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# In-vitro Evaluation of the Inhibitory Effect of Selected Natural Essential Oils on Different Samples

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***Abstract*:** The interest in using antifungal and inhibitory effect against fungi instead of chemical preservatives in fruits and food products has been increasing in recent years. In regard to essential oils natural and liquid secondary plant metabolites are gaining importance of their use in the protection of foods. Since, they are safe and healthy. Although research studies indicate the inhibitory effect and activity of natural spice essential oils. Cinnamon oil, clove oil, eucalyptus oil are selected essential oil. A review provides an overview of the current literature on essential oils mainly on inhibitory effect, antifungal activity, antibacterial activity, antioxidant activity. Different samples are strawberry and chapatti.

***Index terms:*** **cinnamon oil, clove oil, eucalyptus oil**

## 1. Introduction

According to distribution of world, strawberry is a widely grown hybrid species of the fragaria. According to lifespan, *Fragaria ananassa* have a very short shelf life because of the high susceptibility to fungal agents. The use of artificial antifungal chemical compounds has been a concern for increasing the shelf life of this fruit. For this reason, it is necessary to use safe methods to control decomposition and maintain the quality of strawberry fruit during storage[13].Its susceptibility to fungal rot, especially Rhizopus stolonifer, as well as high respiration rate, high water content (about 91%), and high metabolic activity have made strawberry one of the most corrosive fruit with short lifespan [13].

*Rhizopus* species, a Zygomycetes has a filamentous growth development. Its filamentous are ceonocytic, they are non septate. It's the only one fungus yet known to obtain rhizoids which penetrate the substratum on host for nutrients [11]. Stolons are present, which connect rhizoids joint. The spore of *R. stolonifer* is very common in atmosphere and this infection of fruits occurs mainly at wound sites during harvest or packaging [10].

EOs are secondary metabolites of plants, it have volatile, natural, and complex characteristics [1].Food products are often contaminated not only with pathogen microorganisms but also with moulds and the toxins produced by these moulds. This contamination can be effected at different stages of the food chain such as post-harvest processing, transportation, and storage stages [7]. Similarly bacterial contamination, fungal growth and mycotoxins lead to quality and quantity defect sand loss of market value in addition to their health risks. Additionally, mould invasion leads to noticeable quality and morphological changes [7] [9].For best of our knowledge, while their inhibitory effect of EOs and anti bacterial [8], antioxidant activities [4] [9], anti inflammatory [3] are well known, antifungal activity [5][2][12][6]of EOs have yet to be investigated deeply. It can be easily degraded by oxygen, temperature fluctuations and light factor [2].

The aim of this research was to test in vitro the effect of 3 essential oils on mycelia growth of two samples (strawberry and chapatti).

## 2. Material and Method

### 2.1Collection of essential oils:

Essential oils were collected from retail shop, Ahmedabad.

**Growth of fungal strains: two sample sample-A strawberry, sample-2 chapatti.**

*Fragaria ananassa* fruits were bought from retail shop; it should be fresh in condition. Samples are sterilized. Strawberries were put in three different sterilized containers in control condition. After two days strawberry due to humidity and moisture level and effect of environment factor temp, light it would start to spoil and we can observe the fungal growth on the surface. The fungal spores are very minute that we cannot see naked eye. Growth of fungus spoilage start firstly mycelia development on strawberry notified at third day. Notified fungus know as Rhizopus rot. This Rhizopus rot is also known as *Rhizopus stolonifer.*

Food chapattis made from home; should be fresh. Chapattis were put in three different sterilized containers. All samples should be kept in control in condition. Due to humid, moist and effect of environment factor after three to four days it would be spoil by

fungal spore. Development of mycelia and colonies were notified at fifth day. Mould fungus infected on chapatti, it known as *Rhizopus stolonifer*. Collection of fungus mainly depends on the fact that fungus usually grow on moist surface or substratum. The fungal spores are very minute that we are not able to know its physiology just by examining via naked eyes. Fungal collection should be very accurate to there would not be any contamination in the samples. Fungal hyphae are very in number so that it is forms the habit and habitat of its fruiting bodies. It can be of different shapes and sizes.

**In vitro assay to check antifungal activity**

All the equipments which were added for performing the experiment were sterilized by using 70% propyl alcohol or Ethanol. Two samples were selected for the Experiment chapattis and strawberry. By surface sterilization, strawberry and chapatti were disinfected. Treatments of Cinnamon oil, clove oil and eucalyptus oil were given to both samples. Samples were incubated inside the sterilized container. Observations were done under room temp and humid condition and given balanced light and dark periods, for a week.

