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Postharvest Microbial Contamination in Oyster Mushroom and their Management using Plant Essential Oils

Hari S. Adhikari and Sanjay Kumar Jha
Central Department of Botany, Tribhuvan University, Kathmandu, Nepal

(Corresponding author: Sanjay Kumar Jha*)*
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ABSTRACT: Micro-contamination is one of the major causes for the postharvest loss which affects the quality and restrict the shelf life of horticultural fresh produce during the supply chain. Mushrooms are more vulnerable as their shelf life is too short and are consumed fresh. The main target of this work is to isolation and control of fungal contaminants in mushrooms during the postharvest storage. Samples of *Pleurotus ostreatus* and *Pleurotus florida* were collected from three major vegetable market of Kathmandu city which revealed presence of 21 fungi. Results exhibited *Aspergillus niger* and *Rhizopus* sp as most abundant contaminants which were treated with different concentrations of essential oils of *Cinnamomum tamala, Mentha spicata, Zanthoxylum armatum* and *Eucalyptus citriodora* using poisoned food technique. The control combination was potato dextrose agar with no oils added. All the EOs were found 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 *A. niger* and *Rhizopus* sp respectively at a concentration of 20 µl/ml. This clearly suggests that EOs could be an alternative to the synthetic chemicals that are currently used to control fungal contamination in mushroom and extend their shelf life.

Key words: Microbial contamination, Postharvest, EOs, Oyster mushroom, shelf life

INTRODUCTION

Postharvest loss (PHL) refers to the measurable quantitative and qualitative degradation of food in postharvest system (de Lucia and Assennato, 1994). Quantitative loss is more concern than qualitative loss in developing countries (Humble and Reneby, 2015). According to the FAO (2015) global food loss of perishable crops is 40-50%. The condition of postharvest losses in perishable crops is worse in the less developing countries (Hodges *et al.,* 2011). As the mushroom is heterotrophic and most perishable in nature, similar instances are prevalent.

Oyster mushroom (*Pleurotus* spp.) belonging to class Basidiomycetes and family Agaricaceae is popularly known as 'Kanye chyau' in Nepal. It is economically important and widely cultivated especially in East Asia (Mandeel *et al.,* 2005). *Pleurotus ostreatus* is the second largest next to *Agaricus bisporus* in the world market (Sanchez, 2010). It is most popular in Nepal due to its ease of cultivation (Parajuli, 2014), high yield potential and high nutritional value (Raut, 2013). Oysters also have medicinal properties such as antioxidant, antimicrobial, immunomodulating and many other therapeutic potentials (Cimerman, 1999).

Many serious postharvest diseases occur rapidly and cause extensive breakdown of food. It is estimated that 36% of the vegetable decay is caused by soft rot bacteria (Sahu and Bala, 2017). Contamination of various mould fungi occur during the growth and postharvest stages of mushroom which adversely affect the mushroom yield and its

shelflife (Sharma *et al.,* 2009; Biswas, 2014). Studies on various aspects of fungal contaminants and diseases of *Pleurotus* spp. were undertaken by different workers (Rai *et al*., 1993; Lopez-arevalo *et al.,* 1996; Thakur *et al.,* 2001, Suada *et al*., 2015) and they reported *Trichoderma* spp, *Aspergillus* spp., *Penicillium* spp., *Monilia* sp, *Fusarium* spp, *Rhizopus* sp, *Mucor* sp etc. were the major contaminants of *Pleurotus* spp. All the studies were focused in the cultivation of mushroom no literature was found regarding the postharvest microbial contamination.

The use of synthetic fungicides for reducing such contamination and loss in mushroom is very common in Nepal (Raut, 2013). The hazardous effects of chemicals in human health and environmental aspect are known (Palmer *et al.,* 2013). Apart from these problems continuous usage of same chemicals may leads towards pest's resistance.

Essential oils are non-water based phytochemicals made up of volatile aromatic compounds (Lawless, 2013). 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 (Prakash *et al.,* 2012). These chemicals are also biodegradable and non-toxic (Adebayo *et al*. 2013). 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 (Tripathi *et al*. 2008). 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.

MATERIALS AND METHODS

A. Isolation and Identification of Microbial contaminants

Samples of *Pleurotus ostreatus* and *Pleurotus florida* were collected from three major vegetable market of Kathmandu city, Nepal. The Surface of sample were cut into 3mm sized pieces and plated into PDA plates. A week later, from the numerous colony of fungi each were isolated and identified using standard literature (Barnett and Hunter, 2003; Watanabe, 2010). Most frequent two fungi were used as the test fungi for assessment of fungi toxic effect of essential oils.

