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Management of Green Mold (*Trichoderma harzianum*) in Oyster Mushroom (*Pleurotus ostreatus*) Cultivation Using Botanicals

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Article info	Abstract
Received: 18 May, 2025 Accepted: 23 June, 2025 Published: 06 July, 2025 Available in online: 10 July 2025 *Corresponding author:  rubelpip@pstu.ac.bd 	Green mold caused by <i>Trichoderma harzianum</i> poses a major threat to oyster mushroom (<i>Pleurotus ostreatus</i>) cultivation, leading to significant crop losses. This study aimed to determine the prevalence of green mold and evaluate the efficacy of botanicals against the pathogen. Several fungi were isolated, including <i>Trichoderma</i> spp., <i>Aspergillus</i> spp., <i>Penicillium</i> spp., and <i>Rhizopus stolonifera</i>. <i>T. harzianum</i> was the predominant contaminant, detected in approximately 82% of infected spawn packets. The antifungal activity of five plant extracts, including onion, aloe vera, neem, lantana, and tulsi, was assessed at 5% and 10% concentrations. Neem extract showed the highest efficacy, inhibiting over 65% of mycelial growth. In a compatibility test of different plant extracts, neem was also the most supportive of mushroom growth and yield. The study suggests that plant extracts, particularly neem, offer a promising, eco-friendly, and residue-free approach to manage green mold in oyster mushroom cultivation. Keywords: Green mold, Oyster mushroom and Botanicals.

Introduction

After button mushrooms, oyster mushrooms (*Pleurotus* spp.) are among the most extensively cultivated mushrooms worldwide (Adejoye et al., 2006). Their adaptability to a wide range of temperatures allows for year-round cultivation in both temperate and tropical regions (Amin et al., 2007). *Pleurotus* species possess strong enzymatic systems that enable efficient degradation of lignocellulosic materials, making them well-suited to grow on diverse agricultural and industrial wastes (Jandaik & Goyal, 1995; Zadrazil & Brunnert, 1981). Their popularity is further enhanced by their high biological efficiency, low production costs, and rich nutritional profile, as they are a good source of proteins, vitamins, and minerals (Mattila et al., 2000; Wasser, 2002). In addition to their nutritional value, oyster mushrooms exhibit medicinal properties, including anti-tumor, anti-cholesterol, and anti-cancer effects (Wang et al., 1995), making them beneficial for individuals with diabetes, hypertension, and hyperacidity. In Bangladesh, oyster mushroom cultivation has grown significantly in recent years.

Despite these advantages, oyster mushroom production faces a major challenge from green mold disease caused by *Trichoderma harzianum*. Once considered a minor contaminant, this pathogen

has become a serious threat to mushroom production globally, causing significant yield losses up to 100% and economic damage (Šašić Zorić et al., 2023; Seaby, 1987; Ospina-Giraldo et al., 1998). Management of this disease is particularly difficult because both the host (*Pleurotus*) and the pathogen (*Trichoderma*) are fungi, making selective control highly challenging.

Chemical fungicides like carbendazim are sometimes applied for disease management; however, their effectiveness is limited due to concerns about cost, environmental impact, and food safety (Shah & Nasreen, 2011). These drawbacks have prompted growing interest in eco-friendly and sustainable alternatives. Among these, botanicals are considered promising due to their safety, cost-effectiveness, and potential to provide sustainable disease control (Tiwari et al., 1988). In this context, the present study was undertaken to identify the major mycoflora associated with oyster mushroom substrates, characterize virulent strains of *T. harzianum*, and evaluate the efficacy of selected botanicals in managing green mold.

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Materials and Methods

Study area

The study was conducted in the Plant Pathology Laboratory of Patuakhali Science and Technology University, Bangladesh.

Collection of Oyster mushroom

Pleurotus ostreatus, which is frequently grown, was chosen for this investigation. The National Mushroom Development and Extension Centre in Sobhanbag, Savar, Dhaka, donated the spawn packets. Isolating tissue from the fruiting bodies in order to prepare mother cultures allowed for the establishment of pure cultures.

