# Evaluation of some essential oils for their fungitoxicity on storage fungi of pulses

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***Abstract***
*Four essential oils such as camphor oil, cinnamon oil, clove oil and rose oil were evaluated for their antifungal activity against three storage fungi isolated from pulses. The storage fungi namely Alternaria sp., Cladosporium sp. and Colletotrichum sp., were isolated from green gram and black gram by standard blotter method. The essential oils were screened against these isolated fungi following poisoned food method. Different concentrations of oils such as 100 ppm, 200 ppm, 300 ppm and 400 ppm were used to evaluate the antifungal effect.*

*All the four oils screened exhibited significant antifungal activity against all the three fungi studied. Comparatively, antifungal activity of clove oil and cinnamon oil was better than rose oil and camphor oil; Alternaria sp. was more susceptible and Cladosporium sp. was least susceptible to the oils screened. The results of this study thus revealed that these four essential oils could be an alternative to synthetic fungicides for the management of post-harvest storage fungi of pulses.*

**Keywords:** Pulses, Storage fungi, Essential oil, Antifungal activity, Fungitoxicity.

## Introduction

Cereals and pulses are important sources of nutrition worldwide and because of their rich chemical composition, they are prone to contamination and spoilage by both bacteria and fungi. Fungi are the major microorganisms involved in the spoilage of foodstuff during storage. By their growth, they make the foodstuff unfit for human consumption by reducing the nutritive value of food as well as by producing mycotoxins. Mycotoxins are well known carcinogens, mutagens, nephrotoxicants and neurotoxicants[8]. Storage fungi are commonly controlled by synthetic chemicals, but most of the synthetic fungicides are reported to have carcinogenicity, teratogenicity and residual toxicity. Regular usage of these chemicals could lead to the emergence of resistant fungi and harm the non-targeted organisms in the environment. Public awareness on the health and environmental hazards associated with synthetic fungicides has therefore generated interest in the use of antifungal substances from natural sources[10,14].

Essential oils represent potential source of alternative and environmental friendly compounds for the management of storage fungi. Biologically active constituents of the essential oils have been found to be less phytotoxic, more systemic and easily biodegradable. Essential oils are very complex mixture of compounds. The main categories of compounds are terpenes and terpenoids; rarely nitrogen and sulphur containing compounds, coumarins and homologues of phenylpropanoids can also be found. Because of rich pool of substances, they can provide a very broad spectrum of action[4,11].

The use of essential oils for the preservation of food is not new to mankind and they have been used since the earliest civilization. Due to their broad antimicrobial properties, many essential oils have been used for the control of microbial spoilage, preservation of food quality and safety and prolongation of shelf-life of food. Essential oils are classified as "Generally Recognised as Safe" (GRAS) by the Food and Drugs Administration (FDA)[11]. The antifungal activity of essential oils is well documented[1,3,8,13]. Owing to these aspects, the present study was conducted to evaluate the antifungal activity of some essential oils against a few fungi of pulses isolated from green gram and black gram.

## Material and Methods

**Isolation of storage fungi from pulses:** Two samples (green gram and black gram) were procured from local market. Isolation of storage fungi from these samples was carried out following standard blotter method (SBM)[6]. A pair of white blotter papers of 8.5 cm diameter was jointly soaked in sterile distilled water placed in pre-sterilized Petri plates. 10 pulses per Petri plate were placed at equal distance on the moist blotters. The upper lid of the Petri dish was covered and appropriately labelled. The Petri plates were incubated for 7 days at 28 ± 1°C with 12 h photoperiod.

Water was sprinkled whenever required aseptically, thus preventing the drying of the blotter paper. After incubation, the seeds were observed for fungal growth and the fungi growing on seeds were identified based on sporulation and mycelial characteristics. The identified fungi were sub-cultured on to potato dextrose agar (PDA, Himedia) plates aseptically incubated at 28 ± 1°C with 12 h photoperiod for seven days. The pure cultures thus obtained were maintained at 4°C.

