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Effect of different essential oils on enzymatic activity of oyster mushroom (*Pleurotus florida*)

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An experiment was carried out to study the effect of different essential oils on enzymatic activity of stored oyster mushroom (*Pleurotus florida*). The harvested fruiting body was treated with four essential oils, i.e. lemongrass oil, citronella oil, mint oil and clove oil at two different concentrations – 5 and 10 µl – to test the total phenol content (TPC) and activity of three important enzymes, viz. phenylalanine ammonia lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) that are involved in post-harvest quality preservation of mushrooms. TPC (0.286 mg/g), PAL content (0.038 µM/g), PPO content (0.042 U/mg) and POD content (0.38 U/mg) were found significant in mint oil-treated mushroom at 10 µl concentration. TPC and PAL content were higher in essential oil-treated mushrooms compared to the control samples, whereas PPO and POD contents were lower in the treated samples, signifying that essential oils treatment had a positive impact on the quality of harvested mushrooms. This preservative technique will help in increasing the shelf-life of harvested fruiting bodies.

Keywords: Enzymes, essential oils, fruiting bodies, *Pleurotus florida*, preservation.

POST-harvest quality is a major concern among mushroom-growers. Mushrooms are a highly perishable commodity that are not suitable for long-term storage and long-distance transportation[1]. Several methods have been developed to increase the post-harvest shelf-life of mushrooms, but only a few have achieved success. Use of essential oils in the storage of mushrooms is a new concept, but has shown positive results in improving quality attributes of harvested fruiting bodies.

The most important quality parameter for assessing the marketability of mushrooms is the colour of the fruiting body which is degraded upon storage due to activity of enzymes such as polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), peroxidase (POD), superoxide dismutase (SOD) and secondary metabolites like phenols and ascorbic acid. The use of essential oils in the preservation of mushrooms is a new concept which is gaining appreciation because of its easy application and negligible side effects. Essential oils are natural volatiles obtained by distillation and have the characteristic aroma of the plant from which they are obtained[2]. Essential oils act on the biochemical processes of mushrooms, and suppress or enhance the concentration of enzymes and secondary metabolites which are involved in quality preservation[3].

Fumigation of the fruiting bodies of mushroom (*Agaricus bisporus*) with three essential oils (clove, cinnamaldehyde and thyme) recorded changes in browning index, weight loss, firmness, percentage of open caps, total phenolics, ascorbic acid, microbial activity and activity of important enzymes such as PPO, PAL and POD. All essential oils inhibited the post-harvest degradation of mushrooms, of which cinnamaldehyde oil (5 µl) was found to be the most efficient[4]. Different concentrations of essential oils of cinnamon, mint, winged prickly ash and eucalyptus improved the post-harvest quality of oyster mushroom (*Pleurotus ostreatus* and *Pleurotus florida*). Cinnamon and mint oil (20 µl) were found to be the most effective against post-harvest microbial losses[5]. Essential oils

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improve the post-harvest quality of freshly cut mushrooms. Mushroom samples were treated with essential oils and stored at 4°C for 5 days. It was observed that maximum inhibition of PPO of 64.50% was obtained by *Origanum vulgare* L. subsp. *hirtum* on the third day of storage[6].

Fruiting bodies of *P. florida* were used for analysis of enzymatic activity upon treatment with essential oils. The freshly harvested mushrooms were kept in clean polythene bags and stored at 5°C for 48 h. Four essential oils, viz. lemongrass, citronella, mint and clove purchased from the market were used for treatment of harvested mushrooms.

The mushrooms were removed after 48 h of storage and visually assessed to confirm uniform size of fruiting bodies and absence of mechanical damage. Next 5 g of tissue was weighed and kept in sterilized polypropylene containers. Filter-paper cutting of the size of the container cap was fitted on the inside portion of cap. Two concentrations of essential oil, i.e. 5 and 10 µl were obtained with the help of micropipette and spotted on to the filter paper. After treatment, the samples were stored at 5°C for 10 days. Upon completion of storage period, the fruiting bodies were transferred into plastic bags and stored at –60°C for 24 h to arrest all enzymatic activities.

The harvested oyster mushrooms were treated with four essential oils at two different concentrations to test the total phenol content (TPC) and activity of three important enzymes, viz. PAL, POD and PPO that are involved in post-harvest quality preservation of mushrooms.

TPC was estimated by taking 2 g of mushroom sample that was blended with 10 ml of 80% methanol and left for 24 h at 5°C. The next day, the mixture was centrifuged at 10,000 rpm and 4°C for 15 min. Then 1 ml of supernatant was taken in a test tube into which 1 ml of folin reagent and 2 ml of Na_2CO_3 were added. The enzyme solution was tapped with 10 ml of distilled water and vortexed. Readings were taken after 15 min at 765 nm and absorbance was recorded. TPC was expressed as gallic acid equivalent in mg/g of fr. wt (ref. 7).

