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# Management of *Sclerotium rolfsii* Sacc. Infection in Oyster Mushroom [*Pleurotus ostreatus* (Jacq.) Kumm.] by Plant Extracts

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## ABSTRACT

**Background:** Successful methods to control *Sclerotium rolfsii* in beds of oyster mushroom cultivation are not presently known. The aim of the present investigation was to determine antifungal potentials of some plant extracts against the infection of *S. rolfsii* associated with the oyster mushroom (*Pleurotus ostreatus*) cultivation.

**Methods:** Aqueous extracts of four different plants parts *viz.* leaf of *Azadirachta indica* (neem), leaf of *Pongamia pinnata* (karanj), bulbs of *Allium cepa* (onion) and cloves of *Allium sativum* (garlic) were evaluated in the laboratory for their efficacy against both *P. ostreatus* mycelium and pathogen. The plant extracts which displaced least adverse effects on the growth of *P. ostreatus* and maximum inhibition of pathogen were evaluated to find out the yield performance of *P. ostreatus* under *in vivo* conditions in mushroom house.

**Results:** Neem (*Azadirachta indica*) leaf extract showed maximum average mycelial and sclerotial inhibition i.e. 47.9 % and 52.4 %, respectively followed by karanj (*Pongamia pinnata*) leaf extract i.e. 38.4 % and 47.9 %, respectively. Maximum yield of oyster mushroom (885.0 g/ kg dry substrate) was also recorded in neem leaf extract followed by karanj leaf extract (867.5 g/ kg dry substrate) and cloves of garlic (846.3 g/ kg dry substrate) which were statistically at par with each other.

**Key words:** Oyster mushroom, Plant extracts, *Pleurotus ostreatus*, *Sclerotium rolfsii* .

## INTRODUCTION

Oyster mushroom (*Pleurotus ostreatus*) belonging to class *Basidiomycetes* and family *Agaricaceae* is popularly known as "*Dhingri*" in India. The popularity of oyster mushroom has been increasing due to its ease of cultivation, high yield potential and high nutritional value (Banik and Nandi, 2004; Gregori *et al.,* 2007).

Mushrooms are known for good quality amino acids, vitamin B complexes, sodium, potassium, iron, dietary fibers and also considered as the primary natural source of ergosterol or pro vitamins, also possess high nutritional and medicinal value and are being widely domesticated (Jr, 2005). From various studies, edible mushrooms are also found to have many pharmacological role like antiviral, antioxidant, anti-tumor, hypo-cholesterolemic and hypoglycemic (Cheung, 2010). Edible mushrooms are also found to be very effective in reducing stress, cholesterol, asthma, diabetes, cancer, insomnia *etc*. (Wani *et al.,* 2010).

Several fungal contaminants of mushrooms have been serving as the major restraining factor in the growing mushroom industry for a long time. Fungal contaminants like *Trichoderma* spp., *Mycogone* spp., *Lecanicillium* spp., *Cladobotryum* spp., *Coprinus* spp., *Sependonium* spp., *Sclerotium rolfsii* and *Cephalothecum roseum* among many, are found to infect mushroom crops at different stages from spawn run period to maturation of fruiting bodies. These contaminants may reduce yield and degrade the quality of fruiting bodies of the mushroom causing economic losses. Most of these contaminants come from poorly sterilized substrates (Ghimire *et al.,* 2021; Fletcher and Gaze, 2008; Biswas and Kuiry, 2013).These contaminants deteriorate quality and damage basidiomes ultimately leading to reduced production and sometimes complete failure of the crop (Gea *et al.,*2021).

*Sclerotium rolfii* Sacc. is a common contaminant of beds of the mushroom *Pleurotus flabellatus* (Berk. and Br.) Sacc. cultivated on paddy straw. This straw-borne contaminant appears in the mushroom beds 5-8 days after spawning and grows as a white fluffy mycelial mass, producing dark brown sclerotia 2-4 days later. Depending on the time of its appearance and the intensity of contamination, a reduction in mushroom yield from 40-100% was noticed by Rajarathnam *et al.* (1992). This

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fungus is characterized by profuse production of cottony; rapid aerial mycelial growth, forming abundant light brown sclerotia; 1.0-1.5 mm in diameter, globose to sub-globose, smooth surface, glossy and compacted (Watanabe, 2002). Rajarathnam *et al.* (1992) reported *Sclerotium rolfsii* as a serious substrate-borne fungal contaminant found during commercial cultivation of *Pleurotus flabellatus* on unfermented rice straw of rice fields. Depending on the degree of contamination, the loss due to *S. rolfsii* in mushroom yield ranges from 80% to 96% (Rajarathnam and Zakia, 1987).

