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Antifungal Activity of Some Essential oil of Aromatic Plants Against Post Harvest Disease of Grapes (*Vitis vinifera*)

[1]Urmila Bista, [2]Dinesh Chaudhary, [3]Dhan Bahadur Bist, [4]Anand Prakash Joshi

[1]Aishwarya Multiple Campus Dhangadhi Kailali, Nepal
[2]Aishwarya Multiple campus Dhangadhi kailali, Nepal
[3]Ministry of Industry, Tourism, Forests and Environment, Sudurpaschim Province, Dhangadhi, Nepal
[4]Aishwarya Multiple campus Dhangadhi kailali, Nepal
Corresponding author email: bhattaraghu2029@gmail.com

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Abstract

As being highly perishable fruit Grapes quality and shelf life is affected by various factors during postharvest among them common loss in caused by fungi. An in-vivo study was carried out to test possibility of the use of some plant essential oil of *Cinnamomum tamala, Cymbopogon flaxuosus, Cinnamomum tenuiple, Mentha spicata* and *Eucalyptus citriodora* to reduce post-harvest loss induced by *fungi. Aspergillus niger* was most frequent fungal pathogen isolated from grapes collected from local markets of Dhangadhi city. Pathogenity test was confirmed by inoculating the pathogen in healthy grapes. Essential oils (EOs) of five essential oil extracted by hydro-distillation using Clevenger oil extracting apparatus. Using various concentration (40, 20, 10, 5 and 2.5 µl/ml) of Eos the fungi was treated by using poisoned food technique. Significant ($p<0.05$) inhibition of mycelial growth was found in fungi by all EOs. Among them, strong inhibitory action of *Mentha spicata* oil was recorded followed by *Cinnamomum tenuipile, Cinnamomum tamala, Cymbopogon flexuous* and *Eucalyptus citriodora*. These results suggest that EOs of five tested plants could be a good alternative to control fungal contaminants and extend the shelf life of grapes in postharvest conditions.

Key words: *In-vitro, mycelial growth, hydro-distillation, inhibition, poisoned food technique.*

Introduction

Grape (*Vitis vinifera)* is one of the very popular fruit crop in the world and it can be cultivated in tropical, subtropical and temperate region. It is one of the earliest fruit known since civilization, popular for its nourishing, delicious and refreshing fruits (Jegadeeswari et al., 2010). It can be consumed in different forms like fresh fruit, drinks as juices, wines, beverages and medicines, and stored as raisin so, has its identity as worldly fruit. (Dahal et al., 2017) In Nepal, grape cultivation started more than 70 years ago at the time of Rana regime (Dahal et al., 2017). Grape cultivation covered an area of about 20 ha with total fresh grapes production of around 76 tonnes annually in Nepal. (Atreya et al. ,2015). Quantitative and qualitative growth in demand of wine shows a very promising scope for local farmers (Acharya and Yang, 2015). In recent years, commercial vineyards are establishing which will certainly increase area and productivity of grapevine in Nepal. In Nepal, various table grape cultivars such as,

'Thompson seedless', 'Perlette', 'Himrod', 'Beauty Seedless', 'Steuben', 'Kyoho', 'Black Olympia' etc. were subsequently introduced from Japan and India (Joshi, 1986).

Postharvest loss refers to the deterioration of food quantity and quality after harvest. this condition is greater for perishable crops in less developed countries (Hodges, 2011). Quantitative loss is more of an issue in these countries than qualitative loss (Humble and Reneby, 2014). The FAO estimates that 40 to 50 percent of the world's horticulture crop yield is lost in postharvest conditions (FAO, 2018). Nepal produces huge number of fruits and vegetables but still post-harvest losses in them is high ranging from 20-50% (Bhattarai et al., 2017). It is estimated that about one-third of the fresh produce harvested worldwide is lost at some point between harvest and consumption (Kitinoja and Kader, 2002)

Essential oils are volatile, natural, complex compound. It has characterized by a strong order. Natural biologically active compounds extracted from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and it has rich source of disease control agents (Tripathi et al., 2008). Essential oils has antifungal effects for both pathogens and spoilage fungi and are a rich source of bio-active compounds (Daferera et al., 2003; Piccaglia et al., 1993). These oils are also eco-friendly as it is biodegradable and non-toxic (Adebayo et al., 2013). This study aimed to evaluate the whether the oils of the aromatic plants were significantly effective to control postharvest rots in grapes and used as alternative method to synthetic pesticides in-vitro conditions.

