

Evaluation of the Biological Efficacy of Fungus and Bacteria Isolated from Mushroom Substrates against Pathogenic Fungi

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Abstract— *In vitro* efficacy of fungus and bacteria isolated from mushroom substrates were evaluated against selected pathogens (*Fusarium oxysporum*, *Sclerotium rolfsii*, and *Colletotrichum corchori*). There were four fungi *Aspergillus flavus*, *Aspergillus niger*, *Trichoderma harzianum*, and *Penicillium* sp and four bacteria *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas* sp. and *Paenibacillus polymyxa* isolated from substrate. Different biochemical and pathogenicity test were performed to confirm their species. In in-vitro dual culture method *T. harzianum* showed the highest inhibition in case of *Fusarium oxysporum* (70.26%) followed by *Sclerotium rolfsii* (59.94%) and *Colletotrichum corchori* (57.02%) whereas *Penicillium* sp showed the least significant result against these pathogens. To inhibit the growth of *Sclerotium rolfsii* and *F. oxysporum*. *Pseudomonas* spp. was significant around 72.19% and 62.62% respectively. On a comparative study among the four isolated bacteria and fungi *Pseudomonas* sp. and *T. harzianum* showed the best significant antagonistic activity against all the selected plant pathogenic fungi.

Keywords— *In vitro*, isolated, mushroom substrates, dual culture, pathogenic fungi.

I. INTRODUCTION

Among the edible fungi, mushroom is a large reproductive structure, which is the most popular nutritious, delicious and medicinal vegetable around the world. Materials containing cellulose, hemicellulose and lignin (i.e., rice and wheat straw, cotton seed hulls, sawdust, waste paper, leaves, and sugarcane residue) can be used as mushroom substrates (Chang, 1989).

Remarkable various factors are responsible for lower mushroom production in Bangladesh. Among the different factors, fungal and bacterial disease and their antagonistic effect on mushroom is one of the major influential factors, which can initiate from the mushroom substrate.

Usually chemical components are not prescribed for the management of diseases because they have negative impression on the environment and human health. Antifungal agents produced by some fungus and bacteria in mushroom have shown to be beneficial to control pathogenic fungi (Chang and Kim, 2007). Some bacteria belonging to the species *Pseudomonas* and *Bacillus* have been reported to exert promoting effects on the growth of mushrooms, including *P. eryngii* (Kim *et al.*, 2007). *Trichoderma harzianum* used as potential biocontrol agents against different *Fusarium* sp. (Marten *et al.*, 2000; Siddiqui, 2005).

Keeping these facts in mind the study was undertaken to evaluate the biological activities of isolated bacteria and fungi against selected pathogenic fungi (*Fusarium oxysporum*, *Sclerotium rolfsii*, *Colletotrichum corchori*).

II. MATERIALS AND METHODS

The experiment was conducted at the Molecular Plant Pathology Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University.

2.1 Collection of mushroom substrate and pathogenic fungi

Mushroom substrates mainly containing Rice and wheat straw, paper and saw dust were collected from mushroom culture centre, Savar, Bangladesh as they are available and cheap to use.

Three pathogenic fungi *Fusarium oxysporum* (the causal agent of dry rot of potato), *Sclerotium rolfsii* (the causal agent of foot and root rot of betel vine), *Colletotrichum corchori* (the causal agent of anthracnose of jute) were collected from MS Laboratory Department of Plant Pathology of Sher-e-Bangla Agricultural University. The fungi were cultured in Potato Dextrose Agar media.

2.2 Isolation of bacteria on NA media

Bacteria were isolated from mushroom substrate by dilution plate method following the technique described by Goszczynska and Serfontein, (1998).

2.3 Biochemical Tests for bacteria

Various biochemical tests were done to confirm the bacterial species. The tests were- KOH solubility test (Suslow *et al.*, 1982), catalase test (Schaad, 1988), oxidase test (Kovacs, 1956), gelatin liquefaction test (Salle, 1961), starch hydrolysis test (Cowan, 1974). *Bacillus Cereus* Agar Base was done to separate different species of *Bacillus* (Harmon (1992). Virulent colonies of *Pseudomonas* were selected on the basis of growth of bacteria on cetrimide agar medium.

2.4 Isolation of fungi on PDA media

Potato Dextrose Agar (PDA) media were prepared to isolate fungi. Dilution plate technique was carried out as described by (Dhingra and Sinclair, 1985) for isolation of mycoflora.

2.4.1 Identification of fungi

Identification was done with the help of different books, manuals and publications following the keys suggested by Barnett and Hunter (1992) Watanabe (2000) Mathur and Kongsdal (2003).