The use of fungicides for controlling the competitor moulds and diseases in oyster mushroom cultivation is very common in India. The hazardous effects of chemicals in human health and environmental aspect are known. Apart from these problems continuous usage of same chemicals may lead towards pest's resistance.

Considering the above, an experiment was conducted to develop a suitable management practice against the competitor molds of *Pleurotus ostreatus* in an eco- friendly manner under the agro-ecological condition of Jharkhand. Medicinal plants are frequently used for the isolation of biologically active compounds widely used in the preparation of various drugs. The use of substrates containing bioactive compounds or supplemented with minerals, as well as postharvest treatments are some of the strategies to increase the nutraceutical value of *Pleurotus* spp. (Carrasco-Gonzalez *et al.,* 2017).

## MATERIALS 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 July to September, 2022 and October to December,2022. The pathogen was isolated from naturally affected bags of oyster mushroom (*Pleurotus ostreatus*) grown during July to September, 2022.

### Isolation and preservation of *Sclerotium rolfsii*

Mushroom bags of *P. ostreatus* bearing contamination of *S. rolfsii* were selected. Straw of these beds were cut into small pieces and surface sterilized by 0.1% mercuric chloride for one minute and rinsed with three changes of sterilized distilled water. Each piece was aseptically transferred to the PDA plates. The inoculated plates were incubated at 25±1°C for 6 days. Pure culture of the fungus was made by single spore isolation and periodic sub-culture was done during the period of investigation. All the pure cultures were kept in refrigerator at 4°C for preservation.

### Collection and preparation of phyto extracts

Leaves of *Azadirachta indica* (neem), leaves of *Pongamia pinnata* (karanj), bulbs of *Allium cepa* (onion) and cloves of *Allium sativum* (garlic) 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, 10 gram plant products were collected and washed thoroughly with distilled water. The rinsed plant parts were shade dried for 24 h at room temperature 24±2°C and homogenized with distilled water (100 ml) by crashing them with electric grinder machine. The extract was 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 which was considered as a standard solution (Biswas *et.al*., 2018).

### Sensitivity of *S. rolfsii* and *P. ostreatus* against phyto extracts (*in vitro*)

Efficacy of aqueous extracts of four different plants parts *viz.* leaf of *Azadirachta indica* (neem), leaf of *Pongamia pinnata* (karanj), bulbs of *Allium cepa* (onion) and cloves of *Allium sativum* (garlic) were evaluated in the laboratory for their efficacy against both *P. ostreatus* mycelium and pathogen. The plant extracts were evaluated *in vitro* through Poison Food Technique (Nene and Thapliyal, 2000).

Plant extracts of all the species were tested at 5.0%, 7.5% and 10.0% concentrations against both *P. ostreatus* mycelium and pathogen *S. rolfsii.* Four replications were maintained for all the treatments. Per cent inhibition of mycelial growth of pathogen and *P. ostreatus* over check was calculated using the following formula of Vincent (1947).

Mycelial inhibition=

$$\frac{\text{Radial growth in check - Radial growth in treatment}}{\text{Radial growth in check}} \times 100$$

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

The botanicals which displaced least adverse effects on the growth of *P. ostreatus* and maximum inhibition of pathogen were evaluated to find out the yield performance of *P. ostreatus in vivo*. The cultivation trials were conducted at Mushroom unit, ICAR-RCER-FSRCHPR, Plandu, Ranchi during July- September,2022 and October- December, 2022.

Paddy straw was used as substrate for cultivation. The chopping of paddy straw was done manually into bits of 3-5 cm in length and were cleaned thoroughly 2-3 times with tap water and then treated in hot water for one hour. The paddy straw bits were then drained off. Mycelial suspension of *S. rolfsii* was obtained from 7 days old culture. *S. rolfsii* raised in PDA plates by repeated washing in 15 ml sterile distilled water with a pen brush under aseptic condition. The washing was taken in a beaker, shaken in a shaker for 30 min to get a homogeneous suspension of mycelium. 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 were sprayed on the mushroom beds at the time of spawning. The paddy straw inoculated with *S. rolfsii* fungus and sprayed with different selected plant extracts of 10.0% concentration filled in polythene bags at the rate of 1.5 kg dry substrates. The untreated bags (devoid of botanicals) were kept as check. Ten bags per replication

were kept in each treatment and all the treatments including check were replicated four times in randomized block design. Spawn of *P. ostreatus* was added for each trial at the rate of 10% on dry weight basis of substrate.

