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Short Communication

Detection of Biocontrol Agents from Contaminated Fungal Culture Plates

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Abstract

Three species of *Aspergillus* viz. *A. flavus*, *A. fumigatus*, *A. niger*, *Penicillium* sp.; *Trichoderma viride*, and two sterile fungi were found potential antagonist against cultured fungal plates in Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka.

There are many species of fungi attacking harmful fungi of agricultural and medical importance. Of these, few species have received much attention with a view use as biological control activities. The mechanisms of control have been comparatively less studied, and many questions remain to be answered. Some fungi are parasitic on other fungi. Fungi often compete strenuously with one another for substrate. Competition between the fungi is one of the biocontrol mechanisms of fungal pathogens has achieved its greatest successes. Species of *Aspergillus* viz. *A. flavus*, *A. fumigatus*, *A. niger*; *Penicillium* sp., *Trichoderma viride* had been reported as biocontrol agents (Madhanraj *et al*. 2010). All the above mentioned genera belong to the class Deuteromycetes. In nature they survive as saprophytes in soil debris, few are air borne, endophytic or pathogenic except *T. viride*. Except few species of *Penicillium* they are first growing and capable of producing numerous light spores within three to five days on substratum in nature or culture medium.

Lot of researches had been carried out on role of fungi as biocontrol agents (Chet 1987 & 1993; Dorner and Cole 2002; Trutemann *et. al*. 1983). Since there is few reports on this regards in Bangladesh (Begum and Begum, 2010; Bashar and Chakma, 2014), recently different isolates of fungi were observed as biocontrol agents in sporulating fungal cultures.

During the tenure of January 2013 to May 2015 about 10 separate research works had been conducted at Mycology and Plant Pathology Laboratory of Botany Department, Dhaka University. Fungi were isolated from infected samples following Tissue planting and Blotter method. Leaf stem or seed samples were used for isolation of fungi. In case of Tissue Planting method, fifty inocula each measuring 2 square mm. were cut with the help of a sterilized scalpel from a particular specimen and kept in a sterile Petri plate. The inocula were washed in sterile water and then surface sterilized by dipping them in 10% Clorox for 3-5 minutes. Then three inocula were placed in each plate containing sterilized Potato Dextrose Agar (PDA) medium, and incubated for 5-7 days at 25 ± 2 ºC.

In Blotter method, moist chambers were made by placing two layers of filter paper on the bottom of the petriplate

and covered with upper plate. In each Petri plate surface sterilized inocula or seed were placed and transferred them to incubation chamber. A total number of 50 inocula were transferred in 10 sterilized plates and incubated under room temperature for 5-7 days. The cultured fungal plates contaminated with a particular fungus or fungi showing antagonistic properties against cultured fungus or fungi were separated for the present study. Antagonism between cultured fungi and antagonist fungi were recorded following Skidmore and Dickson (1976) and Madhanraj *et al*. (2010). In the present investigation inhibitory action of antagonistic fungus over cultured fungi were calculated following Table 1 such as ++++ = 100% controlled (when antagonistic fungus completely grown over cultured fungus), +++ = 75% -80% controlled (when antagonistic fungus showed 75 -80 % growth over cultured fungus , ++ = 50% controlled (when antagonistic fungus showed 50 % growth over cultured fungus) , and + =25% or less than 25% controlled (when antagonistic fungus showed 25 to less than 25 % growth over cultured fungus).

The isolated fungi were identified based on morphological characteristics observed under a compound microscope following standard keys (Barnett and Hunter 2000, Booth 1971, Ellis 1971, 1976, Ellis and Ellis 1997 and Sutton 1980).

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During the tenure of January 2013 to May 2015, fourteen isolates of *Aspergillus niger*, two isolates of *A. flavus*, four isolates of *A. fumigates*, two isolates of *Penicillum* sp. and three isolates of *Trichoderma viride* were found to be potential biocontol agents obtained from contaminated culture plates in the laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka. Results of the experiments are presented in Plate 1-7 and Table 1.

Three isolates of *Aspergillus niger* were capable of controlling 50% growth of *A. terreus*, *A. fumigatus* and *A. flavus* colonies. *Aspergillus niger* with sclerotia was capable of controlling *A. niger* colony (Plate 1).

