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Antifungal activity test of fungal bio-agents against *Colletotrichum gloeosporioides* causing anthracnose of mango

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Abstract
Colletotrichum gloeosporioides, obtained from anthracnose symptoms on mango fruits. The disease infected mangoes were collected and isolated with confirming test. Bio-agents were applied in *in vitro* against *C. gloeosporioides* isolated from anthracnose affected mango. The study investigates to appropriate natural management. Three fungal bio-agents *Aspergillus* sp., *Pestalotia* sp. and *Trichoderma* sp. were used. Among the bio-agents, significantly highest mycelia growth inhibition 74.52% was achieved by *Aspergillus* sp. followed by 61.54% and 56.25% from *Pestalotia* sp. and *Trichoderma* sp. respectively over control. However, the present study suggested that *Aspergillus* sp. may use in controlling anthracnose of mango as bio-safety tools of integrated disease management.

Keywords: *Colletotrichum gloeosporioiedes* fungal bio-agents, anthracnose, mango

Introduction
Colletotrichum gloeosporioides is the causal agent of anthracnose and is particularly damaging on tropical fruits (Jeffries, *et al.*, 1990) [5]. This pathogen produces depressed lesions on fruits and blight like on leaves. The disease can destroy inflorescences and are most common in ripe mangoes during post-harvest. The productivity is hampered mainly due to the associated anthracnose, powdery mildew, die back, Phoma blight, bacterial canker and red rust diseases. Of these diseases anthracnose is economically the most important, causing heavy losses in mango production (Nelson, 2008) [7]. According to the researcher Akem (2006), anthracnose is presently recognized as field and post-harvest disease of mango worldwide. Disease symptoms appear as slightly, black, sunken irregular shape lesions, which gradually enlarge and developed, leaf spotting, blossom blight, fruit staining, dark brown sunken lesions on mango fruits, leaves and stems and may also cause twig die back and rot (Ploetz, 1999).
Several options exist for the control of mango anthracnose which include mango orchards in drier areas, farm sanitation and the application of fungicides (These methods are usually applied together in an integrated pest management programme (Nelson, 2008) [7]. Fungicide application to control mango anthracnose has been done both at the pre-and postharvest periods. However, they have been reported as being less effective under high disease pressure (Arauz, 2000) [3] and may also be phytotoxic to flowers (Dodd *et al.*, 1997) as well as health hazards. Therefore, it is important to find a practical, cost effective and non-toxic method to prevent fungal decaying of mango. Thus the experiment needs to develop natural disease control measures as alternative to chemicals. Use of biological agents provides an opportunity to avoid or minimize chemical preservatives. In context with above facts the present investigation was to find out the appropriate control of anthracnose of mango.

Material and Methods
The experiment was conducted in the Plant Disease Clinic (PDC) and Plant Pathology Laboratory under the Department of Plant Pathology of Patuakhali Science and Technology University (PSTU), Dumki, Patuakhali during the period from March 2019 to March 2020.

Collection and Isolation of *C. gloeosporioides*
Anthracnose affected mangoes were collected from in highly mango growing district Satkhira (Figure 1a). Mangoes with sunken lesion showing symptoms of anthracnose were preferred and isolated through Alam, *et al.*, 2017 [1]. The areas showing sunken lesion were cut by using a flame sterilized surgical scissors. Pieces of surface cut (5 ×5 mm) were put onto PDA containing petri plates and incubated at 25°C for 5 days (Figure 1b). The fungal hyphal tips growing from the diseased plant materials were transferred to fresh PDA containing petri plates for pure cultures and the fungal cultures were stored at 4°C in a freeze (Figure 1c).

