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SYMPTOMATOLOGY OF FUNGAL COMPETITORS ON OYSTER MUSHROOM'S SPAWN PACKETS AND *IN VITRO* EVALUATION USING PHYTOEXTRACTS AND A FUNGICIDE

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Abstract

An experiment was conducted to find out the fungal competitors and symptom studies in damaged Oyster Mushroom spawn packets at National Mushroom Development and Extension Center, Savar, Dhaka, Bangladesh. A total of nine fungal competitors of oyster mushroom were isolated and identified namely- *Trichoderma harzianum* Rifai, *T. viride* Pers. (Green strain), *T. viride* Pers. (Yellow strain), *T. koningii* Oudem, *Mucor hiemalis* Wehmer, *Papulaspora byssina* Hotson, *Neurospora* sp. Shear and B.O. Dodge., *Aspergillus flavus* Link., and *Botryodiplodia theobromae* Pat. on the basis of microscopic, morphological and cultural characteristics. To produce oyster mushroom in an eco-friendly manner and to find out their antifungal potency, 23 plant species belonging to 19 families were screened out against isolated nine fungal competitors of oyster mushroom. Among 23 extracts, the maximum (44%) mycelial inhibition of *T. harzianum* was found due to *Aegle marmelos* whereas *Eclipta alba* showed the highest mycelial inhibition (62%) of *T. viride* (Green strain); in case of *T. viride* (Yellow strain), *Cassia tora* exhibited the highest mycelial inhibition (39%); *Diospyros cordifolia* showed the maximum mycelial inhibition (48%) of *T. koningii*; *Curcuma longa* (rhizome) gave the maximum mycelial inhibition (90%) of *Neurospora* sp. There were no significant effects found to control of *P. byssina*, *B. theobromae*, *M. hiemalis* and *A. flavus* due to 23 different types of botanicals tested. *Trichoderma harzianum, T. viride* (Green strain), *T. viride* (Yellow strain), *T. koningii*, *A. flavus*, *Neurospora* sp. and *P. byssina* was successfully inhibited by 30, 50 and 70 ppm of fungicide-Bavistin 50 WP but *B. theobromae* and *M. hiemalis* were not affected by Bavistin at mentioned concentration.

Keywords: Oyster Mushroom, Fungal Competitors, Plant Extracts.

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Introduction

Oyster mushroom [*Pleurotus ostreatus* (Jacq.) P. Kumm.] is one of the popular and widely cultivated mushroom varieties in Bangladesh and cheaply available named as PO-2 at National Mushroom Development and Extension Centre (NAMDEC) and cultivated all the year round. A number of competitor moulds have been reported to occur in the substratum, which used for oyster mushroom production. Variations in the types of moulds are mainly due to the use of a diversity of substrates, different methods of substrate preparation and the conditions and containers used for cultivation. Different saprophytic and plant pathogenic fungi occurring in the substrate and competing with mushroom mycelium for space and nutrition are *Aspergillus niger*, *A. flavus, Alternaria alternata, Drechslera bicolor, Fusarium moniliforme, Mucor* sp., *Penicillium* sp., *Rhizopus* spp., *Rhizpus stolonifer, Sclerotium rolfsii,, Trichoderma viride* (Sharma *et al*., 2007; Sharma and Kumar, 2011). There might be an interaction between *Trichoderma* sp. and the mushroom due to the enzymatic action on substrate by mushroom that favors green mold fungal growth (Colavolpe *et al*., 2015). Antifungal activity of different plant extracts have been reported earlier by several investigators against a number of plant pathogens (Ashrafuzzaman *et al*., 1990). Present study was undertaken with the aim to investigate the symptoms produced due to fungal competitors of mushroom during mushroom production; isolation, identification of competitor fungi of Oyster mushrooms, to evaluate *in vitro* antifungal potency of several phytoextracts and fungicide against the fungal competitors.

Materials and Methods

Symptomatological study of damaged spawn packets, isolation and identification of fungal competitors

On the basis of visual observation range of different symptoms were noticed in spawn packets where the mycelium of mushroom were damaged or dominated by the competitors. The symptoms and signs were closely and carefully observed. A total of ten infected spawn packets were taken randomly to isolate the mushroom competitors responsible for damaging as methods given by Dhingra and Sinclair (1985). Individual isolates were identified following Commonwealth Mycological Institute description as described by Barnett (1960) for imperfect fungi, Alexopolus *et al.* (1996) for perfect fungi.

