Antifungal Activity of Plant's Essential Oils against Post Harvest Fungal Disease of Apple Fruit

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Abstract : Bioactive natural compounds are developed as alternatives to synthetic fungicides for the control of rot diseases of apple fruit. The antifungal activity of essential oils exudes from five plants, namely, *Cinnamomum tamala*, *Lantana camara*, *Ageratina adenophora*, *Citrus limetta* and *Eucalyptus citriodora* were evaluated in vitro against *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Alternaria alternata* causing postharvest rot disease in apple fruits. The pathogens were isolated from infected apple fruits collected from local markets of Kathamandu, Nepal. The essential oils were extracted through hydro-distillation process using Clevenger apparatus. The pathogenicity test was confirmed by inoculating pathogen into healthy apple fruit. The assessment of fungi toxicity was carried out by poison food technique using five different concentrations: 2.5µl/ml, 5µl/ml, 10µl/ml, 20µl/ml and 40µl/ml and controls were set to determine percentage inhibition of mycelial growth to test fungi. Among tested five essential oils, *Cinnamomum tamala* showed most effective antifungal activity against all three pathogens, which inhibited mycelium growth by 100% at 40 µl/ml concentrations. However, *Eucalyptus citriodora* showed all three pathogens inhibited mycelium growth by 65.87%, 73.17% and 86.91%, respectively at 40 µl/ml concentration.

Keywords: Fungitoxicity, hydrodistillation, pathogenicity, bioactive compounds, rot disease in apple

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Introduction

Apple has antioxidant, antimicrobial, anticancer and many other beneficial effects thus it is used in food, pharmaceutical and cosmetic industries (Kalinowska et al. 2014). The major post-harvest disease caused by fungi and bacteria in fruits and infection occurs during pre-harvest stage at field or after harvesting mainly during storage and transportation (Singh and Sharma 2018). Rot diseases in apple are economically significant which is caused by several fungi with worldwide distribution like brown rot by *Monilinia fructigena*, grey mould by *Botrytis cinerea, Fusarium* rot by *Fusarium* species, bitter rot by *Colletotrichum* spp., bull's eye rot by *Neofabraea* spp., and blue mold decay caused by *Penicillium expansum* (Grantina-Ievina 2015). The large number of plant pathogen belongs to division Deuteromycota and Ascomycota (Moore 1996). Bitter rot is one of the serious post-harvest apple disease caused by *Collitotrichum gloeosporiode*, its sexual stage *Glomerella cingulata, Collitotrichum acutatum* and *Collitrotrichum* fragariae (Velho et al. 2014). Some species of fungal pathogens are responsible for pre-harvest as well as post-harvest diseases such *Fusarium, Rhizoctonia, Phytophthora* and *Pythium* (Tewoldemedhin et al. 2011). The post-harvest diseases in harvest fruits cause large economic losses, and chemical fungicides are the main agents to control the disease (Nunes 2012).

The chemical fungicides are responsible to cause different diseases, such as acute and chronic neurotoxicity, lung damage, chemical burns, infant methemoglobinemia, immunologic abnormalities, adverse reproductive and varieties of cancers (Weisenburger 1993). Biologically active natural products have the potential to replace synthetic fungicides, and exploitation of some natural products, such as flavour compounds, acetic acid, jasmonates, glucosinolates, propolis, fusapyrone and deoxyfusapyrone, chitosan, essential oils and plant extracts for the management of fungal rotting of fruits (Tripathi and Dubey 2004). Essential oils are rich source of biologically active compound with antifungal effects against both pathogen and spoilage fungi (Piccaglia et al. 1993). Plants contain thousands of the constituents and are valuable sources of new and biologically anti-microbial property, such as tannin, allicin, essential oils, etc. (Gurjar et al. 2012). Essential oils consist of volatile molecules, such as terpenoids, terpenes and phenol derived aliphatic and aromatic compounds, which are antiviral, bactericidal and fungicidal. Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential diseases control agents (Tripathi et al. 2008). Essential oils have application in folk medicine, food preservation, and as feed additive (Kurade et al. 2010). The present study aims to isolate rot fungi and test *in-vitro* antifungal

activity of some plant's essential oils against post-harvest pathogens of apple fruit.