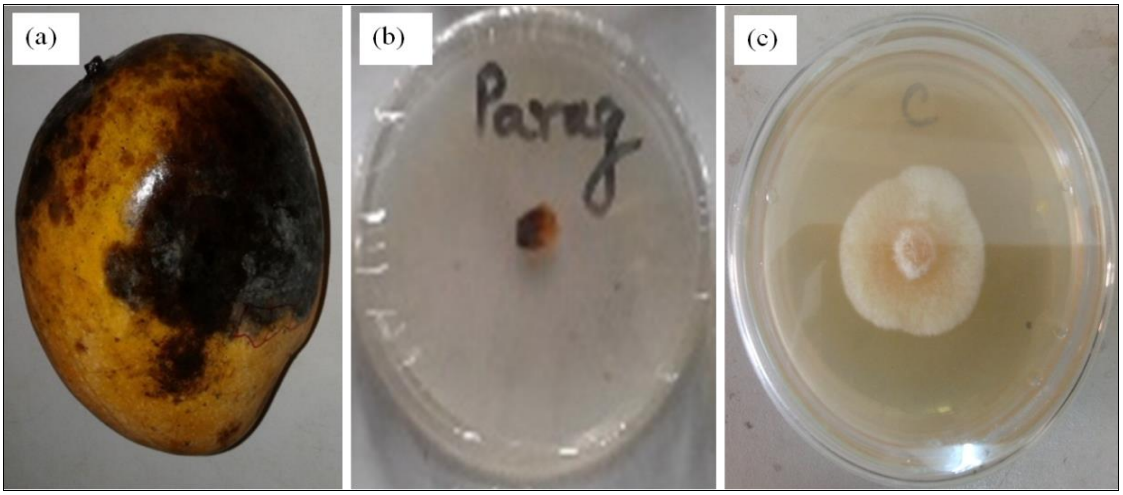

Fig 1(a-c): Isolation of *C. gloeosporioides* a) Anthracnose affected mango, b) Infected tissue planted on PDA medium and c) Pure culture of *C. gloeosporioides*

Collection and pure culture of bio-agents

The identified three bioagents (*Trichoderma* sp., *Aspergillus* sp., *Pestalotia* sp.) were collected from Ispahani Agro Limited and Plant Disease Clinic in the department of Plant Pathology, PSTU. Cultured the fungal bio-agents were performed through standard procedure as earlier I described (Figure 2). The highly first growing fungus was *Trichoderma harzianum* (Figure 2a). The medium growth fungus *Aspergillus* sp. was shown in figure 2b. The slow growth fungus *Pestalotia* sp. was found in figure 2c as compare to *Trichoderma*.

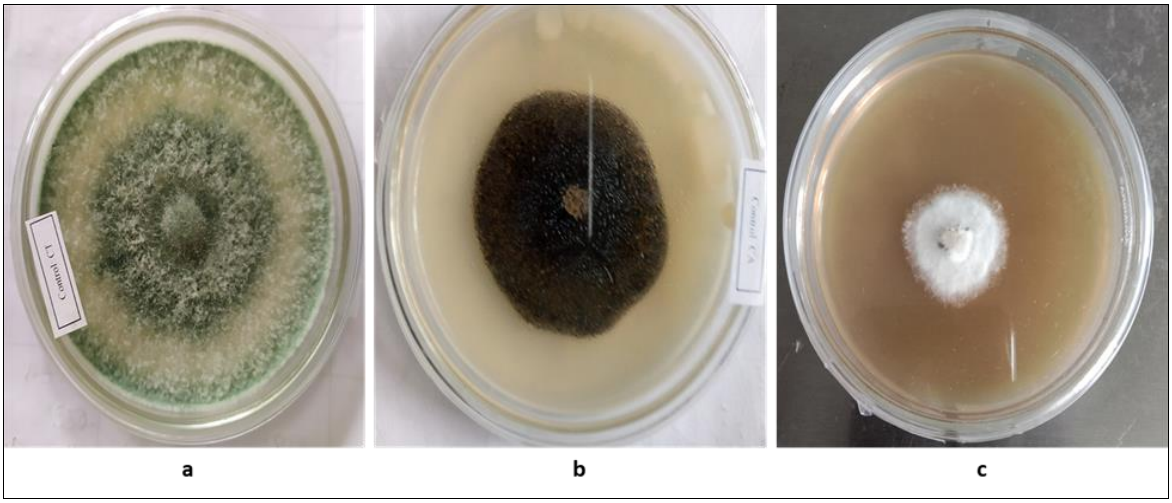

Fig 2(a-c): Pure culture of three fungal bio-agents a) *Trichoderma* sp. b) *Aspergillus* sp. and c) *Pestalotia* sp.

In vitro* evaluation of bio-agents against *C. gloeosporioides

The antagonistic activity of bio-control agents against *C. gloeosporioides* was determined by dual culture technique under in vitro (Thahir *et al.,* 2010) [11]. The cultured Petri plates were sterilized by Autoclave at 121^0c for 20 minutes and then incubated room temperature at 28±2°C with five replicates and without bio agents as control. The percent inhibition of fungal growth was estimated by using the formula given by Alam, *et al.,* (2017) [1].

$$I = \frac{C-T}{C} \times 100$$

Where
I is the percent inhibition,
C is the colony diameter in control, and
T is the colony diameter in treatment.