In vitro evalulation of phytoextracts and fungicide-Bavistin 50 WP

A total of twenty three plant species belonging to 19 families were collected from different locations of Jahangirnagar University campus namely- *Aegle marmelos* (L.) Correa., *Axonopus* compressus (Sw) P. Beauv., *Blumea lacera* (Burm. f) DC., *Bougainvillea glabra* Choisy, *Calamus viminalis* Willd., *Cassia tora* L., *Catharanthus roseus* L., *Curcuma longa* (leaf) L., *Curcuma longa* (rhizome) L., *Diospyros cordifolia* Roxb., *Eclipta alba* L., *Hemidesmus indicus* Br., *Hollarhena antidysenterica* (Linn) Wall., *Ixora coccinea* L., *Lantana camara* L., *Melastoma malabathicum* L., *Mesua nagesarium* Kost., *Mucuna pruriens* L., *Pimenta acris* Wt., *Pteris* sp. L., *Rungia pectinata* (L.) Nees, *Catunaregam spinosa* (Thunb) Tirveng., *Zingiber offinale* Rosc. Ethanol was used to extract the active constituent of plant materials. Filtration of extract through Membrane filters was carried out as described by Cappuccino and Sherman (1998). The extracts were tested by introducing 0.5 ml of filtrate spreaded onto 20 ml PDA media containing Petri plate and incubated at 30°C for five days. In a sterile Petri plate, 20 ml of PDA was poured and 2 wells of 5 mm were dug at two sides. 100 µl of each botanical were poured into these wells using sterile micropipette. Fungal discs (5 mm) were punched from 5 days old cultures of the test fungus and placed at the centre of the Petri plates to evaluate the efficacy of the extracts. Petri dish containing PDA medium with each fungal inoculums alone served as control. The plates were incubated at room temperature (28±2°C) for 7 days. The mean radial growth of the fungal colony was recorded after 7 days. The efficacy of extract was determined by comparing the radial growth in treatment (T) with the control (C). The inhibition percentage (I) was calculated using the formula given by Vincent (1947):

$$\text{Mycelial inhibition (\%)} = \frac{C-T}{C} \times 100$$

Three different concentrations (30, 50, 70 ppm) of a recommended fungicide-Bavistin 50 WP (Carbendazim) were used in the experiment. PDA medium served with requisite amount of distilled water and poured in sterile petri plate and inoculated with test fungus served as control. Each treatment was replicated thrice and kept at room temperature (28±2°C) for 7 days. The inhibition percentages (I) of fungicides were calculated using the formula given by Vincent (1947). The data obtained from different treatments related to phytoextracts and fungicide were analyzed statistically to find out the variation resulting from experimental treatments using SPSS-18 programme.

Results and Discussion

Symptomatological of fungal competitor in oyster spawn packets

The symptoms appeared in the spawn packets and observed damaging mushroom mycelium were distinctly different from each other depending on different causal competitors. The different symptoms appeared have been described in Table 1. A total of nine fungal competitors were identified in oyster spawn packets namely *Trichoderma viride* (green strain), *Trichoderma viride* (yellow strain), *T. harzianum*, *T. koningii*, *Papulaspora byssina, Mucor hiemalis, Botrydiplodia theobromae*, *Aspergillus flavus, Neurospora* sp.

Table 1. Different symptoms appeared in Oyster spawn packets due to different competitors.

Causal organisms	Symptoms
Trichoderma harzianum	Appeared white in color and compete mushroom mycelium, distinctly showed the green sporulation and ceased the growth of mushroom.
Trichoderma viride (Green strain)	Deep green and compact sporulation found growing over the mushroom mycelium and covered the whole packet.
Trichoderma viride (Yellow strain)	Creamy white or yellowish, light green sporulation appeared over the spawn packet.
Trichoderma koningii	Green sporulation found spreaded over spawn packet.
Mucor hiemalis	Pinheaded mold became mature vigorously and run over mushroom for space and nutrition.
Botrydiplodia theobromae	Destroyed the spawn packet substrate and black acervuli appeared.
Aspergillus flavus	Olive green powdery sporulation observed.
Neurospora sp.	Pink colored vigorously growing mycelium observed covered mushroom.
Papulaspora byssina	Brown powdery substance recorded which completely covered the spawn packet space.

