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**Short Communication**

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# Efficacy of *Trichoderma* spp. and fungicides against *Lasiodiplodia theobromae*

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## Abstract

Experiments were carried out to find out the bio-efficacy of four *Trichoderma* species, viz. *Trichoderma harzianum*, *T.koningii*, *T.viride* (green strain), *T.viride* (yellow strain) against canker pathogen *Lasiodiplodia theobromae.* Bioassay of antagonist against test pathogens conducted by dual culture techniques at different temperatures; volatile, non volatile and naturally untreated metabolites of isolates were examined. *T. koningii* and *T.viride* (yellow strain) exhibited maximum inhibition in controlling the pathogens. Fungicides, viz. Bavistin and Dithane M-45 used where Bavistin found little effective but Dithane M-45 showed no effects on pathogen. *Trichoderma viride* showed better performance to control *Lasiodiplodia theobromae* than commercial fungicides used during present investigation.

**Keywords**: *Trichoderma* spp; Biological control; *Lasiodiplodia theobromae*

## Introduction

Species of the *Botryosphaeriaceae* have a cosmopolitan distribution which occurs on a wide range of monocotyledonous, dicotyledonous and gymnospermous hosts, as well as on lichen thalli. *Lasiodiplodia theobromae* (Syn. *Botryodiplodia theobromae*) belongs to Botryosphaeriaceae, are associated with different symptoms such as shoot blights, stem cankers, fruit rots, die-back, gummosis (Ciesla *et al*., 1996), canker and die-back, followed by kino exudation, and in severe cases tree death (Shearer *et al*. 1987; Smith *et al*. 1994; Old & Davison 2000; Roux *et al*. 2001). Ciesla *et al*., (1996) reported that species of the *Botryosphaeriaceae* are generally regarded as weak pathogens that invade stressed or wounded plants after drought, hail, wind, frost or insect damage and was also cited that the *Botryosphaeriaceae* occur in asymptomatic tissue as latent pathogens in trees such as *Eucalyptus*, *Pinus* and *Syzigium* (Pavlic *et al*., 2004). Hence, present investigation was carried out to investigate the efficacy of biological agents; *Trichoderma* spp. and fungicides against *L.theobromae* causing disease in plants.

## Material and Methods

Four species of *Trichoderma* namely, *Trichoderma harzianum*, *T.koningii*, *T.viride* (green strain), *T.viride* (yellow strain) were isolated from spent (infected) mushroom spawn packets of *Pleurotus ostreatus* (Jacquin *ex* fr.) Kummer, during December'2010 to February'2011. *Lasiodiplodia theobromae* was also isolated from wood samples (saw dust) which used as raw materials for spawn packets preparation to grow commercial mushroom at National Mushroom Development and Extension Centre, Savar, Dhaka. After Surface sterilized samples were inoculated on PDA plates and incubated at three different temperatures viz. 20±2ºC, 28±2ºC, 35ºC. Radial growth of mycelium were measured. Mycelium of the pathogens was spread over the whole plate after 3 days and sub-cultured on PDA slants and incubated for further growth. Cultural and microscopic characteristics were observed under microscope.

*In vitro assay of antagonists by Dual culture technique*

*Trichoderma* isolates were evaluated against *Lasiodiplodia theobromae* by dual culture technique as described by Kunz (2007). A 5 mm diameter mycelial disc from the margin of the 7 days-old culture of *Trichoderma* isolates and the *Lasiodiplodia theobromae* was placed on the PDA media at opposite of the plate at equal distance from the periphery. In control plates, (without *Trichoderma*), a sterile agar disc was placed at centre of the plates. Inoculated plates were incubated at 28±2ºC, 32 ± 2ºC, and 35ºC until the end of the incubation period of 7 days. Inhibition percent was calculated (Kunz, 2007) by the following formulae:

$$\% \text{ inhibition} = C\text{-}T/C \times 100$$

Where,

C = Radial growth of control plates.

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T = Radial growth of treated plates.

