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Inhibitory effect of different plant extracts and antifungal metabolites of *Trichoderma* strains on the conidial germination and germ tube growth of *Colletotrichum capsici* causing chili anthracnose

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Abstract

Antifungal activities of twenty four plant extracts and secondary metabolites of five *Trichoderma* strains were tested against conidial germination and germ tube growth of *Colletotrichum capsici*. Hundred percent inhibition of conidial germination and shortest germ tube formation of *Colletotrichum capsici* were recorded in *Azadiracta indica* (leaf), *Ocimum sanctum* (leaf) and *Curcuma longa* (rhizome) extracts after 4 to 24 hours of incubation. *Lantana camara* (leaf) and *Colocasia antiquorum* (leaf) extracts exhibited less inhibitory against the pathogen. Different concentrations and different day-old metabolites of five *Trichoderma* strains were significantly affected to inhibit of conidial germination and germ tube growth of *C. capsicci*. The hundred percent inhibitions of conidial germination and shortest germ tube formation was exhibited at 2000 mg/l concentration of 30-day-old metabolites of *T. harzianum* IMI-392433 and the lowest inhibition of conidial germination and longest germ tube formation was recorded at 1000 mg/l concentrations of 10-day-old metabolites of *T. pseudokoningii* IMI-392431after 4 to 24 hours of incubation. This study showed that the plant extracts of *Azadiracta indica* (leaf), *Ocimum sanctum* (leaf) and *Curcuma longa* (leaf) posses high ability to inhibit conidial germination and germ tube formation of *C. capsici* and antifungal metabolites of *T. harzianum* IMI-392433 have good antagonistic activity on *C. capsicci* spore germination and germ tube formation and that can be used as potential biological control agent to control the Anthracnose disease of the pathogen.

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Introduction

Anthracnose disease caused by *Colletotrichum capsici* is one of the major diseases for chili. This fungus causes severe damage on chili fruits in both pre and post harvest stages and these infections together account for more 50% of the crop losses (Pakdeevaraporn *et al*., 2005). The fungus is both internally and externally seed-borne (Ramachandran *et al*., 2007). The black wound found on infected fruits will expand very quickly under high moisture condition, especially in tropical countries. Fruiting bodies and spores of *C. capsici* will be abundantly produced on those black lesions. Sowing such contaminated seeds results in pre emergent and post emergent damping-off of seedlings in nursery and field. The fungus survives in an active form on the stems and branches causing die-back symptoms. There are various methods in controlling the disease, but the two common ones are chemical using benomyl and bio control (Intana *et al*., 2007). Since the 1940s, a wide range of pesticides have been used in the vegetable industry. These include products comprising all groups of pesticides from organic to synthetic agrochemicals. Vegetable growers generally apply pesticides as preventive measure to control insect pests and diseases. The indiscriminate and over usage of a wide range of pesticides is prevalent and many undesirable problems have emerged, i.e. development of pest resistance, food poisoning, residue contamination in market produce, environmental pollution, and escalating costs in vegetable production. One of the options overcome these problems is to replace the unilateral chemical approach with the search for alternative control methods. The control of chili anthracnose fruits rot has, for many years, relied on chemicals and resulted in many undesirable problems. There was a need to incorporate alternative control components which are effective at farm level. Biological control of fruit rot and die-back of chili with plant products tested in many laboratories and field trials showed that the crude extract from rhizome, leaves and creeping branches of sweetflag (*Acorus calamus* L.), palmorosa (*Cymbopogon martinii*) oil, *Ocimum sanctum* leaf extract, and neem (*Azadirachia indica*) oil could restrict growth of the anthracnose fungus and were tested for their efficacy to reduce fruit rot of capsicum (Jeyalakshmi and Seetharama, 1998; Korpraditskul *et al*., 1999). Biological control of plant pathogens by microorganisms has also been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). *Trichoderma* and its products have been studied and used for bio control in many countries. The fungi *Trichoderma* has been an exceptionally good model to study bio control because it is ubiquitous, easy to isolate and culture, grows rapidly on many substrates, affects a wide range of plant pathogens, acts as a mycoparasite, competes well for food and growth sites, produces antibiotics and has an enzyme system capable of attacking a wide range of plant pathogens (Islam *et al.,* 2008). Furthermore, *Trichoderma* inhibit or degrade pectinases and other enzymes that are essential for plant pathogenic fungi, such as *Botrytis cinerea*, to penetrate leaf surfaces (Zimand *et al*., 1996). Most of effective *Trichoderma* strains produced both cell wall lytic enzymes and secondary metabolites against the disease causing fungi (Rahma *et al*., 2009). Therefore, the purposes of this research work were to observe the effect of different plant extracts and antifungal metabolites extracted from selective five *Trichoderma* strains for inhibition of spore germination and germ tube growth of *C. capsici*.