**Study of the antifungal activity of essential oils on the isolated storage fungi of pulses:** Four essential oils such as camphor oil, cinnamon oil, clove oil and rose oil (Table 1) were studied for their antifungal effect on *Alternaria* sp., *Cladosporium* sp. and *Colletotrichum* sp. The evaluation of

antifungal effect of essential oils on the growth of fungi was carried out by poisoned-food technique[2]. Essential oils were separately dissolved in acetone to get 10,000 ppm stock solution. The oils were then added to PDA medium after sterilization under aseptic conditions to have different concentrations such as 100, 200, 300 and 400 ppm. The oil amended medium was poured into sterile 90 mm diameter Petri plates (20 ml / plate).

The PDA medium with only acetone without any oil served as control. The mycelial agar disc (5.0 mm) of each test fungi obtained from the margin of seven-day-old culture was inoculated at the centre of the Petri plates having both control and essential oil amended PDA medium. The inoculated Petri plates were incubated at 28 ± $2^0$C with 12 h photoperiod for seven days. After incubation, the colony diameter was measured. The per cent mycelial growth inhibition (PI) with respect to the control was computed using formula PI = (C-T / C) X 100 where C is the colony diameter of the control and T is that of the treated combinations. The experiment was repeated three times.

## Results

**Isolation of storage fungi from pulses:** The species *of Alternaria* was isolated from black gram while species of *Cladosporium* and *Colletotrichum* were isolated from green gram by standard blotter method (Figure 1 and 2). Sub-culturing on PDA yielded pure culture of these fungi (Figure 3) that were maintained at 4°C.

**Table 1**
**Essential oils tested against phytopathogenic fungi for antifungal activity**

| Essential oil | Plant source | Family | Plant part used |
|---|---|---|---|
| Camphor oil | *Cinnamomum camphora* L. | Lauraceae | Chipped wood, root stumps and branches |
| Cinnamon oil | *Cinnamomum zeylanicum* Blume | Lauraceae | Bark and leaves |
| Clove oil | *Syzygium aromaticum* L. | Myrtaceae | Bud, leaves and stem |
| Rose oil | *Rosa damascene* Mill. | Rosaceae | Flowers |

**Table 2**
**Effect of essential oils on *Alternaria* sp.**

| Concentration | Mycelial growth (cm) | | | |
|---|---|---|---|---|
| | Camphor oil | Cinnamon oil | Clove oil | Rose oil |
| Control | 5.96 ± 0.12 | 6.13 ± 0.09 | 6.13 ± 0.16 | 4.23 ± 0.54 |
| 100 ppm | 5.06 ± 0.04 | 5.1 ± 0.08 | 4.2 ± 0.08 | 3.9 ± 0.14 |
| 200 ppm | 3.1 ± 0.16 | 3.23 ± 0.04 | 3.96 ± 0.16 | 3.46 ± 0.12 |
| 300 ppm | 2.03 ± 0.04 | 0 | 0 | 3.2 ± 0.08 |
| 400 ppm | 1.36 ± 0.12 | 0 | 0 | 2.8 ± 0.08 |

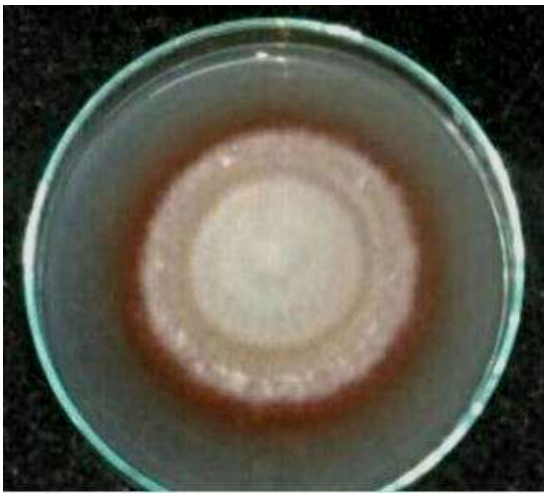
Values given are means of three replicates ± standard error

**Figure 1: *Alternaria* sp. isolated from black gram by SBM**

**Figure 2: *Cladosporium* sp. and *Colletotrichum* sp. isolated from green gram by SBM**

**A**

**B**



**C**

**Figure 3: Pure cultures of (A) *Alternaria* sp., (B) *Cladosporium* sp. and (C) *Colletotrichum* sp., on PDA (7-days-old)**