2.5 Dual culture method to evaluate the antagonistic effect of isolated bacteria and fungi against pathogenic fungi

Isolated antagonist was screened for their ability to suppress the mycelial growth of fungal *in vitro* dual culture assays on potato dextrose agar media. Each combination of pathogen and antagonist was replicated three times and plates were randomly placed in the dark chamber and incubated at 25°C for 7 days. The radial mycelial growth of pathogenic fungus towards the antagonist (T) and that on a control plate (C) were measured and the mycelial growth inhibition was calculated according to the formula (Amadioha 2004):

$$\% \text{ inhibition of growth} = \frac{C-T}{C} * 100$$

Data collected during experiment period were tabulated and analyzed following Statistical package MSTAT-C. Treatment means were compared with Duncan's Multiple Range Test (DMRT) (Gomez, K.A. and Gomez, 1984).

III. RESULTS AND DISCUSSION

3.1 Antagonistic effect of isolated bacteria against three selected pathogenic fungi

Biological efficacy of bacteria isolated from mushroom substrate against three pathogenic fungi were studied and found significant variations in terms of percent inhibition of radial mycelial growth of pathogenic fungi (Table 1). In case of *Fusarium oxysporum* the highest inhibition observed against *Pseudomonas* spp. (62.62 %) and the lowest against *Paenibacillus polymyxa* (48.00 %). In case of *Sclerotium rolfsii* the highest inhibition observed against *Pseudomonas* spp. (72.19 %) and the lowest against *Bacillus subtilis* (20.14 %). In case of *Colletotrichum corchori* the highest inhibition observed against *Pseudomonas* spp. (48.15 %) and the lowest against *Bacillus subtilis* (20.15). In this study it has been observed that among the bacterial antagonists used against pathogenic fungi the most effective was *Pseudomonas* sp.

TABLE 1
BIOLOGICAL EFFICACY OF BACTERIA ISOLATED FROM MUSHROOM SUBSTRATE AGAINST THREE PATHOGENIC FUNGI.

Bacterial isolates	% Inhibition of mycelial growth		
	<i>Fusarium oxysporum</i>	<i>Sclerotium rolfsii</i>	<i>Colletotrichum corchori</i>
<i>Bacillus subtilis</i>	62.21 b	20.14 d	20.15 c
<i>Bacillus cereus</i>	54.00 c	40.18 c	38.14 b
<i>Paenibacillus polymyxa</i>	48.00 d	48.85 b	35.28 b
<i>Pseudomonas</i> sp.	62.62 a	72.19 a	48.15 a
LSD(0.50)	2.71	2.19	3.27

3.2 Antagonistic effect of isolated fungi against three selected pathogenic fungi

Biological efficacy of fungal isolated from mushroom substrates against three pathogenic fungi were studied and found significant variation in terms of percent inhibition of radial mycelial growth of pathogenic fungi. In case of *Fusarium oxysporum* the highest inhibition was observed against *T.harzianum* (70.62%) and the lowest against *Penicillium* sp (28.58%). In case of *Sclerotium rolfsii* highest inhibition observed against *T. harzianum* (59.94%) and the lowest against *Penicillium* sp (20.15%). In case of *Colletotrichum corchori* highest inhibition observed against *T. harzianum* (57.02%) and the lowest against *Penicillium* sp (39.59%).

TABLE 2
BIOLOGICAL EFFICACY OF FUNGAL ISOLATED FROM MUSHROOM SUBSTRATE AGAINST THREE PATHOGENIC FUNGI.

Fungal isolates	% Inhibition of mycelial growth		
	<i>Fusarium oxysporum</i>	<i>Sclerotium rolfsii</i>	<i>Colletotrichum corchori</i>
<i>Penicillium</i> sp	28.58 d	20.15 d	39.59 c
<i>Aspergillus flavus</i>	32.37 c	57.67 b	42.69 c
<i>Aspergillus niger</i>	49.23 b	45.37 c	47.69 b
<i>Trichoderma harzianum</i>	70.62 a	59.94 a	57.02 a
LSD(0.50)	2.62	2.39	3.32

IV. CONCLUSION

The present study was based on the presence of bacteria and fungus on mushroom substrate and their antagonistic effect against *Fusarium oxysporum*, *Sclerotium rolfsii* and *Colletotrichum corchori*.

On a comparative study among the four isolated fungi *Trichoderma harzianum* isolated from different mushroom substrate was found effective against the selected pathogenic fungi. Thus, *Trichoderma harzianum* could be used as bio-control agent against those pathogenic fungi. Further works need to be conducted to research the method of application of *Trichoderma harzianum* against those fungi. Among the four isolated bacteria *Pseudomonas* sp. showed the best significant antagonistic activity against all the selected plant pathogenic fungi. After *Pseudomonas* sp. *Bacillus subtilis* had the most significant antagonistic activity.

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