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## *In vitro* antifungal activities of five plant essential oils against *Botrytis cinerea* causing gray mold of orange

**Sania Pallabi Reang, JP Mishra and Rajendra Prasad**

**Abstract**

Five essential oils i.e. Clove (*Syzygium aromaticum* L.), Thyme (*Thymus vulgaris* L.), Lavender (*Lavandula angustifolia* L.), lemongrass (*Cymbopogon citratus*) and peppermint (*Mentha piperita* L.) were evaluated against mycelial growth of *Botrytis cinerea* at 0.5%, 1% and 1.5% concentration under *in vitro* condition by using Poisoned Food Technique. Among the five essential oils Thyme oil showed maximum growth inhibition of *Botrytis cinerea*. The next best essential oil which inhibited the mycelial growth of pathogen was Clove oil.

**Keywords:** Essential oils, antifungal activity *Botrytis cinerea*, gray mold

**Introduction**

Citrus is the collective term containing a number of species and varieties of fruits, known to world over for their characteristic flavor and attractive range of colours. The orange (*Citrus sinensis*) is the fruit of citrus species. *Citrus sinensis* is also called as sweet orange which is differentiate it from the bitter orange (*Citrus aurantium*). The juice of orange includes about 12 percent sugars, 0.5-1.5 percent titrable acidity and other soluble solids, 1 percent citric acid, about 50 mg vitamin C per 100 ml of juice and about 70-90 percent water.

The origin of most citrus fruits are to be the Southern slope of Himalayan region, the entire north-eastern region of India and adjacent China. Oranges have their origin in China.

The major producers of oranges are Mexico, USA, India and Argentina. Oranges are extensively grown in Nagpur regions of Maharashtra, Coorg of Karnataka, Darjeeling of West Bengal, Sikkim, North-Eastern region of India, Himachal Pradesh and Punjab.

Orange is attacked by number of fungal, bacterial, and viral diseases out of which Gray mold is measured as most important disease for low yields of oranges which is caused by fungus. Gray mold is also identified as *Botrytis rot* and bunch rot. It is prevalent all over the world where oranges are grown. Gray mold disease is affected by fungus *Botrytis cinerea*. Under favorable condition, this fungus attacks all the fleshy tissues having high sugar, however, maximum damage is noticed in fruits at the harvest time along with during transport and storage. By the attack of *Botrytis* the mycelium colonizes the fruit coating and destroys epidermal cells thereby agree too much dehydration of water through cuticle.

The most prominent symptoms of the disease are found on the fruits. In fruits the first signs are small, circular, faintly cleared spot. Infected part of fruits become grey coloured and show hairy mycelium in grey coloured all above the surface of fruits. Often the fungus can be seen developing with the cracks and splits on the fruits. Bunches of conidiophores and conidia swell from stomata and peri-stomatal cracks on the skin of the fruits.

**Materials and Methods****Isolation of the pathogen**

Collected infected fruits were used for isolation. Infected part of the fruits were transferred directly by niddle to petriplates containing sterilized PDA medium. These plates were incubated at 22±1 °C for four days to obtain good growth of the pathogen.

**Identification of the fungus**

The greyish mycelium was observed under the high power (40X) microscope from the infected fruits of orange for confirmation the identity of the fungus. Under microscope the branches of conidiophores are shown which have rounded apical cells bearing clusters of conidia. The conidiophores and clusters of conidia look like a grape shape cluster and they were compared with original description of the fungus.

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**In vitro efficacy of plant essential oils**

The five selected oils were tested against *B. cinerea*. All five oils were evaluated at 0.5%, 1% and 1.5% concentration against *B. cinerea* by using Poisoned Food Technique. In order to make dilution of plant oils, organic solvent like tween 20 (1:10ml) was pipetted and added in sterilized molten agar media along with the required concentration of plant oils separately which was mixed in PDA medium thoroughly and poured into sterilized petri dishes. After solidification, the 4 mm test pathogen was kept on the centre of petri dishes and control plates were kept without oil. For each treatment four replication were maintained and incubated at 22±1°C for some days until the mycelial growth of the pathogen was fully covered in the control petridishes.