*Volatile metabolites from antagonists on Lasiodiplodia theobromae*

The effect of released volatile metabolites of *Trichoderma* isolates on the mycelial growth of the pathogen was evaluated as methods described by Dennis and Webster (1971). Test pathogens were inoculated at the centre on PDA plates with 5 mm diameter mycelial growth and *Trichoderma* inoculated plates were inverted on the top of the test pathogens plates and held together by adhesive tape. Radial growths of the pathogens were recorded at 24 hours interval at room temperature (28 ± 2ºC).

*Effects of non volatile metabolites on Lasiodiplodia theobromae*

The method was followed as described by Kaur *et al*. (2006). Three mycelial agar blocks, each having 5mm diameter of four individual fungal antagonists, were cut off from the advanced margins of 5 day old culture and inoculated into a 500 ml conical flask containing 250 ml potato dextrose broth medium. The inoculated flasks were allowed for 15 days incubation period at 28 ± 2ºC. After incubation, the culture broth of each antagonist was filtered through a double ring filter paper (11cm) and finally through a millipore filter paper under suction pump to obtain cell and bacteria free extracts under aseptic conditions. All plates were incubated at room temperature 28 ± 2ºC and percent inhibition in mycelia growth was calculated. The effects of natural untreated metabolites by dipping culture disc method was followed as described by Ashrafuzzaman and Aminur (1992).

*In vitro assay of fungicides*

The effect of fungicides, namely Bavistin and Diathane-M 45 were used to examine the effectiveness against *L. theobromae* on PDA medium using 30 ppm, 50 ppm, 70 ppm concentration of each fungicides. Three replicated PDA plates were used for each dose of fungicides. PDA plate received no fungicide was served as control. The inoculated plates were incubated at 28±2ºC and percent of inhibition was calculated.

**Results and Discussion**

*T. harzianum* was characterized based on morphology such as colonies, hyphae, conidiophores, phialides and conidia according to Choi In-Young *et al.* (2003). Other strains of *Trichoderma* in the present study were characterized as described by Bernet (1960); Choi In-Young *et al.* (2010).