Two isolates of *Aspergillus flavus* also arrested the growth of *Alternaria alternata* and *Fusarium moniliforme* (Plate 4).

Plate 2. showed that four isolates of *A. niger* were capable of controlling growth of *Colletotrichum gloeosporioides*, *Cladosporium* sp., *A. flavus* and *A. fumigatus* colony.

Table 1. List of fungi found as biocontrol agent obtained from contaminated fungal culture plate

Fungal isolates (No.)	Controlled fungi on culture plates	Remarks
Plate-1		
An 1. *Aspergillus niger*	*A. terreus*	++
An 2. *Aspergillus niger*	*A. fumigatus*	++
An 3. *Aspergillus niger*	*A. flavus*	++
An 4. *Aspergillus niger* (with sclerotia)	*A. niger*	+
Plate-2		
An 5. *Aspergillus niger*	*C.gloeosporioides*	++
An 6. *Aspergillus niger*	*Cladosporium* sp.	+++
An 7. *Aspergillus niger*	*A. flavus* and *A. fumigatus*	++
An 8. *Aspergillus niger*	*A. fumigatus*	++
Plate-3		
An 9. *Aspergillus niger*	*Rhizopus* sp.	++ ++
An 10.*Aspergillus niger*	*Penicillium* sp.	++++
An 11.*Aspergillus niger*	*S. sclotiorum*	++
An 12.*Aspergillus niger*	*A. fumigatus*	+ +
An 13.*Aspergillus niger*	Sterile fungi	+
An 14.*Aspergillus niger*	*S. rolfsii*	+
Plate-4		
A fa 1.*Aspergillus flavus*	*Alternaria alternata*	+++
A fa 2.*Aspergillus flavus*	*F.moniliforme*	++
Plate-5		
Afu 1. *A. fumigatus*	*Fusarium* sp.	++ +
Afu 2. *A. fumigatus*	*Alternaria alternata*	++
Afu 3. *A. fumigatus*	*A. flavus*	+
Afu 4. *A. fumigatus*	*C. gloeosporioides*	+
Afu 5. *Aspergillus flavus*	*C.gloeosporioides*	+
P 1. *Penicillum* sp.	*A. flavus*	+
P 2. *Penicillum* sp.	*A. fumigauus*	+
Plate-6		
Tv 1. *Trichoderma viride*	*Curvularia lunata*	+++
Tv 3. *Trichoderma viride*	*A. alternata*	+++
Tv *Trichoderma viride*	*S. sclerotiorum*	++++
Plate -7		
Sterile fungus$_1$	*A. niger*	+++
Sterile fungus$_2$	*C. gloeosporioides*	++

++++= 100% controlled, +++ = 75% -80% controlled, ++ = 50% controlled and + =25% or less than 25% controlle.

Plate-1

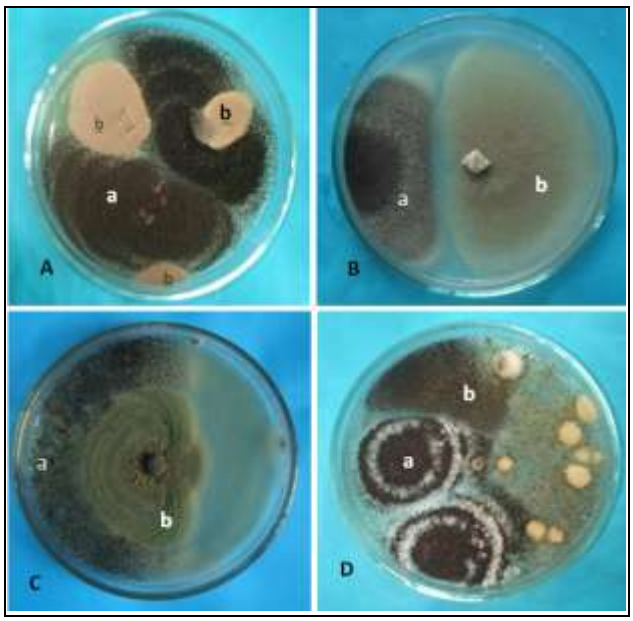

Plate-1. A. a. *Apergillus niger* (An1) controlling, b..*A. terreus* colonies, B. a. *A. niger* (An2) controlling b. *A fumigatus* colony, C. a. *A. niger* (An3) controlling b. *A. flavus* colony, and D. a. *A. niger* (An4) (with sclerotia) controlling b. *A. niger* colony.

