

Management of green mould infestation in oyster mushroom crop (*Pleurotus ostreatus* (Jacq.) Kumm.) using plant extracts

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ABSTRACT

Green mould caused by *Trichoderma harzianum* is a serious threat in oyster mushroom cultivation causing heavy crop losses. This study is an attempt to manage green mold fungus *Trichoderma harzianum* using plant extracts. *In vitro* results showed maximum mycelial growth inhibition of 45.80 % in *T. harzianum* using neem leaf extract at 10% concentration followed by karanj leaf extract (42.40 %) at the same concentration. Mycelial growth inhibition of *P. ostreatus* in neem leaf extract was 9.8% only and was statistically at par with karanj leaf extract (8.70%). Neem leaf extracts showing maximum efficacy against pathogen and minimum inhibition against oyster mushroom (*P. ostreatus*) was further used in mushroom cultivation experiment to check its efficacy in crop and yield performance of *P. ostreatus*. Spraying of 10 % neem leaf extract on the pasteurized paddy straw before spawning resulted minimum first harvest days (28.2 days), maximum fruit weight (11.5 g/fruit) and maximum yield of mushroom (723.3 g/kg dry substrate) closely followed with karanj leaf extract. Mushroom yield obtained in neem and karanj leaf extract was statistically at par with each other. Carbendazim (0.1%), sprayed during spawning as control treatment, produced 525.0 g/kg dry substrate. Yield recorded in untreated bags were 200.5 g/kg dry substrate. Use of plant extracts proved promising in managing green mould in oyster mushroom crop, which can be economical, long lasting and free from residual side effects.

Key words: *Pleurotus ostreatus*, oyster mushroom, green mold, *Trichoderma harzianum*, plant extracts

The mushroom cultivation is a biotechnological process for conservation of various lignocellulosic agricultural, industrial, forestry, horticultural waste or their by-products in to quality proteins. Mushroom cultivation is a viable alternative venture for minimizing the ever-increasing protein malnutrition gap and multitude of allied problems in developing countries (Eswaran and Ramabadrana, 2000).

White button mushroom (*Agaricus bisporus*), Paddy straw mushroom (*Volvariella* sp) and Oyster mushroom (*Pleurotus* sp.) are the mushroom most

commonly grown in India (Munshi and Ghani, 2003). Oyster mushroom is known for its culinary and nutritional properties, and can grow under varied agro-climatic conditions (Baysal *et al.*, 2003). They are rich in proteins, vitamins and minerals (Mattila *et al.*, 2000). The proximate composition reveal 85-95% water, 3% protein, 4% carbohydrates, 0.1% fats, 1% minerals and vitamins on fresh weight basis (Wasser, 2002). They contain minerals like potassium, phosphorous, copper and iron but little amount of calcium (Vetter, 2007). Oyster mushroom cultivation can help in managing organic wastes, whose disposal

is very complicated and time consuming (Das and Mukherjee, 2007).

Pleurotus ostreatus (Jacq.) Kumm., commonly known as oyster mushroom, is commercially one of the most important edible mushroom grown in Jharkhand. The cultivation of Oyster mushroom has picked up in Jharkhand due to easy availability of paddy straw as a substrate and easy cultivation process. Jharkhand has great potential for oyster mushroom cultivation since it requires limited space, low initial investment and the raw materials used are cheap and easy to acquire. These facts about the oyster mushroom cultivation make it most affordable to small and marginal farmers in order to generate extra income during the dry months.

Just like other crops, diseases and pests also affect mushroom crops. The diseases and pests happen to be devastating and perpetuate easily from one season to another. A number of competitor molds appear in oyster mushroom crop due to substrates, methods of substrate preparation, growing conditions and containers used for cultivation (Sharma *et al.*, 2007; Sharma and Kumar, 2011). The most serious problem in oyster mushroom crop is *Trichoderma harzianum* causing green mold *Pleurotus sajor-caju* (Sharma and Jandaik, 1980). The pathogen inhibits the growth of mushrooms and hinders the production of fruiting bodies. Reports are available about the interaction between *Trichoderma* sp. and the mushroom due to the enzymatic action on substrate by mushroom that favours green mould fungal growth (Colavolpe *et al.*, 2015). Heavy crop losses up to 70% is reported due to the contaminant mould in the mushroom crop (Vijay *et al.*, 1986).