The bags were then incubated inside the cropping room, where temperature ranged between 25-2°C and relative humidity 80-85% maintained. Room having spawn running bags was kept in dark for 15-18 days till complete colonization of the substrate with fungal mycelium. The polythene bags were cut open when the substrate was completely colonized with mycelium. Finally, total yield of first three flushes was recorded.

## RESULTS AND DISCUSSION

All the four plant extracts more or less significantly inhibited mycelial growth and sclerotial formation of *Sclerotium rolfsii* at all the tested concentrations. Maximum mycelial inhibition percentage and least mycelial growth of *S. rolfsii* was observed in the neem leaf extract (47.9%, 46.1 mm) followed by karanj leaf extract (38.4%, 55.4 mm), cloves of garlic (28.0%, 64.8 mm) and bulbs of onion (15.7%, 75.9 mm). Maximum sclerotial inhibition percentage and least number of scletotia formation of *Sclerotium rolfsii* was observed in the neem leaf extract (52.4%, 8.3) followed by karanj leaf extract (47.9%, 9.1), cloves of garlic (32.4%, 11.8) and bulbs of onion (21.7%, 13.7) (Table 1 and 2). It was further observed that the effect of all three concentrations (5.0,7.5 and 10.0 per cent) on mycelial growth inhibition and sclerotial inhibition of *S. rolfsii* varied significantly *i.e.*, with the increase in concentrations from 5.0 to 10.0 per cent, there was an increase in the inhibition of mycelial growth and sclerotial formation of pathogen as well. Efficacy of onion extract was also recorded against fungal contaminants and disease due to presence of inhibitory compounds like cycloallin and carbohydrate propenyl sulphonic acid (Rastogi and Mehrotra, 1969; Chauhan and Singh, 1991 and Siddique *et al.,* 2004).

All the four plant extracts showed least toxicity to *P. ostreatus*. Least mycelial inhibition percentage and maximum mycelial growth of *P. ostreatus* was recorded in the bulbs of onion (6.4%, 84.4 mm) followed by cloves of garlic (7.1%, 83.6 mm), karanj leaf extract (7.9%, 82.9 mm) and neem leaf extract (9.5%, 81.5 mm) which were statistically at par with each other (Table 3 and 4).

In this bioassay, the plant extracts which displayed 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 and bulbs of onion.

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

It is evident from the Table 5 that there was significant difference between the influences of plant extracts on time taken for first harvest of *P. ostreatus*. The average number of the days required for first harvest of *P. ostreatus* was significantly less (24.7 days) in neem leaf extract followed

**Table 1:** Effect of different plant extracts at different concentrations on the mycelial growth and sclerotia formation of Sclerotium rolfsii.

| Phyto extracts | Mycelial growth (mm) and sclerotia formation (no.) of S. rolfsii at different concentrations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5.0 % | | 7.5 % | | 10 % | | Mean | |
| | Mycelial growth (mm) | No. of sclerotia (after 10 Days) | Mycelial growth (mm) | No. of sclerotia (after 10 Days) | Mycelial growth (mm) | No. of sclerotia (after 10 Days) | Mycelial growth (mm) | No. of sclerotia (after 10 Days) |
| Azadirachta indica (neem) leaf extract | 49.6 | 10.0 | 47.3 | 8.5 | 44.0 | 6.5 | 46.1 | 8.3 |
| Pongamia pinnata (karanj) leaf extract | 59.3 | 10.3 | 56.5 | 9.5 | 50.5 | 7.5 | 55.4 | 9.1 |
| Allium sativum (garlic) cloves | 76.0 | 13.0 | 60.0 | 12.0 | 58.3 | 10.5 | 64.8 | 11.8 |
| Allium cepa (onion) bulbs | 83.0 | 15.3 | 76.3 | 14.3 | 68.5 | 11.5 | 75.9 | 13.7 |
| Check | 90.0 | 17.5 | 90 | 17.5 | 90 | 17.5 | 90.0 | 17.5 |
| CD (0.05) | 5.6 | 3.7 | 6.1 | 2.9 | 5.9 | 2.2 | 5.8 | 2.9 |

by karanj leaf extract (25.9 days), cloves of garlic (27.2 days) and bulbs of onion (27.3 days) which were statistically at par with each other. The average number of the days for first harvest was significantly more (32.2 days) in Check-II (With pathogen and without toxicants). Weight of fruiting bodies was also recorded maximum (14.8g/fruit) in case of neem leaf extract followed by karanj leaf extract (13.4 g/fruit) and cloves of garlic (12.8g/fruit) which were statistically at par