Materials and methods

Isolation and pathogenicity test of the pathogen

C. capsici, a causal agent of anthracnose was isolated from nine anthracnose infected chili fruits obtained, from the different chili planting areas in Pabna and Rajshahi districts, Bangladesh. Anthracnose fungus was isolated by tissue transplanting method described by Agrios (2005). For pathogenicity test, each of nine isolates of *C. capsici* was cultured on PDA for 3 days. Then 0.7 cm agar plug contained with mycelia of *C. capsici* was placed on pierced area on chili fruit (*Capsicum annum* L. var. annum) obtained from chili plantations area at Agriculture field, Khorkhori, Rajshahi district. All inoculated fruits were incubated in moist plastic chamber, kept at room

temperature (27±°C). Disease severity of anthracnose infection was recorded at 5 days after incubation by measuring size of diseased lesion on chili fruit. The percentage of disease severity was calculated by using the formula; $((R_T-R_C)/R_T) \times 100$, when R_T was the mean of diseased lesion radius on chili in the tested treatment and R_C was the mean of diseased lesion radius on chili in the control (placed with an agar plug without C. *capsici*). For each treatment, there were four replicates and 5 chili fruits were used in each replicate. Then a most pathogenic isolate was used for further.

Preparation of plant extracts

Leaf extraction of *Azadiracta indica*, *Andrographis paniculata*, *Cassia alata*, *Targates erecta*, *Ocimum sanctum*, *Rauwolfea serpentina*, *Piper betel* L*, Adhatoda vasica, Acalypha indica*, *Piper longum*, *Vinca rosea*, *Lantana camara*, *Datura matel*, *Coccinia cordifolia*, *Curcuma longa*, *Colocasia antiquorum*, *Salmalia malabaricum*, *Calotropis procera*, *Centella asiatica*, *Moringa oleifera*; and rhizome extractions of *Curcuma longa*, *Zingiber officinales* and bulb extraction of *Allium sativum* and *Allium cepa* tissues in alcohol was done following the method described by Mohadevan and Sridhar (1982). Five gram tissue was cut in pieces and immediately plunged in to boiling 80 % ethyl alcohol in a beaker and allowed to boil for 5-10 minutes, using 5-10 ml of alcohol for each gram tissue. The extraction was carried out on top of a steam bath. It was cooled in a pan of cold water. The tissues were crushed thoroughly in a mortar with a pestle and then passed through two layers of cheese cloth and re-extracted the ground tissues in hot 80 % alcohol for 3 minutes, using 2-3 ml of alcohol for per gram of tissues. Extracts were cooled and passed through cheese cloth and filtered through Whatman No. 1 filter paper. Extracts were evaporated on a stem bath to dryness and made 5 % concentration with sterilized distilled water for experiment.