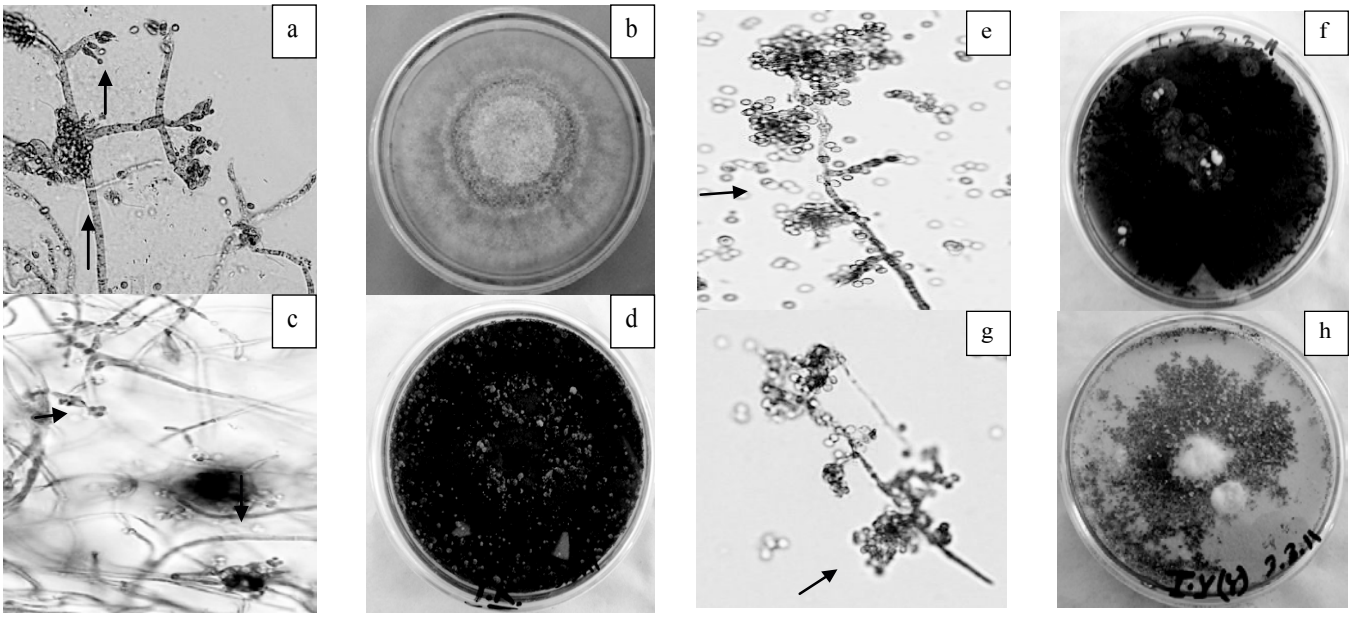


**Plate. Photographs showing cultural and microscopic features of *Trichoderma* spp. (**magnification 40X)
**a & b.** Microscopic features and colony of *Trichoderma harzianum* showing phialides, conidia
**c & d.** Microscopic features and colony of *Trichoderma koningii* showing phialides, conidia,coiling
**e & f.** Microscopic features and colony of *Trichoderma viride* (green strain) showing spores
**g & h.** Microscopic features and colony of *Trichoderma viride* (yellow strain) showing spores

The cultural and microscopic observation of the mycelia, spores of *L. theobromae* was confirmed, as described by Kunz (2007). Off-white colored immature colony appeared which turned into black color within 2-3 weeks. Colonies were luxuriant with regular fast growth. Black septate mycelium with colourless and unicellular spores was found at young stage. Upon maturity, spores became brown colored, distichously and thick walled. Spores were elliptical and larger in sized. The cultural and microscopic observation of the mycelia, spores of *L. theobromae* was confirmed, as described by Kunz (2007).


**Fig. (a) Septed mycelium of L.theobromae**

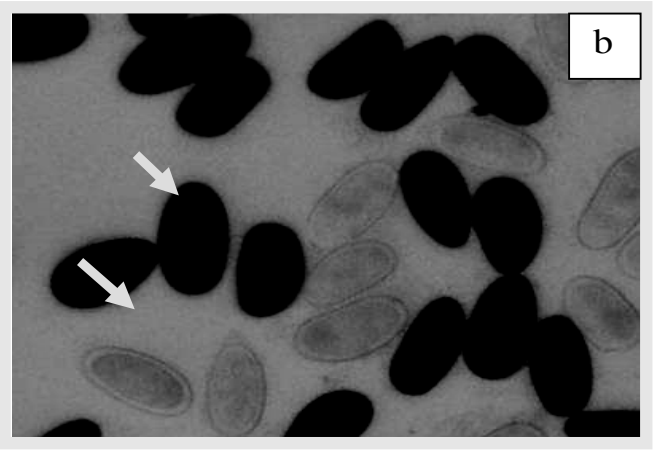


**Fig. (b) Bi-celled mature spore and single celled immature spore of L.theobromae**

Findings of the dual culture tests demonstrated that all the *Trichoderma* isolates tested showed inhibitory effects against *Lasiodiplodia theobromae* ranged from 60-75% at 28±2 ºC temperature whereas the maximum inhibition (80%) was exhibited by both *T.koningii* and *T.viride* (green strain) at 32±2 ºC and 35°C temperature (Table I). In case of volatile metabolites, *T.viride* (green strain) showed maximum inhibition (33.3%) whereas non volatile and naturally untreated metabolites of fungal cultures did not perform any significant reduction of mycelial growth of *L. theobromae* (Table 1). The mode of action of *Trichoderma* spp. showed mycoparasitism and competition for space and nutrients in dual culture which are in agreement with Kotze (2008). The antagonistic potentiality of *Trichoderma* spp. against *Lasiodiplodia theobromae* was also reported by earlier workers (Mortuza and Ilag, 1999, Yadav & Majumdar, 2005, Kunz, 2007). Mortuza and Ilag