Isolation and identification of associated mycoflora

Samples of pathogens were collected from green mold-infected substrate poly bags and spawn bottles from Mushroom polli, Savar, Dhaka. On potato dextrose agar (PDA), little amounts of the contaminated substrate were cultivated, and they were incubated for eight days at 27 °C. In accordance with the descriptions provided by Barnett (1998), Maren (2002), and CMI (Cell Mediated Immunity), fungal colonies were purified and identified using morphological characteristics. 200 g of potato was boiled, the volume was adjusted to 1 L, and 20 g of dextrose and 18 g of agar were added to create PDA. After being sterilised for 15 minutes at 121°C and 1 kg/cm², the medium was transferred into sterile petri plates (Tuite, 1969).

Collection and isolation of *Trichoderma harzianum*

In Savar Upazila, Dhaka, ten packets of *P. ostreatus* spawn infested with green mould were gathered from mushroom culture houses. The hyphal tip approach was used to isolate pure cultures of *Trichoderma harzianum*, which were then kept at 10°C on PDA slants (Hyakumachi, 1994).

Screening of *Trichoderma harzianum*

The dual culture method on PDA was used to evaluate the pathogenicity of ten *T. harzianum* isolates against *P. ostreatus* (Tuite, 1969). Each *T. harzianum* isolate's 3-day-old mycelial discs (5 mm) were positioned at one edge of a PDA plate, while a *P. ostreatus* disc of the same size was positioned on the other edge. For seven days, plates were incubated at 25 ± 3 °C. Inhibition percentage of the radial growth of *T. harzianum* and *P. ostreatus* was calculated after seven days of incubation, following the formula as suggested by Sunder et al. (1995).

$$\% \text{ inhibition} = \frac{X - Y}{X} \times 100$$

Where,

X= Mycelial growth (mm) of pathogen in the absence of antagonists.

Y= Mycelial growth of pathogen in the presence of antagonists.

The plates were arranged in Completely Randomized Design (CRD) with three replications.

In vitro evaluation of plant extracts against *T. harzianum*

The poisoned food technique was used to evaluate ethanol extracts of eight plants (*Allium cepa*, *Allium sativum*, *Curcuma longa*, *Aloe vera*, *Azadirachta indica*, *Lantana camara*, *Ocimum sanctum*, and *Datura stramonium*) against five isolates of *T. harzianum* (Nene & Thapliyal, 2000). In 9-cm Petri dishes, 2.5 ml of each extract (at 5% and 10% concentrations) was combined with 20 ml of PDA. Following solidification, 3-day-old *T. harzianum* cultures were used to inoculate 5-mm mycelial discs. As a control, plates with no extracts were used. Radial growth inhibition was determined in accordance with Sunder et al. (1995), and incubation

was carried out at 27 ± 1 °C. The poisoned food technique was used to test each extract at 5% and 10% concentrations (Begum, 2006).

Preparation of the mother culture of *P. ostreatus*

18 x 25 cm polypropylene bags weighing 200 g each were filled with a substrate consisting of sawdust and wheat bran (2:1), 0.2% calcium carbonate, and 65% moisture. Cotton-plugged necks were attached to the bags, which were then sterilised for an hour at 121°C and cooled for a whole day. After being aseptically injected, pure *P. ostreatus* mycelium was cultured at 25 ± 2 °C. Within 15–16 days, full colonisation took place, creating mother cultures for the preparation of spawn.

Preparation of spawn packets of *P. ostreatus*

Substrates treated with 5% leaf extracts of onion, aloe vera, neem, lantana, and tulsi were used to create spawn packets. Fungal inocula were combined with 500 g of substrate in each 22.5 x 30 cm polypropylene bag. The same protocol used for mother cultures was followed for packet preparation, sterilisation, and incubation. Two teaspoonfuls of *P. ostreatus* were added to each package, and the cells were incubated at 20 to 25 °C until they were fully colonised.

Evaluation of botanicals on *P. ostreatus* yield

The mycelium on the surface of the spawn packets was scraped off, soaked in water, drained, and then put in a culture house that was regularly irrigated, lit, and ventilated. Cap edges curled after the mushrooms were plucked by twisting them. Following the initial harvest, only scraping was needed for successive flushes; soaking and scraping were repeated.