B. Extraction of Essential Oils

Leaves of *Cinnamomum tamala*, *Mentha spicata, Eucalyptus citriodora* and *Zanthoxylum armatum* were collected from garden of CDB, TU and Kirtipur. 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 >10ºC until use and analysis.

C. Assessment of fungi toxicity

The fungi toxicity of the essential oils were assessed by poisoned food technique (Grover & Moore 1962) for the mycelial growth. Oils were diluted into different concentrations of 1.25, 2.5, 5, 10 and 20µl/ml with 50 % Acetone (Rao & Srivastava, 1994). At first, 1ml of each concentration of essential oil was poured into sterilized petriplates followed by addition of 9 ml of melted PDA. Each 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 mycelial growth calculated as;

Inhibition of MG (%) = [(gc–gt)/ gc] × 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 of EOs on spore germination of test fungi. Five replicates were maintained and observed after 24 hrs of incubation. The percentages of spore germination was calculated as (Kiraly *et al.,* 1974);
Spore germination (%) = (Sg/St) × 100

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

D. Data analysis

MS Excel 2016 was used for entering data, drawing charts and required graphs. The data were analysed with the help of ANOVA followed by Post-Hoc; Tukey HSD[a] test at (p<0.05) using Software statistical package for social science (SPSS) version 23.

RESULTS AND DISCUSSION

A. Postharvest microbial contamination in 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 (Fig. 1).

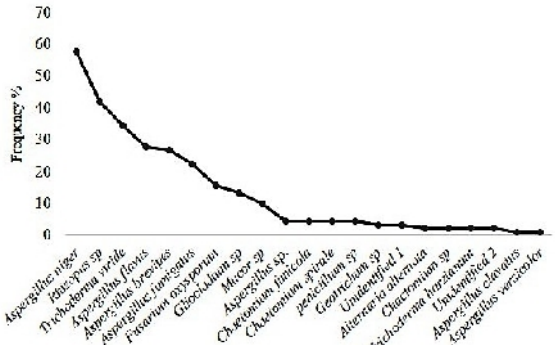

Fig. 1. Frequency rank curve of fungal contaminants in oyster mushroom.

Table 1: Occurrence of fungal contaminants in Oyster Mushroom.

S. No.	Fungal contaminants	Mushroom type
1.	*Mucor* sp	PO and PF
2.	*Rhizopus* sp	PO and PF
3.	*Chaetomium spirale* Zopf.	PO and PF
4.	*Chaetomium funicola* Cooke	PO
5.	*Chaetomium* sp	PO
6.	*Alternaria alternate* (Fr.) Keissl.	PO
7.	*Asperigillus fumigates* Fresen.	PO and PF
8	*Aspergillus niger* van Teigh.	PO and PF
9.	*Aspergillus flavus* Link	PO and PF
10.	*Aspergillus clavatus* Desm.	PF
11.	*Aspergillus brevipes* Smith	PO and PF
12.	*Aspergillus versicolor* (Vuillemin) Tiraboschi	PO and PF
13.	*Aspergillus* sp	PF
14.	*Fusarium oxysporum* Schltdl.	PO and PF
15.	*Gliocladium* sp	PO and PF
16.	*Giotrichum* sp	PF
17.	*Penicillium* sp	PO and PF
18.	*Trichoderma harzianum* Rifai.	PO
19.	*Trichoderma viride* Pers.	PO and PF
20.	Unidentified Species 1	PF
21.	Unidentified Species 2	PF

((Note: PO: *Pleurotus ostreatus* PF: *Pleurotus florida*)

B. Antifungal bioassay of essential oils

The results shows that all four essential oils has significant antifungal effect (p<0.05) over mycelial growth of both test fungi. *Cinnamomum tamala* oil has best effects over *Aspergillus niger* than *Rhizopus* sp. As, the mycelial growth of *A. niger* was (0.7±0.04cm) at 20 µl/ml concentration (Fig. 2A). Meanwhile, *Mentha spicata* oil has better effects over *Rhizopus* sp (0.85±0.05cm) than *A. niger* (0.93±0.00cm) at concentration of 20µl/ml.

