

## In Vitro Evaluation of the Performance of Different Plant Extracts Against the Growth of Mycoflora Associated with the Substrate of Oyster Mushroom

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**Abstract:** This study was conducted to know firstly about the prevalence of different mycoflora or weed molds associated with the substrate of oyster mushroom (*Pleurotus ostreatus*) and to evaluate the performance of different plant extracts against the isolated mycoflora. Ten weed molds including *Aspergillus flavus*, *Aspergillus niger*, *Penicillium italicum*, *Penicillium notatum*, *Rhizopus stolonifer*, *Absidia sp.*, *Fusarium sp.*, *Chaetomium globosum*, *Chaetomium elatum*, and *Trichoderma harzianum* were isolated and identified. From a number of 400 contaminated spawn packets Average 63.25% spawn packets were found contaminated with *T. harzianum* and remaining 36.75% spawn packets were found contaminated by other isolated mycoflora. An attempt was taken to determine antifungal potentials of different plant extracts viz. Onion (*Allium cepa*), Garlic (*Allium sativum*), Turmeric (*Curcuma longa*), Aloe vera (*Aloe Barbadensis*), Neem (*Azadirachta indica*), Lantana (*Lantana camara*), Tulsi (*Ocimum tenuiflorum*), Datura (*Datura stramonium*) at 5% concentration against isolated weed molds. Neem leaf extract at the concentration of 5% inhibits the percent radial growth of all mycoflora (ranging from 55.26% to 67.87%) radial growth inhibition which perform the best. In compatibility test of different plant extracts at 5% concentration with *P. ostreatus*, it is revealed that extracts of neem, lantana and datura were found to be compatible with *P. ostreatus*. Turmeric and onion were highly incompatible and significantly inferior.

**Keywords:** Mycoflora, Oyster mushroom, Plant extracts.

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### I. Introduction

Mushrooms belong to the kingdom of Fungi, a group very distinct from plants, animals and bacteria. Oyster mushroom (*Pleurotus sp.*) belonging to the Class Basidiomycetes and Family Agaricaceae [1]. Mushrooms of *Pleurotus sp.* are commonly known as oyster mushrooms which occupy the second most among cultivated edible mushrooms worldwide because of their nutritional and medicinal values [2]. The mushrooms are affected adversely by a huge number of biotic and abiotic factors. Weed molds have been considered as a major factor contributing to low productivity of mushroom in India particularly under seasonal cultivation [3]. The management of mycoflora associated with substrate of mushroom is very difficult because both the host and the parasites are fungi. Application of chemicals to mushroom substrate is practiced frequently worldwide. Many chemicals are using in the control of these pests are highly toxic hazardous to the environment, health of men [4]. The use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment [5]. In a study it is reported that, 38% farmers of Patuakhali district in Bangladesh feel vomiting and headache during pesticide application [6]. Constant use of fungi-toxic chemicals adds to the food chain poisoning, environmental pollution and increase the chance of resistance development. Consequently, efforts are under way to find out alternatives to chemical fungicides. The uses of plant extracts have opened a new avenue for the control of plant diseases. Besides, being safe and generally non-phytotoxic, the plant extracts are known to be effective against various plant pathogens [7]. Extracts of many plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trial. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and Danger to consumers in contrast to synthetic pesticides.[8] In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world . Plant products as antimicrobial agents[9]. The present study was carried out to identify the major mycoflora associated with the substrate of oyster mushroom and evaluate the inhibition efficacy of different plant extracts against associated mycoflora.

### II. Methodology

Widely cultivated species *Pleurotus ostreatus* of oyster mushroom was selected to conduct the present experiment. Commercial spawn packet of oyster mushroom (*Pleurotus ostreatus*) was collected from National Mushroom Development and Extension Centre, Sobhanbag, Savar, Dhaka, Bangladesh. Pure culture was grown

by taking tissues from the fruiting bodies and subsequently mother culture was prepared by using the pure culture. A number of 400 contaminated *Pleurotus ostreatus* (Oyster mushroom) growing substrate packets were collected from different mushroom farms at Savar Upazilla. Pure cultures of different associated mycoflora were isolated by inoculating a small portion of contaminated substrates to PDA media. Eight commonly available plants viz. Onion, Garlic, Turmeric, Aloe vera, Neem, Lantana, Tulsi and Datura were collected as inhibition agents and were prepared extracts for evaluating inhibition efficiency against mycoflora associated with the substrate of oyster mushroom. 5% concentration of different plant extracts viz. Onion, Garlic, Turmeric, Aloe vera, Neem, Lantana, Tulsi and Datura were tested *in vitro* to evaluate their effect on the radial growth of isolated fungi. Efficiency of plant extracts were evaluated on the basis of percent inhibition of the radial colony growth of the isolated fungi, and computed as a proportional measurement of colony diameter on control plate expressing in percentage, following the given formula.

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

Where, X= radial growth (mm) of control plates,

Y = radial growth (mm) of fungicide treated plates.

5% concentration of Onion, Garlic, Turmeric, Aloe vera, Neem, Lantana, Tulsi and Datura were used to determine their effect on growth of *P. ostreatus* following poison food technique.