Materials and methods

Isolation and identification of post-harvest mycobial contaminants:

Infected grapes were collected from major market of Dhangadhi. Firstly, infected portion is surface sterilized using 70% ethanol and then by distilled water. They were transferred to the petriplate containing PDA medium and was incubated at 25± 2ºC for one week. After one week, the mycelial growth of fungal colony was observed and were identified based on micromorphological and cultural characteristics by following standard literature (Barnett and Hunter, 1972; Watanabe, 2010). The fungi *Aspergillus niger* were most abundant were selected as test fungi to evaluate the extent to which EOs were inhibitory to fungi.

Extraction and source of plant essential oil:

Leaves of *Cinnamomum tamala,* and *Eucalyptus citriodora* were collected from Jai, Dhangadhi and *Cymbopogon flaxuosus, Cinnamomum tenuiple, Mentha spicata* were collected from Dewahariyan Botanical garden, Dhangadhi. 100 gm of shade dried leaves were taken and it was surface sterilized with 70% ethanol and washed thoroughly with distilled water. Then the leaf sample was taken for hydro-distillation for 6-8 hours in Clevenger's apparatus containing 1000ml water. After that processed hydro-distillation oil was collected and dehydrated over anhydrous sodium sulphate and stored at low temperature (4-10ºc) (Rao and Srivastava, 1994).

Antifungal effect of plant essential oil:

The poisoned food technique was used to evaluate the antifungal activity of EOs on the in vitro growth and development of fungal contaminants (Grover and Moore, 1962). Five different concentrations of

EOs viz, 2.5, 5, 10, 20 and 40 µl/ml were prepared with 80% acetone. First, 1 ml of each concentration of essential oil was added to sterilized petriplates, and then 9 ml of melted PDA was added. The test fungus of 4 mm diameter, which was growing aggressively, was then inoculated into each petriplates. Positive and Negative control were maintained in this regard. In positive control set, 80% Acetone was used, whereas in negative control no essential oil was used. After a week, observations were made. Five replications were maintained and fungi toxicity of Essential oils was assessed by measuring the percentage of mycelial growth that was inhibited, which was computed as;

Inhibition of Mycelial Growth (%) = [(Mc–Mt)/ Mc] × 100

[Where; Mc= mean colony diameter in control sets and Mt= mean colony diameter in treatment sets].

Data analysis

Data entry, chart diagram and graph was done by using Microsoft Excel 2017. The data were analyzed by ANOVA by using SPSS v.20 (Confidence level 95%, 5%significance level). To compare the differences, Post - Hoc; Bonferroni test was done comparing differences

Result

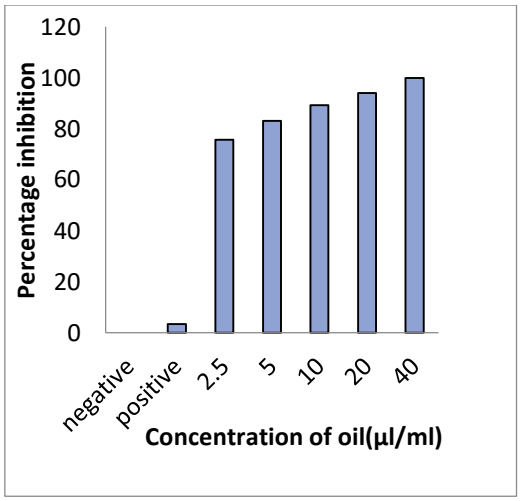
Various fungi are observed, among them most common is Aspergillus niger was identified. After inoculation to healthy grapes, *Aspergillus niger* first appears at first as a water-soaked spots injured fruits. The spots enlarge to about 2-3cm in diameter within 2 days and the center turns black due to the presence of profuse conidial heads. The fruit gets decayed within 5-7 days with profuse exudation of the sap.