For estimation of POD activity, 0.1 g of mushroom sample was homogenized with 2 ml of potassium phosphate buffer and centrifuged at 16,000 rpm and 4°C for 15 min. After centrifugation, 0.05 ml of supernatant was mixed with 1.5 ml guaicol. Then 0.5 ml of H_2O_2 was added to the mixture and absorbance was recorded immediately after colour change (orange colour) at 420 nm. The readings were taken at 30 sec intervals for 3 min. The specific activity was expressed as U/min/mg fr. wt (ref. 8).

PPO analysis was done by taking 4 g of mushroom sample that was homogenized with 12 ml of potassium phosphate buffer and centrifuged at 16,000 rpm and 4°C for 15 min. Next, 0.5 ml of enzyme extract was incubated with 2.5 ml of buffered mixture which contains 0.1 M of sodium phosphate (pH 6.4) and 0.05 M catechol. The absorbance was recorded at 398 nm for 3 min at an interval of 30 sec. The activity of PPO was expressed as U/g protein fr. wt (ref. 9).

For analysis of PAL activity, 0.1 g of mushroom sample was homogenized with 2 ml of sodium phosphate buffer and centrifuged at 16,000 rpm and 4°C for 15 min. The reaction mixture consisted of 0.2 ml supernatant, 0.5 ml borate buffer, 1.3 ml distilled water and 1 ml phenylalanine. This mixture was incubated at room temperature (25°–30°C) for 30 min. The reaction was terminated with 0.5 ml of trichloro acetic acid (TCA). The absorbance was recorded at 240 nm. The activity of the enzyme was expressed in terms of trans cinnamic acid/g fr. wt (ref. 10).

TPC of treated mushrooms was higher than the untreated ones. Among the four treatments, i.e. lemongrass oil, citronella oil, mint oil and clove oil, mint oil showed maximum accumulation of phenolic compounds which was 0.250 and 0.286 mg/g of gallic acid equivalent at 5 and 10 µl concentration respectively. TPC in mushrooms treated with clove oil was 0.232 and 0.269 mg/g at 5 and 10 µl respectively. This was followed by lemongrass oil in which TPC was 0.173 mg/g at 5 µl and 0.226 mg/g at 10 µl. The lowest TPC was observed in citronella oil-treated mushrooms, which was 0.168 and 0.189 mg/g of gallic acid equivalent at 5 and 10 µl respectively.

It was also observed that the concentration of phenol compounds was higher in mushrooms treated with 10 µl of essential oil compared to those treated with 5 µl of the same. Figure 1 shows the effect of the essential oils on TPC of stored mushroom.

PAL content was higher in essential oils-treated mushrooms compared to control treatment, because more phenols were synthesized by PAL enzyme under stress. At 5 µl concentration of essential oils, the highest PAL content was recorded in mint oil-treated oyster mushroom (0.038 µM/g) followed by clove oil (0.033 µM/g) and lemongrass oil (0.018 µM/g), while the lowest PAL content was observed in the treatment with citronella oil

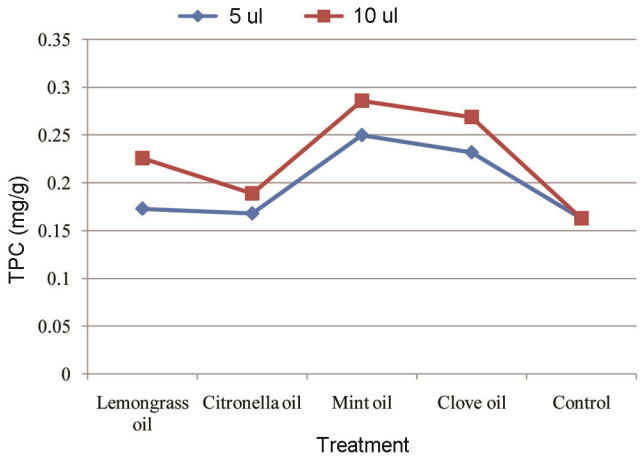

Figure 1. Effect of essential oils on total phenol content (TPC) of stored mushroom.

(0.014 µM/g). Moreover, at 10 µl concentration of essential oils, maximum PAL content was observed in the treatment with mint oil (0.042 µM/g) followed by clove oil (0.035 µM/g) and lemongrass oil (0.029 µM/g), whereas minimum PAL content was found in citronella oil (0.025 µM/g) (Figure 2).