The symptoms appeared and time of expression varied with the different species. *Trichoderma* spp. initially found to produce the denser compact mycelia compared to *Pleurotus,* which gradually turned green in color due to heavy sporulation, within two to three days, a characteristic symptom of green mold disease (Table 1). *Trichoderma* spp. having a green, green-yellow, or white color on the mushroom compost, compete with other mushrooms for nutrients, cause parasitic damage and no fruit bodies observed in infected spawn packets. The occurrence of different species of *Trichoderma* on *Pleurotus* spawn packets, the incidence of *T. harzianum* was the highest at low temperature. The incidence of *T. harzianum* became lower while temperature raised but incidence of other *Trichoderma* spp. increased. The findings of the present study are in agreement with those described by Choi *et al*. (2003). Mushrooms infected with *T. harzianum* developed larger, light brown spots (Dano, 2000); *T. koningii* developed reddish spots (Fletcher *et al*., 1989), *T. viride* developed dark brown spots (Rinker and Wuest, 1994), which are not similar to present findings. During present study, it was observed that *T. harzianum* and *T. viride* (green) caused maximum damage in mushroom production. Dano (2000) also reported the similar findings and cited that *T. harzianum* and *T. viride* are more severe than *T. koningii.* The present findings are in agreement with the results of Sharma and Kumar (2011) who found the severe incidence of Green moulds (*Trichoderma viride, T. harzianum, T. hamatum, T. koningii, Aspergillus* spp., *Penicillium cyclopium*) and *P. byssina* in mushroom cultivation. Different nutrient sources like carbon and nitrogen, percent of high relative humidity (RH), hot temperatures, a fluctuation of mentioned factors, and the absence of light during spawn run are considered as an ideal environmental conditions for the growth of moulds which can easily lead to a contamination (Chen and Moy, 2004). Sharma *et al.* (2007) reported a number of fungi (namely-*Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp., *Mucor* spp., *Rhizopus* spp., *Fusarium* spp., and *Papulospora* spp.) in compost and casing soil during the cultivation of white button mushroom. Our results are also supported by Chinara and Mohopatra (2014) who observed a number of fungal competitors' namely-*Aspergillus flavus*, *A. niger*, *Mucor* sp., *Penicillium* sp., *Sclerotium rolfsii* and *Trichoderma* sp.

In vitro evaluation of botanicals against mushroom competitors

The present investigation revealed the antifungal activity of some botanicals against the isolated fungal competitors of oyster mushroom. Among 23 botanical extracts, *Aegle marmelos* showed the highest mycelia growth inhibition (44%) of *T. harzianum,* followed by *Zingiber offinale* (12%) while rest of 21 botanical extract did not show any inhibitory effect on green mould- *T. harzianum* (Table 2). In our study, *Eclipta alba*showed the highest inhibition (62%) of *T. viride* (green strain), which was followed by *Pteris* sp. (44%), *Curcuma longa* (44%), *Diospyros cordifolia* (44%). The other 6 phytoextracts of *Aegle marmelos*, *Lantana camara*, *Cassia tora*, *Rungia pectinata*, *Pimenta acris*, showed statistically similar inhibitory effects on *T. viride* (green strain) (Table 3) while the rest of the 11 phytoextracts had no significant effects on *T. viride*. On the other hand, *Cassia tora* showed the highest mycelia inhibition (39%) against *T. viride* (yellow strain) which was followed by *Rungia pectinata*, *Aegle marmelos*, *Lantana camara* and *Pimenta acris* (Table 4). The maximum inhibition (48%) of *T. koningii*was found due to phytoextracts of *Diospyros cordifolia*, followed by *Cassia tora* (41.4%), *Blumea lacera* (40%) and *Mucuna pruriens* (37%) (Table 5); other botanical extract of *Rungia pectinata*, *Pimenta acris*, *Aegle marmelos* and *Curcuma longa* showed similar significant inhibition effect on *T. koningii* while the rest of the 15 botanicals extract did not show any inhibitory effect on *T. koningii*. There was a number of reports on green mould management by onion, garlic, neem, *Juglans regia* whereas in our study, *Aegle marmelos, Eclipta alba, Pteris sp., Cassia tora*, *Diospyros cordifolia* gave substantial mycelial inhibition of the green mould (*T. harzianum, T. koningii, T. viride*) associated with oyster mushroom substrate. Siddique *et al.* (2004) found the maximum inhibition due to the extract of onion (*Allium cepa*), followed by the extracts of *Aegle marmelos* and *Wedelia chinensis*. Shah *et al*. (2011) recorded the maximum mycelial inhibition (51.9%) of *Trichoderma* due to *Juglans regia*, followed by *Azadiracta indica* (34.1%), *Allium sativum* (28.4%). Mishra (2009) found the effective control of *Trichoderma viride* by the use of Neem leaf extract, Neem cake solution and Neem saw dust. Narzari *et al*. (2007) reported that complete mycelial inhibition of *T. harzianum* was found by 0.4% concentration of *Allium sativum* (garlic) extract. Inam-ul-Haq *et al*. (2010) found that *Azadirachta indica,* and *Citrus lemon* was capable of controls pathogenic microbes (*T. harzianum*) in oyster mushroom cultivation and increasing mushroom yield. Parvez *et al*. (2012) recorded the maximum mycelial inhibition (51.25%) of green mould (*T. harzianum*) of mushroom substrate due to extract of *Lantana camara*, followed by *Azadirachta indica* (47.75%), *Allium cepa* (34.85% and *A. sativum* (28.95%).

