased to 30.75 and then to 40.00 in control, but seven *Trichoderma* isolates recorded PDI values significantly less than that of control on the third observation. Least PDI value of 25.75 was recorded in *T. viride* (NAIMCC-1817) treatment which was significantly less than that in all other isolates, except that of *T. harzianum* (NAIMCC-1965). The other isolates which recorded low PDI values were *T. koningii* (NAIMCC-1938), *T. pseudokoningii* (NAIMCC-1775), *T. harzianum* 5R, *T. harzianum* (NAIMCC-1744) and *T. hamatum* (NAIMCC-1717).

The most effective isolate viz. *T. viride* (NAIMCC-1817) had shown maximum percent degradation of protoplasm *in vitro* (Table 1) and also exhibited most pronounced coiling around the hyphae of *C. gloeosporioides*. Incidentally all the seven isolates which gave significant control of the disease in field, had recorded more than 70 percent degradation of protoplasm *in vitro* (Table 1), except *T. harzianum* 5R which had recorded a marginally lower percentage at 67.50 percent. But, this isolate had shown maximum establishment on leaves, which also could have contributed to its higher efficacy. Although, three other isolates *viz. T. harzianum* (NAIMCC 1961), *T. viride* (NAIMCC 1823) and *T. koningii* (NAIMCC 1762) showed more than

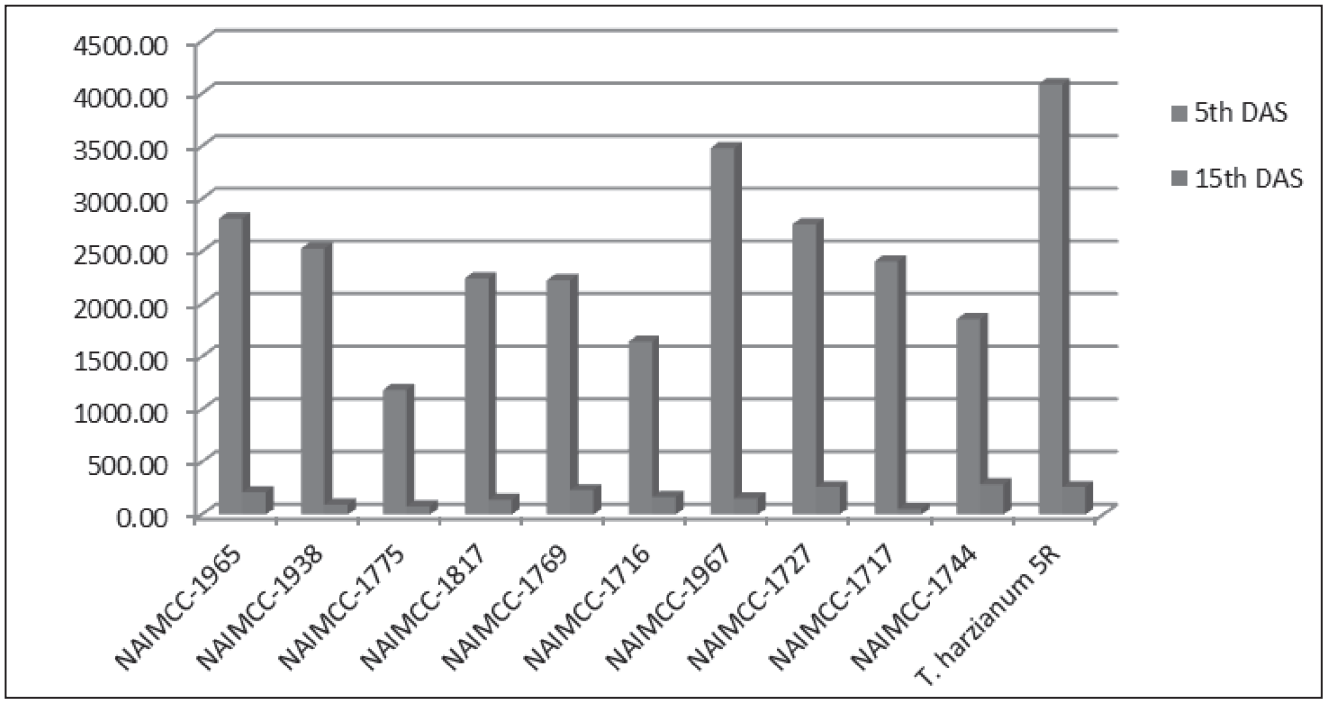

Fig 2. *Trichoderma* cfu per cm^2 of leaf area after 5 and 15 days of application