Plate-2

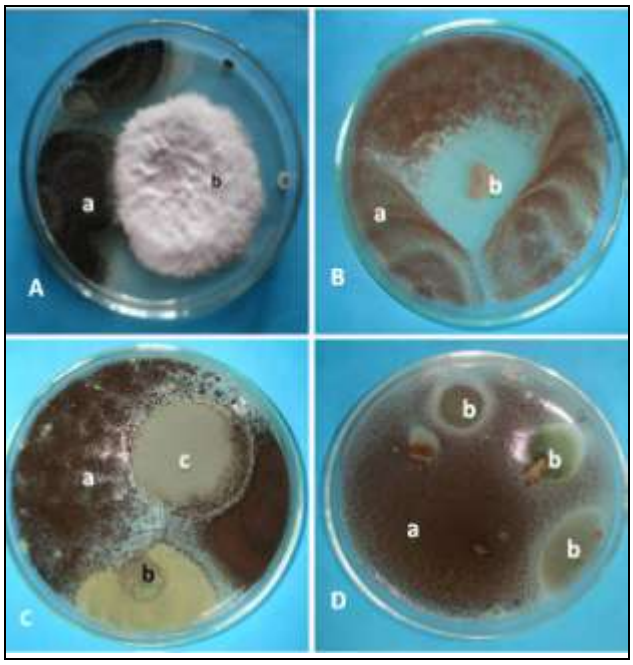

Plate -2. A. a. *Apergillus niger* (An5) controlling b..*Colletotrichum* sp. colony, B. a. *A. niger* (An6) controlling b. .*Cladosporium* colony. *C.* a. *A. niger* (An7) controlling b. *A. fluvus* and c. *A. fumigatus* colony, D. a. *A. niger* (An8) controlling b. *A. fumigatus* colony.

Plate-3

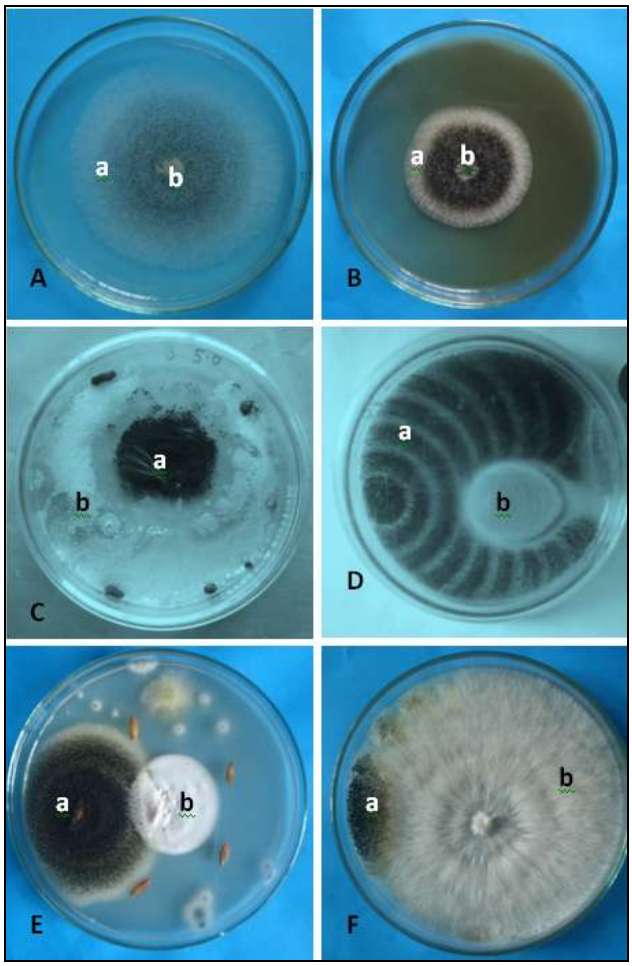

Plate -3. A. a. *A. niger* (An9) controlling b. *Rhizopus* sp., B. a. *A. niger* (An10) controlling *Penicillium* sp.,C. a. *A. niger* (An11) controlling b. *Sclerotinia sclerotiorum*, D. a. *A. niger* (An12*)* controlling b. *A. fumigatus*, E. a. *A. niger* (An13) controlling , b. a sterile fungus and F. a. *A. niger* (An14) controlling b. *Sclerotium rolfsii*.

