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Microbial contamination on oyster mushroom after harvest and their management using some essential oils in Azamgarh (U.P.) India

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ABSTRACT

Mushrooms are more vulnerable as their self life is too short and are consumed fresh. The main study of this work was isolation and control of fungal contaminants in mushrooms during and after harvest storage. Samples of *Pleurotus ostreatus* and *Pleurotus florida* were collected from three vegetable markets of Azamgarh city which revealed presence of 21 fungi. Results exhibited *Aspergilus niger* and *Rhizopus sp.* as most abundant contaminants which were treated with *Zanthoxylum armatum* and *Eucalyptus citrodora* using poisoned food technique. The control combination was potato dextrose agar with no oils added. All the essential oils significantly inhibit ($p>0.05$) the growth and spore germination of both test fungi. A strong inhibitory action of Cinnamon oil and Mentha oil was recorded against *Aspergilus niger* and *Rhizopus* sp respectively at a concentration of 20 ml/ml. This clearly suggests that essential oil could be an alternative to the synthetic chemicals that are currently used to control fungal contamination in mushroom and extend their shelf life.

Figure : 00 References : 26 Table : 01
KEY WORDS : Essential oils, Microbial contamination, Oyster mushroom, Shelf life.

Introduction

Oyster mushroom (*Pleurotus spp.*) belonging to class Basidiomycetes and family Agaricaceae is popularly known as Khumbhi or Khukhari in India. It is economically important and widely cultivated especially in East Asia[12]. *Pleurotus ostreatus* is the second largest next to *Agaricus bisporus* in the World market. It is most popular in India due to its ease of cultivation[14], high yield potential and high nutritional value[16]. Oysters also have medicinal properties such as antioxidant, antimicrobial, immunomodulating and many other therapeutic potentials[5].

After harvest loss (AHL) refers to the measurable quantitative and qualitative degradation of food in after harvest system[6]. Quantitative loss is of more concern than qualitative loss in developing countries[9]. The condition of postharvest losses in perishable crops is worse in the less developing countries[8]. As the mushroom is heterotrophic and most perishable in nature, similar instances are prevalent.

Many serious after harvest diseases occur rapidly and cause extensive breakdown of food. It is estimated that 36% of the vegetable decay is caused by soft rot bacteria[19]. Contamination of various mould fungi occur during the growth and postharvest stages of mushroom which adversely affect the mushroom yield and its shelf life[4,20]. Studies on various aspects of fungal contaminants and diseases of *Pleurotus spp*. were undertaken by

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different workers[22,23] and they reported *Trichoderma* spp., *Aspergillus* spp., *Penicillium* spp., *Monilia* sp., *Fusarium* spp., *Rhisopus* sp., *Mucor* sp. *etc.* All the studies were focused in the cultivation of mushroom. No literature was found regarding the after harvest microbial contamination.

The use of synthetic fungicides for reducing such contamination and loss in mushroom is very common in Nepal[16]. The hazardous effects of chemicals in human health and environmental aspect are known[20]. Apart from these problems continuous usage of same chemicals may lead towards pest's resistance.

Essential oils are non-water based phytochemicals made up of volatile aromatic compounds[11]. Essential oil bearing plants constitute a rich source of bioactive chemicals, which have been well reported to have fungicidal property against a wide range of fungi[16]. These chemicals are also biodegradable and non-toxic[1]. Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease-control agents[24]. Considering the above, an attempt was made to develop a suitable management practice against the microbial contamination in oyster mushroom after harvest in an eco-friendly atmosphere.

Materials and Methods

(a) Isolation and identification of microbial contaminants : Samples of *Pleurotus ostreatus* and *Pleurotus florida* were collected from three vegetable markets of Azamgarh (U.P.) India. The surface of samples were cut into 3 mm sized pieces and plated into PDA plates. A week later, from the numerous colony of fungi each were isolated and identified using standard literature[25]. Most frequent two fungi were used as the test fungi for assessment of fungi toxic effects of essential oils.

(b) Extraction of Essential Oils : Leaves of *Cinnamomum tamala* (Dalchini), *Mentha spicata* (Pudina), *Eucalyptus citriodora* (Neelgiri or Safeda) and *Zanthoxylum armatum* (Tejbal or Tejpat) were collected from garden of Chandeshwar, Kuba and Kaptanganj (Azamgarh). They were air-dried and stored at room temperature in darkness until distillation. The air-dried materials were subjected to hydro distillation for 6-8 hours using Clevenger's apparatus. The essential oils were collected, dehydrated using anhydrous sodium sulphate (Na_2SO_4) and stored at temperature >10^0C until use and analysis.

(c) Assessment of fungi toxicity : The fungi toxicity of the essential oils were assessed by poisoned food technique[7] for the mycelia growth. Oils were diluted into different concentrations of 1.25, 2.5, 5, 10 and 20 ml/ml with 50% Acetone[17]. At first, 1 ml of each concentration of essential oil was poured into sterilized petriplates followed by addition of 9 ml of melted PDA. All petriplates were then inoculated by a 4 mm diameter of the actively growing test fungus. In control sets, distilled water and 50% Acetone were used instead of essential oil. Observations were recorded on 7th day. Five replications were maintained and fungi toxicity was measured in terms of percent inhibition of mycelia growth calculated as;

$$\text{Inhibition of mycelial growth (\%)} = \left(\frac{gc - gt}{gc}\right) \times 100$$

Where; gc = mean colony diameter in control sets, and gt = mean colony diameter in treatment sets.