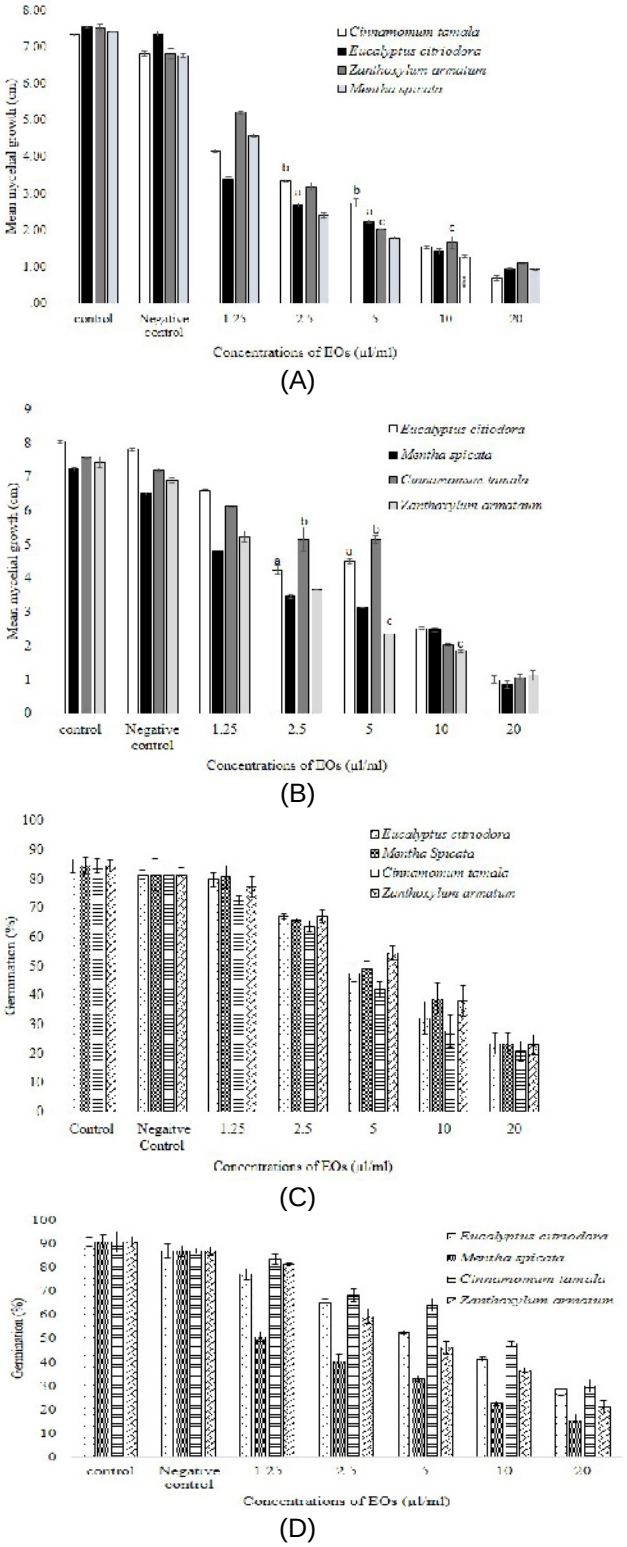

Fig. 2. Fungi toxic effect of different EOs against the mycelial growth (A and B) and spore germination (C and D) of *A. niger* (A and C) and *Rhizopus* sp (B and D).

In all concentration of cinnamon oil better effect was found in *A. niger* than *Rhizopus* sp. (Fig. 2: A and B).

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 mycelial growth of *Aspergillus niger* was (1.09±0.09cm) whereas (1.13±0.15cm) in *Rhizopus* sp at 20µl/ml concentration (Fig. 2 A and B). But among all EOs, *Zanthoxylum armatum* oil has the less effect on the mycelialgroth of both fungal contaminants. Except the *Mentha spicata* EOs of all plants have greater effect on the *A. niger* than *Rhizopus* sp.

Regarding the effect of EOs on spore germination selected fungal contaminants, similar result was observed as in the effect on mycelial growth at 20 µl/ml of oil concentration but in other concentration it was different. *Cinnamomum tamala* showed best inhibition effect whereas *Mentha spicata* showed least antifungal effect in controlling *Aspergillus niger* among all four oils (Fig. 2C). It contains eugenol, cinnamaldehyde, cinnamyl alcohol, cinnamylacetate and cinnamic acid and many other responsible for the observed antifungal properties (Pandey *et al*., 2012). At 20µl/ml oil concentration, *C. 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, *C. tamala* showed highest inhibition (84.48%) followed by *Zanthoxylum armatum* (78.80%), *C. camphora* (83.35%), *Eucalyptus citriodora* (71.02%) and *Cinnamomum tamala* (69.80%) at 20µl/ml oil concentration respectively.

These results more or less supported by various research (Prakash *et al.,* 2012; Barbosa et al., 2016; Zaidi and Dahiya, 2015). The difference in fungi toxicity at same concentration in different essential oils may be due to different chemical composition of the oils (Singh *et al*.1983).

CONCLUSION

This study concludes that various microbial contaminants are responsible for the postharvest decay of oyster mushroom. Four different EOs extracted from Four different plants can be promising in management of such postharvest 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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