Table 2. Bio-efficacy of *Trichoderma* isolates in management of anthracnose on Thompson Seedless

Accession No.	Species	PDI of anthracnose on leaves		
		After 7 days	After 14 days	After 21 days
NAIMCC-1727	*T. harzianum*	13.75 (21.62)	26.25 (30.72)	40.75 (39.61)
NAIMCC-1744	*T. harzianum*	17.75 (24.70)	28.00 (31.92)	33.25 (35.18)
NAIMCC-1965	*T. harzianum*	14.75 (22.49)	20.75 (27.04)	26.75 (31.12)
NAIMCC-1967	*T. harzianum*	16.50 (23.86)	34.75 (36.07)	40.75 (39.63)
–	*T. harzianum* 5R	11.00 (19.24)	23.75 (29.13)	31.75 (34.26)
NAIMCC-1817	*T. viride*	13.50 (21.47)	20.50 (26.85)	25.75 (30.42)
NAIMCC-1938	*T. koningii*	18.50 (25.25)	25.00 (29.83)	30.75 (33.56)
NAIMCC-1775	*T. pseudokoningii*	17.50 (24.61)	23.00 (28.59)	30.75 (33.63)
NAIMCC-1716	*T. hamatum*	17.50 (24.65)	36.75 (37.27)	46.50 (42.97)
NAIMCC-1717	*T. hamatum*	15.00 (22.65)	23.75 (29.11)	34.75 (36.06)
NAIMCC-1769	*T. asperellum*	16.25 (23.61)	28.50 (32.21)	36.00 (36.83)
–	Control	18.00 (25.08)	30.75 (33.61)	40.00 (39.20)
CD (0.05)	2.90	2.63	2.79	

Figures in parentheses are arcsine transformed values

70 percent degradation of protoplasm *in vitro* but it could not reduce PDI *in vivo (*Table 1). Further studies are required to find the reasons for their reduced bio-efficacy.

One each of these isolates was originally obtained from soil of western Ghats of Kerala, Darjeeling, Kodagu and Thiruvananthapuram and three from Andaman and Nicobar islands. Thus, these seven efficient isolates were originally obtained from four geographically diverse locations from different climatic zones and ecosystems. Further these seven effective isolates belonged to five different species of *Trichoderma*.

Out of the five isolates of *T. harzianum* evaluated in the field, two were not effective. Earlier, Gopalkrishnan *et al*. (2003) evaluated 12 *Trichoderma* isolates belonging to *T. viride, T. harzianum, T. virens* and *T. hamatum* isolates for their efficacy in controlling cotton seed rot and damping off and found that \one isolate was more effective.

Earlier, a number of *Trichoderma* species have been evaluated and found effective for the control of diseases caused by *Colletotrichum* species e.g. *T. viride* for the control of brown blotch of cowpea caused by *C. truncatum* (Bankole and Adebanjo, 1996); *T. harzianum, T. hamatum, T. atroviride* and *T. longibrachiatum* for the control of anthracnose of strawberry caused by *C. acutatum* (Freeman *et al.*, 2004) and yam anthracnose (Michereff *et al.*, 1995). In grapes, *T. harzianum* isolates have been tested and found effective for the control of postharvest rot caused by *Botrytis cinerea* (Elad, 1994; Harman *et al*., 1996) and of pruning wounds (Halleen *et al.,* 2010) and though the potential of *T. harzianum* 5R for control of anthracnose was shown by Sawant and Sawant (2008) to the best of our knowledge this is the first study on systematic selection and evaluation of *Trichoderma* from a large geographically diverse population. This finding also emphasizes the need for systematic evaluation of the large collection of bio-control isolates available in various institutions in India for control of different diseases on various crops. The evaluation may identify an isolate with wide host range and climatic adaptability, like isolate T39 of *T. harzianum* (Elad, 2000) which would help in commercialization.