Inhibition of conidial germination by plant extracts

Conidia of *C. capsici* Cca-AKR-01 were taken from the culture on PDA plates and conidial suspension (10×10^3 conidia/ml) was made separately with different concentrations of plant extracts. A drop of treated conidial suspension (10×10^3 conidia/ml) from different plant extract was taken on grove slides in a moisture chamber for 2-24 hours of incubation. After that a drop of lacto phenol cotton blue was put over the conidial suspension on the slides. The slides were examined under high power microscope (× 400) for observing the inhibition percentage of conidial germination of *C. capsici* Cca-AKR-01 using the following formula of Akhter *et al*. (2006). Where, Inhibition (%) of conidial germination = Total no. of conidia-No. of germinated conidia/Total no. of conidia ×100. The length of germ-tube was also measured with the help of lactophenol cotton blue staining under light micro-scope and compared with a control treatment.

Sources of Trichoderma strains

Five *Trichoderma* strains namely; *T. virens* IMI-392430, *T. pseudokoningii* IMI-392431 and *T. harzianum* IMI-392432, *T. harzianum* IMI-392433 and *T. harzianum* IMI-392434 were used in this study which was collected from Biotechnology and Microbiology Laboratory, Department of Botany, Rajshahi University, Bangladesh. These strains were isolated and identified from decomposed garbage and soil by Rahman (2009) and were previously verified by CABI Bioscience, Surrey, U.K.

Extraction of antifungal metabolites from Trichoderma strains

Five *Trichoderma* strains were cultured on PDA for 3 days, before the margin of colony of each strain was cut by 0.7 mm cork borer. Twenty-five plugs of each *Trichoderma* strain were put into 3 liter flask containing 1 liter potato dextrose broth (PDB) and incubated at room temperature for 10, 20 and 30 days. After incubation of 10, 20 and 30 days, conidia and mycelia of *Trichoderma* strains were removed from broth culture by filtration. The culture filtrate was used for extraction of antifungal metabolites. The extraction of antifungal metabolites was sequences with 350 ml of ethyl acetate. Separation of the two phases was facilitated by the addition of 5 g of sodium chloride to the first extraction. Then ethyl acetate was

evaporated at 40°C in a rotary evaporator. Dry weights of antifungal were recorded (Intana, 2003).

Inhibition of spore germination of C. capsici by metabolites of Trichoderma strains

Spore suspension of *C. capsici* Cca-AKR-01 was prepared from 7-day-old culture growing on PDA, using PDB as diluents. Concentration of spores in a suspension was determined and adjusted with haemacytometer to10 x 10^3 spores/ml. Then, 5.0 ml of spore suspension was mixed with 5.0 ml of 1000, 1500 and 2000 mg/l of 10, 20 and 30 days old each antifungal metabolite in a test tube, respectively and kept for 24 h at room temperature before the sample was stained with lacto phenol cotton blue. Germinating spores and the length of germ-tube were observed and recorded under compound light microscope and compared with a control treatment. The percentage inhibition of conidial germination of *C. capsici* Cca-AKR-01 was calculated by using the formula; $((R_C-R_T)/R_C) \times 100$, when R_T was the mean of spore germination on the tested treatment and R_C was the mean of spore germination on the control treatment (Yenjit *et al*., 2004).

Statistical Analysis

All data were analyzed by DMRT using the help of computer package program SPSS (SPSS Inc., Chicago, IL, USA).

Results and discussion

Isolation and pathogenicity test of the pathogen

The results showed that all strains of *C. capsici* could cause disease on chili fruits at various levels of percent infection. Isolates Cca-AKR-01 and Cca-AKR-02 collected from Agriculture field, Khorkhori, Rajshahi provided 82.69 and 81.78 % of infection. While isolate Cca-ARU-01 provided only 48.12 % of infection (Table 1). Therefore isolate Cca-AKR-01 was used in this experiment.