Percentage inhibition of test fungi by different essential oil

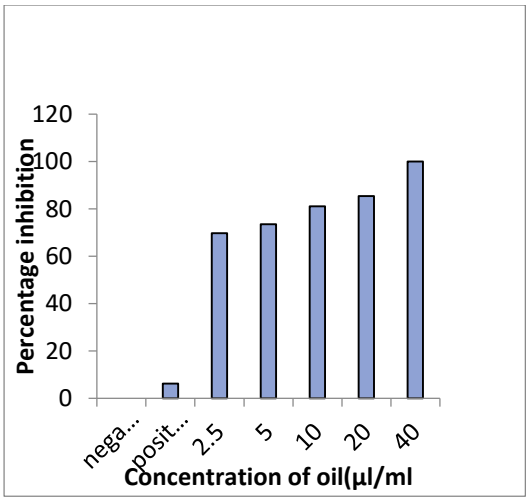
Both the negative control (without essential oil) and positive control (80% acetone) doesn't showed impact on mycelial growth. At the concentration of 2.5 µl/ml, 5 µl/ml, 10 µl/ml, 20 µl/ml and 40 µl/ml, the inhibition percentage of *Mentha spicata* were 71.29%, 78.71%, 86.14%, 90.58%, and 100% respectively (figure 1A). Similarly, at the concentration 2.5 µl/ml, 5 µl/ml, 10 µl/ml, 20 µl/ml and 40 µl/ml, the inhibition percentage of *Cymbopogon flaxuosus* were 69.93%, 73.64%, 81.01%, 85.47%, 100% respectively(figure1B). Again at the concentration of 2.5 µl/ml, 5 µl/ml, 10 µl/ml, 20 µl/ml and 40 µl/ml, the inhibition percentage of *Cinnamomum tenuipile* were 71%, 75.74%, 83.75%, 88.25, and 100% respectively(fig*ure1C).* At the concentration of 2.5 µl/ml, 5 µl/ml, 10 µl/ml, 20 µl/ml and 40 µl/ml, the inhibition percentage of *Cinnamomum tamala* were 71%, 75.74%, 83.75%, 88.25%, and 100% respectively (Figure1D). At the concentration of 2.5 µl/ml, 5 µl/ml, 10 µl/ml, 20 µl/ml and 40 µl/ml, the inhibition percentage of *Eucalyptus citriodara* were measured at 66.83%, 72.48%, 80.59%, 85.01 %,99.01 respectively (Figure 1E).

All the plants essential oil showed in all concentration significant impact on the growth of mycelial growth of Aspergillus niger. The essential oil of Mentha spicata showed the maximum inhibition of mycelial growth of Aspergillus niger followed by Cinnamomum tenuipile, Cinnamomum tamala, Cymbopogon flexuosus whereas oil of Eucalyptus citriodora showed the minimum (figure 2).

Two way ANOVA (table. 2) showed there was significant difference on different types of oil and different concentrationon treatment with Aspergillus niger at 0.05 level of significance and could be well described that various plant essential oils and their graded concentration level are significantly effective on controlling the antifungal activities and giving the positive significance in controlling the Aspergillus rot in grapes. Acording to Bonferroni test all the essential oil are significantly different from each other ($P<0.005$) while oil of *Cinnamomum tamala* and *Cymbopogon flexuosus* are not significantly different($p>0.05$)(table 1)



A



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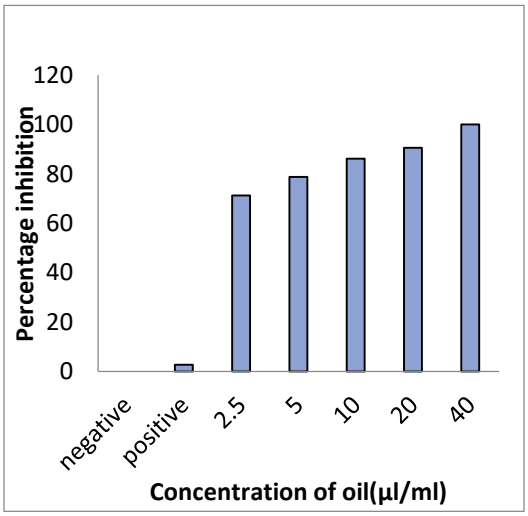