Statistical Analysis

The experiment was conducted with CRD (Completely Randomized Design) method with 5 replicates. Data were analyzed by using Minitab software version 17 and means were compared Tukeys method at 95% confidence level.

Results and Discussion

Identification of *Colletotrichum gloeosporioides*

Pure Cultures of the fungal pathogen *C. gloeosporioides* were confirmed for their pathogenicity using Koch's postulate technique. Conidia were observed under compound microscope with 100X magnification. The acervulus with conidia and black colored tapering shape setae were observed in figure 3a. The oval shape, both ends are round hyaline conidia were found which is identical as the conidia of the genus *Colletotrichum* (Figure 3b). The researcher Sutton, 1992 revealed that the above characteristics of the fungal genus are as *Colletotrichum*.

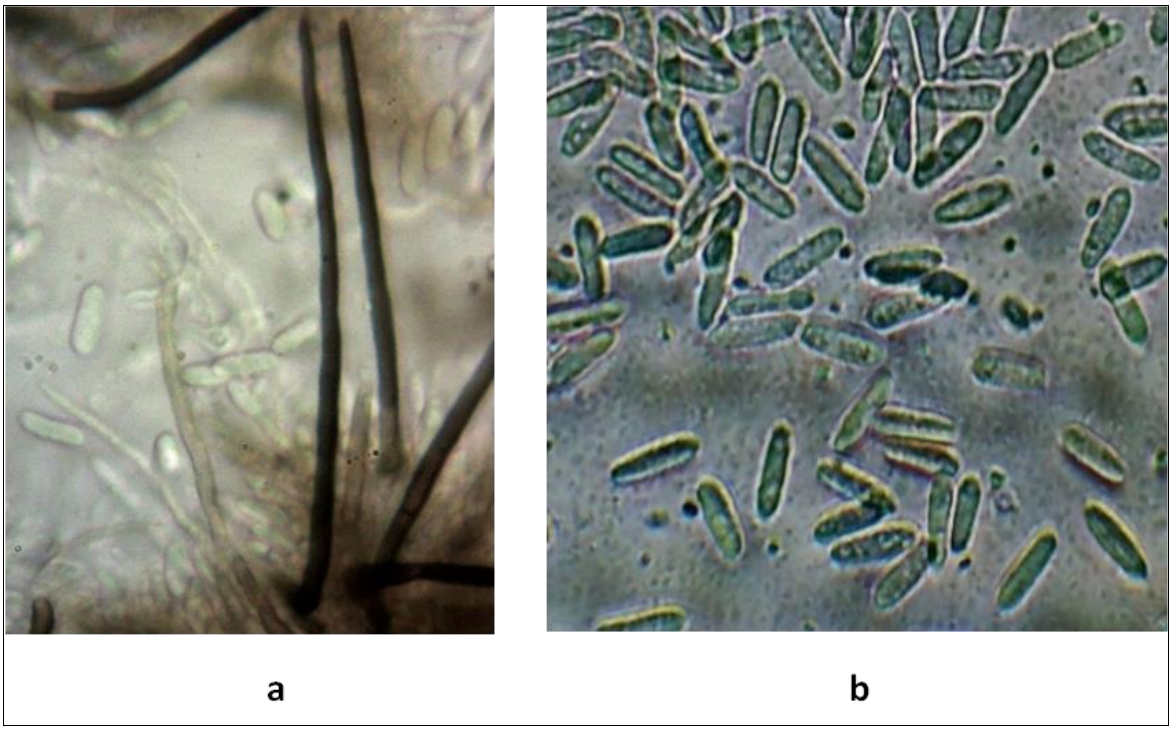

Fig 3(a-b): Pure culture of *Colletotrichum gloeosporioides* a) Conidia with setae, b) Oval shape, round end hyaline conidia

In vitro* evaluation of selected fungal bio-control agents against *C. gloeosporioides

The fungal bio-control agents were evaluated against *Colletotrichum gloeosporioides* in *in vitro* condition. Significant (at p<05) inhibited was found in controlling *C. gloeosporides* at different mean mycelia growth with standard deviation. The highest mean mycelial growth 60.0±0.816 mm was recorded from fungal bio agents *Aspergillus* sp. and followed by 50.0±1.826 mm and 45.0±1.225 mm in plate with *Pestalotia* sp. and *Trichoderma* sp. respectively. The lowest co-efficient of variance (CV%) was found in bio-agents *Aspergillus* sp. (Table 1).