Table 2. Effect of selected plant extracts on vegetative growth of *T. harzianum.*

Sl. No.	Plant name	Mycelial inhibition (%)
1.	*Aegle marmelos* (L.) Correa.	44±0.58 a
2	*Zingiber offinale* Rosc	12±0.28 b
3-23.	Others 21 botanical extracts	Nil

Data recorded at 7 days of incubation, Data represents mean ±SE of three replications, Column having the different letters differ significantly at 5% level of significance.

Table 3. The effect of plant extracts on the vegetative growth of *Trichoderma viride* (Green strain).

Sl. No.	Plant name	Mycelial inhibition (%)
1.	*Eclipta alba* L.	62±0.60 a
2.	*Pteris* sp. L.	49±0.44 b
3.	*Diospyros cordifolia* (Roxb.)	44±0.32 c
4.	*Curcuma longa* (leaf) L.	44±0.53 c
5.	*Lantana camara* L.	31±1.15 d
6.	*Pimenta acris* Wt.	31±2.01 d
7.	*Rungia pectinata* L.(Nees)	29±0.20 d
8.	*Cassia tora* L.	26±1.20 d
9.	*Aegle marmelos* (L.) Correa.	26±0.58 d
10.	*Curcuma longa* (Rhizome) L.	17±2.5e
11-23.	Others 13 botanical extracts	Nil

Data recorded at 7 days of incubation, Data represents mean ±SE of three replications, Column having the same letters do not differ significantly at 5% level of significance.

Table 4. Effect of plant extracts on the vegetative growth of *Trichoderma viride* (Yellow strain).

Sl. No.	Plant name	Mycelial inhibition (%)
1.	*Cassia tora* L.	39±2.30 a
2.	*Rungia pectinata* (L.) Nees	37±1.58 a
3.	*Aegle marmelos* (L.) Correa.	31±0.60 b
4.	*Pimenta acris* Wt.	30±.80 b
5.	*Eclipta alba* L.	29±0.57 b
6-23.	Others 18 botanical extracts	Nil

Data recorded at 7 days of incubation, Values represents mean ± SE of three replications; Columns having the same letters do not differ significantly at 5% level of significance.

Table 5. Effect of plant extracts on the vegetative growth of *Trichoderma koningii* (Yellow strain).

Sl. No.	Plant name	Mycelial inhibition (%)
1.	*Diospyros cordifolia* (Roxv.)	48±2.05 a
2.	*Cassia tora* L.	41.4±1.56 b
3.	*Blumea lacera* (Burm f)	40±0.60 b
4.	*Mucuna pruriens* L.	37±1.20 b
5.	*Rungia pectinata* (L.) Nees	33±0.58 c
6.	*Pimenta acris* Wt.	33±0.72 c
7.	*Aegle marmelos* (L.) Correa.	33±2.63 c
8.	*Curcuma longa* (leaf)	27±0.60 c
9-23.	Others 15 botanical extracts	Nil

Data recorded at 7 days of incubation, Values represents mean ± SE of three replications, Columns having the same letters do not differ significantly at 5% level of significance.

The highest mycelial inhibition (90%) of *Neurospora* sp. was recorded due to botanical extracts of *Curcuma longa* (rhizome), followed by *Pteris* sp. (83%), *Bougainvillea glabra* (80%) (Table 6) and others six phytoextracts namely- *Diospyros cordifolia*, *Rungia pectinata*, *Pimenta acris*, *Cassia tora*, *Eclipta alba*, and *Blumea lacera* showed significantly positive inhibitory effect on mycelial growth of *Neurospora* sp as well. The record of controlling measure of *Neurospora* sp. through botanicals was not available as to the knowledge of the author. In the present investigation, none of the 23 botanicals showed any significant effect against the mycelia growth inhibition of *A. flavus*, *B. theobromae*, *Mucor hiemalis* and *P. byssina*.