**Table 3**
**Effect of essential oils on *Cladosporium* sp.**

| Concentration | Mycelial growth (cm) | | | |
|---|---|---|---|---|
| | Camphor oil | Cinnamon oil | Clove oil | Rose oil |
| Control | 5.86 ± 0.18 | 6.43 ± 0.124 | 7.56 ± 0.449 | 7.26 ± 0.24 |
| 100 ppm | 2.93 ± 0.24 | 3.53 ± 0.24 | 2.96 ± 0.124 | 3.73 ± 0.124 |
| 200 ppm | 2.56 ± 0.04 | 3.2 ± 0.18 | 1.65 ± 0.108 | 2.4 ± 0.163 |
| 300 ppm | 2.43 ± 0.12 | 1.3 ± 0.081 | 1.4 ± 0.081 | 2.06 ± 0.047 |
| 400 ppm | 2.16 ± 0.09 | 1.0 ± 0.04 | 0.8 ± 0.081 | 0.9 ± 0.08 |

Values given are means of three replicates ± standard error

**Table 4**
**Effect of essential oils on *Colletotrichum* sp.**

| Concentration | Mycelial growth (cm) | | | |
|---|---|---|---|---|
| | Camphor oil | Cinnamon oil | Clove oil | Rose oil |
| Control | 6.23 ± 0.89 | 5.236 ± 0.281 | 7.25 ± 0.89 | 8.26 ± 0.281 |
| 100 ppm | 3.45 ± 0.458 | 4.23 ± 0.453 | 4.35 ± 0.458 | 3.25 ± 0.123 |
| 200 ppm | 1.056 ± 0.25 | 2.76 ± 0.281 | 1.6 ± 0.25 | 1.56 ± 0.256 |
| 300 ppm | 0.967 ± 0.01 | 1.23 ± 0.281 | 0.76 ± 0.01 | 0.523 ± 0.120 |
| 400 ppm | 0 | 0 | 0 | 0 |

Values given are means of three replicates ± standard error

**Antifungal activity of essential oils on the isolated storage fungi of pulses:** All the essential oils screened exhibited significant antifungal activity on all the fungi studied such as *Alternaria* sp., *Cladosporium* sp. and *Colletotrichum* sp. isolated from pulses (Table 2 – 4).