**Table 2:** Effect of different plant extracts at different concentrations on percentage inhibition on mycelial growth and sclero formation of *S. rolfsii.*

| Phyto extracts | Percentage inhibition mycelial growth and sclerotia formation of *S. rolfsii* at different concentrations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5.0 % | | 7.5 % | | 10 % | | Mean | |
| | Mycelial growth | No. of sclerotia | Mycelial growth | No. of sclerotia | Mycelial growth | No. of sclerotia | Mycelial growth | No. of sclerotia |
| *Azadirachta indica* ( neem) leaf extract | 45.2 | 42.9 | 47.4 | 51.4 | 51.1 | 62.9 | 47.9 | 52.4 |
| *Pongamia pinnata* (karanj) leaf extract | 34.2 | 41.1 | 37.2 | 45.7 | 43.8 | 57.1 | 38.4 | 47.9 |
| *Allium sativum* (garlic) cloves | 15.5 | 25.7 | 33.3 | 31.4 | 35.3 | 40.0 | 28.0 | 32.4 |
| *Allium cepa* (onion) bulbs | 7.8 | 12.6 | 15.3 | 18.3 | 23.9 | 34.3 | 15.7 | 21.7 |
| Check | - | - | - | - | - | - | - | - |
| CD (0.05) | 6.2 | - | 8.5 | - | 6.6 | - | 7.1 | - |

**Table 3:** Effect of different plant extracts at different concentrations on the mycelial growth of *Pleurotus ostreatus.*

| Phyto extracts | Mycelial growth (mm) of *P. ostreatus* at different concentrations | | | |
|---|---|---|---|---|
| | 5.0 % | 7.5 % | 10 % | Mean |
| *Azadirachta indica* ( neem) leaf extract | 84.0 | 82.0 | 78.5 | 81.5 |
| *Pongamia pinnata* (karanj) leaf extract | 86.3 | 82.0 | 80.5 | 82.9 |
| *Allium sativum* (garlic) cloves | 86.5 | 83.0 | 81.3 | 83.6 |
| *Allium cepa* (onion) bulbs | 87.5 | 85.3 | 80.3 | 84.4 |
| Check | 90.0 | 90.0 | 90.0 | 90.0 |
| CD (0.05) | 2.8 | 3.4 | 3.3 | 3.2 |

**Table 4:** Effect of different plant extracts at different concentrations on percentage mycelial growth inhibition of *Pleurotus ostreatus.*

| Phyto extracts | Percentage mycelial growth inhibition of *P. ostreatus* at different concentrations | | | |
|---|---|---|---|---|
| | 5.0% | 7.5% | 10% | Mean |
| *Azadirachta indica* ( neem) leaf extract | 6.7 | 8.9 | 12.8 | 9.5 |
| *Pongamia pinnata* (karanj) leaf extract | 4.2 | 8.9 | 10.6 | 7.9 |
| *Allium sativum* (garlic) cloves | 3.9 | 7.8 | 9.7 | 7.1 |
| *Allium cepa* (onion) bulbs | 2.9 | 5.4 | 10.8 | 6.4 |
| Check | - | - | - | - |
| CD (0.05) | 3.1 | 3.8 | 3.6 | 3.5 |