This study has brought out the possibility of biological control of anthracnose disease of grapes and indicates the need for large scale field trials with the selected efficient isolates of *Trichoderma* as alternative to or in combination with the traditional use of chemicals in management. Studies on improving the efficiency of the effective biocontrol isolates by approaches like i) making

effective and stable formulations, ii) prophylactic applications before onset of the disease incidence to build up the populations to the required levels (Elad and Kirshner, 1993a) and identification of suitable micro-climate for the establishment and proliferation (Elad and Kirshner, 1993b), iii) coupling with soil applications of isolates which can elicit ISR (De Meyer *et al.*, 1998), and iv) integrating or alternating *Trichoderma* applications with safer fungicides (Elad, 1994; Harman *et al*., 1996) are required to further improve level of the disease control in the field.

ACKNOWLEDGEMENTS

The authors are thankful to the Indian Council of Agricultural Research (ICAR), New Delhi for funding the research under the AMAAS Project. The authors also gratefully acknowledge the National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, for providing the *Trichoderma* cultures.

REFERENCES

Ajith PS, Lakshmidevi N. 2010. Effect of volatile and non-volatile compounds from *Trichoderma* spp. against *Colletotrichum capsici* incitant of anthracnose on bell peppers. *Nature Sci.* **8**: 265–269. (ISSN: 1545–0740).

Anees M, Tronsmo A, Edel-Hermann V, Hjeljord LG, Heraud C, Steinberg C. 2010. Characterization of field isolates of *Trichoderma* antagonistic against *Rhizoctonia solani. Fungal Biol.* **114**: 691–701.

Asran-Amal A, Abd-Elsalam KA, Omar MR, Aly AA. 2005. Antagonistic potential of *Trichoderma* spp. against *Rhizoctonia solani* and use of M13 micro-satellite-primed PCR to evaluate the antagonist genetic variation. *J Plant Dis Prot.* **112**: 550–561.

Bankole SA, Adebanjo A. 1996. Biocontrol of brown blotch of cowpea caused by *Colletotrichum truncatum* with *Trichoderma viride. Crop Prot.* **15**: 633–636.

De Meyer G, Bigirimana J, Elad Y, Hofte M. 1998. Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea. European J Pl Pathol.* **104**: 279–286.

Dennis C, Webster J. 1971a. Antagonistic properties of species-groups of *Trichoderma*: I. Production of non-volatile antibiotics. *Trans British Mycol Soc.* **57**: 25–39.

Dennis C, Webster J. 1971b. Antagonistic properties of species-groups of *Trichoderma*: II. Production of volatile antibiotics. *Trans British Mycol Soc.* **57**: 41–48.

Dennis C, Webster J. 1971c. Antagonistic properties of species-groups of *Trichoderma*: III. Hyphal interaction. *Trans British Mycol Soc.* **57**: 363–369.

Elad Y. 1994. Biological control of grape grey mould by *Trichoderma harzianum. Crop Prot.* **13**: 35–38.

Elad Y. 2000. *Trichoderma harzianum* T39 preparation for biocontrol of plant diseases-control of *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Cladosporium fulvum*. *Biocontrol Sci Tech.* **10**: 499–507.

Elad Y, Chet I, Henis Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soils. *Phytoparasitica* **9**: 59–67.

Elad Y, Kirshner B. 1993a. Establishment of an active *Trichoderma* population in the phylloplane and its effect on grey mould (*Botrytis cinerea*). *Phyto-parasitica,* **22,** (supplement 1): 137s–141s.