**The growth inhibition percent of mycelium was calculated by following formula.**

$$I=100(C-T)/C$$

**Where**

I - Per cent inhibition in mycelial growth  
C - Linear mycelial growth in control (mm)  
T - Linear mycelial growth in control (mm)

**Statistical analysis**

The data obtained as mycelial growth inhibition of essential oils were subjected to analysis of variance for completely randomized design by using OPSTAT and charts were drawn

by Excel 2013 software.

**Table 1:** List of five plant oils tested for antifungal activity against the pathogen

| S.no | Common name | Botanical name                   | Family   |
|------|-------------|----------------------------------|----------|
| 1    | Clove       | <i>Syzygium aromaticum</i> L.    | Myrtacea |
| 2    | Thyme       | <i>Thymus vulgaris</i> L.        | Lamiacea |
| 3    | Lavender    | <i>Lavendula angustifolia</i> L. | Lamiacea |
| 4    | Lemongrass  | <i>Cymbopogon citratus</i>       | Poacea   |
| 5    | Peppermint  | <i>Mentha piperita</i> L.        | Lamiacea |

**Results**

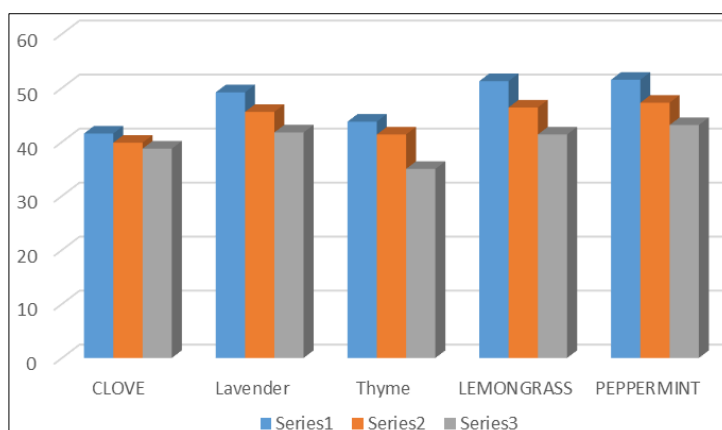
The five plant essential oils with three different concentrations was evaluated by using the poison food technique under *in vitro* condition. The results presented in the following table revealed that among the five essential oils at 1.5 percent concentration maximum mycelial growth inhibition showed in Thyme oil (50%). The next best essential oil which inhibited the mycelial growth of pathogen was Clove and showed 44.65% growth inhibition at 1.5% concentration. The other essential oil Lemongrass, Lavender and Peppermint showed 40.89%, 40.35%, 38.39% inhibition of mycelial growth at 1.5% concentration. However, Clove at 1% concentration showed the growth inhibition of 43.07%. The least growth inhibition was observed in peppermint at 0.5% concentration with the growth inhibition of 26.42%, followed by same concentration of Lemongrass showed the growth inhibition of 26.78%, Peppermint have a low capability to control *B. cinerea* at lower concentration.