Carbendazim is frequently used as a protectant against the occurrence of *T. harzianum* in oyster mushroom crop, but residual toxicity of chemicals is of great concern. Constant use of chemicals results in food chain poisoning, environmental pollution besides increasing the chance of resistance development.

Thus, use of plant extracts has opened a new avenue for control of mushroom moulds and diseases as they are safe and non-toxic (Tiwari *et al.*, 1988).

MATERIAS AND METHODS

The present experiment was carried out at Mushroom unit, ICAR- RCER, Farming System Research Centre for Hills and Plateau Region, Ranchi, Jharkhand during the season July to September, 2021 and October to December, 2021.

Isolation and preservation of *Trichoderma* sp

Mushroom bags of *P. ostreatus* naturally infected with typical green mold were used to isolate the causal organism. The isolated fungi were identified using microscopy. Straw from infected bed was taken and cut into small pieces. Serially diluted using sterile distilled water, inoculated on the Malt extract agar plates and incubated at $25 \pm 1^\circ \text{C}$ for 6 days. Fungal culture was purified by single spore isolation and periodic sub-culture. All the pure cultures were kept in refrigerator at 4°C for till further use.

Collection and preparation of phyto extracts

Leaves of *Azadirachta indica* (neem), *Pongamia pinnata* (karanj), *Lawsonia inermis* (mehndi), *Ocimum sanctum* (tulsi), *Lantana camara* (putus), bulbs of *Allium cepa* (onion), cloves of *Allium sativum* (garlic), rhizomes of *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) were collected from the nearby forest area as well as local market and agricultural farm of ICAR-RCER, FSRCHPR, Ranchi, Jharkhand.

For the preparation of phyto-extracts, plant products were washed thoroughly with distilled water and were shade dried for 24 hrs at room temperature ($24 \pm 2^\circ \text{C}$). 10 gram of each plant products were homogenized with distilled water (100 ml) by crashing them with electric grinder machine. The extracts were filtered through double-layered muslin cloth and

centrifuged at 4000 rpm for 10 minutes. The supernatant was collected and filtered through Whatman No.1 filter paper (Biswas *et.al.*, 2018).

$$\text{Mycelial inhibition (\%)} = \frac{\text{Radial growth in check} - \text{Radial growth in treatment}}{\text{Radial growth in check}} \times 100$$

Sensitivity of *T. harzianum* and *P. ostreatus* against phyto-extracts (*in vitro* evaluation)

Efficacy of aqueous extracts of all the nine different plant products were evaluated in the laboratory for their efficacy against both *P. ostreatus* and pathogen (*T. harzianum*) mycelium. The plant extracts were evaluated *in vitro* using poison food technique (Nene and Thapliyal, 2000). Plant extracts of all the species were tested at 1.0 %, 2.0 %, 3.0%, 5.0%, 7.5% and 10.0% concentrations. The efficacy of phyto extracts was compared with carbendazim at 0.1% concentration. Five replications were maintained for all the treatments. Per-cent inhibition of mycelial growth of green mold fungus over check was calculated using the following formula of Vincent (1947).

Efficacy of plant extracts on mushroom yield (*in vivo* evaluation)

The botanicals showing least adverse effects on the growth of *P. ostreatus* and maximum inhibition of pathogen were evaluated for yield performance of *P. ostreatus in vivo*. Paddy straw was used as substrate for cultivation. Chopped paddy straw of 3-5 cm in length was cleaned thoroughly for 2-3 times with tap water and treated in hot water (70-75°C) for one hour followed by draining excess water. Spore of *T.harzianum* was obtained from a 7 day old culture and homogeneous suspension of spores was prepared with a concentration of 1×10^7 Conidia /ml of water using a haemocytometer. The inoculum suspension was sprayed over the hot water treated pasteurized

paddy straw before spawning @40ml/ kg dry weight of substrate and thoroughly pulverized (Narzari *et al.*, 2007). Selected plant extracts of 10.0 % concentration was sprayed on the mushroom beds at the time of spawning. Inoculated and treated paddy straw substrate was filled in polythene bags at the rate of 1.5 kg dry substrates. The untreated bags (no spray of plant extracts) were kept as control along with a carbendazim treated control. Ten bags per replication with 10 replications were kept for all the treatments including check in Randomized Block Design. Spawn of *P. ostreatus* was added at the rate of 10 % on dry weight basis of substrate.