Table 1: Evaluation of fungal bio-agents against *C. gloeosporioides*

Sl No.	Bio-agents (Fungi)	Mycelial growth (mm) Mean±SD	Inhibition (%)	CV (%)
1	*Aspergillus* sp.	60.0±0.816	74.52 ^{a*}	1.36
2	*Pestalotia* sp.	50.0±1.826	61.54 ^{b}	3.65
3	*Trichoderma* sp.	45.0±1.225	56.25 ^{c}	2.72

Note: * Significantly highest percent inhibition. Same letters of the same column are not significantly different. Data were compared at 5% level of significance.

In the current study, the antifungal activities of fungal genus were studied. The three bio-agents were used where *Aspergillus* sp. was found the most dominant on *Colletotrichum gloeosporioides* as compared to *Pestalotia.* The scientist Bugni, *et al.,* 2000 reported that metabolites of *Aspergillus* showed antibacterial and antifungal potential. The researcher Johnny with his co-authors, 2011 revealed that there are a variety of plant extracts that were used to control anthracnose disease.

In case of percent inhibition of mycelial growth over control, the best performance was achieved by *Aspergillus* sp. (74.52%) and the lowest percent inhibition (56.25%) of mycelial growth over control was recorded from *Trichoderma* sp and followed by *Pestalotia* sp. (61.54%) (Figure 4).

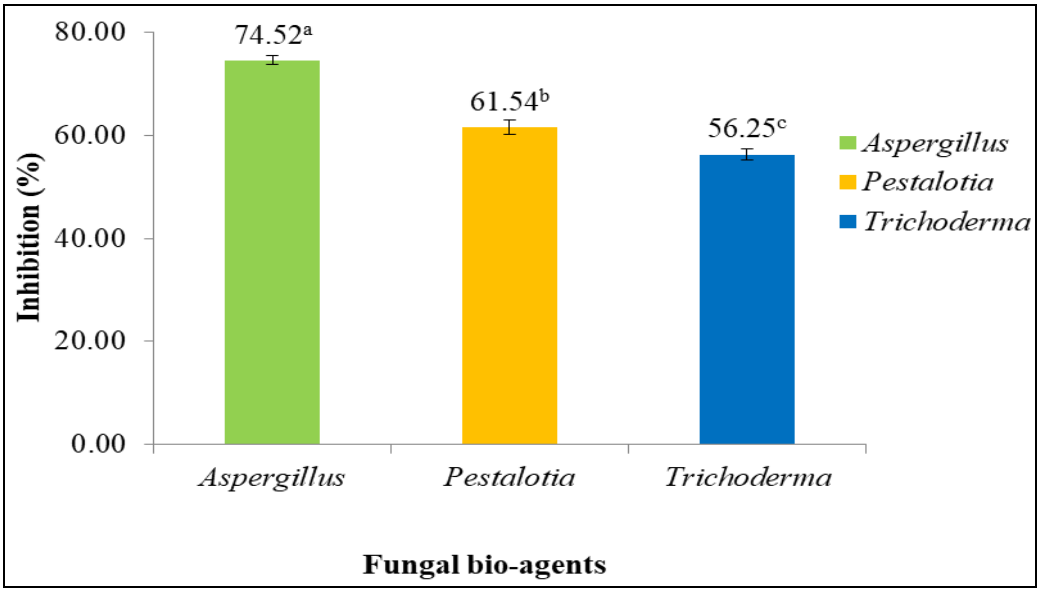

Fig 4: Effect of fungal bio-agents on inhibition of mycelial growth of *C. gloeosporioides*

In a previous study, *C. gloeosporioides* isolates from mango had the similar results against plant extracts (Silva, *et al.*, 2008) [9]. The bio-agent *Aspergillus* whose antifungal activity has already been established in the literature (Bugni, *et al.*, 2008) [4]. Other plant pathogenic fungi and bacteria could also be inhibited the mycelia growth (Prapassorn, *et al.*, 2012) [8].

All the fungal bio-agents were not performed equally (Figure 5). Out of three fungal bio agents, *Aspergillus* sp. exhibited significantly inhibited against *C. gloeosporioides* (Figure 5b), while *C. gloeosporioides* in control treatment (PDA without bio agents figure 5a) grew well and reached the other side of the test medium. In figure 3c shown that there is modest effect between the *Pestalotia* sp. against *C. gloeosporioides. Trichoderma* sp. grew fast as compared to *C. gloeosporioides* but it less inhibit than *Pestalotia* sp. (Figure 5d).