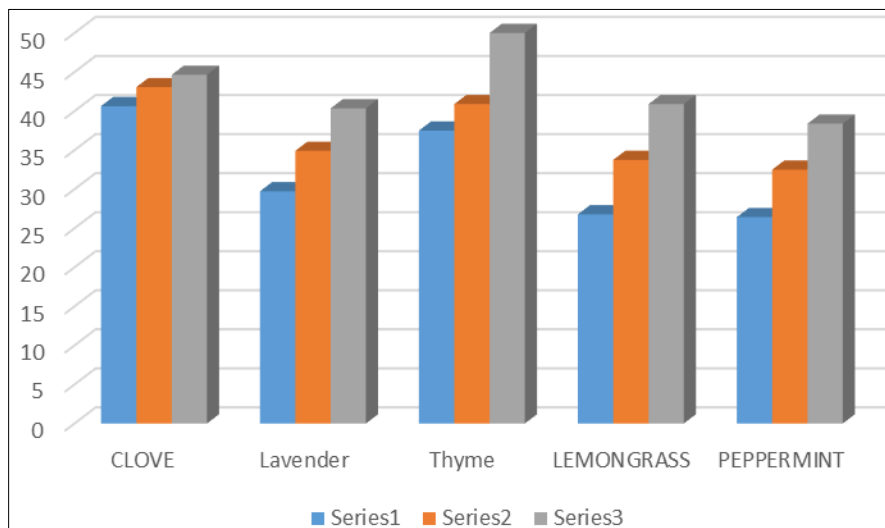
**Table 2:** Effect of essential oils on colony growth of *Botrytis cinerea*

| Colony growth (mm*) on Essential Oils |       |          |       |            |            |
|---------------------------------------|-------|----------|-------|------------|------------|
| Concentration %                       | Clove | Lavender | Thyme | Lemongrass | Peppermint |
| 0.5                                   | 41.55 | 49.18    | 43.75 | 51.25      | 51.5       |
| 1                                     | 39.85 | 45.56    | 41.37 | 46.37      | 47.25      |
| 1.5                                   | 38.75 | 41.75    | 35    | 41.37      | 43.12      |
| Control                               | 70    | 70       | 70    | 70         | 70         |
| SE(m)                                 | 0.25  | 0.63     | 0.92  | 0.59       | 0.69       |
| CD                                    | 0.81  | 2.04     | 2.98  | 1.92       | 2.23       |

**Table 3:** Effect of essential oils on growth inhibition of *Botrytis cinerea*

| Growth Inhibition % |       |          |       |            |            |
|---------------------|-------|----------|-------|------------|------------|
| Concentration %     | Clove | Lavender | Thyme | Lemongrass | Peppermint |
| 0.5                 | 40.65 | 29.73    | 37.5  | 26.78      | 26.42      |
| 1                   | 43.07 | 34.91    | 40.89 | 33.75      | 32.5       |
| 1.5                 | 44.65 | 40.35    | 50    | 40.89      | 38.39      |
| Control             | 00    | 00       | 00    | 00         | 00         |
| SE(m)               | 0.36  | 0.90     | 1.32  | 0.85       | 0.98       |
| CD                  | 1.16  | 2.91     | 4.26  | 2.75       | 3.18       |

**Fig 1:** Effect of essential oils on colony growth of *Botrytis cinerea*



**Fig 2:** Effect of essential oils on mycelial growth inhibition of *Botrytis cinerea*

### Discussion

Five essential oils Clove, Lavender, Thyme, Lemongrass and Peppermint were tested against *B. cinerea*. Among these five essential oils maximum mycelial growth inhibition showed in Thyme oil (50%). The next best essential oil which inhibited the mycelial growth of pathogen was Clove and showed 44.65% growth inhibition. The other essential oil Lemongrass, Lavender and Peppermint showed 40.89%, 40.35%, 38.39% inhibition of mycelial growth.

Abd-AIIA M.A. *et al.* (2011) [4] reported that thyme essential oil showed best effective against gray mold of strawberry fruits. Mehdi Behdani *et al.* (2012) also proved that Thyme and Clove oil showed best growth inhibition against *B.cinerea* causal agent of postharvest apple rot.

Sirirat Siripornvisa *et al.* (2009) reported that Clove and Lemongrass showed best growth inhibition against gray mold (*Botrytis cinerea*).

### Conclusion

In this study we revealed that Thyme and Clove essential oil gave best effective mycelial growth inhibition of *Botrytis cinerea* and it may be used for the control of gray mold of orange as a natural fumigant in container or packaging.

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