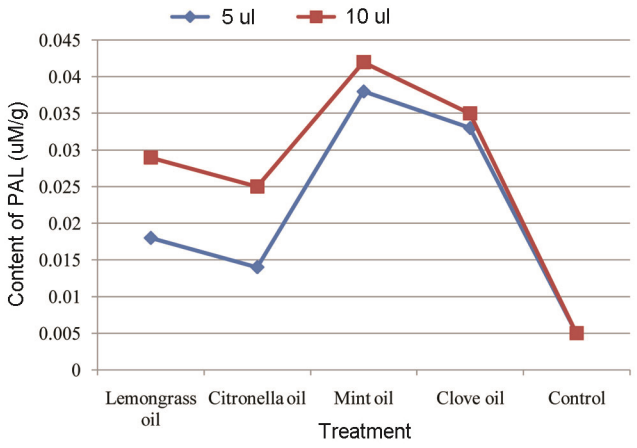

Figure 2. Effect of essential oils on phenylalanine ammonia lyase (PAL) content of stored mushroom.

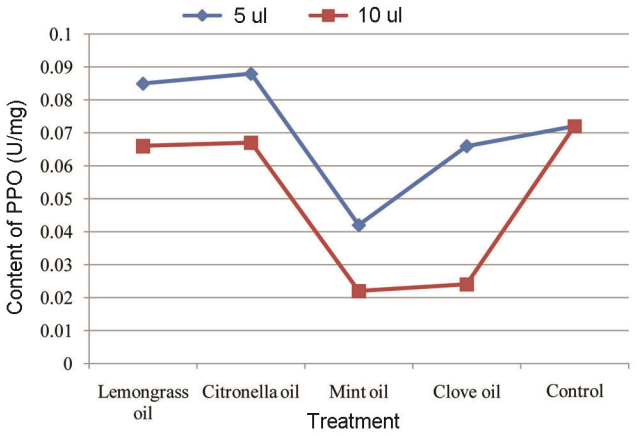

Figure 3. Effect of essential oils on polyphenol oxidase (PPO) content of stored mushroom.

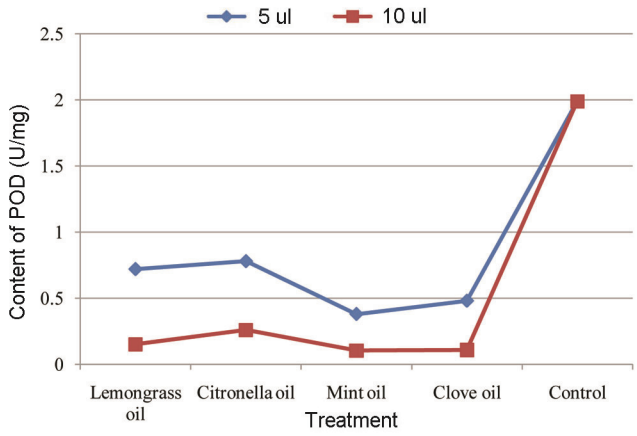

Figure 4. Effect of essential oils on peroxidase (POD) content of stored mushroom.

The PPO content was lower in essential oils-treated mushrooms compared to the untreated samples. At 5 µl concentration of essential oils, the PPO content was lowest in mint oil-treated oyster mushrooms (0.042 U/mg) followed by clove oil (0.066 U/mg), while PPO content was highest in samples treated with citronella oil (0.088 U/mg). The PPO content in lemongrass oil-treated mushrooms was 0.085 U/mg. Similarly, at 10 µl concentration of essential oils, minimum PPO content was observed in the treatment with mint oil (0.022 U/mg) followed by clove oil (0.024 µM/g) and lemongrass oil (0.066 U/mg), whereas maximum PPO content was found in the mushrooms treated with citronella oil (0.067 U/mg) (Figure 3).

The POD content was lower in essential oils-treated mushrooms compared to control because this enzyme is responsible for spoilage of mushrooms. At 5 µl concentration of essential oils, minimum POD content was recorded in mint oil-treated mushrooms (0.38 U/mg) followed by clove oil (0.48 U/mg) and lemongrass oil (0.72 U/mg), while the highest POD content was observed in the treatment with citronella oil (0.78 U/mg). At 10 µl concentration of essential oils, the minimum POD content was observed in treatment with mint oil (0.105 U/mg) followed by clove oil (0.110 U/mg) and lemongrass oil (0.153 U/mg), whereas maximum POD was recorded in the citronella oil treatment (0.261 U/mg) (Figure 4).

Several studies have shown similar results, indicating that essential oils increase PAL content and total phenol content whereas they decrease the production of PPO and POD[11]. It was also observed that increasing the concentration of essential oils had a positive effect on the quality attributes of mushrooms[12].

In this study, it was observed that mint oil performed best among all the essential oils in increasing the storage life of harvested mushrooms. It was also observed that 10 µl concentration of all essential oils was more effective in regulating enzymatic activity of harvested mushrooms. Essential oils fumigation of mushrooms is an emerging technology that utilizes the oxidizing and reducing properties of essential oils to enhance the post-harvest quality of mushrooms. This preservative technique will help in increasing the shelf-life of harvested fruiting bodies and improving the marketability of oyster mushrooms.

Conflict of interest: The authors declare that they have no conflict of interest.

Statement on human and animal rights: This article does not contain any studies with human participants or animals performed by any of the authors.

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