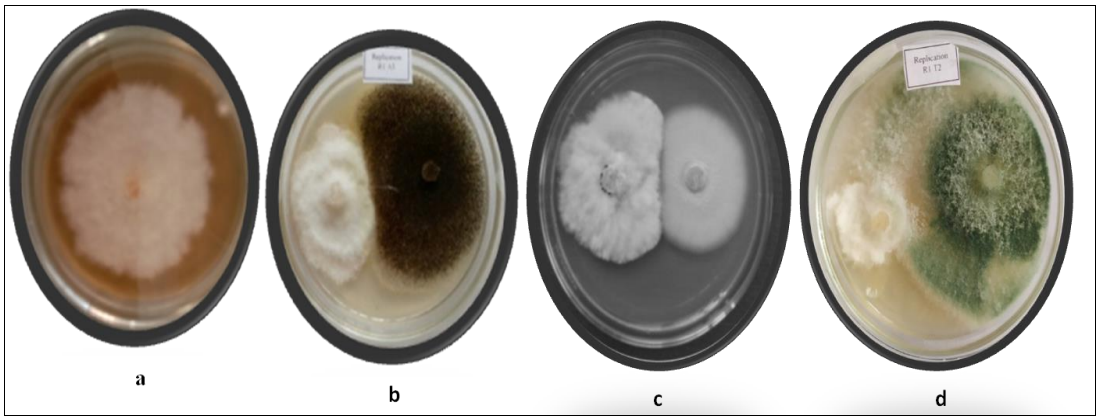

Fig 5: Duel culture of fungal bio-control agents inhibits the mycelial growth of *C. gloeosporioides* a) Control, b) *Aspergillus* sp, c) *Pestalotia* sp., and d) *Trichoderma* sp. inhibit the mycelial growth.

Conclusion

The mycelial growth of tested fungi *Colletotrichum gloeosporioides* was highly affected by the bio-control agents. Among the employed bio-control agents *Aspergillus* sp was the most effective in restricting the growth of the test fungi followed by *Pestalotia* sp. and *Trichoderma* sp. *in vitro*. *Aspergillus* sp. could significantly inhibit the radial growth of certain given fungi. The proper use of bio-control agents may be useful in controlling anthracnose of mango as bio-safety tools. It paves the way of control plant disease in the field as well as storage life of the fruit at post-harvest preservation.

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References

1. Alam A, Adhikary SK, Ahmed. "Effectiveness of Selected Homeopathic Medicines against *Colletotrichum gloeosporioides"*, Asian Journal of Plant Pathology,2017:11:118-129.

2. Akem, C.N. “Mango anthracnose disease present status and future research priorities”, Journal of Plant Pathology,2006:5:266-273
3. Arauz LF. “Mango anthracnose: Economic impact and current options for integrated management”, Plant Disease,2000:84(6):600-611.
4. Bugni TS, Abbanat D, Bernan VS, Maiese WM, Greenstein M, Wagoner RMC *et al*. “Novel metabolites from a marine isolate of *Aspergillus niger”*, Journal of Organic Chemistry,2000:65:195-200.
5. Jeffries p, Jeger MJ, Plumbley RA. “The Biology and control of Colletotrichum species on tropical fruit crops”, Plant Pathology,1990:39:343-366.
6. Johnny L, Yusuf UK, Nulit R. “Antifungal activity of selected plant leaves crude extracts against a pepper anthracnose fungus, *Colletotrichum capsici* (sydow) butler and bisby (Ascomycota: Phyllachorales)”, African Journal of Biotechnology,2011:10(20):4157-4165.
7. Nelson SC. “Mango anthracnose (*Colletotrichum gloeosporioides*)”, College of Tropical Agriculture and Human Resource, 2008, (48).
8. Prapassorn B, Namsena P, Rattanasena P, Chandrapatya A. “Effect of Crude Leaf Extracts on *Colletotrichum gloeosporioides* (Penz.)Sacc.”, Hindawi Publishing Corporation, Psyche, 2012, 1-6.
9. Silva PA, Oliveira DF, Prado NRT, Carvalho DA, Carvalho GA. “Evaluation of the antifungal activity by plant extracts against *Colletotrichum gloeosporioides* PENZ”, Cienc. agrotec., Lavras,2008:32(2):420-428.
10. Sutton BC. “The genus *Glomerella* and its anamorph *Colletotrichum*. In: *Colletotrichum* Biology, Pathology and Control”, CAB International, Wallingford, UK, 1992, 1-26.
11. Thahir B, Suvarna S, Hemalatha JTM, Eswara, Reddy NP. “Compatibility of native potential bioagents with different fungicides against *Colletotrichum gloeosporioides* Penz. Causing mango anthracnose”, *The Bioscan*,2010:5(1):19-20.