Materials and Method

Essential Oil Isolation

Five different test plants species were collected from Kathmandu, Nepal, namely, *Eucalyptus citriodora*, *Cinnamomum tamala*, *Lantana camara*, *Ageratina adenophora* and *Citrus limetta*.

The collected leaves of plants were shade dried for 24 hours at normal temperature. Then the leaf sample was taken for hydro-distillation for 6-8 hours in Clevenger's apparatus. Two layers - upper aromatic layers of essential oil and lower colorless liquid were observed. The aromatic layer was collected and dehydrated over sodium sulphate and stored at low temperature (4-10°C) (Rao and Srivastava 1994).

Isolation and Identification of Fungi Pathogens

The disease infested apple fruits were collected from local market in Kathmandu during winter season. The fruits were surface sterilized using 70% ethyl alcohol. The small pieces of infected parts from the infected apples were inoculated in the petri-plate containing PDA media and incubated at 25±2°C temperature for a week. After a week, the mycelial growth of fungal colony was observed. Three fungal pathogens, viz. *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Alternaria alternata* were identified by observing under microscope, and compared their characters by using standard literatures (Barnett and Hunter 1972; Ellis 1971; Watanabe 2010.)

Pathogenicity Test

To confirm the pathogenicity of fungi, healthy apple fruits were washed with distilled water and surface sterilized with 70% ethyl alcohol for one minute, and ultimately, washed with sterilized water. Some fruits were wounded with the help of a sterilized cork borer. The fungus inoculums were inoculated in the wounded part and wrapped with clean polythene bag, and were kept at 25±2°C for a week. After a week, characteristics symptoms were produced which were found to be similar as that of the previous one. The fungi pathogens were isolated, studied under microscope and compared with previously isolated fungus.

Assessment of Fungi-toxicity

Fungi-toxicity of essential oils was assessed by poisoned food technique. The media was poisoned with oil and extracted which showed the antifungal effect.

0.5 ml of each concentration of essential oil was aseptically poured into sterilized petri-plates followed by addition of 9.5 ml of PDA. When the media got solidification, 4 mm diameter of test fungus was inoculated by upside down at the center aseptically. The positive and negative controls were maintained. In positive control set, 60% acetone was used whereas in negative control set no essential oil was used. The cultured petri-plates were incubated for a week at 25±2°C temperature. Finally, average diameter was calculated on the seventh day of incubation, and percentage of mycelial growth inhibition was deliberated.

Calculation of Mycelial Growth Inhibition

The fungi-toxicity of distinct concentrations of five different essential oils was studied in terms of percentage inhibition of mycelial growth of test fungus (Rao and Srivastava 1994) as follows:

Percentage inhibition = $(G_c-G_t)/G_c \times 100$

Where, G_c= growth of mycelial colony after incubation in control set (diameter of colony in control set - diameter of inoculum disc)

G_t= growth of mycelial colony after incubation period in treatment set (diameter of colony in treatment set- diameter of inoculum disc.)

Data Analysis

For the data analysis, Excel 2013 was used for entering data, drawing charts and graphs. The data was analyzed with the help of ANOVA using SPSS version 20 and Bonferroni test was done for comparing the differences, post- Hoc.

Results and Discussion

Results

Mycelial Growth of Test Fungi by Different Essential Oils

The essential oil of *Cinnamomum tamala* showed antifungal effect over the three tested fungi. It showed significant ($p<0.05$) result against *Alternaria alternata* followed by *Fusarium oxysporum* and then *Colletotrichum gloeosporioides*. At 2.5, 5, 10, 20 µl/ml oil concentration the mycelial growth of *Colletotrichum gloeosporioides* was relatively higher than that of *Fusarium oxysporum* and *Alternaria alternata*. At 40 µl/ml oil concentration all fungi were completely inhibited (Figure 1).