These methods are repeated for more than 5-6 days. At the end of incubation period, antifungal activities were evaluated. The strain of fungus which was observed while performing it was – *Rhizopus stolonifer (*black and bread mould).The infective stage was accelerated on the 5$^{th}$ to 6$^{th}$ day. This performing method is totally because oil is having the different level of antifungal activities. Both samples are treated for 5days by spray bottle. Control plates (without the treatment of essential oils) were inoculated following the same protocol. Three replicates were maintained for each sample. As the above method have taken to know about the fungal activity of the different specimens, the fungus which they have inhibited was just because of the properties of different oil which are used in this. Both samples were divided into three distinct phases.

Phase1 ($P_0$)- control
Phase2 (P-2)- cinnamon oil
Phase3( P-3)- eucalyptus oil } Strawberry.

Phase1($P_0$)- control
Phase2 (P-2) - cinnamon oil
Phase3( P-3)- clove oil } Chapatti.

Essential oils sprayed on samples by spray bottle. OD treatment given to samples for 5day. 1ml sprayed on samples for 5 days through OD treatment (table-2, 7). Control specimens were infected on 4$^{th}$ day on sample-A and 5$^{th}$ day on specimen-B

**3. Result and Discussion**

The result showed significant effects of essential oils on both samples, the antifungal activities of essential oils were significantly inhibited to different samples.

**Sample-A**

According to study on sample-A strawberry, cinnamon oil and eucalyptus oil have antifungal properties, it inhibited fungal mycelia growth. Cinnamon oil have the moderate antifungal activity has medium in between) and eucalyptus oil highest inhibitory activity against the fungal infection on strawberry. Expected ratio of cinnamon oil and eucalyptus oil (30%:70%). Absent of fungal strains and growth on P-1 and P-2, and present of fungal strains and growth on $P_0$.

Table-1: To give treatment of cinnamon oil and eucalyptus oil on *Fragaria ananassa* fruit.

| Day | Control ($P_0$) | P-2 | P-3 |
|---|---|---|---|
| 1 | - | - | - |
| 2 | - | - | - |
| 3 | - | - | - |
| 4 | - | + | + |
| 5 | - | + | + |
| 6 | - | + | + |
| 7 | - | + | + |
| 8 | - | + | + |
| 9 | - | * | * |
| 10 | - | * | * |

[-]= no treated, [+] = treated, [*]= stable,
[$P_0$] control, [P-2] cinnamon oil, [P-3], eucalyptus oil

Table-2: Inhibitory effect of essential oils on the mycelia growth on *Fragaria ananassa* fruit.

| Treatment | Dose (ml/day) | Mycelia growth |
|---|---|---|
| Cinnamon oil | 1ml (day/day) | I |
| Eucalyptus oil | 1ml (day/day) | I |
| Control | No treatment | $I_o$ |

(I-Inhibit, $I_o$- Infected)

Table-3: Observations.

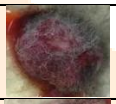
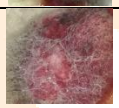
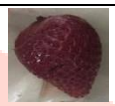
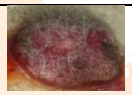
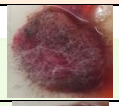
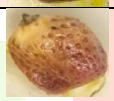
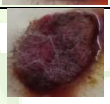
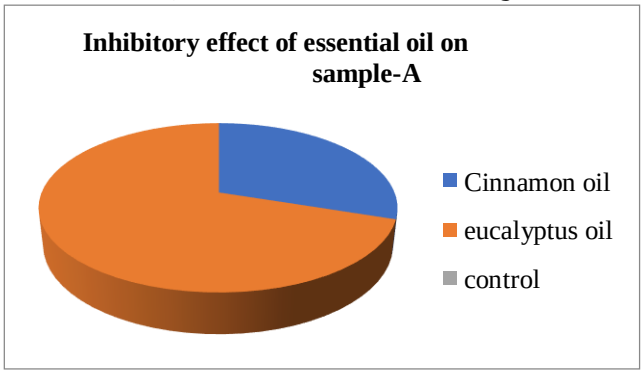
| Day | Strawberry | | |
|---|---|---|---|
| | Control | P-1 | P-2 |
| 1 | - | - | - |
| 2 | - | - | - |
| 3 |  |  |  |
| 4 |  |  |  |
| 5 |  |  |  |
| 6 |  |  |  |
| 7 |  |  |  |
| 8 |  |  |  |
| 9 |  |  |  |
| 10 |  |  |  |

Table-4: Result for sample-A

| Sample-A Strawberry) | Control | Cinnamon oil | Eucalyptus oil |
|---|---|---|---|
| Antifungal activity | No | Yes | Yes |
| Level of barrier to infection | Very less | Medium in between | Strongly highest |

Figure -1: %of inhibitory effect of essential oils on *Fragaria ananassa* fruit




**Sample-B**

According to research study for sample-B chapatti, cinnamon oil and clove oil have antifungal properties, it inhibited fungal mycelia growth. Cinnamon oil has medium in between and clove oil has strongly highest in level of barrier of fungal infection on

Chapatti. Expected ratio of cinnamon oil and clove oil (30%:70%). Absent of fungal strains and growth on P-1and P-2, and present of fungal strains and growth on $P_0$.