Plate-4

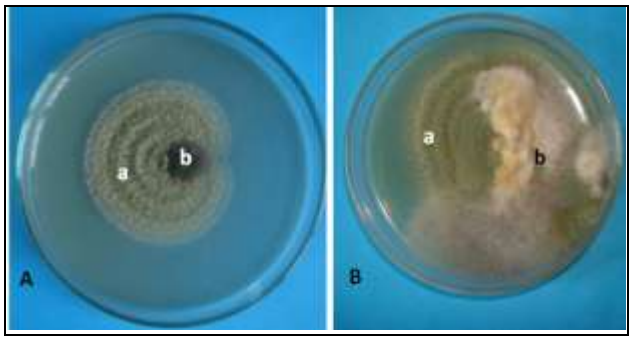

Plate-4. A. a. *Aspergillus flavus* (Af1) controlling b. *Alternaria alternata* colony and B. a. .*A. flavus* (Af2) controlling b . *Fusarium moniliforme* colony.

Plate 3 showed that six isolates of *A. niger* were capable of controlling of growth of *Rhizopus* sp., *Penicillium* sp., *Sclerotinia sclerotiorum, A. fumigatus*, sterile fungus and *Sclerotium rolfsii*.

Plate 5 showed that four isolates of *A. fumigatus* were capable of controlling growth of *Fusarium* sp., *A. alternata*, *A. flavus* and *C. gloeosporioides*. In the same culture plate *A. flavus* also controlled growth of *C. gloeosporioides.* Two isolates of *Penicillum* sp. was controlled colonial growth of *A. flavus* and *A. fumigatus* colony.

Plate 3. A. a. *A. niger* (An9) controlling b. *Rhizopus* sp., B. a. *A. niger* (An10) controlling *Penicillium* sp.,C. a. *A. niger* (An11) controlling b. *Sclerotinia sclerotiorum*, D. a. *A. niger* (An12*)* controlling b. *A. fumigatus*, E. a. *A. niger* (An13) controlling , b. a sterile fungus and F. a. *A. niger* (An14) controlling b. *Sclerotium rolfsii*.

Plate-5

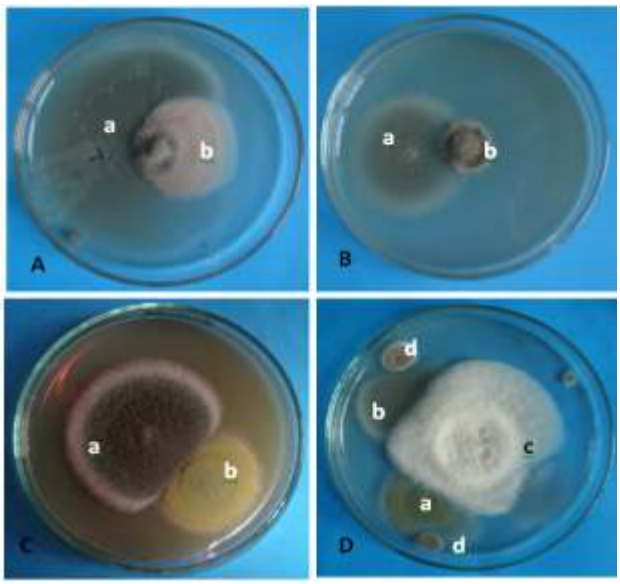

Plate-5. A. a. *Aspergillus fumigatus* (Afu1)controlling b. *Fusarium* sp., B. a. *A. fumigatus* (Afu2) controlling b. *Alternaria alternata* colony , C. a. *A. fumigatus* (Afu3) controlling b. *Aspergillus f*lavus colony D. a. *Aspergillus f*lavus (Af3) controlling c. *Colletotrichum gloeosporioides*, *b. A. fumigatus* (Afu5) controlling c. *C. gloeosporioides,* d. *Penicillum* sp. (Pn1) controlling a. *A. flavus*, d. *Penicillum* sp. (Pn2) controlling b. *A. fumigatus* colony

Three isolates of *Trichoderma viride* were capable controlling growth of *Curvularia lunata A. alternata* and *Sclerotinia sclerotiorum* (Plate 6).