The bags were incubated inside the cropping room at temperature between 25 -28 ° C and relative humidity 80-85% maintained. Spawn run room was kept in dark for 15-18 days till complete colonization of the substrate with fungal mycelium. The polythene bags were cut open and diffused light with fresh air was introduced for fruiting. Total yield of first three flushes was recorded.

RESULTS AND DISCUSSION

All the nine plant extracts more or less significantly inhibited mycelial growth of *Trichoderma harzianum* at all the tested concentrations. The maximum mycelial inhibition percentage and least mycelial growth of *T. harzianum* was observed in neem leaf extract (33.9 %, 59.5 mm) followed by karanj (28.8%, 64.1 mm) (Table 1). The least mycelial growth inhibition was exhibited in extracts of mehndi leaves (6.7 %) followed by lantana (7.9%) and tulsi leaves (13.2%) treatment. Similarly, 95.4 % inhibition of the mould was recorded in case of carbendazim (0.1%). It was further observed that the effect of all six concentrations tested on mycelial growth inhibition of *T. harzianum* varied significantly for each treatment. There was a linear increase in the inhibition of mycelial growth of pathogen was recorded with the increase in concentration.

Table 1. Effect of different plant extracts at different concentrations on the mycelia growth of *Trichoderma harzianum* and per cent growth inhibition

S. No.	Phyto extracts/chemical	1.0 %		2.0 %		3.0 %		5.0 %		7.5 %		10 %		Mean	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B
1.	<i>Azadirachta indica</i> (neem) leaf extract	66.2	26.4	65.6	27.1	65.2	27.6	59.4	33.9	51.4	42.9	48.8	45.8	59.5	33.9
2.	<i>Allium cepa</i> (onion) bulbs	79.8	11.3	77.4	13.9	72.6	19.3	69.2	23.1	64.8	27.9	60.0	33.3	70.6	21.5
3.	<i>Allium sativum</i> (garlic) cloves	77.6	13.8	75.6	15.9	71.2	20.9	64.8	27.9	59.6	33.8	54.0	39.9	67.1	25.4
4.	<i>Curcuma longa</i> (turmeric) rhizomes	81.6	9.3	80.8	10.2	79.0	12.2	73.4	18.4	66.8	25.8	62.6	30.4	74.0	17.7
5.	<i>Zingiber officinale</i> (ginger) rhizomes	87.4	2.9	85.4	5.1	82.4	8.4	78.0	13.3	74.4	17.3	73.4	18.4	80.2	10.9
6.	<i>Pongamia pinnata</i> (karanj) leaf extract	73.4	18.4	70.4	21.8	67.6	24.9	65.0	27.8	56.4	37.3	51.8	42.4	64.1	28.8
7.	<i>Lawsonia inermis</i> (mehndi) leaf extract	89.0	1.1	87.8	2.4	85.2	5.3	83.4	7.3	81.0	9.9	77.2	14.2	83.9	6.7
8.	<i>Ocimum sanctatum</i> (tulsi) leaf extract	85.2	5.3	83.4	7.3	81.0	9.9	76.8	14.7	73.4	18.4	68.6	23.8	78.1	13.2
9.	<i>Lantana camera</i> (putus) leaf extract	88.2	1.9	86.4	3.9	83.4	7.3	81.6	9.3	80.2	10.9	77.2	14.2	82.8	7.9
10.	Carbendazim (0.1 %) (check-I)	6.0	93.3	4.2	95.3	3.2	96.4	3.6	95.9	3.8	95.8	4.0	95.5	4.1	95.4
11.	Without any treatment (check-II)	90	-	90	-	90	-	90.0	-	90.0	-	90.0	-	90.0	-
	CD (0.05)	4.0	-	4.2	-	3.7	-	3.9	-	4.1	-	3.9	-	4.0	-

 A - Mycelial growth (mm) of *T. harzianum* at different concentrations; B - Percentage mycelial growth inhibition