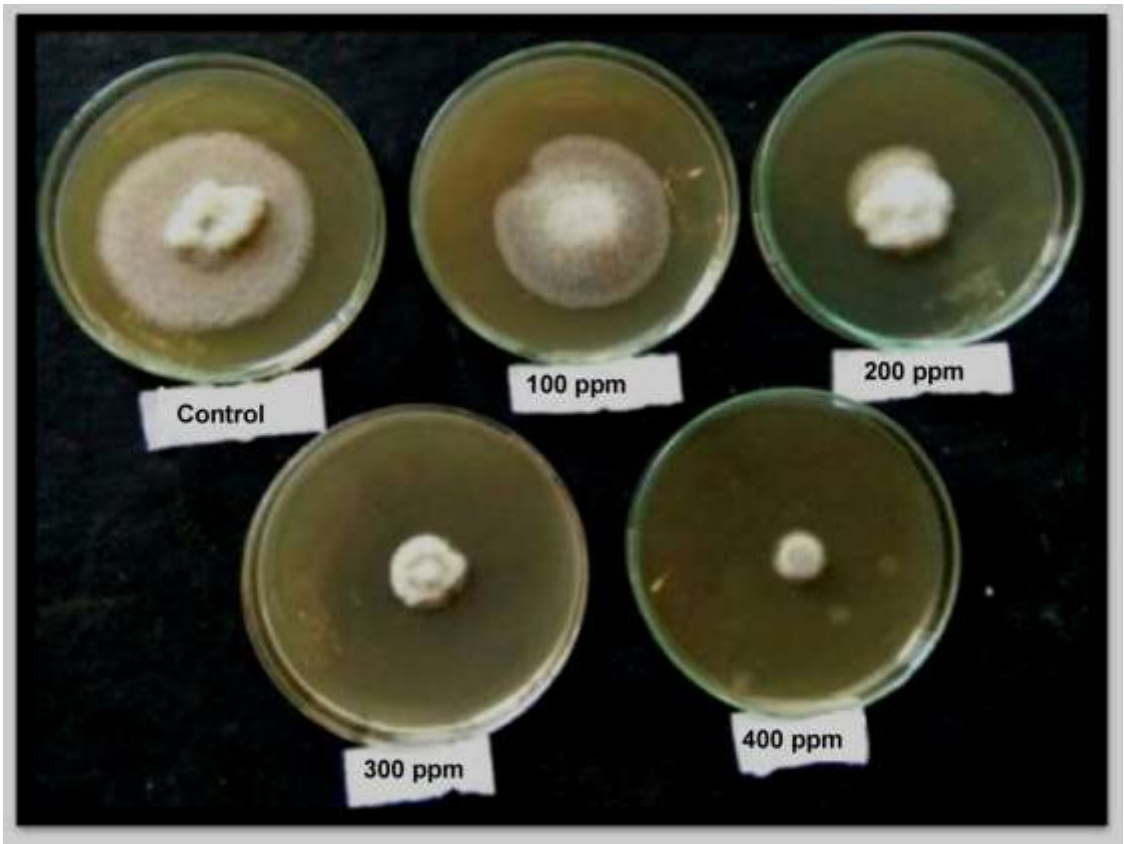



**Figure 4: Antifungal activity of Camphor oil on *Alternaria* sp.**

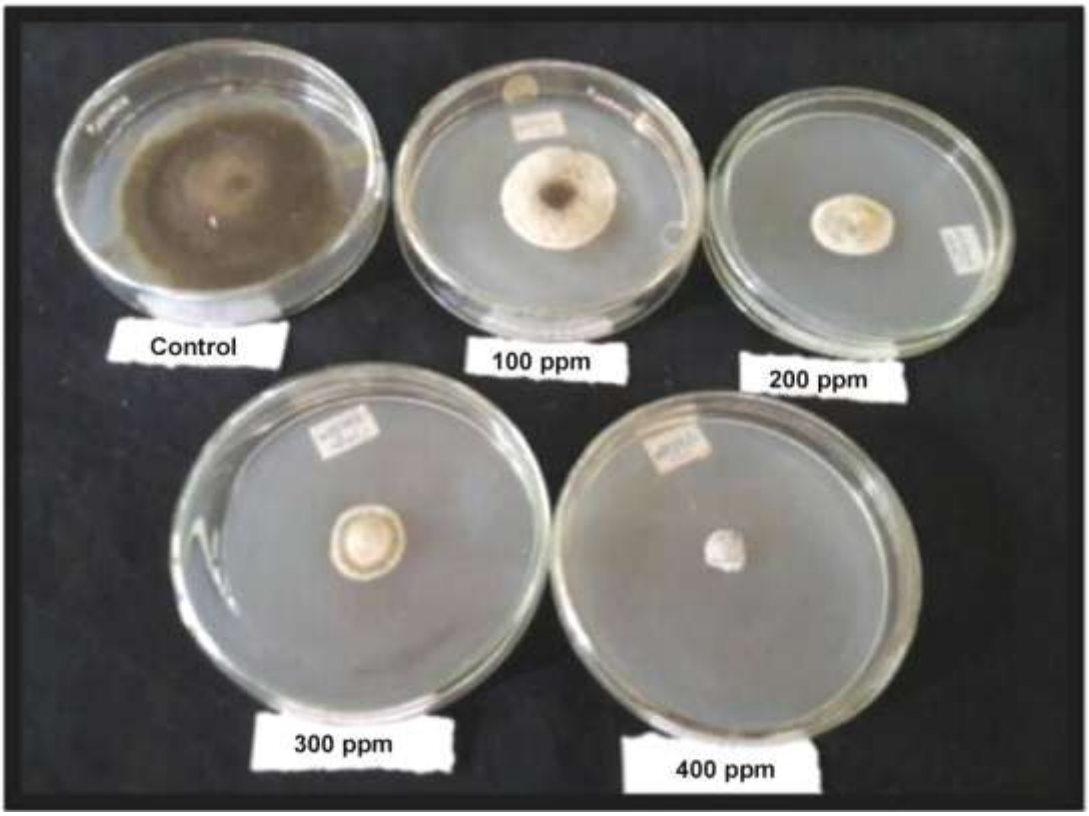



**Figure 5: Antifungal activity of Clove oil on *Cladosporium* sp.**

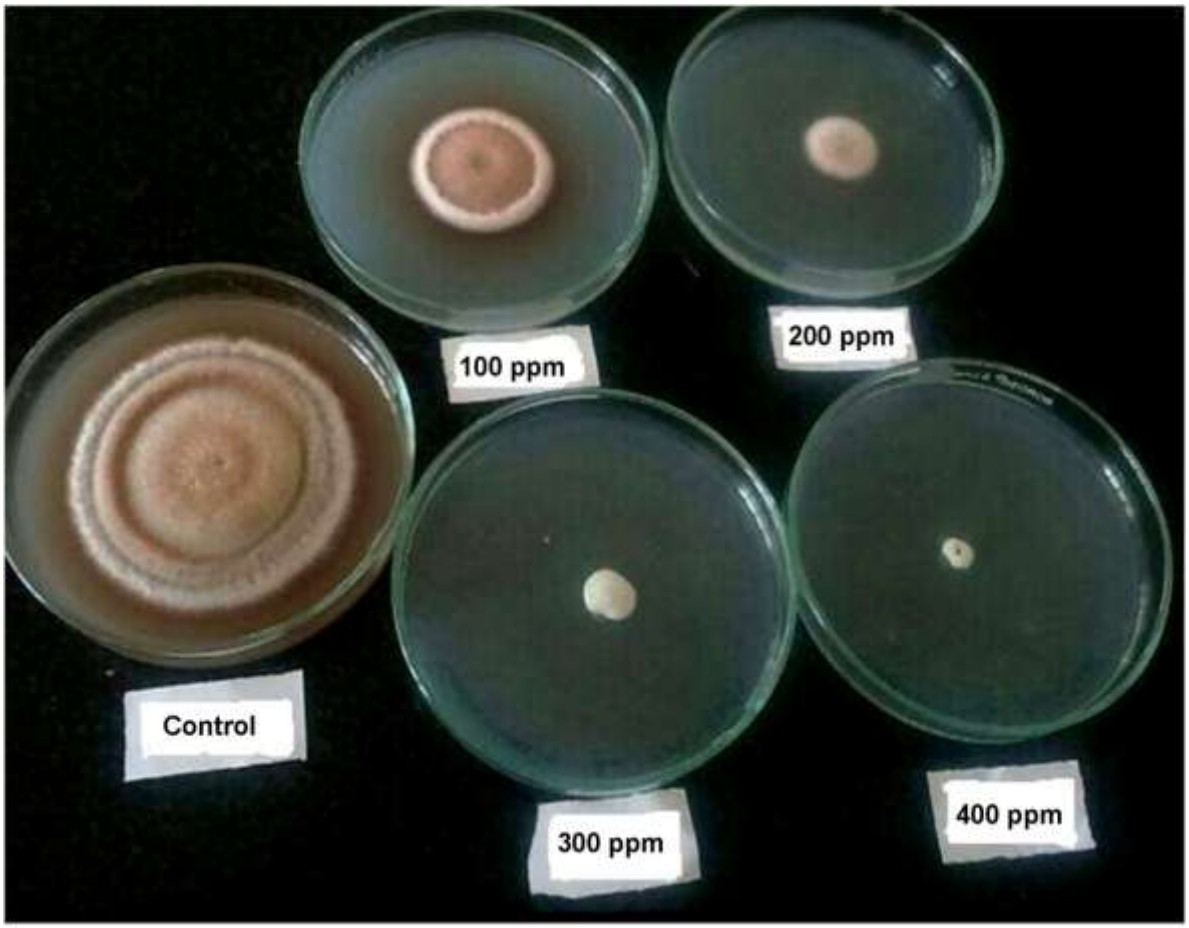



**Figure 6: Antifungal activity of Rose oil on *Colletotrichum* sp.**

Effects of camphor oil on the mycelial growth of *Alternaria* sp., clove oil on the mycelial growth of *Cladosporium* sp. and the rose oil on the mycelial growth of *Colletotrichum* sp. are presented in figures 4 to 6. A gradual decrease in the mycelial growth of all the three tested fungi with increased concentration of oils is evident. Similar results were observed with all other oils screened against each of the fungi tested.

