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# Antifungal activity of essential oils derived from some medicinal plants against grey mould (botrytis cinerea)

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## Abstract

Preventing fungal decay in organic fruits after harvest is an increasingly challenge and novel preservation approaches which comply with organic food standards need to be developed. Preliminary experiments have shown that the essential oils of various plants possess some antifungal activities. In this study, the essential oils derived from four medicinal plants were evaluated and compared for their antifungal efficacies against *Botrytis cinerea*, an aggressive postharvest pathogen on a wide range of fruit. According to a modified in vitro micro-atmospheric test, vapors of clove oil, cinnamon oil and lemongrass oil exhibited strong inhibitory effects on *B. cinerea*, with a MIQ (minimal inhibitory quantity) equal to 15 µL. However the headspace vapors of galingale oil exhibited weaker inhibitory effect (MIQ = 25 µL). The results of the nature of this inhibition of these oils indicated that clove oil, cinnamon oil and lemongrass oil all exhibited fungicidal effect on the pathogen, while galingale oil exhibited some fungistatic properties. These results have shown that the essential oils derived from clove, cinnamon and lemongrass might be used as alternative options for the control of gray mould on postharvest organic fruits.

**Keywords :** antifungal activity; biological control; *Botrytis cinerea*; essential oil

## Introduction

Gray mould decay caused by the fungus *Botrytis cinerea* is a serious worldwide problem in flower, fruit and vegetable production (Masih and Paul, 2002). Gray mould can cause severe

postharvest losses, particularly in organic produces due to the prohibition of synthetic fungicides. Therefore, the developments of alternative non-synthetic chemical strategies, which better comply with organic food standards, are needed. Among the various strategies proposed, biological control using natural products such as plant extracts and essential oils show most promise.

Many plant essential oils and their volatile constituents have been reported to possess potent antifungal activities. The advantage of plant essential oils is their bioactivity in the vapor phase, which makes them attractive as possible fumigants for the postharvest control of decay fungi in fruit and grains (Paster et al., 1995; Hammer et al., 1999; Tripathi et al., 2008). For example, peppermint and sweet basil oils have reported to be effective fumigants to the control of decay fungi in stored peach fruits (Ziedan and Farrag, 2008). Tripathi et al. (2008) recently reported the effective control of the gray mould fungi using essential oils derived from *Ocimum sanctum*, *Prunus persica* and *Zingiber officinale*. In this study, the essential oils derived from four medicinal plants (clove, cinnamon, lemongrass and galingal) were evaluated for efficacy against *B. cinerea* under a modified atmosphere environment *in vitro*.

## Materials and Methods

### *Pathogen*

*B. cinerea*, was isolated from decayed strawberry fruits and maintained on potato dextrose agar (PDA) at 4 °C. Fresh cultures of the fungus were prepared by subculture of mycelia onto new PDA plate and the incubated at 23 °C for 3-5 days.

### *Extraction of essential oils*

Essential oils of selected plants including clove, cinnamon, lemongrass and galingale, were obtained by hydro-distillation in a Clevenger-type apparatus as described by Tripathi et al., (2008). The extracted crude essential oils were stored in sealed glass bottles or flasks, and protected from the light by wrapping in aluminum foil and stored at 4 °C.

### *Antifungal bioassay*

The volatile component of the essential oils from clove, cinnamon, lemongrass and galingale were tested for antifungal activity against *B. cinerea* by using modified micro-atmospheric method (*in vitro*) previously described by Singh et al. (1999) with some modification.

PDA plates were prepared using 5 cm Petri dishes containing 5 mL of PDA. A 4 mm (diameter) agar disc of *B. cinerea* was cut from the periphery of the active growth culture (3 - 5 days old) and the mycelial surface was placed upside down on the centre of the dish. The Petri dish was then inverted and a small paper disc (6 mm diameter, Whatman No.1) was placed inside on the lid of each Petri dish. An aliquot amount (5, 10, 15, 20 and 25 µl) of each essential oil was applied to the paper disc. Incubation of the fungus and test was conducted in a growth chamber at 23 °C with 12/12 dark-light regime. Each test was replicated for three times. The antifungal activity was determined after 3 day incubation by means of the percentage of inhibited redial growth as following equation:

$$\% \text{ inhibition} = \frac{\Delta d_O - \Delta d}{\Delta d_O} \times 100$$

Where $\Delta d_o$ and $\Delta d$ are the average diameter of the fugal colonies in the control and treatment sets, respectively

### *Determination of the nature of the inhibition of essential oil*

To determine the nature of the inhibition of each essential oil, the mycelial discs which were totally suppressed by the essential oil were transferred to a PDA plate which was not

supplemented with the essential oil. The treatment was fungistatic if the growth of the fungus began again and was considered fungicidal if the fungus did not re-grow.

**Results and Discussion**

Of the four essential oils tested, clove oil, cinnamon oil and lemongrass oil exhibited strong inhibitory effects on *B. cinerea*, since they completely suppressed the mycelial growth of the fungus at 15 µL (MIQ = 15 µL). However the headspace vapors of galingale oil exhibited weaker inhibitory effect (MIQ = 25 µL) (Table 1).

The lemongrass oil and clove oil appeared to possess the strongest antifungal activity against *B. cinerea* since it allow only minimal growth of the fungi at 10 µL and a few growth at 5 µL dose (Table 1). Lee et al. (2007) tested lemongrass oil (*Cymbopogon citrates*) as a fumigant against *B. cinerea*, but in contrast to this study their results were negative. This may be because Lee et al. (2007) only applied 1 µL of oil for each treatment, which less than the MIQ level we found in this study.