Efficacy of onion extract was also recorded against fungal contaminants and diseases due to presence of inhibitory compounds like cycloallin and carbohydrate propenyl sulphonic acid (Rastogi and Mehrotra, 1969; Chauhan and Singh, 1991; Ahmed *et al.*, 2000; Siddique *et al.*, 2004). Siddique (2000) reported the effectiveness of aqueous extract of nine different plants against *T. harzianum*. He observed maximum inhibition of *T. harzianum* both *in vitro* and *in vivo* with onion extract (*Allium cepa*) while Narzari *et al.* (2007) reported complete inhibition of *T. harzianum* by 0.4% *Allium sativum* (garlic) extract. Mishra (2009) used neem leaf extract, neem cake solution and neem saw dust against *Trichoderma viride* and found similar kind of results. Garlic extracts were effective against *Trichoderma* sp. might be due to presence of inhibiting compounds like ajoene, diallyl disulphide oxide (allicin) and S-allyl cysteine (Narzari *et al.*, 2007).

All the nine plant extracts showed low toxicity to *P. ostreatus*. Least mycelial inhibition percentage and maximum mycelial growth of *P. ostreatus* was recorded in the turmeric rhizome extract (2.8 %, 87.5 mm) followed by lantana leaf extract (3.2 %, 87.1 mm), which were statistically at par with each other. Mycelial growth of *P. ostreatus* in neem leaf extract (84.5 mm) was statistically at par with karanj leaf extract (85.2 mm) (Table 2).

In this bioassay, the plant extracts showed the maximum efficacy against pathogen and least adverse effect on the growth of *P. ostreatus* were further evaluated *in vivo*. The plant extracts selected for *in vivo* trial were: neem leaf extract, karanj leaf extract, cloves of garlic, bulbs of onion and rhizomes of turmeric along with three controls (Table 3). For comparing the efficacy of plant extracts in the present study, carbendazim was also taken as check and applied in the beds as a protectant against *T. harzianum* as the efficacy of this fungicide against *Trichoderma* sp is well established. But, a judicious use of carbendazim is highly preferred to avoid residual

Table 2. Effect of different plant extracts at different concentrations on the mycelial growth of *Pleurotus ostreatus* and percentage mycelial growth inhibition

S. No.	Phyto extracts/chemical	1.0 %		2.0 %		3.0 %		5.0 %		7.5 %		10 %		Mean	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B
1.	<i>Azadirachta indica</i> (neem) leaf extract	88.6	1.6	86.6	3.8	84.4	6.2	84.2	6.4	82.0	8.9	81.2	9.8	84.5	6.1
2.	<i>Allium cepa</i> (onion) bulbs	89.6	0.4	89.4	0.7	89.0	1.1	87.8	2.4	85.0	5.6	81.0	9.9	86.9	3.4
3.	<i>Allium sativum</i> (garlic) cloves	89.4	0.7	88.4	1.8	87.2	3.1	85.6	4.8	84.4	6.2	80.8	10.1	85.9	4.6
4.	<i>Curcuma longa</i> (turmeric) rhizomes	90.0	0.0	89.4	0.7	89.2	0.9	88.8	1.3	86.2	4.2	81.6	9.3	87.5	2.8
5.	<i>Zingiber officinale</i> (ginger) rhizomes	88.4	1.8	87.6	2.7	86.8	3.6	86.0	4.4	85.4	5.1	84.0	6.7	86.4	3.9
6.	<i>Pongamia pinnata</i> (karanj) leaf extract	88.4	1.8	87.2	3.1	86.0	4.4	85.0	5.6	82.6	8.2	82.2	8.7	85.2	5.3
7.	<i>Lawsonia inermis</i> (mehndi) leaf extract	89.8	0.2	88.0	2.2	86.4	3.9	84.8	5.8	84.0	6.7	83.0	7.8	86.0	4.4
8.	<i>Ocimum sanctum</i> (tulsi) leaf extract	89.4	0.7	89.0	1.1	87.8	2.4	86.2	4.2	85.0	5.6	83.6	7.1	86.8	3.6
9.	<i>Lantana camera</i> (putus) leaf extract	89.8	0.2	89.0	1.1	87.6	2.7	86.4	3.9	85.4	5.1	84.4	6.2	87.1	3.2
10.	Carbendazim (0.1 %) (check-I)	69.4	22.9	69.4	22.9	67.6	24.9	65.6	27.1	68.8	23.5	69.8	22.4	68.4	23.9
11.	Without any treatment (check-II)	90.0	-	90.0	-	90.0	-	90.0	-	90.0	-	90.0	-	90.0	-
	CD (0.05)	1.9	-	2.2	-	2.2	-	2.7	-	2.6	-	2.5	-	2.4	-