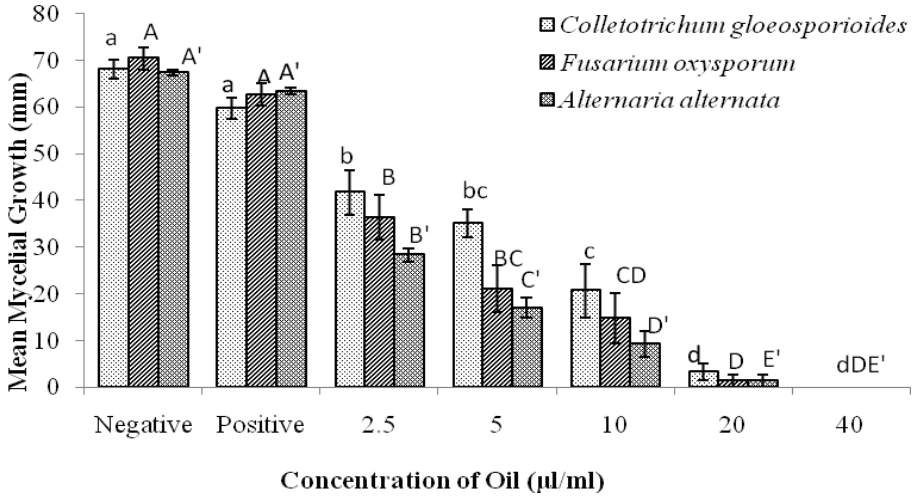

Figure 1 : Antifungal Properties of *Cinnamomum tamala* against *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Alternaria alternata*

The essential oil of *Lantana camara* showed the best antifungal activity against the *Alternaria alternata* among tested fungi followed by *Fusarium oxysporum*. The essential oil of *Lantana camara* showed significant (P<0.05) result against *Fusarium oxysporum* and *Alternaria alternata* in comparison to *Colletotrichum gloeosporioides* (Figure 2).

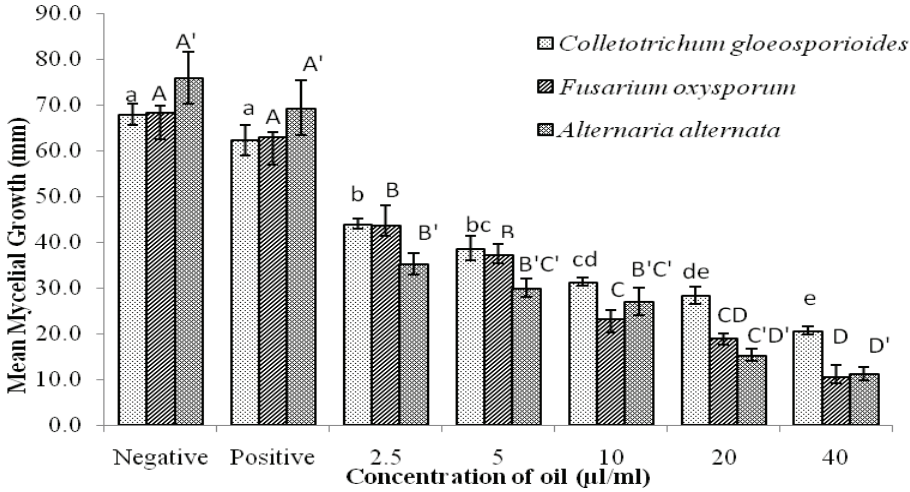

Figure 2 : Antifungal Properties of *Lantana camara* against *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Alternaria alternate*

The essential oil of *Citrus limetta* showed significant ($p<0.05$) antifungal activity against the test fungi. It showed the best effect against *Alternaria alternata* and *Fusarium oxysporum* followed by *Colletotrichum gloeosporioides* at 40 µl/ml. At all concentration growth of *Colletotrichum gloeosporioides* was high in comparison to *Fusarium oxysporum* and *Alternaria alternata* (Figure 3).