The recorded data for each character from the experiment were analyzed statistically using MSTAT C 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).

### III. Result and Discussion

#### 3.1 Isolation and identification of associated weed molds

A total number of 10 fungi including *Aspergillus flavus*, *Aspergillus niger*, *Penicillium italicum*, *Penicillium notatum*, *Rhizopus stolonifer*, *Absidia* sp., *Fusarium* sp., *Chaetomium globosum*, *Chaetomium elatum* and *Trichoderma harzianum* were isolated and identified (Table 1). The highest 63.25% number of spawn packets were contaminated with *Trichoderma harzianum*. The similar findings also found by Sinden et al (1953). They reported that *Trichoderma* species with the respective compost has been known for a long time to limit commercial production [10].

**Table 1.** Prevalence of associated weed moulds on oyster mushroom spawn packet

| Associated mycoflora         | % Contaminated packets with weed molds |
|------------------------------|--|
| <i>Aspergillus flavus</i>    | 5.39                                   |
| <i>Aspergillus niger</i>     | 3.85                                   |
| <i>Penicillium italicum</i>  | 1.58                                   |
| <i>Penicillium notatum</i>   | 2.97                                   |
| <i>Rhizopus stolonifer</i>   | 7.95                                   |
| <i>Absidia</i> sp.           | 2.85                                   |
| <i>Fusarium</i> sp.          | 3.85                                   |
| <i>Chaetomium globosum</i>   | 5.82                                   |
| <i>Chaetomium elatum</i>     | 2.49                                   |
| <i>Trichoderma harzianum</i> | 63.25                                  |

Result of the laboratory evaluation of different plant extracts viz. 5% concentration of Onion, Garlic, Turmeric, Aloe vera, Neem, Lantana, Tulsi, Datura are presented in the Table 2. All the plant extract inhibited the weed molds. Among the plant extracts, Neem was superior in inhibiting the radial growth of all the tested 10 weed molds where inhibition occur 57.34% in *Aspergillus flavus*, 55.26% in *Aspergillus niger*, 62.54% in *Penicillium italicum*, 66.28 % in *Penicillium notatum*, 64.73 % in *Rhizopus stolonifer*, 66.38% in *Absidia* sp, 65.53% in *Fusarium* sp, 67.87 % in *Chaetomium globosum*, 66.33% in *Chaetomium elatum* and 65.33 % in *Trichoderma harzianum*.

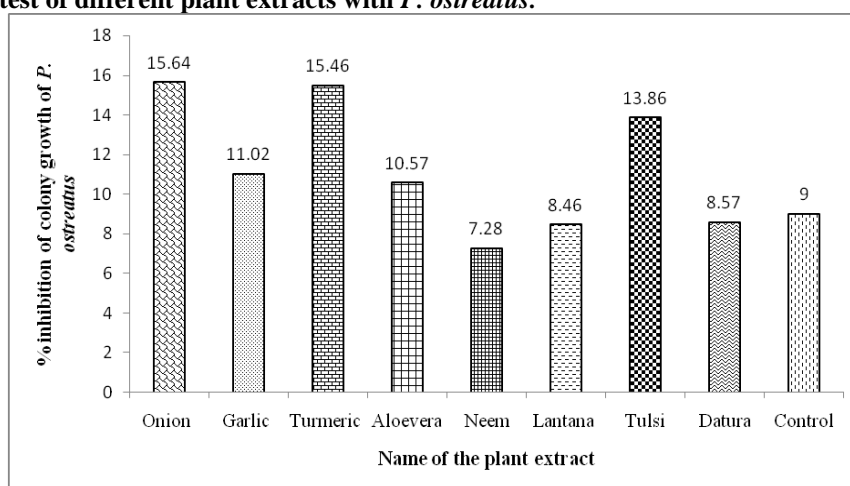
The percent inhibition produced by *Azadiracta indica* against *Trichoderma* was recorded 34.1% in vitro [11]. *Azadiracta indica* (neem) showed its supremacy and exhibited maximum inhibitory effect ranging 54.1 % to 71.6 % Against *Aspergillus* sp, *Trichoderma* sp., *Coprinus* sp., and *Penicillium* sp. and was found to be less effective against *Sclerotium rolfsii* [12] Lantana also showed good performance in inhibiting the radial growth .Onion and turmeric showed poor performance in inhibiting radial growth of all weed molds. However, the results of the present study revealed that 5% neem inhibit the radial growth of all weed molds which perform the best.