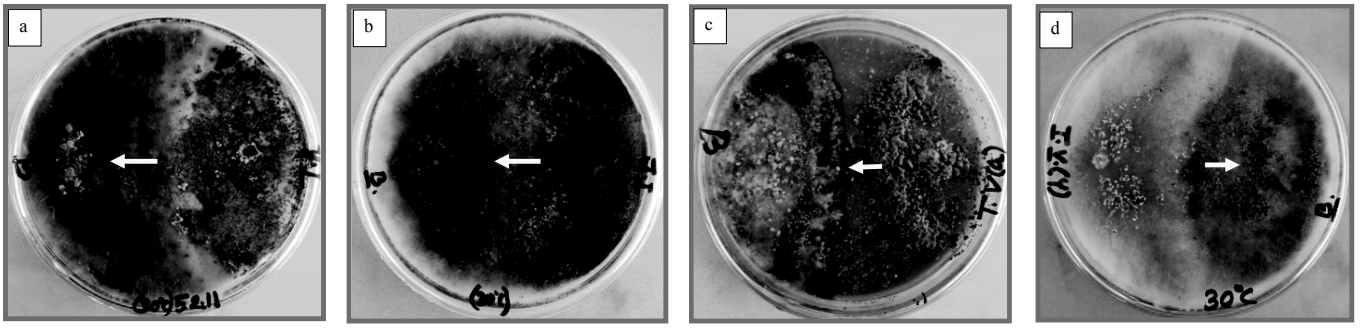


**Plate. Photographs showing growth and antagonistic activity of four *Trichoderma* spp. against *Lasiodiplodia theobromae*** on PDA medium

a. Overgrowth of *T.harzianum* to *Lasiodiplodia theobromae*
b. Overgrowth of *T.koningii* to *Lasiodiplodia theobromae*
c. Inhibittion zone between colonies of *Lasiodiplodia theobromae* and *T.viride* (yellow strain )
d. Overgrowth of *T.viride* (yellow strain) to *Lasiodiplodia theobromae*

(1999) cited that *T.harzianum* exhibited the greatest inhibition in dual culture, whereas Yadav & Majumdar (2005) reported that *T. viride* was more effective than *T. harzianum.*

Volatile metabolites from *Trichoderma viride* only inhibit (33.3%) the growth of fungi, *Lasiodiplodia theobromae*. Present findings have partially conformity with the results of Kotze (2008) who reported 23.6% inhibition by *T. atroviride*. During present study, non volatile metabolites had no effects on *Lasiodiplodia theobromae* which contradict to results cited by John *et al.* (2004).

During present investigations fungicide Bavistin found effective to control *Lasiodiplodia theobromae* at 70 ppm than others used, whereas Dithane M-45 showed no significant effect at any concentration (Table 2). These findings are contradictory with Yadav & Majumdar

**Table I. *In vitro* percent of inhibition of *Lasiodiplodia theobromae* by four *Trichoderma* spp. at different temperatures (7 days after incubation)**

| Antagonists | % of inhibition of *Lasiodiplodia theobromae* | | | | | |
|---|---|---|---|---|---|---|
| | Dual culture | | | Volatile | Non volatile | Naturally untreated |
| | 28±2 ºC | 32±2 ºC | 35ºC | 28±2 ºC | 28±2 ºC | 28±2 ºC |
| ***T. harzianum*** | 60 | 75 | NE | NE | NE | NE |
| ***T. koningii*** | 75 | 80 | 80 | NE | NE | NE |
| ***T. viride* (green strain)** | 60 | 75 | NE | 33.3 | NE | NE |
| ***T. viride* (yellow strain)** | 70 | 80 | 80 | NE | NE | NE |

Note:NE = No effect

**Table II. Effect of different concentration of Bavistin and Diathane M-45 on mycelia growth of *L. theobromae* at 28±2ºC temperature**

| Concentration of fungicides | % of growth inhibition of *L. theobromae* | |
|---|---|---|
| | Bavistin | Diathane M-45 |
| **30 ppm** | 0.58±0.58 c | NE |
| **50 ppm** | 0.06±0.06 b | NE |
| **70 ppm** | 0.44±0.44 a | NE |

• Data recorded after 7 days of incubation

(2005).

The aggressiveness of *Trichoderma* spp. studied varies more or less to previously mentioned workers. This might be due to difference in the characteristics of *Trichoderma*.