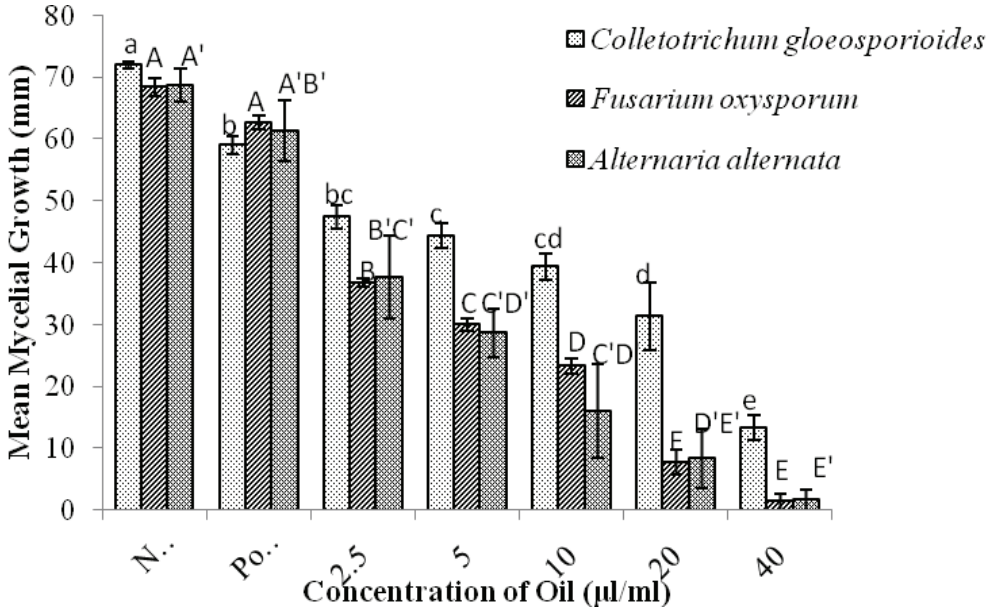

Figure 3 : Antifungal Properties of *Citrus limetta* against *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Alternaria alternata*

The *Ageratina adenophora* oil showed significant ($p<0.05$) activity against *Alternaria alternata* with all concentrations whereas it was less effective against *Colletotrichum gloeosporioides* in comparison to *Fusarium oxysporum* and *Alternaria alternata*. All concentrations showed effective results against *Alternaria alternata* followed by *Fusarium oxysporum* and then *Colletotrichum gloeosporioides* (Figure 4).

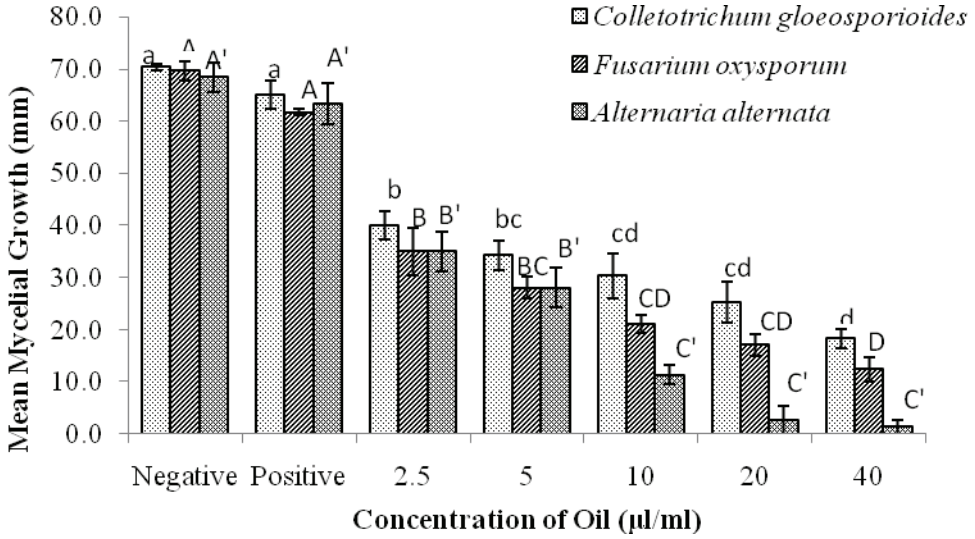

Figure 4 : Antifungal Properties of *Ageratina adenophora* against *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Alternaria alternata*

The *Eucalyptus citriodora* oil showed significant antifungal activity over all the test fungi. It showed significant ($p<0.05$) results against the *Alternaria alternata, Colletotrichum gloeosporioides* and *Fusarium oxysporum*. At 2.5, 5, 10, 20 and 40 µl/ml concentrations the mycelial growth of *Colletotrichum gloeosporioides* and *Fusarium oxysporum* were significantly higher than that of *Alternaria alternata*. The inhibition effect on *Fusarium oxysporum* was higher than that of *Colletotrichum gloeosporioides* at 5, 10, 20 and 40 µl/ml concentrations (Figure 5).