Elad Y, Kirshner B. 1993b. Survival in the phylloplane of an introduced bio-control agent (*Trichoderma harzianum*) and populations of the plant pathogen *Botrytis cineria* as modified by abiotic conditions. *Phytoparasitica* **21**: 303–313.

Eziashi EI, Uma NU, Adekunle AA, Airede CE. 2006. Effect of metabolites produced by *Trichoderma* species against *Ceratocystis paradoxa* in culture medium. *African J Biotech.* **5**: 2196–2201.

Faheem A, Razdan VK, Mohiddin FA, Bhat KA, Sheikh PA. 2010. Effect of volatile metabolites of *Trichoderma* species against seven fungal plant pathogens *in-vitro*. *J Phytol.* **2**: 34–37.

Freeman S, Minz D, Kolesnik I, Barbul O, Zveibil A, Maymon M, Nitzani Y, Kirshner B, Rav-David D, Bilu A, Dag A, Shafir S, Elad Y. 2004. *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea* and survival in strawberry. *European J Pl Pathol.* **110**: 361–370.

Gopalakrishnan C, Ramanujam B, Prasad RD, Rao N S, Rabindra R J. 2003. Screening and selection of potential *Trichoderma* isolates for the control of cotton seed rot and damping off. *J Bio Control* **17**: 161–165.

Halleen F, Fourie PH, Lombard PJ. 2010. Protection of grapevine pruning wounds against *Eutypa lata* by

biological and chemical methods. *South African J Ecology Viticulture* **31**: 125–132.

Harman GEA, Latorre B, Agosin E, Martin RS, Riegel DG, Nielsen PAA, Tronsmo A, Pearson RC. 1996. Biological and integrated control of *Botrytis* bunch rot of grape using *Trichoderma* spp. *Biol Control* **266**: 259–266.

Michereff SJ, Silveira NSS, Reis A, Mariano RLR. 1995. Greenhouse screening of *Trichoderma* isolates for control of *Curvularia* leaf spot of yam. *Mycopathologia* **130**: 103–108.

Pandey RR, Arora DK, Dubey RC. *1993.* Antagonistic interactions between fungal pathogens and phylloplane fungi of guava. *Mycopathologia,* **124**: 31–39.

Rini CR, Sulochana KK. 2007. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporium* infecting tomato. *J Trop Agric.* **45**: 21–28.

Sawant IS, Sawant SD. 2008. Potential of *Trichoderma* spp. for management of anthracnose disease of grapevines. *Acta Horticulturae* **785**: 301–304.

Sawant IS, Sawant SD. 2010. *Anthracnose of grapes*. Published by IIHR, Bangalore under ICAR-ORP ALCOCERA (ORP on Leaf spot diseases of field and horticultural crops). 14 p.

Shovan LR, Bhuiyan MKA, Begum JA, Pervez Z. 2008. *In vitro* control of *Colletotrichum dematium* causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma harzianum*. *International J Sustainable Crop Production* **3**: 10–17.

Sivakumar D, Wilson WRS. 2000. Antagonistic effect of *Trichoderma harzianum* on postharvest pathogens of rambutan (*Nephelium lappaceum*). *Phytoparasitica* **28**: 240–247.

Sobowale AA, Odeyingbo OA, Egberongbe HO, Feyisola RT, Ayinde OA, Adesemowo A. 2010. Growth inhibition (*in vitro*) of *Colletotrichum gloeosporioides* isolated from cassava (*Manihot esculenta*) using *Trichoderma longibrachiatum. African J Microbiol Res.* **4**: 2196–2201.

Soytong K, Srinon W, Rattanacherdchai K, Kanokmedhakul S, Kanokmedhakul K. 2005. Application of antagonistic fungi to control anthracnose disease of grape. *J Agril Biotech.* **1**: 33–41.

Tronsmo A. 1978. Effect of temperature on antagonistic properties of *Trichoderma* species. *Trans British Mycol Soc.* **71**: 469–474.

Verma M, Brar SK, Tyagi RD, Surampalli RY, Valero JR.2007. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochemical Eng J.* **37**: 1–20.