Effect of plant extracts on conidial germination of C. capsici

Twenty four plants extracts tested for the inhibition percentage of conidial germination of *C. capsici* Cca-AKR-01 and the results are presented in Table 2. Inhibition percentage of conidial germination of *C. capsici* Cca-AKR-01 was significantly affected by the application of different plant extract after 2 to 24 hours of incubation. Among the plant extracts tested, hundred percent of conidial germination was inhibited with the application of *Azadiracta indica* (leaf), *Curcuma longa* (rhizome) and *Ocimum sanctum* (leaf) and the lowest inhibition of conidial germination (%) was recorded in *Lantana camara* and *Colocasia antiquorum* after 2 to 24 hours of incubation.

Table 1. Percent infection on chili fruits by *Colletotrichum capsici* isolated from anthracnose infected chili fruits in various locations.

Strains	Location of isolates	Infection (%)
Cca-AIP-01	Agriculture field, Ishurdi, Pabna	61.47 e
Cca-AIP-01	Agriculture field, Ishurdi, Pabna	57.49 f
Cca-AKR-01	Agriculture field, Khorkhori, Rajshahi	82.69 a
Cca-AKR-02	Agriculture field, Khorkhori, Rajshahi	81.78 a
Cca-AKR-03	Agriculture field, Khorkhori, Rajshahi	75.24 b
Cca-ABR-01	Agriculture field, Budpara, Rajshahi	72.18 c
Cca-ABR-02	Agriculture field, Budpara, Rajshahi	70.48 cd
Cca-AMR-01	Agriculture field, Meahearchondi, Rajshahi	68.79 d
Cca-ARU-01	Agriculture research field, Rajshahi University campus, RU.	48.12 g

Means in a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

Among the plant extracts tested, the most promising fungi toxic effect was also recorded in *Curcuma longa* (leaf), *Zingiber officinales* (rhizome), *A. sativum* (bulb) and *A. cepa* (bulb) after 24 hours of incubation, which corresponded to 99.62, 98.47, 95.47 and 93.47 % inhibition of conidial germination, respectively. The germ tube formation was significantly affected by the application of different plant extract after 2 to 24 hours of incubation. The longest germ tube formation was found in *Lantana camara* (leaf) extract and the shortest was *Azadiracta indica* (leaf) extract after 2 to 24 hours of incubation, respectively.

Table 2. Effect of different plant extracts on the inhibition of conidial germination and germ-tube elongation of *C. capsici* Cca-AKR-01 within 24 hr of incubation.