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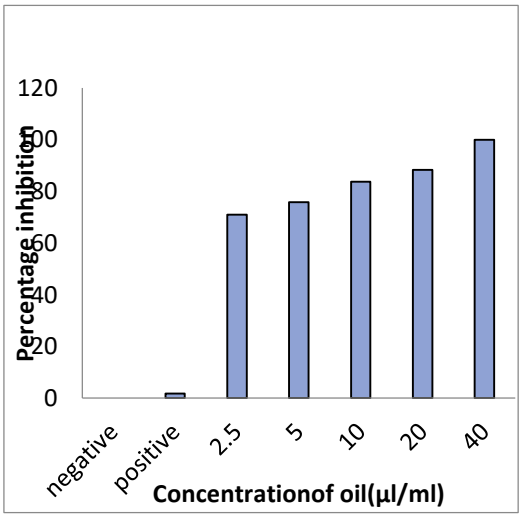

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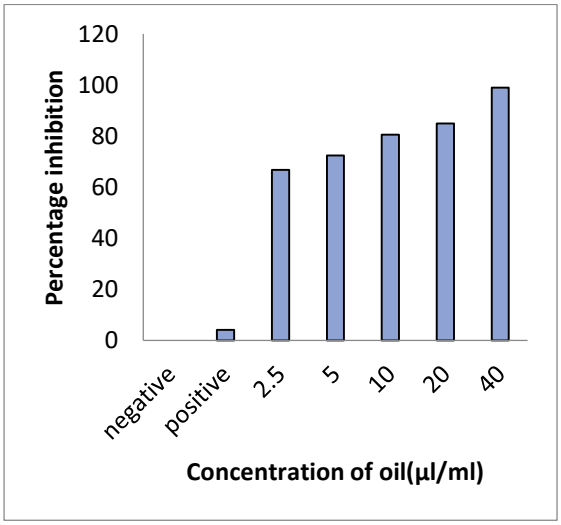

E

Figure 1. Antifungal activity of (A) *Mentha spicata* (B) *Cymbopogon flexuosus* (C) *Cinnamomum tenuipile* (D) *Cinnamomum tamala* and (E *Eucalyptus citriodara* against *Aspergillus niger*

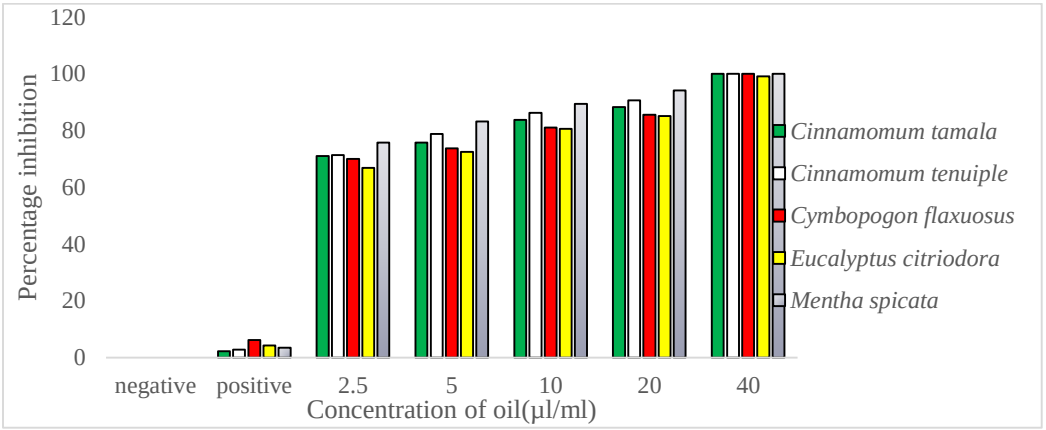

Figure 2:Fungitoxicities of different essential oils at different concentrations against *Aspergillus niger*.