So, to control the pathogen by using *Trichoderma* isolates is an environment friendly and non hazardous approach over chemical control.

**References**


Ashrafuzzaman, M.H. and Aminur, R. K. 1992. Antifungal activity *in vitro* of some plant extract on *Rhizoctonia solani*. *Bangladesh J.Sci.Res.* **10(2)**: 243-244.

Bernet, H.L. 1960. *Illustrated Genera of Imperfect Fungi*. Second Edition. Burgees Pub. Co. Minneapoils, U.S.A.

Choi In-Young., Choi, J.-N., Praveen, K. S. and Lee. W. H. 2003. Molecular and Morphological Characterization of Green Mold, *Trichoderma* spp. isolated from Oyster Mushrooms. *The Korean Society of Mycology*. **31(2)**: 74-80.

Choi In-Young., Choi, J.-N., Praveen, K. S. and Lee. W. H. 2010. Isolation and Identification of Mushroom Pathogens from *Agrocybe aegerita*. *The Korean Society of Mycology*. **38(4)**: 310-315.

Ciesla, W.M., Diekmann, M. and Putter, C.A.J. 1996. *Eucalyptus spp.* FAO/IPGRI technical guidelines for the safe movement of germplasm No. 17. Rome, Food and Agriculture Organization of the United Nations & International Plant Genetic Resources Institute. pp.66.

Dennis, C. J. and J. Webster, 1971. Antagonism properties of species groups of *Trichoderma*, III. Hyphal interaction. *Transactions British Mycological Society*. **57**: 363-369.

John, J., Joy, M. and Abhilash, E. K. 2004. Inhibitory effects of Tamarind (*Tamarindus indica* L.) on polypathogenic fungi. *Allelopathy J.* **14(1)**: 43-49.

Kaur, M., Sharma, O.P. and Sharma, P.N. 2006. *In vitro* effect of *Trichoderma* species on *Colletotrichum capsici* causing fruit rot of chilli (*Capsicum annum* L.).*Indian Phytopathology*. **59(2)**: 243-245.

Kotze, C. 2008. Biological control of the grapevine trunk disease of pathogens:pruning wound protection. M.S. thesis in agriculture, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Kunz, R. 2007. Control of Post Harvest Disease (*Botryodiplodia* sp.) of Rambutan and *Annona* Species by Using a Bio-Control Agent (*Trichoderma* sp.). Experiments were undertaken by the Industrial Technology Institute (ITI) in cooperation with the International Centre for Underutilised Crops (ICUC).pp. 1-44.

Mortuza, M., Ilag, G. and Lina, L. 1999. Potential for Biocontrol of *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. in Banana Fruits by *Trichoderma* Species. *Biological Control.* **15 (3)**: 235-240.

Old, K.M. and Davison, E.M. 2000. Canker diseases of Eucalypts. In: *Diseases and pathogens of Eucalypts* (Keane PJ, Kile GA, Podger FD, Brown BN, eds). CSIRO Publishing, Australia.

Pavlic, D., Slippers, B., Coutinho, T.A., Venter, M. and Wingfield, M.J. 2004. *Lasiodiplodia gonubiensis* sp. nov, a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Studies in Mycology* **50**: 313-322.

Roux, J., Coutinho, T.A., Mujuni, B. D. and Wingfield, M.J. 2001. Diseases of plantation *Eucalyptus* in Uganda. *South African Journal of Science*. **97**: 16–18.

Shearer, B.L., Tippett, J.T. and Bartle, J.R. 1987. *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiate* in species selection trials. *Plant Disease*. **71**: 140−145.

Smith, H., Kemp, G.H.J. and Wingfield, M.J. 1994. Canker and die-back of *Eucalyptus* in South Africa caused by *Botryosphaeria dothidea*. *Plant Pathology*. **43**: 1031−1034.

Yadav, R.K. and Majumdar. V.L. 2005. Efficacy of Plant Extracts, Biocontrol Agents and fungicides Against *Lasiodiplodia theobromae* Incited Die –back of Guava (*Psidium guajava* L.) *J.Mycol.Pl.Pathol*. **35(2)**: 352-353.

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