Name of plants	Used part	Inhibition of conidial germination (%) after various incubation period *						Length of germ tube (µm) *
		2 h	4 h	6 h	8 h	12 h	24 h	
Azadiracta indica	Leaf	100 a	100 a	100a	100a	100a	100 a	4.18 q -25.38 t
Andrographis paniculata	Leaf	64.16m	65.58 m	67.78m	69.19lmn	70.13lmn	74.48ijkl	16.78 j -45.59 m
Cassia alata	Leaf	45.56vw	46.12 v	46.88vw	47.18y	48.63vw	50.56vwx	37.18 c -69.94 d
Targates erecta	Leaf	83.23e	84.36 e	85.76f	87.14e	89.38de	91.81cd	9.19 m -37.45 o
Ocimum sanctum	Leaf	98b	99 a	100a	100a	100a	100a	4.98 pq -27.71 s
Rauwolfea serpentine	Leaf	62.16n	63.36 n	64.45n	66.68o	68.89mn	71.17klmn	18.89 i - 48.27 l
Piper betle	Leaf	47.14u	48.27 u	49.26tu	51.14xy	52.26tu	54.53tuv	32.23 d -65.58 e
Adhatoda vasica	Leaf	80.16f	81.34 f	82.94g	84.88f	86.61e	88.91de	10.98 l -39.32 n
Acalypha indica	Leaf	53.36r	54.16 r	56.31q	57.14uv	58.26qr	61.17qrs	25.53 g -58.88 h
Piper longum	Leaf	51.17st	52.21s	53.34r	55.28vw	56.14rs	59.96qrst	27.73 f -60.57 g
Vinca rosea	Leaf	66.96jk	67.75jkl	69.18lm	71.28kl	73.38jkl	76.18ijkl	14.48 k - 44.64 m
Moringa oleifera	Leaf	49.17u	50.43t	51.14s	53.36wx	54.19st	57.76rstu	30.15 e -62.23f
Datura matel	Leaf	60.14o	61.13o	62.27o	63.35pq	64.49o	68.27mnop	20.38 i -50.25k
Coccinia cordifolia	Leaf	58.12p	59.94op	60.27p	61.12rs	62.29op	65.17opq	22.12 h -54.43 j
Curcuma longa	Rhizome	100a	100a	100a	100a	100a	100a	5.18pq -29.18 s
Curcuma longa	Leaf	90.58c	92.98b	95.91b	96.32b	98.74a	99.62a	5.84 opq -32.12 r
Colocasia antiquorum	Leaf	42.16w	43.32w	44.11xy	45.21y	46.36wx	47.88wxy	41.14 b -74.43 b
Salmalia malabaricum	Leaf	41.13xy	41.94wx	42.28yz	45.96y	46.68wx	48.22wx	39.91b -72.12 c
Calotropis procera	Leaf	70.12i	72.14i	73.74j	76.11ij	77.73ghi	82.21fgh	11.12 l -40.28 n
Centella asiatica	Leaf	55.17q	57.16q	59.92p	60.28st	61.24opq	63.34pqr	24.12 g -56.68 i
Lantana camara	Leaf	40.31xy	41.12x	41.84yz	42.26yz	43.34xy	47.78wxy	43.34 a -76.68 a
Zingiber officinales	Rhizome	89.26c	90.63c	91.57c	93.73c	95.27b	98.47ab	6.65op -34.22 q
Allium sativum	Bulb	87.27d	88.48d	89.21d	91.18d	92.74bc	95.47abc	7.18no - 35.58 pq
Allium cepa	Bulb	84.56e	86.93d	87.53e	89.32d	90.37cd	93.47bcd	8.71mn -36.47op

* mean of three replications
Means in a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

Table 3. Efficacy of different days and different concentrations antifungal metabolites (1000, 1500 and 2000 mg/l) from five *Trichoderma* strains on the inhibition of spore germination and germ-tube elongation of *Colletotrichum capsici* Cca-AKR-01within 24 hr of incubation.