Figure 3:Grapes infected with Aspergillus niger

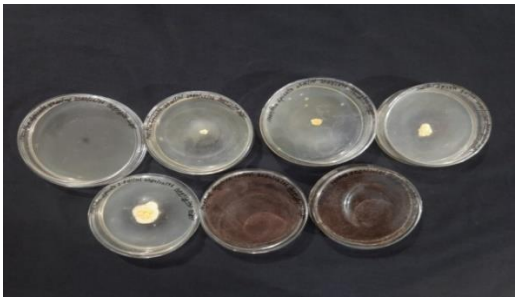

Figure 4:Antifungal activity of *Mentha spicata* gainst *Aspergilus niger*

Discussions

In the present study we found Aspergillus niger was most frequent fungal disease in grapes. Dos santos et a.,2012, de soudu et. al.,2013 reported grapes are susceptible to fungal infection especially from *Aspergilus niger* which is one of major causes of postharvest loss in table grapes. This can be concluding that *Aspergilus niger* play major role in postharvest loss in grapes.

Table 1: Bonferroni test applied to compare different plant essential oil

Plant oil		sig
Cinnamomum tamala	*Cinnamomum tenuiple*	0.029
	Cymbopogon flaxuosus	.877
	Eucalyptus citriodora	.000
	Mentha spicata	.000
Cinnamomum tenuiple	*Cinnamomum tamala*	.029
	Cymbopogon flaxuosus	.000
	Eucalyptus citriodora	.000
	Mentha spicata	.000
Cymbopogon flaxuosus	*Cinnamomum tamala*	.877
	Cinnamomum tenuiple	.000
	Eucalyptus citriodora	.043
	Mentha spicata	.000
Eucalyptus citriodora	*Cinnamomum tamala*	.000
	Cinnamomum tenuiple	.000
	Cymbopogon flaxuosus	.043

Mentha spicata	*Mentha spicata*	.000
	Cinnamomum tamala	.000
	Cinnamomum tenuiple	.000
	Cymbopogon flaxuosus	.000
	Eucalyptus citriodora	.000

In this study, the most active oil for reducing Aspergilus niger was oil of Mentha spicata in all tested concentration. This may be due to presence of different bioactive constituents characterized by Methone, Carvone, Limonene, 1,8-Cineole and ß-myrecene in mentha (Sokovie et al.,2009). The antifungal activity of *Cinnamomum tenuipile* oil may be due to presence of Eucalyptol (24.17%), Methyl cinnamate (52.18), Camphor (19.57) (Bhatta et al.,2019). the fungitoxicity of *Cinnamomum tamala* oil may be due to bioactive compound eugenol (65-92%) , β-caryophyllene, linalool, safrole, cinnamic aldehyde and cinnamic acetate (Senanayake and Wijesekera, 1989). Similarly, Myrcene, α-citral and β- citral (Onawunmi et al., 1984), Citrol, geraniol and geranyl acetate (Gupta et al., 2015) may be chemical constituent responsible for antifungal property of *Cymbopogon flexuosus.* the antifungal activity may be due to citronella and citronellal as main component from leaf of Eucalyptus citriodara (Su et al.,2006).

Table 2: Two way ANOVA test for the analysis of anti-fungal activities and their responses

Source	Test applied	df	Sig.
Plant oil	Two Way ANOVA	4	<.001
Concentration		6	<.001
Plant oil * Concentration		24	<.001

Conclusion

Plant essential oil used in present research are effective to manage the post harvest disease of grapes fruit rot caused by Aspergilus niger. All oil *Mentha spicata, Cinnamomum tenuipile, Cinnamomum tamala , Cymbopogon flexuous* and *Eucalyptus citriodora* showed significant impact on mycelial growth in vitro. Among them, Mentha spicata oil showed maximum inhibition. Therefore, the application of essential oil to control the grapes rot in post- harvest is better than the use of synthetic fungicides. So, futher research could be carried out in vi vivo condition to see its impact.

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