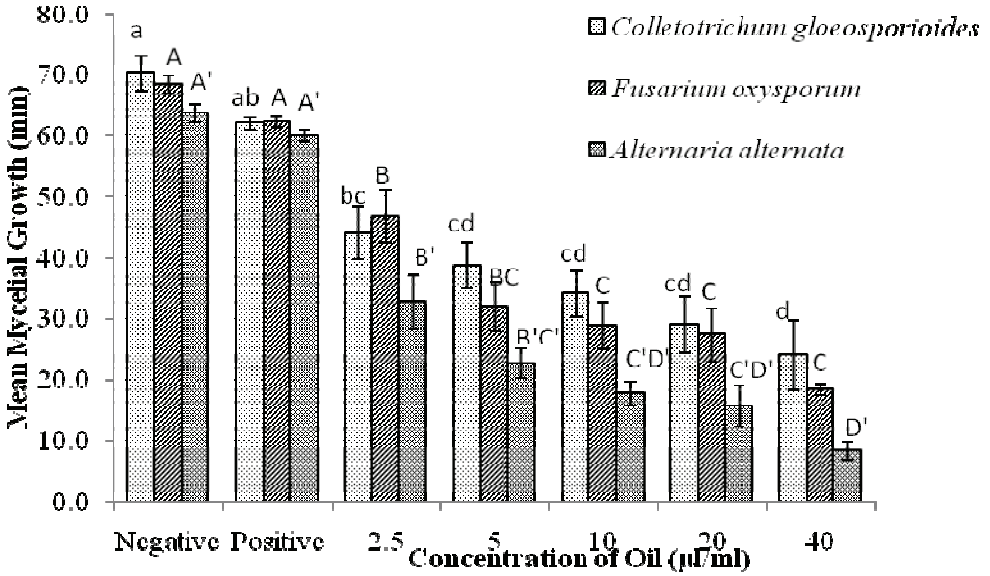

Figure 5 : Antifungal Properties of *Eucalyptus citriodora* against *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Alternaria alternata*

Comparative Inhibition (percentage) of Test Fungi by Essential Oils

All essential oils showed the antifungal activity against *Colletotrichum gloeosporioides*. At 40 µl/ml concentration, *Cinnamomum tamala* showed the maximum inhibition (100%) followed by *Citrus limetta* (81.46%), *Ageratina adenophora* (73.93%), *Lantana camara* (69.61%) and *Eucalyptus citriodora* (65.87%). Among all essential oils, *Cinnamomum tamala* oil showed the most effective antifungal activity against *Colletotrichum gloeosporioides.* At 20 µl/ml concentration, *Cinnamomum tamala* showed the highest inhibition (95.10%) followed by *Ageratina adenophora* (63.98%), *Eucalyptus citriodora* (58.76%), *Lantana camara* (58.33%) and *Citrus limetta* (56.48%). At 10 µl/ml concentration, *Cinnamomum tamala* showed maximum inhibition (69.61%) followed by *Ageratina adenophora* (56.87%), *Lantana camara* (53.92%), *Eucalyptus citriodora* (51.65%) and *Citrus limetta* (45.83%). At 5 µl/ml concentration, *Ageratina adenophora* showed the highest inhibition (51.18%) followed by *Cinnamomum tamala* (48.52%), *Eucalyptus citriodora* (45.03%), *Lantana camara* (43.14%) and *Citrus limetta* (31.03%). Again at 2.5 µl/ml concentration, *Ageratina adenophora* showed maximum inhibition (43.12%) followed by *Cinnamomum tamala* (38.72%), *Eucalyptus citriodora* (37.44%), *Lantana camara* (35.29%) and *Citrus limetta* (25%) (Figure 6).