Concentrations (mg/l)	Strains of *Trichoderma*	Inhibition of conidial germination (%) after various incubation period *						Length of germ tube (µm)*
		2 h	4 h	6 h	8 h	12 h	24 h	
		10 days old metabolites						
1000	***T. virens*** IMI-392430	21.97z	42.18yz	45.12 z	47.82 z	52.82 y	55.15 v	71.18 b - 96.68 b
	T. pseudokoningiiIMI-392431	22.92z	41.18z	44.29 z	46.18 z	51.17 z	53.23 w	73.38 a - 98.44 a
	T. harzianum IMI-392432	26.62x	43.12y	48.81 y	50.24 y	55.92 x	58.86 tu	58.12 cd - 91.14 d
	T. harzianum IMI-392433	27.75wx	44.98x	49.92 x	52.26 y	56.68 x	59.92 t	57.18 de - 90.12 d
	T. harzianum IMI-392434	25.55y	42.48yz	47.74 y	49.48 y	55.84 x	57.77 u	59.46 c - 93.32 c
1500	***T. virens*** IMI-392430	29.16w	47.97w	53.12 wx	56.68 w	58.86 yz	61.98 s	55.58 e - 87.33 e
	T. pseudokoningiiIMI-392431	28.82w	46.32x	50.21 x	54.46 x	57.96 yz	60.14 t	56.15 e - 89.92 e
	T. harzianum IMI-392432	31.21v	51.17v	54.48 wx	59.98 tu	61.14 uv	63.38 rs	52.28 f - 84.47 f
	T. harzianum IMI-392433	32.17v	52.28v	55.48 w	60.84 tu	62.26 tu	64.64 qr	50.16 g - 82.28 g
	T. harzianum IMI-392434	30.92v	49.94w	53.22 xy	58.88 v	60.84 vw	63.12 rs	53.37 f - 86.64 e
2000	***T. virens*** IMI-392430	36.48 t	54.22 u	59.82 u	62.28 t	64.45 tu	66.12 q	58.86 g - 79.98 h
	T. pseudokoningiiIMI-392431	34.28 u	54.12 u	57.74 v	61.94 t	63.36 tu	65.58 q	59.98 g - 81.12 gh
	T. harzianum IMI-392432	41.17 r	59.94 s	62.19 st	65.54 s	67.84 rs	70.89 op	55.54 h - 76.68 i
	T. harzianum IMI-392433	42.28 qr	60.14 s	63.27 rs	67.74 r	68.44 rs	71.12 op	53.35 i - 74.48 j
	T. harzianum IMI-392434	39.98 s	57.92 t	61.12 tu	64.45 s	66.99 s	69.72 p	56.64 h - 77.38 i
		20 days old metabolites						
1000	***T. virens*** IMI-392430	45.59 p	64.48 r	65.48 p	69.92 q	71.14 q	73.34 n	49.54 jk - 72.28 kl
	T. pseudokoningiiIMI-392431	43.32 q	61.34 s	64.47 qr	68.24 r	69.33 r	72.18 no	51.16 j - 73.39 jk
	T. harzianum IMI-392432	49.89 n	66.18 qr	67.12 p	71.18 pq	72.12 q	76.68 r	47.22 lm - 69.78mn
	T. harzianum IMI-392433	50.42 n	67.75 pq	69.86 o	72.25 q	74.48 p	77.17 lm	46.29 m - 68.24 n
	T. harzianum IMI-392434	47.24 o	65.29 r	66.48 p	70.94 pq	71.98 q	75.58 m	48.18 kl - 71.12 lm
1500	***T. virens*** IMI-392430	53.38 m	69.98 no	71.18 o	75.54 n	76.12 o	80.18 k	43.12 no - 65.54 op
	T. pseudokoningiiIMI-392431	52.12 m	68.48 op	70.44 o	73.34 o	75.54 op	78.44 l	44.46 n - 66.48 o
	T. harzianum IMI-392432	56.92 kl	72.12 lm	74.26 mn	77.12 n	80.12 mn	82.18 j	41.19 pq - 62.28 q
	T. harzianum IMI-392433	57.12 kl	73.34 kl	75.54 m	79.94 m	81.21 lm	83.38 ij	40.12 qr - 61.12 qr
	T. harzianum IMI-392434	55.78 l	71.18 mn	73.33 n	76.58 n	79.29 n	81.94 j	42.28 op - 64.64 p
2000	***T. virens*** IMI-392430	60.47 j	75.54 j	79.98 k	81.17 lm	83.38 k	85.54 gh	37.82 s - 57.72 s
	T. pseudokoningiiIMI-392431	58.38 k	74.92 jk	77.74 l	80.26 m	82.21 kl	84.46 hi	39.48 r - 59.94 r
	T. harzianum IMI-392432	62.18 i	78.44 i	82.22 ij	83.44 jk	85.54 j	87.12 fg	34.48 t - 54.48 tu
	T. harzianum IMI-392433	63.38 hi	79.58 hi	83.48 hi	84.18 ij	86.64 ij	88.27 ef	33.18 tu - 53.37 uv
	T. harzianum IMI-392434	61.94 ij	76.39 j	81.12 jk	82.22 kl	85.14 j	86.98 fg	36.69 s - 55.44 t
		30 days old metabolites						
1000	***T. virens*** IMI-392430	67.12 g	81.18 gh	85.48 fg	86.11 h	88.84 h	90.12 d	29.18 v - 48.86 w
	T. pseudokoningiiIMI-392431	64.26 h	80.33 gh	84.28 gh	85.32 hi	87.72 hi	89.24 de	32.27 u - 52.44 v
	T. harzianum IMI-392432	70.89 f	84.12 f	86.23 f	88.68 fg	91.27 fg	92.12 c	24.16 w – 46.18 w
	T. harzianum IMI-392433	71.29 f	85.58 ef	86.47 f	89.73 fg	92.18 f	93.28 c	22.18 x - 39.14 y
	T. harzianum IMI-392434	68.37 g	81.39 g	86.18 f	88.47 g	90.47 g	91.89 c	26.26 w – 47.58 w
1500	***T. virens*** IMI-392430	74.24 e	87.34 cd	88.47 e	92.24 e	95.52 de	96.46 b	17.78 x - 32.34 x
	T. pseudokoningiiIMI-392431	72.14 f	86.32 de	86.61 f	90.18 f	94.29 e	95.44 b	19.18 x - 36.65 x
	T. harzianum IMI-392432	78.92 cd	88.76 bc	91.18 d	95.58 cd	97.32 c	99.12 a	12.29 y - 28.88 y
	T. harzianum IMI-392433	80.14 c	89.86 b	93.13 c	96.14 c	98.17 bc	99.86 a	10.19 y - 26.69 yz
	T. harzianum IMI-392434	77.38 d	87.79 cd	90.48 d	94.42 d	96.64 cd	98.47 a	14.97 x - 29.22 y
2000	***T. virens*** IMI-392430	84.14 b	90.39 b	96.94 b	99.94 a	100 a	100 a	7.87 y - 22.72 yz
	T. pseudokoningiiIMI-392431	83.28 b	90.18 b	94.16 c	97.84 b	99.38 a	100 a	9.89 xy - 24.78 yz
	T. harzianum IMI-392432	80.14 a	96.28 a	100 a	100 a	100 a	100 a	4.65 z - 17.48 z
	T. harzianum IMI-392433	89.23 a	98.78 a	100 a	100 a	100 a	100 a	3.18 z - 16.78 z
	T. harzianum IMI-392434	89.14 a	95.76 a	99.24 a	100 a	100 a	100 a	5.18 z - 19.44 z