Two sterile isolates were capable of controlling the growth of *A. niger* and *C. gloeosporioides* (Plate 7).

Plate-6

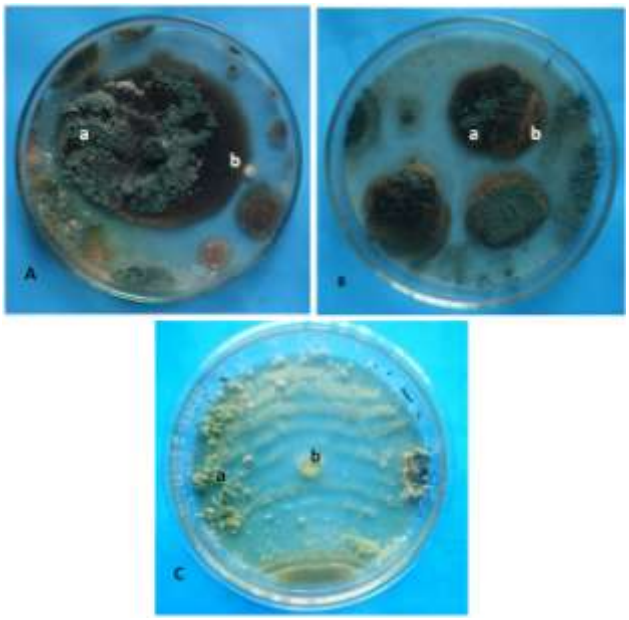

Plate-6. A. a. *Trichoderma viride* (Tv1) controlling b.*Curvularia lunata* colony, B. a. *T. viride* (Tv2) controlling *Alternaria alternata* colony and C. a. *T viride* (Tv3) controlling b. *Sclerotinia sclerotiorum* colony.

Plate-7

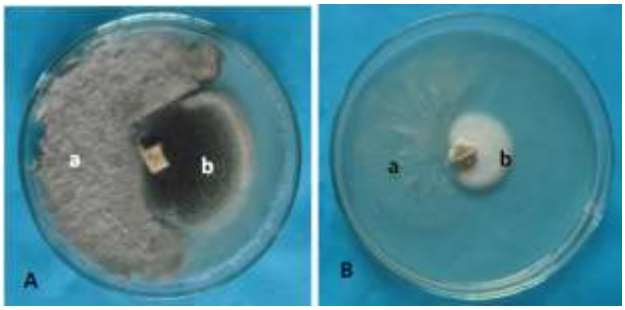

Plate 7. A. a. sterile fungus 1 controlling b. *Aspergillus niger* colony and B. a sterile fungus2 controlling b. *Colletotrichum gloeosporioides* colony.

Seven fungi *viz. Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Penicillium* sp., *Trichoderma harzianum* and *T. viride* associated with the rhizosphere, non-rhizosphere and rhizoplane of brinjal plants were selected to observe their antagonistic potential against the test fungi *Fusarium oxysporum* and *F. solani* . Out of seven soil fungi *T. harzianum* was found most effective to

control the growth of both the test fungi (Bashar and Chakma 2014).

Tiwari *et al*. (2012) reported the potential of *Aspergillus niger* and *Trichoderma viride* as biocontrol agents of wood decay fungi.

Present investigation indicates that *Aspegillus niger* (An 9 and An10) was capable of controlling 100% colonial growth of *Rhizopus* sp. and *Penicillium* sp. respectively. *Trichoderma viride* (Tv3) also controlled 100% colonial growth of *S. sclerotiorum* on PDA culture. Two isolates of *T. viride* (Tv1 and Tv2) were also capable of controlling 80% colonial growth of *A. alternata* and *C. lunata*.

Present finding will be helpful for selecting biocontrol agents against pathogenic fungi. Extensive research works in this regard can be carried out to confirm the present investigation.

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