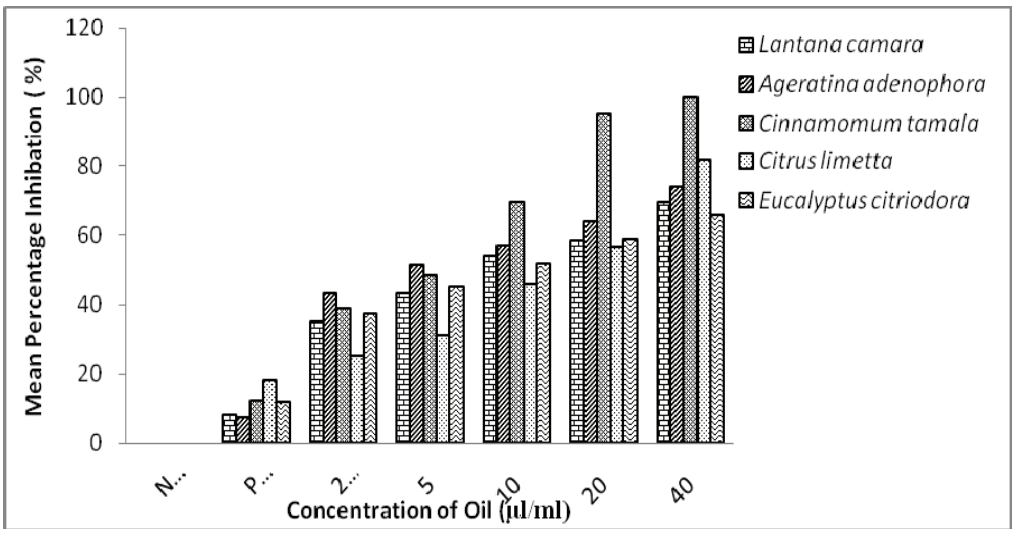

Figure 6 : Fungi-toxicities of Different Essential Oils at Different Concentrations against *Colletotrichum gloeosporioides*

Among all essential oils, *Cinnamomum tamala* oil showed maximum inhibition against *Fusarium oxysporum*. At 40 µl/ml concentration, *Cinnamomum tamala* showed the peak inhibition (100%) followed by *Citrus limetta* (98.05%), *Lantana camara* (84.39%), *Ageratina adenophora* (82.29%) and *Eucalyptus citriodora* (73.17%). At 20 µl/ml concentration, *Cinnamomum tamala* showed the highest inhibition (98.10%) followed by *Citrus limetta* (88.78%), *Ageratina adenophora* (75.59%), *Lantana camara* (72.19%) and *Eucalyptus citriodora* (60%). At 10 µl/ml concentration, *Cinnamomum tamala* showed the highest inhibition (79.15%) followed by *Ageratina adenophora* (69.85%), *Citrus limetta* (65.88%), *Lantana camara* (65.85%) and *Eucalyptus citriodora* (58.05%). At 5 µl/ml concentration, *Cinnamomum tamala* showed the highest inhibition (70.14%) followed by *Citrus limetta* (60.98%), *Ageratina adenophora* (59.80%), *Eucalyptus citriodora* (53.16%) and *Lantana camara* (45.36%). At 2.5 µl/ml concentration, *Ageratina adenophora* showed the maximum inhibition (49.75%) followed by *Cinnamomum tamala* (48.43%), *Citrus limetta* (46.34%), *Lantana camara* (36.10%) and *Eucalyptus citriodora* (31.7%) (Figure 7).