Cinnamon and clove oil were highly effective against *Alternaria* sp. by completely inhibiting the fungal mycelial growth at 300 ppm itself followed by camphor oil and rose oil. Against *Cladosporium* sp., best activity was shown by clove oil and rose oil followed by cinnamon oil and camphor oil. In the case of *Colletotrichum* sp., all the oils completely inhibited the mycelial growth of fungus at 400 ppm. Comparatively rose oil and clove oil exhibited better activity than camphor oil and cinnamon oil.

Over all, comparatively clove oil and cinnamon oil showed better antifungal activity than rose oil and camphor oil. *Alternaria* sp. was found to be most susceptible being inhibited at 300 ppm by cinnamon oil and clove oil followed by *Colletotrichum* sp. which was inhibited at 400 ppm by all the oils tested, while *Cladosporium* sp. was comparatively less susceptible to the oils screened which exhibited little growth even at the highest concentration (400 ppm) screened (Figure 7, tables 2 – 4).

## Discussion

A major reason for the extensive analysis of biological activity of essential oils in recent years is the concern of human health. Chemical drugs, synthetic fungicides and preservatives have been found to have carcinogenic and teratogenic effects as well as residual toxicity[7]. This has led to the finding of eco-friendly alternative approaches for the management of plant pathogens. The demand for the natural products has been intensified as most of the natural products are socially acceptable[11,14]. Many researchers have documented the antifungal activity of essential oils including eucalyptus oil, pepper oil, coriander oil, clove oil, lemongrass oil, thyme oil and oregano oil against different fungal species[2,11,14,18].

The present study evaluated the effect of four essential oils namely rose oil, clove oil, cinnamon oil and camphor oil on storage fungi of pulses such as *Alternaria*, *Cladosporium* and *Colletotrichum* species. Although all the four essential oils significantly inhibited the growth of fungi at different concentrations, rose oil and clove oil proved to be more potent in inhibiting the mycelial growth. Among the different concentration of essential oils tested, 300 - 400 ppm appeared to be most effective range.