Hanging drop method was used to test the effect replicates maintained and observed after 24 hrs. of incubation. The percentages of spore germination was calculated as[10].

$$\text{Spore germination (\%)} = \left(\frac{Sg}{St}\right) \times 100$$

Where; Sg = number of spore germinated per microscopic field, and St = number of spore per microscopic field.

Results and Discussion

(a) Microbial contamination in after harvest Oyster mushroom : Altogether 21 fungal contaminants were isolated among which 19 were identified but two were not (Table-1) *Aspergillus niger* and *Rhizopus sp.* were the most frequent.

(b) Antifungal bioassay of essential oils : The results show that all four essential oils have significant antifungal effect ($p<0.05$) over mycelia growth of both test fungi. *Cinnamomum tamala* oil has best effects over *Aspergillus niger* than *Rhizopus sp.* As, the mycelia growth of *Aspergillus niger* was (0.7 ± 0.04 cm) at 20 ml/ml concentration. Meanwhile, *Mentha spicata* oil has better effects over *Rhizopus sp.* (0.85 ± 0.05 cm) than *Aspergillus niger* (0.93 ± 0.00 cm) at concentration of 20 ml/ml. In all concentrations of cinnamon oil better effect was found in *Aspergillus niger* than *Rhizopus* sp.

Eucalyptus citriodora oil has greater effects over *Aspergillus niger* than *Rhizopus*. Similarly, *Zanthoxylum armatum* oil has better effects over *Aspergillus niger* than *Rhizopus* sp. The mycelia growth of *Aspergillus niger* was (1.09 ± 0.09 cm) whereas (1.13 ± 0.15 cm) in *Rhizopus* sp at 20 ml/ml concentration. But among all essential oils, *Zanthoxylum armatum* oil has the less effect on the mycelia growth of both fungal contaminants. Except the *Mentha spicata* essential oils of all plants have greater effect on the *Aspergillus niger* than *Rhizopus* sp. Regarding the effect of essential oils on spore germination

TABLE-1 : Occurrence of fungal contaminants in Oyster Mushroom

S. No.	Fungal contaminants	Mushrooms type
1.	*Alternaria alternate*	PO
2.	*Aspergillus brevipes*	PO and PF
3.	*Aspergillus clavatus*	PF
4.	*Aspergillus flavus*	PO and PF
5.	*Aspergillus fumigates*	PO and PF
6.	*Aspergillus niger*	PO and PF
7.	*Aspergillus* sp.	PF
8.	*Aspergillus versicolor*	PO and PF
9.	*Chaetomium funicola*	PO
10.	*Chaetomium* sp.	PO
11.	*Chaetomium spirale*	PO and PF
12.	*Fusarium oxysporum*	PO and PF
13.	*Gliocladium* sp.	PO and PF
14.	*Gliotrichum* sp.	PF
15.	*Mucor* sp.	PO and PF
16.	*Penicillium* sp.	PO and PF
17.	*Rhizopus* sp.	PO and PF
18.	*Trichoderma harzianum*	PO
19.	*Trichoderma viride*	PO and PF
20.	Unidentified Species 1	PF
21.	Unidentified Species 2	PF

Note : PO = *Pleurotus ostreatus*, PF = *Pleurotus florida*

selected fungal contaminants. Similar result was observed as in the effect on mycelia growth at 20 ml/ml of oil concentration but in other concentrations it was different. *Cinnamomum tamala* showed best inhibition effect whereas *Mentha spicata* showed least antifungal effect in controlling *Aspergillus niger* among all four oils. It contains eugenol, cinamaldehyde, cinnamyl alcohol, cinnamylacetate and cinnamic acid and many other responsible for the observed antifungal properties[14]. At 20 ml/ml oil concentration, *Cinnamomum tamala* showed highest inhibition (79.30%) followed by *Zanthoxylum armatum* (76.94%), *Eucalyptus citriodora* (76.60%) and *Mentha spicata* (76.56%), respectively. But in case of the spore germination of *Rhizopus* sp, *Cinnamomum tamala* showed highest inhibition (84.48%) followed by *Zanthoxylum armatum* (78.80%), *Cinnamomum camphora* (83.35%), *Eucalyptus citriodora* (71.02%) and *Cinnamomum tamala* (69.80%) at 20 ml/ml oil concentration respectively.

These results more or less are supported by various researches[2,16,26]. The difference in fungi toxicity at same concentration in different essential oils may be due to different chemical composition of the oils[21].

Conclusion

This study concludes that various microbial contaminants are responsible for the after harvest decay of oyster mushroom. Four different essential oils extracted from four different plants can be promising in management of such after harvest degradation of mushroom especially in controlling two moulds fungi namely *Aspergillus niger* and *Rhizopus* sp. The oil of *Cinnamomum tamala* and *Mentha spicata* showed the most effective antifungal activity against *Aspergillus niger* and *Rhizopus* sp respectively. The results suggest their possible use as an alternative inputs of synthetic compounds. Further studies on *in-vivo* suitability of such phyto-fungicides are needed.

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