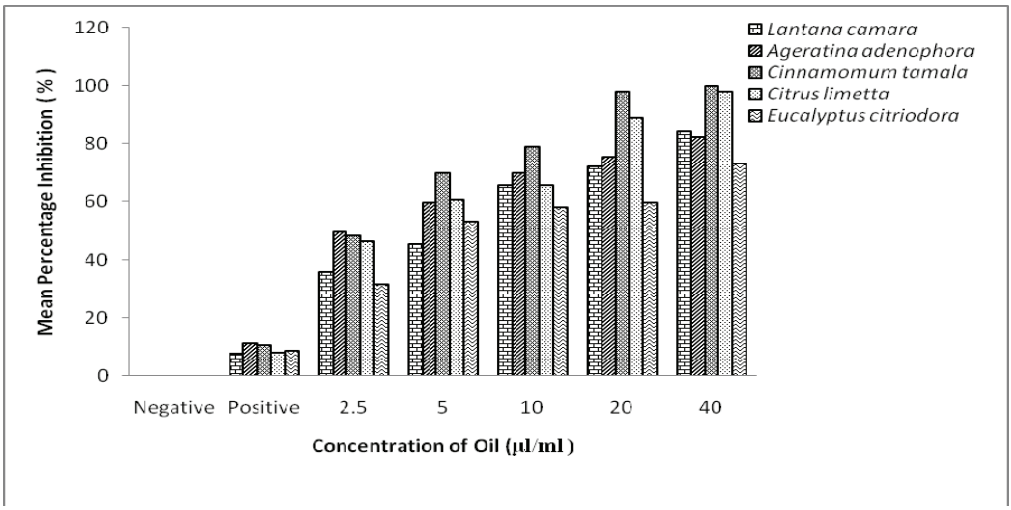

Figure 7 : Fungi-toxicities of Different Essential Oils at Different Concentrations against *Fusarium oxysporum*

At 40 µl/ml concentration, *Cinnamomum tamala* showed the highest inhibition (100%) followed by *Ageratina adenophora* (98.05%), *Citrus limetta* (97.58%), *Eucalyptus citriodora* (86.91%) and *Lantana camara* (85.09%). At 20 µl/ml concentration, *Cinnamomum tamala* showed the highest inhibition (98.02%) followed by *Ageratina adenophora* (96.10%), *Citrus limetta* (87.86%), *Lantana camara* (79.82%) and *Eucalyptus citriodora* (75.40%). At 10 µl/ml concentration, *Cinnamomum tamala* showed the maximum inhibition (86.14%) followed by *Ageratina adenophora* (83.41%), *Citrus limetta* (76.69%), *Eucalyptus citriodora* (72.25%) and *Lantana camara* (64.47%). At 5 µl/ml concentration, *Cinnamomum tamala* showed the highest inhibition (74.75%) followed by *Citrus limetta* (65.54%), *Eucalyptus citriodora* (64.40%), *Lantana camara* (60.52%) and *Ageratina adenophora* (59.02%). At 2.5 µl/ml concentration, *Cinnamomum tamala* showed the maximum inhibition (57.92%) followed by *Lantana camara* (53.51%), *Ageratina adenophora* (48.77%), *Eucalyptus citriodora* (48.69%) *and Citrus limetta* (45.15%) (Figure 8).

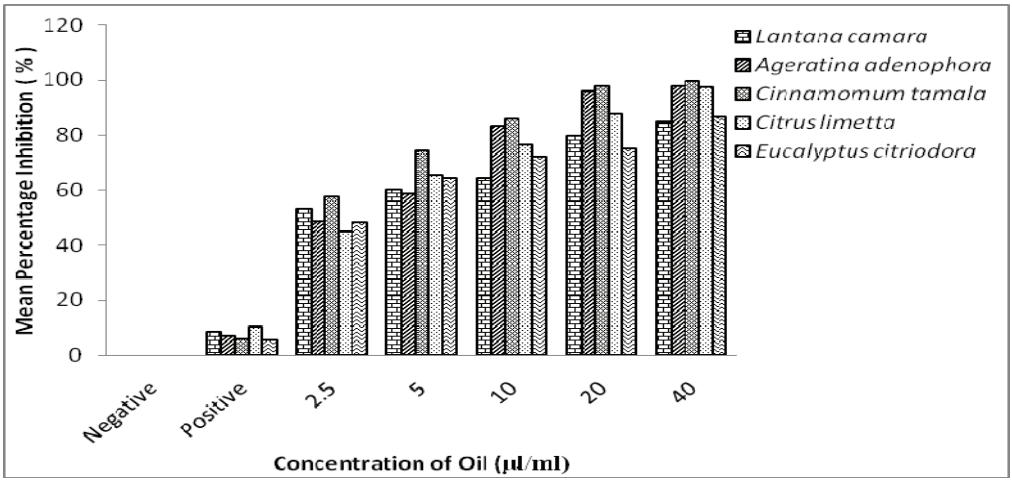

Figure 8 : Fungi-toxicities of Different Essential Oils at Different Concentrations against *Alternaria alternata*