Table-5: To gives treatment of cinnamon oil and clove oil on chapatti.

| **Day** | **Control ($P_0$)** | **P-1** | **P-2** |
|---|---|---|---|
| **1** | - | - | - |
| **2** | - | - | - |
| **3** | - | - | - |
| **4** | - | - | - |
| **5** | - | + | + |
| **6** | - | + | + |
| **7** | - | + | + |
| **8** | - | + | + |
| **9** | - | + | + |
| **10** | - | * | * |

**[-]= no treated, [+]= treated, [*]= stable**

[$P_0$] control, [P-1] cinnamon oil, [P-2], clove oil

Table -6: Morphological and microscopically evidences.

| Day | Morphological evidences | | | | | | | | | Microscopic evidences | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | Cinnamon oil | | | Clove oil | | | Control | Cinnamon oil | Clove oil |
| | **D** | **O** | **C** | **D** | **O** | **C** | **D** | **O** | **C** | **Colonies number** | | |
| 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | + | + | + | - | + | + | - | + | - | - | - | - |
| 5 | + | + | + | - | + | + | - | + | - | 5-15 | 1-2 | 1-2 |
| 6 | + | + | + | - | + | + | - | + | - | 20-25 | * | * |
| 7 | + | + | + | - | + | + | - | + | - | 30-35 | * | * |
| 8 | + | + | + | - | + | + | - | + | - | 40-50 | * | * |
| 9 | + | + | + | + | + | + | + | + | + | 50-60 | * | * |
| 10 | + | + | + | + | + | + | + | + | + | More than 60 | * | * |

(D- Dry, O-Odour, C-color changes) **[+]** = present**, [-]** = absent, **[*]**= stable

Table-7: Inhibitory effects of essential oils on the mycelia growth on chapatti.

| **Treatment** | **Dose (ml/ spray)** | **Mycelia growth** |
|---|---|---|
| Cinnamon oil | 1ml | I |
| Clove oil | 1ml | I |
| Control | No treatment | $I_o$ |

(I-Inhibit, $I_o$- Infected)

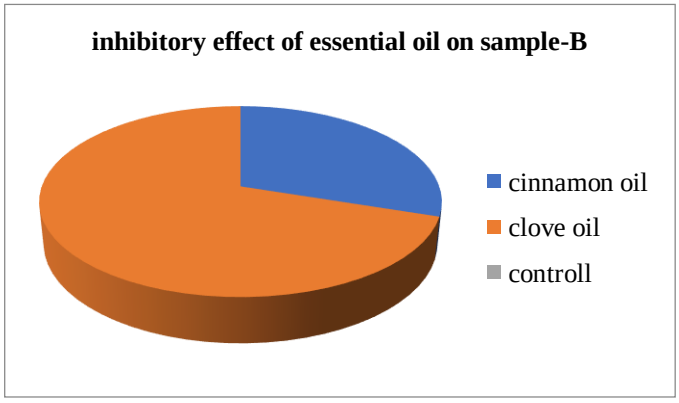
Table-8: Observation.

| Day | Chapatti | | |
|---|---|---|---|
| | Control | Cinnamon oil | Clove oil |
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  |  |  |
| 5 |  |  |  |
| 6 |  |  |  |
| 7 |  |  |  |
| 8 |  |  |  |
| 9 |  |  |  |
| 10 |  |  |  |

Table-9: Result for sample-B

| Specimen 1 (chapatti) | Control | Cinnamon oil | Clove oil |
|---|---|---|---|
| Antifungal activity | No | Yes | Yes |
| Level of barrier to infection | Very less | Medium in between | Strongly highest |

Figure -2: %of inhibitory effect of essential oils on chapatti




**Conclusion:**

According to sources, the result of this study showed that eucalyptus oil was seen to be the most active inhibitor and cinnamon oil has moderate inhibitory activity against *Rhizopus stolonifer* tested in in-vitro condition by different treatments by spraying oils. For sample-B chapatti, clove oil was seen to be the most active inhibitor and cinnamon oil was seen to be medium active inhibitor against *Rhizopus stolonifer* tested in in-vitro condition by different treatments by spraying oils. And both essential oils have

antifungal activity and it inhibited to the destruction of the Rhizopus fungal growth for sample-A *Fragaria ananassa* and sample-B chapatti. Cinnamon oil, clove oil and eucalyptus oil are the finest spice oil for phytochemicals screening, research and pharmaceutical practices. This natural essential oil has strong bioactivity mainly antifungal activity and high inhibitory effect.

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