Statistical analysis

The data from the experiment were analyzed statistically using SPSS program. The mean from all the treatments were calculated and analysis of variance of characters under study was performed by F variance test. The mean differences were evaluated by Duncan's Multiple Range Test (DMRT).

Results and Discussions

Isolation and identification of associated weed molds

A total number of 7 fungi such as *Trichoderma harzianum*, *Trichoderma viridae*, *Aspergillus flavus*, *A. niger*, *Penicillium citrinum*, *Penicillium sp.*, and *Rhizopus stolonifer*, were isolated and identified according to the standard key as described by Barnett (1980) (Table 1). On average, 82% of the spawn packets were contaminated with *Trichoderma harzianum*. Nussbaum et al. (1997) observed that *Trichoderma* spp. were the most abundant contaminant in Oyster mushroom (*Pleurotus ostreatus*) cultivation.

Table 1. Percentage incidence of different associated mycoflora in contaminated packets of oyster mushrooms.

Associated mycoflora	% Contaminated packets with		
	Mother	Commercial	mean
<i>Trichoderma harzianum</i>	79	85	82
<i>Trichoderma viridae</i>	3	0	1.5
<i>Aspergillus flavus</i>	3	2	2.5
<i>A. niger</i>	5	3	4
<i>Penicillium citrinum</i>	3	4	3.5
<i>Penicillium sp.</i>	2	2	2
<i>Rhizopus stolonifer</i>	3	4	3.5

Growth inhibition of *Pleurotus ostreatus* by different isolates of *Trichoderma harzianum*

Ten isolates of *Trichoderma harzianum* were tested against *P. ostreatus* on PDA by the dual culture technique, and the results of the screening experiment are presented in Table 2. All the tested isolates of *Trichoderma* showed more than 50% inhibition of the radial growth of *P. ostreatus* over the control. Isolate Th1 showed the highest 76.04% reduction of the radial growth, followed by Th9, Th10 and Th6 isolates, which are statistically similar. The lowest radial growth inhibition 50.37% was observed by the isolate Th3. Significant reduction of mycelial growth of *P. ostreatus* in the presence of *Trichoderma harzianum* was also reported by several researchers (Narzari et al., 2007; Kredics and Koromoczi, 2005; Magdalena and Staniaszek, 2008).

Table 2. Inhibition of radial growth of *P. ostreatus* on PDA culture by selected 10 isolates of *Trichoderma harzianum*.

<i>Trichoderma harzianum</i> isolates	% inhibition of colony growth of <i>P. ostreatus</i>
Th1	76.04 a
Th2	60.33 d
Th3	50.37 e
Th4	62.41 cd
Th5	59.33 d
Th6	66.71 bc
Th7	65.43 c
Th8	59.34 d
Th9	70.44 b
Th10	70.21 b
Control	9.00 cm

Values within a column with the same letter do not differ significantly (P=0.01) by DMRT

In vitro evaluation of plant extract against the radial growth of isolate Th1 of *T. harzianum*

Results of the in vitro evaluation of different botanicals viz. 5% and 10% concentrations of Onion, Aloe vera, Neem, Lantana, and Tulsi were presented in Table 3. All the botanicals inhibited the green molds. The study revealed that significantly the highest 65.33 % and 67.78% hyphal growth inhibition of *T. harzianum* isolate Th1 were observed by the treatment of neem extract with the concentration of 5% and 10%, respectively. The second highest 56.74% and 57.52% hyphal growth inhibition of *T. harzianum*, respectively, were obtained by treating onion extract with the concentrations of 5% and 10%, followed by Aloe vera and Lantana. Tulsi leaf extract appeared significantly inferior in inhibiting the radial growth of the pathogens. The results of the current study are in with several other investigators who reported that many plant extracts can inhibit the pathogen mycelial growth (Shovan 2008, Gupta et al., 1981, Singh et al., 1997, Gomathi and Kannabiran 2000, Chitra and Kannabiran 2001, Bagri et al., 2004).

Table 3. In vitro evaluation of Plant extract on inhibition of radial growth of *T. harzianum*.