Discussion

The study focused on fungal pathogen responsible for causing post-harvest diseases, and identified three fungal rot diseases caused by *Fusarium oxysporum, Colletotrichum gloeosporioides* and *Alternaria alternate.* The study also focused on their isolation, pathogenicity test, and examined antifungal activities of five plants essential oils against these three fungi. The *Colletotrichum gloeosporioides* was found as a post-harvest pathogen, which caused bitter rot disease in apple fruits. These results are supported by various researches (Grammen et al. 2019; Moreira et al. 2019 and Velho et al. 2014). *Fusarium* rot and *Alternaria* rot caused by *Fusarium oxysporum* and *Alternaria alternata,* respectively were found during the study. The *Fusarium oxysporum* was responsible for post-harvest apple fruit decay (Chutia et al. 2009; Tewoldemedhin et al. 2011). The *Alternaria alternata* caused fruit rot in apple fruits (Chutia et al. 2009; Gur et al. 2018). The essential oil of *Cinnamomum tamala* showed the most effective anti-fungal activity against three test fungi, viz. *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Alternaria alternata*. This result is supported by Pawar and Thaker (2007) and Pandey et al. (2012) who evaluated anti-*Fusarium oxysporum,* anti-*Alternaria* and anti-fungal activity against food spoilage fungi. Yulia et al. (2006) reported that the essential oils of *Cinnamomum* showed effective anti-fungal effect against *Colletotrichum gloeosporioides* and completely inhibited its spore germination. Phenylpropanoids are the main component in *Cinnamomum tamala* (Kumar et al. 2012). These chemical components of *Cinnamomum tamala* oil may show the effective anti-fungal properties than other four plant essential oils. Essential oil of *Lantana camara* showed effective anti-fungal activity against *Alternaria alternata*

followed by *Fusarium oxysporum* and *Colletotrichum gloeosporioides* at different concentration which is supported by Bhattarai, Jha (2016), Deena, Thoppil (2000) and Saraf et al. (2011) who showed that essential oil of *Lantana camara* remarkably inhibited the growth of *Colletotrichum, Fusarium* and *Alternaria* spp. This is due to the presence of different bioactive constituents such as Eicosane, squalene, β-ionone, caryophyllene oxide, β-caryophyllene, hexanoic acid and tiglic acid, a mixture of lantanilic, camaric acids, and lantadene (Delgado-Altamirano et al. 2019). The *Ageratina adenophora* oil at different concentrations revealed against the *Alternaria alternata, Fusarium oxysporum* and *Colletotrichum gloeosporioides*. This outcome is supported by Zheng et al. (2018) in which *Ageratina adenophora* oil showed strong anti-fungal activity against *Alternaria alternata, Fusarium oxysporum*, *Colletotrichum gloeosporioides* and *Rhizoctonia solani*. The anti-fungal activity of *Ageratina adenophora* may be due to the presence of different types of components, such as benzofuran derivatives (Zheng et al. 2018); pinene, camphene, verbenene, menthene, dehydro-1, 8-cineole, limonene, ocimen. It is evident from this study that *Citrus limetta* has significant fungi-toxic properties on all three tested fungi. These results have been supported by Chutia et al. (2009) and Muzna et al. (2014). The *Eucalyptus citriodora* oil showed anti-fungal activity at different concentrations against *Alternaria alternata*, *Fusarium oxysporum* and *Colletotrichum gloeosporioides*. Ramezani et al. (2002) found that essential oil of *Eucalyptus citriodora* possessed strong fungicidal activity against *Colletotrichum lindemuthianum, Alternaria triticina* and *Fusarium oxysporum*. Lee et al. (2007) suggested that *Eucalyptus citriodora* oil has a potential as anti-fungal preservatives for the control of storage diseases such as *Colletotrichum gloeosporioides, Fusarium oxysporum and Rhizoctonia solani.* The antifungal activity of *Eucalyptus citriodora* oil may be due to the presence of different types of components such as α-Pinene, Myrcene, Limone, 1,8-Cineole and Linalool (Batish et al. 2006).

Conclusion

From the results it can be concluded that the essential oils of *Eucalyptus citriodora*, *Cinnamomum tamala*, *Lantana camara*, *Ageratina adenophora* and *Citrus limetta* should be used for the control of post-harvest apple fruit diseases. Especially three rot diseases viz. Bitter rot, *Fusarium* rot and *Alternaria* rot can be effectively controlled by these essential oils. Different types of selected plant essential oils showed significant control of these fungi among which *Cinnamomum tamala* showed the most effective anti-fungal activity against the test fungi.

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