**Table 5:** Effect of botanicals on yield attributing characters of *Pleurotus ostreatus.*

| Phyto extracts@ 10.0% Conc. | DFFH ( Days for first harvest) | | | WOFB (weight of fruiting bodies) (g/ fruit) | | |
|---|---|---|---|---|---|---|
| | July to Sep. 22 | Oct to Dec. 22 | Mean | July to Sep. 22 | Oct to Dec. 22 | Mean |
| *Azadirachta indica* ( neem) leaf extract | 24.8 | 24.5 | 24.7 | 13.8 | 15.8 | 14.8 |
| *Pongamia pinnata* (karanj) leaf extract | 26.3 | 25.5 | 25.9 | 12.5 | 14.3 | 13.4 |
| *Allium sativum* (garlic) cloves | 27.5 | 26.8 | 27.2 | 11.8 | 13.8 | 12.8 |
| *Allium cepa* (onion) bulbs | 27.5 | 27.0 | 27.3 | 11.0 | 13.0 | 12.0 |
| With pathogen and without toxicants (check-II) | 33.3 | 31.0 | 32.2 | 7.0 | 8.0 | 7.5 |
| Without pathogen and without plant extracts (check-I) | 29.3 | 29.0 | 29.2 | 10.5 | 11.5 | 11.0 |
| CD(0.05) | 3.3 | 2.9 | 3.1 | 2.4 | 2.7 | 2.6 |

**Table 6:** Effect of botanicals on yield of *Pleurotus ostreatus.*

| Phyto extracts @ 10.0% Conc. | Yield (g/kg dry substrate) | | | Biological efficiency (%) | | |
|---|---|---|---|---|---|---|
| | July to Sep. 22 | Oct to Dec. 22 | Mean | July to Sep. 22 | Oct to Dec. 22 | Mean |
| *Azadirachta indica* ( neem) leaf extract | 857.5 | 912.5 | 885.0 | 85.8 | 91.3 | 88.5 |
| *Pongamia pinnata* (karanj) leaf extract | 842.5 | 892.5 | 867.5 | 84.3 | 89.3 | 86.8 |
| *Allium sativum* (garlic) cloves | 815.0 | 877.5 | 846.3 | 81.5 | 87.8 | 84.6 |
| *Allium cepa* (onion) bulbs | 742.5 | 842.5 | 792.5 | 74.3 | 84.3 | 79.3 |
| With pathogen and without toxicants (check-II) | 155.0 | 180.0 | 167.5 | 15.5 | 18.0 | 16.8 |
| Without pathogen and without plant extracts (check-I) | 732.5 | 827.5 | 780.0 | 73.3 | 82.8 | 78.0 |
| CD(0.05) | 51.9 | 45.2 | 48.6 | - | - | - |

with each other. Small sized fruiting body (7.5g/fruit) was recorded in Check-II (With pathogen and without toxicants).

**Yield and biological efficiency of *P. ostreatus***

It was revealed that there was a significant difference between the influences of the plant extracts on the total yield of *P. ostreatus* (Table 6). Maximum yield (885.0g/kg dry substrate) with 88.5% biological efficiency was recorded in neem leaf extract followed by karanj leaf extract (867.5 g/kg dry substrate) with 86.8 % biological efficiency and cloves of garlic (846.3 g/kg dry substrate) with 84.6 % biological efficiency which were statistically at par with each other. Minimum yield (167.5 g/kg dry substrate) with 16.8% biological efficiency was recorded in Check-II (With pathogen and without toxicants). Inam-ul-Haq *et al.* (2010) investigated that certain active components of *Eucalyptus camaldulensis*, *Azadirachta indica, Citrus lemon* and *Cymbopogon marginatus* were capable of increasing mushroom yield and controls pathogenic microbes in oyster mushroom cultivation which again supports the present investigation. It supports the present investigation that neem increases the yield and suppresses the infection by *S. rolfsii*. The 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).

## CONCLUSION

Four different plants parts *viz.* leaves of *Azadirachta indica* (neem), leaves of *Pongamia pinnata* (karanj), bulbs of *Allium cepa* (onion) and cloves of *Allium sativum* (garlic) were evaluated in the laboratory for their efficacy against both *P. ostreatus* mycelium and pathogen, *S.rolfsii*. The plant extracts which displaced least adverse effects on the growth of *P. ostreatus* and maximum inhibition of pathogen were evaluated to find out the yield performance of *P. ostreatus* under *in vivo* condition in mushroom house. Neem (*Azadirachta indica*) leaf extract showed maximum average mycelial and sclerotial inhibition *i.e.* 47.9% and 52.4%, respectively followed by karanj (*Pongamia pinnata* ) leaf extract *i.e.* 38.4% and 47.9%, respectively. Maximum yield of oyster mushroom (885.0 g/ kg dry substrate) was also recorded in neem leaf extract followed by karanj leaf extract (867.5 g/ kg dry substrate) and cloves of garlic (846.3 g/ kg dry substrate). Use of plant extracts can be effective, economical, long lasting and free from residual side effects.

**Conflict of interest**

All authors declared that there is no conflict of interest.

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