Although cinnamon oil exhibited lower antifungal activity, it maybe a better potential candidate as fumigant as it is more widely available. Moreover, it essential oils may be used in mixtures to improve their efficacy. Sukatta et al. (2008) previously showed that mixing of clove oil and cinnamon oils at appropriate ratios result in an improvement of the efficacy against the postharvest decay fungi of grapes *Aspergillus niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Phomopsis viticola* and *Rhizopus stolonifer*.

The results of the nature of this inhibition of the oils tested indicated that clove oil, cinnamon oil and lemongrass oil exhibited fungicidal effect on the pathogen, while galingale oil exhibited fungistatic properties.

**Table 1:** Effect of essential oils on the mycelial growth of *B. cinerea*.

| Treatment | Dose (µL/disc) | mycelial growth (mm) |
|---|---|---|
| clove oil | 5 | 15.4 ± 3.71 |
| | 10 | N/M |
| | 15 | I |
| | 20 | I |
| | 25 | I |
| cinnamon oil | 5 | 30.8 ± 5.54 |
| | 10 | 20.2 ± 3.20 |
| | 15 | I |
| | 20 | I |
| | 25 | I |
| galingale oil | 5 | 29.0 ± 2.55 |
| | 10 | 24.8 ± 2.39 |
| | 15 | 24.0 ± 4.53 |
| | 20 | 26.4 ± 3.85 |
| | 25 | I |
| lemongrass oil | 5 | 12.0 ± 5.10 |
| | 10 | N/M |
| | 15 | I |
| | 20 | I |
| | 25 | I |
| Control | No treatment | 31.4 ± 2.41 |

Note: N/M = Minimal growth was observed but can not measure.
I = Total inhibition

It should be noted that the diameter of the fungus growth on the treated plates were nearly the same diameters as those of the untreated controls (Fig. 1). However the treated colony appeared flatter and was considerably thinner in appearance. This observation suggests that the current assessment of essential oils fumigant efficacy against fungi by measurement of growth diameter alone is not suitable. We proposed that alternative method, such as the measurement of mycelial dried weight would be a better measure of efficacy.

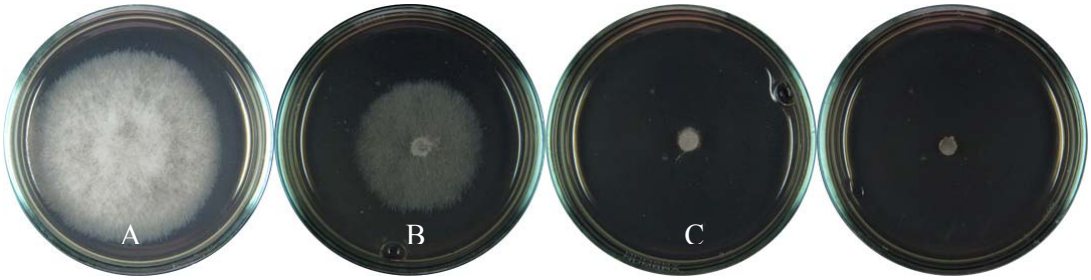


**Figure 1:** Radial growth of *B. cinerea* after 3 days incubation with different dosage of clove oil. (A) untreated control, (B) 5 µL treatment : the fungal colony is flatter and thinner, (C) 10 µL treatment : little growth was observed but the colony sized is unmeasurable, (D) 5 µL treatment : totally inhibited

We also investigated the effect of clove, lemongrass and cinnamon oils by direct investigation under light microscope. The results showed that the oils caused cutotoxicity to the fungus resulting in abnormal development of hyphae of the target fungus (Fig. 2).

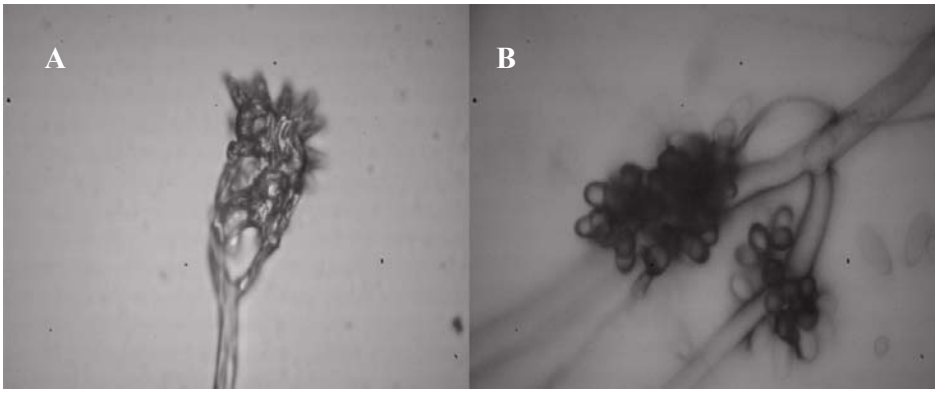


**Figure 2.** Abnormal growth of *B. cinerea* treated with lemongrass oil (A) compared with the untreated (B).

## Conclusions

In conclusion this preliminary study showed that the essential oils derived from clove, cinnamon and lemongrass maybe used as alternative for the control of gray mould on postharvest organic fruits especially as a natural fumigant in closed container or packaging.

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