A - Mycelial growth (mm) of *Pleurotus ostreatus* at different concentrations; B - Percentage mycelial growth inhibition**Table 3.** Effect of botanicals on yield attributing characters of *Pleurotus ostreatus*

S. No.	Phyto extracts@ 10.0% Conc./chemical	DFFH (Days)			WOFB (g/fruit)			Yield (g/kg dry substrate)			Biological efficiency (%)		
		A	B	Mean	A	B	Mean	A	B	Mean	A	B	Mean
1.	<i>Azadirachta indica</i> (neem)	29.8	26.6	28.2	11.1	11.9	11.5	660.5	786.1	723.3	66.1	78.6	72.3
2.	<i>Pongamia pinnata</i> (karanj)	31.4	28.5	29.9	10.4	11.7	11.1	650.0	761.0	705.5	65.0	76.1	70.6
3.	<i>Allium sativum</i> (garlic)	32.9	30.3	31.6	10.2	10.9	10.6	600.5	716.0	658.3	60.1	71.6	65.8
4.	<i>Allium cepa</i> (onion)	33.9	31.3	32.6	9.4	10.2	9.8	572.5	689.0	630.8	57.3	68.9	63.1
5.	<i>Curcuma longa</i> (turmeric)	35.9	31.9	33.9	9.7	9.4	9.6	531.8	619.0	575.4	53.2	61.9	57.5
6.	Carbendazim (0.1 %) (check-III)	37.1	35.0	36.1	10.0	8.9	9.5	507.5	542.5	525.0	50.8	54.3	52.5
7.	Inoculated and untreated (check-II)	40.2	40.7	40.5	9.5	7.8	8.7	196.0	205.0	200.5	19.6	20.5	20.1
8.	Uninoculated and untreated (check-I)	28.5	27.1	27.8	12.0	13.6	12.8	744.5	820.5	782.5	74.5	82.1	78.3
	CD(0.05)	1.9	2.2	2.1	1.5	1.4	1.5	33.1	35.4	34.3	-	-	-

A- July to Sep.21; B- Oct to Dec.21; DFFH- Days For First Harvest; WOFB – Average weight of fruiting bodies

toxicity in mushroom and also to obtain good mycelial growth of mushroom fungus. Dubey *et al.* (1994) recorded with very poor mycelial run of oyster mushroom when carbendazim (0.1%) was used in the sterilized straw and beds.

Time taken for first harvest and weight of fruiting bodies in *P. ostreatus*

It is evident from the (Table 3) that first harvest days in *P. ostreatus* differed significantly among the plant extracts. The average number of the days required for first harvest of *P. ostreatus* was significantly less (28.2 days) using neem leaf extract followed by karanj leaf extract (29.9 days), which were statistically at par with each other. The average number of the days for first harvest was significantly higher (40.5 days) in Check-II (inoculated and untreated). Weight of fruiting bodies was also recorded to be the maximum (11.5 g/fruit) in case of neem leaf extract followed by karanj leaf extract (11.1 g/fruit). Small size fruiting body (8.7 g/fruit) was recorded in Check-II (inoculated and untreated).

Yield and Biological efficiency (%) of *P. ostreatus*

Significant difference was recorded in the effect of the plant extracts on the total yield of *P. ostreatus* (Table 3). The maximum yield (723.3 g/kg dry substrate) with 72.3 % biological efficiency was recorded in neem leaf extract followed by karanj leaf extract (705.5 g/kg dry substrate) with 70.6 % biological efficiency while the minimum yield (200.5 g/kg dry substrate) with 20.1 % biological efficiency was recorded in Check-II (inoculated and untreated). Inam-ul-Haq *et al.* (2010) observed increased yield and reduced disease incidence in oyster mushroom cultivation with the active components of *Eucalyptus camaldulensis*, *Azadirachta indica*, *Citrus lemon* and *Cymbopogon marginatus*, which supports the results of the present investigation. The positive effects of azadirachtin (C₃₅H₄₄O₁₆), the principle active ingredient of *Azadirachta indica* (neem oil),

on the yield and biological efficiency (109.25%) of oyster mushroom was also observed by Sharma and Jandaik (1994) and Biswas (2015).

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