**Table 2.** Evaluation of different plant extracts for inhibiting weed molds in vitro condition

| Plant extracts (5%) | % inhibition     |                 |                    |                   |                      |                    |                     |                    |                  |                     |
|---------------------|------------------|-----------------|--------------------|-------------------|----------------------|--------------------|---------------------|--------------------|------------------|---------------------|
|                     | <i>A. flavus</i> | <i>A. Niger</i> | <i>P. italicum</i> | <i>P. notatum</i> | <i>R. stolonifer</i> | <i>Absidia</i> sp. | <i>Fusarium</i> sp. | <i>C. globosum</i> | <i>C. elatum</i> | <i>T. harzianum</i> |
| Onion               | 24.46 f          | 27.54 e         | 30.23 f            | 28.48 f           | 28.59 e              | 30.64 e            | 24.26 f             | 26.58 f            | 26.26 f          | 29.41 f             |
| Garlic              | 45.24 c          | 44.64 e         | 47.34 c            | 51.16 c           | 53.57 b              | 47.28 e            | 50.65 c             | 51.26 e            | 46.58 c          | 49.59 c             |
| Turmeric            | 34.35 e          | 26.84 e         | 33.65 e            | 32.54 e           | 35.26 d              | 32.26 f            | 35.45 e             | 27.86 f            | 32.56 e          | 34.41 e             |
| Aloe vera           | 46.34 c          | 48.35 c         | 53.24 b            | 48.36 cd          | 45.85 c              | 52.94 b            | 51.24 c             | 44.67 d            | 51.21 c          | 50.74 c             |
| Neem                | 57.34 a          | 55.26 ab        | 62.54 a            | 66.28 a           | 64.73 a              | 66.38 a            | 65.53 a             | 67.87 a            | 66.33 a          | 65.33 a             |
| Lantana             | 56.33 a          | 58.45 a         | 61.52 a            | 62.39 b           | 54.41 b              | 52.38 b            | 57.65 b             | 58.72 b            | 55.53 b          | 56.74 b             |
| Tulsi               | 41.52 d          | 38.25 d         | 43.33 d            | 45.24 d           | 36.35 d              | 42.65 d            | 46.54 d             | 36.27 e            | 38.52 d          | 43.33 d             |
| Datura              | 50.26 b          | 52.65 b         | 54.86 b            | 48.68 cd          | 47.46 c              | 53.43 b            | 47.34 d             | 51.58 c            | 54.68 b          | 57.15 b             |
| Control             | 0                | 0               | 0                  | 0                 | 0                    | 0                  | 0                   | 0                  | 0                | 0                   |

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

**Compatibility test of different plant extracts with *P. ostreatus*:**

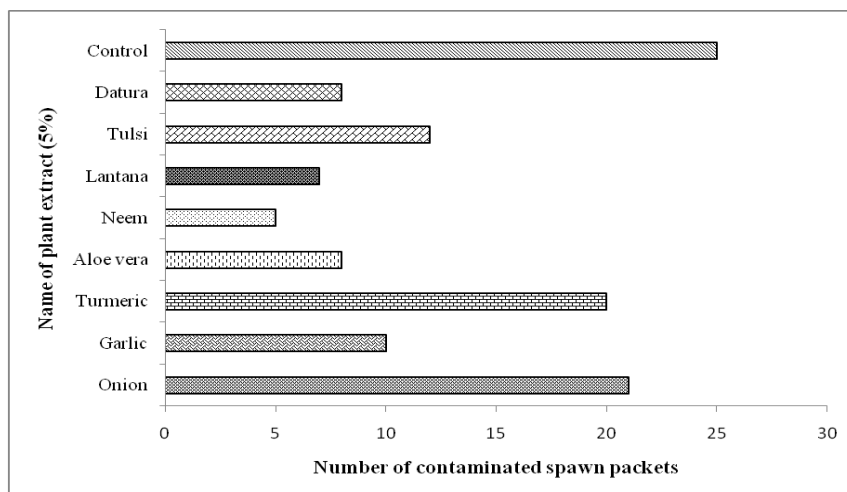


**Figure 4.1** Compatibility test of different plant extracts with *P. ostreatus*

Among all plant extracts only 7.28% colony growth inhibition of *P. ostreatus* were obtained by neem followed by 8.46% and 8.57% inhibition of colony growth in case of lantana and datura. Neem, lantana and datura were found to be compatible with *P. ostreatus* and turmeric and onion were highly incompatible and significantly inferior.

**Effect of different plant extracts in contaminating on spawn packets**

In this experiment different number of contaminated spawn packets were found as the effect of different treatments on spawn packets. The highest percent contaminated spawn packets (25.0%) were found in control treatment. The minimum number of contamination (5.0%) was found in neem treated spawn packets which were followed by 7.0%, 8.0% and 10.0% in case of lantana, aloe vera and garlic, respectively.



**Figure 4.2:** Effect of different plant extracts on contaminated spawn packets

#### IV. Conclusion

Currently, the use of inhibitory botanicals is one of the most possible methods for controlling some plant pathogens. A total number of 10 weed molds namely, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium italicum*, *Penicillium notatum*, *Rhizopus stolonifer*, *Absidia* sp., *Fusarium* sp., *Chaetomium globosum*, *Chaetomium elatum*, and *Trichoderma harzianum* were isolated, identified and found to infect surveyed Oyster mushroom substrate. This study found most promising botanic Neem (*Azadirachta indica*), at 5% neem was found highly effective in inhibiting significantly the growth of all the associated weed molds. For Neem extract contamination spawn packets is only 5%. On the other hand, 5% neem extract is also compatible for colony growth of *Pleurotus ostreatus*.

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