* mean of three replications

Means in a column followed by the same letter(s) are not significantly different according to DMRT (P=0.05).

In a similar study Imtiaj *et al* (2005) were tested 13 plant extracts against conidial germination of *Colletotrichum gloeosporioides* and observed that conidial germination of *C. gloeosporioides* was completely inhibited by *Curcuma longa* (leaf and rhizome), *Tagetes erecta* (leaf) and *Zingiber officinales* (rhizome), while *Vinca rosea* (leaf) and *Z. officinales* (rhizome) gave the longest and shortest germ tube formation of *C. gloeosporioides,* respectively. Akhter *et al.* (2006) was tested eight ethanolic plant extracts and ten aqueous plant extracts in combination with cow urine to inhibition of conidial germination of *Bipolaris sorokiniana* causing leaf blight disease of wheat and recorded that *Adhatoda vasica* (leaf) and *Zingiber officinalis* (rhizome) extracts were most effective in inhibition of conidial germination at 2.5 % concentration where, most cases *Ocimum sactum* extracts exhibited less inhibitory effect. Alam *et al.* (2002) tested the effect of ten plant extracts on conidial germination of *Colletotrichum gloeosporioide* and recorded that *Tagates erecta* (leaf) and *Azadirachta indica* (bark) extracts were most effective in inhibition of conidial germination at 5:1.5 (w/v) concentration. The fungi toxic effect of *Vinca rosea* and *A. indica* has been reported earlier by several investigations against *Botrytis sorokiniana*, *Fusarium oxysporium* f. sp. *vasinfectum*, *Rhizopus artocarpi* (Alam *et al*., 2004) and *Alternaria tenuis* (Alam *et al*. 1999).