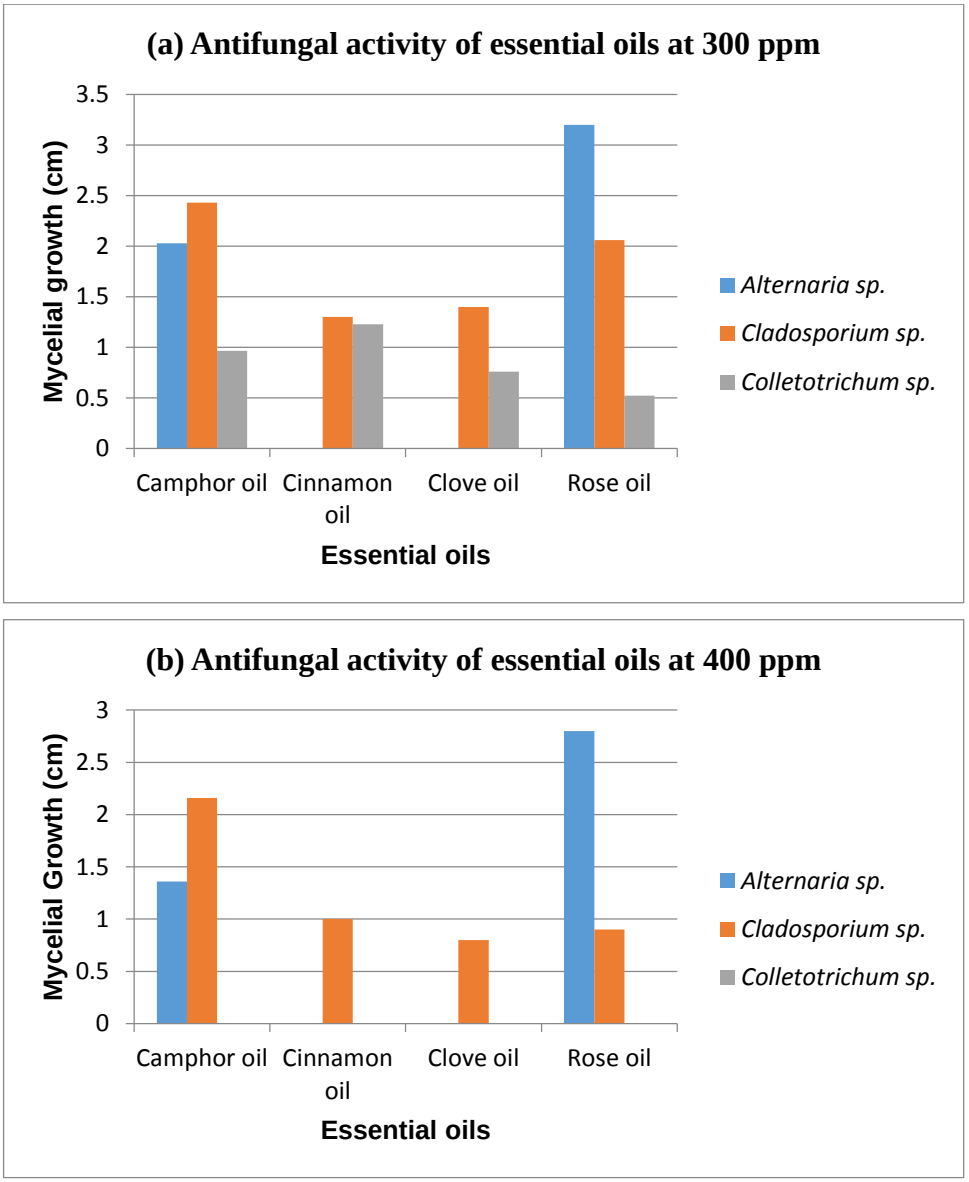



**Figure 7: Antifungal activity of essential oils at (a) 300 ppm and (b) 400 ppm**

Recently, there has been great interest in employing the essential oils from aromatic plants for controlling plant pathogens[3,4,11]. Essential oils are rich sources of bioactive compounds such as monoterpenes, eugenol, cinnamic aldehyde, thymol, terpenes and phenolic compounds that have antimicrobial activity[12,15]. Essential oils can inhibit mycelial growth of fungi or kill fungal cell by various mechanisms[3].

Essential oils can kill fungi via interfering with their membrane-integrated or associated enzymes by stopping their production or activity and also by acting as uncoupling agents and interrupting ADP phosphorylation[9]. They may cause loss of rigidity and integrity of the cell wall of fungal hyphae, resulting in the death of mycelium[15]. The essential oils can inhibit the growth of fungi by damaging their cell wall and cell membrane to various degrees causing cytoplasm leakage and also by partial inhibition of DNA, RNA and protein synthesis[9,10]. The essential oils through the permeabilization of inner and outer mitochondrial membranes may cause the cell apoptosis or necrosis leading to fungal cell death[16]. Essential oils may also act against fungi by the inhibition of ergosterol biosynthesis[5]. Essential oils can inhibit ATPase leading to intracellular acidification and fungal cell death as well as mitochondrial dysfunction-induced ROS accumulation causing the death of fungal cell[17]. The inhibition of fungal growth observed in the present study may be attributed to some of these fungitoxic effects of the essential oils.

## Conclusion

The results of this study indicated that plant essential oils (camphor oil, cinnamon oil, clove oil and rose oil) possess effective antifungal activity and could be exploited as an ideal treatment for future storage fungi management programs eliminating food spoilage as well as fungal spread. However, this is a preliminary investigation to know the potential of some essential oils against storage fungi. Further studies are warranted to know the active compounds present in these oils and their mode of action.

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