Plant extract	Conc. (%)	% inhibition of colony growth of <i>T. harzianum</i>
Onion	5	56.74 b
	10	57.52 b
Aloevera	5	50.74 c
	10	51.33 c
Neem	5	65.33 a
	10	67.78 a
Lantana	5	42.59 d
	10	50.33 c
Tulsi	5	30.41 f

	10	38.52 e
Control		9.00 g

Values within a column with same letter do not differ significantly (P=0.01) by DMRT

Compatibility test of Plant extracts with *P. ostreatus*

By compatibility test of botanicals, the inhibitory effect of neem leaf extract at all concentrations showed the highest compatibility with *P. ostreatus* (Table 4). Only 15.35% and 20.78% colony growth inhibition of *P. ostreatus* were obtained by neem leaf extract at the concentrations of 5% and 10%, respectively. Aloe vera leaf extract was found to be compatible, which is statistically similar to neem leaf extract. Lantana, onion and tulsi leaf extracts at all the concentrations were highly incompatible and significantly inferior to neem and Aloe vera leaf extract. Neem leaf extract at a concentration of 5% was the most compatible with *P. ostreatus*. According to Bagwa (2010), neem leaf extract can enhance the growth of the mycelium of *P. ostreatus*.

Table 4. Compatibility test of Plant extracts with *P. ostreatus*

Plant extract	Conc. (%)	% inhibition of colony growth of <i>P. ostreatus</i>
Onion	5	26.55 b
	10	30.58 a
Aloevera	5	16.42 d
	10	18.33 c
Neem	5	15.35 d
	10	20.78 c
Lantana	5	21.42 c
	10	22.78 c
Tulsi	5	27.41 b
	10	30.52 a

Values within a column with same letter do not differ significantly (P=0.01) by DMRT

Effect of botanicals on yield attributes and total yield of Oyster mushroom

The effect of botanicals on different yield attributing characters, including number and weight of fruiting bodies, length and diameter of stalk, diameter and thickness of pileus and total yield of Oyster mushroom, was studied, and the results are presented in Table 5. Different botanicals had a significant influence in the yield contributing characters and total yield of mushrooms. The number of fruiting bodies grown on different treated spawn packets differed significantly. The significantly highest number of fruiting bodies per packet was 81.33 on the Neem leaf extract-treated spawn packet, followed by the Aloe vera leaf extract-treated spawn packet, producing 80.67 mean number of fruiting bodies. Lantana, onion, and tulsi leaf extract-treated spawn packets produce 70.33, 68.33, and 65.67 mean number of fruiting bodies, respectively, which are significantly higher in comparison to the untreated control (37.33 fruiting bodies).

The highest yield of fruiting bodies per spawn packet was observed in neem leaf extract treated spawn packet (680.50 g) followed by Aloe vera leaf extract treated packet which statistically similar to neem leaf extract treated spawn packet and also significantly higher than other botanicals treated packets.

Conclusion

This study revealed that green mold caused by *Trichoderma harzianum* is the predominant contaminant in oyster mushroom

Table 5. Effect of botanicals on yield attributing characters and yield of Oyster mushroom

Treatments	No. of fruiting body	Wt. of fruiting body (g)	Length of stalk (cm)	Diameter of stalk (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield per spawn packet (g)
Neem	81.33 a	3.93 b	2.51 a	0.79 a	4.67 a	0.83 a	680.50 a
Lantana	70.33 b	3.97 b	2.28 b	0.74 b	4.41 b	0.78 b	475.00 b
Aloe vera	80.67 a	3.93 b	2.51 a	0.78 a	4.67 a	0.83 a	655.50 a
Onion	68.33 b	3.97 b	2.48 a	0.74 b	4.43 b	0.78 b	468.00 b
Tulsi	65.67 b	4.30 a	2.31 b	0.73 b	4.16 c	0.72 c	420.00 b
control	37.33 c	3.22 c	2.11 c	0.71 c	4.14 c	0.69 d	340.00 c

Values within a column with the same letter do not differ significantly ($P=0.01$) by DMRT

substrates, affecting over 80% of contaminated spawn packets. Among the tested botanicals, neem leaf extract showed the highest antifungal activity against green mold and was most compatible with oyster mushroom cultivation, resulting in significantly improved yield. These findings highlight neem extract as a promising, eco-friendly alternative for managing green mold in oyster mushroom production. Further research on large-scale application and formulation development is recommended to ensure practical adoption by growers.

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