Effect of secondary metabolites of *Trichoderma* on conidial germination of *C. capsici*

Percent inhibition of conidial germination of *C. capsici* Cca-AKR-01 varied significantly different ($p \leq 0.05$) by the application of different day's old and different concentrations metabolites of *Trichoderma* strains at different hours. The highest percent inhibition of conidial germination was achieved at 2000mg/l concentration after 2 to 12 hr by 30-day-old metabolites of *T. harzianum* IMI-392433 followed by *T. harzianum* IMI-392432, *T. harzianum* IMI-392434, *T. virens* IMI-392430 and *T. pseudokoningii* IMI-392431, respectively. The lowest percent inhibition of conidial germination of *C. capsici* Cca-AKR-01 was recorded at 1000mg/l concentration after 2 to 12 hours of 10-day-old metabolites of *T. pseudokoningii* IMI-392431 (Table 3). Antifungal metabolites extracted from all *Trichoderma* strains at 2000 mg/l concentration provided markedly high inhibitive activities on germ-tube growth of *C. capsici* Cca-AKR-01. The strongest inhibition of germ tube growth of *C. capsici* Cca-AKR-01 was recorded in 30-day-old metabolites of *T. harzianum* IMI-392433 at 2000 mg/l concentration and the lowest inhibition of germ tube growth was recorded at 1000mg/l concentration in 10-day-old metabolites of *T. pseudokoningii* IMI-392431 after 2 to 12 hours (Table 4) of incubation. This finding complies with many reports which asserted that *T. harzianum*, *T. virens* and *T. hamatum* are very effective at inhibiting mycelial growth of soil borne, seed borne, phyllosphere and storage plant pathogens on PDA (Chet, 1987; Papavizas, 1985; Tronsmo, 1986; Rahman *et al*., 2009). During plant pathogens attack, *Trichoderma* secreted many cell wall-degrading enzymes such as endochitinase, chitobiosidase, N-acetyl-β-glucosaminidase and glucan 1, 3-β-glucosidase. These enzymes strongly inhibit spore germination (or cell replication) and germ tube elongation (Tronsmo and Hjeljord, 1997). *Trichoderma* spp. can also produce different antibiotics against fungal phytopathogens. Among these antibiotics, the production of gliovirin, gliotoxin, viridin, pyrones, peptaibols and others have been described (Vey *et al*., 2001). Gliovirin from *T. virens* was active against *Pythium ultimum*, gliotoxin from *T. virens* was very active against *Rhizoctonia solani* (Howell *et al.,* 1993). There are many reports of successful use of antifungal metabolite extracted from *Trichoderma* spp. to control disease causing fungi such as *Sclerotium rolfsii* causing disease on vegetables (Maiti *et al*., 1991), *Ceratocystis paradoxa* causing pine apple disease of sugarcane (Rahman *et al*., 2009), *Pythium aphanidermatum* causing wilt of cotton and watermelon (Ordentlich *et al*., 1992) and damping-off of cucumber (Intana, 2003) and *Phytophthora* sp. causing various plant diseases (Wilcox *et al*., 1992). This research indicated an additional successful use of antifungal metabolites from *Trichoderma* strains in controlling anthracnose on chili fruits caused by *C. capsici*. Higher

efficacy of the metabolites of *Trichoderma* strains to inhibit spore germination and germ-tube growth of *C. capsici* usually resulting in better control of chili's anthracnose.

Conclusion

In conclusion, the present study demonstrated that the plant extracts of *Azadiracta indica* (leaf), *Ocimum sanctum* (leaf) *and Curcuma longa* (rhizome) posses high ability to inhibit conidial germination and germ tube formation of *C. capsici*, which might be used for controlling phytopathogen of plants. Secondary metabolites of *T. harzianum* IMI-392433 were also most effective to inhibit of conidial germination and germ tube formation of *C. capsicci*. This strain can be also used as potential biological control agent to control the Anthracnose disease of chili.

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