Table 6. The effect of plant extracts on the vegetative growth of *Neurospora* sp.

Sl. No.	Plant name	Mycelial inhibition (%)
1.	*Curcuma longa* (rhizome)	90±1.20 a
2.	*Pteris* sp	83±1.60 b
3.	*Bougainvillea glabra* Choisy	80±1.62 c
4.	*Blumea lacera* (Thunb)	66±1.80 d
5.	*Eclipta alba* L.	66±1.95 d
6.	*Cassia tora* L.	48±0.90e
7.	*Diospyros cordifolia* (Roxv.)	48±1.30 e
8.	*Rungia pectinata* (L.) Nees	37±2.32 f
9.	*Pimenta acris* Wt.	33±1.50 g
10-23.	Others 14 botanical extracts	Nil

Data recorded at 7 days of incubation, Values represents mean ± SE of three replications, Columns having the same letters do not differ significantly at 5% level of significance.

In-vitro effect of fungicide on vegetative growth of test fungus

A fungicide- Bavistin 50 WP (Carbendazim) was applied against the selected competitors to observe the antifungal efficacy (Table 7). *Trichoderma harzianum* was controlled at all of the concentration of Bavistin 50 WP used. *Trichoderma viride* (Green strain), *Trichoderma viride* (Yellow strain), *Trichoderma koningii*, *Aspergillus flavus*, *Neurospora* sp. and *Papulaspora byssina* were inhibited by 30, 50 and 70 ppm of Bavistin 50WP. However, best results obtained due to 70 ppm concentration. Our results are supported by previous findings. The maximum mean inhibition (90.8%) of *Trichoderma harzianum* and the least mycelial inhibition (24.9%) of *Pleurotus sajor-caju* was recorded in Carbendazim (Shah *et al*., 2013).

Table 7. Effect of Bavistin 50 WP on the mycelial growth of fungal competitors of mushroom.

Sl. No.	Name of fungal competitors of mushroom	Concentration (ppm)	Mycelial inhibition (%)
1.	*Trichoderma harzianum*	30	99.50±0.28a
		50	99.50±0.28a
		70	99.50±0.28a
2.	*Trichoderma viride*, (green strain)	30	84.44±0.00c
		50	91.11±0.00b
		70	97.77±0.00a
3.	*Trichoderma viride*, (yellowstrain)	30	82.00±0.06c
		50	91.11±0.57b
		70	97.77±0.00a
4.	*Trichoderma koningii*	30	88.80±0.05c
		50	93.30±0.05b
		70	97.70±.0.05a
5.	*Aspergillus flavus*	30	77.00±0.57c
		50	91.00±0.57b
		70	97.00±0.00a
6.	*Neurospora* sp	30	80.00±0.00c
		50	92.50±0.00b
		70	100.00±0.00a
7.	*Papulaspora byssina*	30	37.77±0.00c
		50	66.66±0.00b
		70	82.22±0.00a
8.	*Botryodiplodia theobromae*	30, 50, 70	-
9.	*Mucor hiemalis*	30, 50, 70	-

Here "-" No mycelial inhbition, Values represents mean ± SE, Data recorded at 5 days of incubation, Columns having the same letters (of the respective fungal competitors) do not differ significantly at 5% level of significance.

Carbendazim was also found to be efficiently inhibiting the mycelial growth green mould isolates (*T. harzianum*) at very low concentrations (0.63 µg mL^{-1} to 5 µg mL^{-1}) and did not influence the growth of Oyster mushroom (*Pleurotus ostreatus* and button mushroom (Hatvani *et al*., 2012; Woo *et al*., 2004). Parvez *et al*. (2009) found that the combination of formalin and Carbendazim (500 mL+ 75 ppm) was the best in inhibiting the mycelial radial growth of all the identified microflora of oyster mushroom substrate. Maurya *et al*. (2013) reported that Carbendazim (0.05%) exhibited strong antifungal properties which inhibited more than 80% mycelial growth of the *T. harzianum* and *P. byssina* but mycelial growth of mushroom

(*Pleurotus florida*) was unaffected against all the test fungicides concentration (0.05, 0.075 and 0.1%). *Botryodiplodia theobromae* and *Mucor hiemalis* were not affected by Bavistin 50 WP at above mentioned concentration used which is in contradictory to Muhammad *et al.* (2009) who reported that Carbendazim showed complete inhibition of *Botryodiplodia theobromae* over at both 50 and 100 ppm doses.

In conclusion, fungicide-Bavistin was found to be effective to control a range of microflora associated with oyster mushroom substrate.

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