Biological Forum – An International Journal **8**(2): 259-263(2016)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Antifungal Effects of some Plant Essential Oils against *Alternaria alternata* (Fr.) Keissl. and *Aspergillus niger* van Tiegh. from Grapes

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(Received 20 August 2016, Accepted 23 September, 2016)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The essential oil of six plants viz. *Murraya koenigii* (L.) Spreng. *Eucalyptus citriodora* Hook. *Artemisia indica* Willd. *Cinnamomum camphora* (L.) J. Presl., *Cinnamomum tamala* (Buch.-Ham.) Nees and Eberm and *Lantana camara* L. were assessed in vitro for their antifungal activity against *Alternaria alternata* (Fr.) Keissl. and *Aspergillus niger* van Tiegh., causing postharvest rots in grapes. The test fungi were isolated from infected grapes. The essential oils were extracted through hydro distillation process using Clevenger's apparatus. For screening of antifungal activity, treatments at concentration of 20, 40, 80, 160 and 320 µl/ml and controls were set to determine percentage inhibition of mycelial growth of two test fungi using poisoned food technique. All the tested oils exhibited significant antifungal effect ($p<0.05$) over tested fungi. Among six essential oils, *Cinnamomum camphora* showed the most effective antifungal activity against *Aspergillus niger* which inhibited the mycelial growth by 81.58 % and 100 % at 20 and 80 µl/ml oil concentration respectively. *Cinnamomum tamala* showed the best antifungal effect in controlling *Alternaria alternata* among all six oils which inhibited the mycelial growth by 93.11 % at 20 µl/ml and by 100 % at 80 µl/ml oil concentrations. With appropriate formulation, these EOs can replace synthetic preservatives used in increasing selflife of grapes.

Key words: Antifungal activity, hydro distillation, mycelial growth, Percentage inhibition, Poisoned food technique.

INTRODUCTION

Eurasian grapevine (*Vitis vinifera* L.) is the most widely cultivated and economically important fruit crop in the world (Mattia *et al*. 2008). Its global production in the year 2012 was 691 Mql (OIV 2013). Numerous studies have indicated that grape consumption may be beneficial in reducing the plasma concentration of cholesterol and preventing atherosclerosis (Hashemi 2014). They are useful in fighting dyspepsia, hemorrhoids, stones in the urinary tract, bile ducts, activating liver functions, ease digestion, help reduce the cholesterol level of the blood and eliminate uric acid (FAO 2005).

Essential oils are non-water based phytochemicals made up of volatile aromatic compounds (Lawless 2013). Essential oil bearing plants constitute a rich source of bioactive chemicals, which have been reported to have various antifungal properties. These chemicals are often active against a limited number of species, including the specific target species. They are also biodegradable and non-toxic (Adebayo et *al*. 2013). 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 disease-control agents (Tripathi *et al*. 2008).

Post-harvest disease account for substantial losses of fruits, vegetables and other plant products during the transit and storage process (Mahmoudi *et al*. 2012). The primary cause of post-harvest loss in table grapes is grey mould disease or *Botrytis cinerea* (Pearson & Goheen 1988, Snowdon 1990). Some other important fungal post-harvest diseases of table grapes include *Aspergillus* rot (*Aspergillus niger*) which doesn't grow below 5ºC, *Alternaria* rot (*Alternaria alternata*), blue mold rot (*Penicillium* spp.), Rhizopus rot (*Rhizopus oryzae; R. stolonifer*), anthracnose (*Elsinoeampelina, Glomerella cingulata*) and others (Snowdon 1990).

MATERIALS AND METHOD

A. Collection of plant samples and extraction

Leaves of *Cinnamomum tamala*, *Cinnamomum camphora* and *Murraya koenigii* were collected from garden of CDB, TU. Similarly leaves of *Eucalyptus citriodora*, *Artemisia indica* and *Lantana camara* were collected from around TU area, Kirtipur and were air-dried and stored at room temperature in darkness until distillation.

The air-dried materials were subjected to hydro distillation for 6-8 h using Clevenger's apparatus. The essential oils were collected, dehydrated using anhydrous sodium sulphate (Na_2SO_4) and stored at temperature >10ºC until use and analysis.

B. Isolation and identification of test fungi (Aspergillus niger and Alternaria alternata)

Some pieces of fungal colony from infected grapes were transferred aseptically on PDA plates. A week later, the observed fungal colony were identified using standard literature (Ellis 1971, Watanabe 2010).

C. Assessment of fungi toxicity

The fungi toxicity of the essential oils were assessed by poisoned food technique (Grover & Moore 1962). Oils were diluted into different concentrations of 20, 40, 80, 160 and 320 µl/ml with 60 % Acetone (Rao & Srivastava 1994). At first, 1ml of each concentration of essential oil was poured into sterilized petriplates followed by addition of 9 ml of melted PDA. Each petriplates were then inoculated by a 4 mm diameter of the test fungus. In control sets, Fungicide namely Mancozeb 75 % WP and 60 % Acetone were used instead of essential oil. Observations were recorded on 7th day.

Five replications were maintained and fungi toxicity was measured in terms of percent inhibition of mycelial growth calculated as; Inhibition of MG (%) = $[(g_c - g_t)/ g_c] \times 100$ [Where; g_c = mean colony diameter in control sets and g_t= mean colony diameter in treatment sets].

D. Statistical analysis

Excel 2013 was used for entering data, drawing charts and required graphs. The data were analysed with the help of ANOVA followed by Post-Hoc; Bonferroni test at (p<0.05) using Software statistical package for social science (SPSS) version 20.

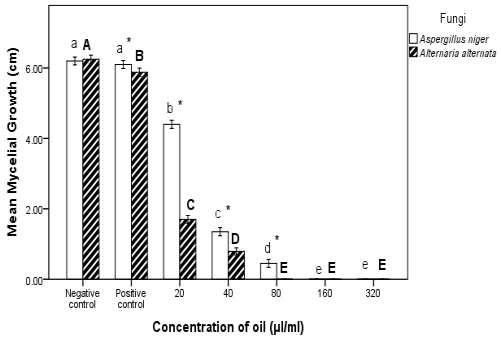
RESULTS AND DISCUSSION

A. Percentage yield of essential oils

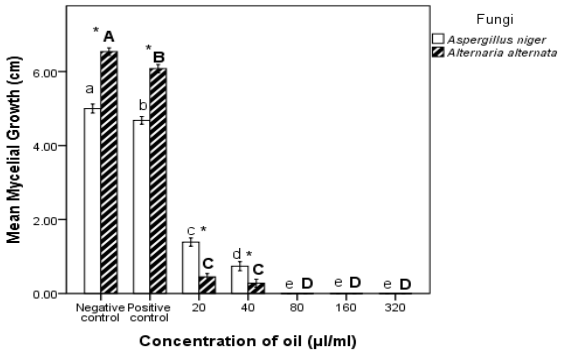
Among six plant samples, *Eucalyptus citriodora* has the highest yield (3%) followed by *Cinnamomum camphora* (2%), *Cinnamomum tamala* (1%), *Artemisia indica* (0.6%), *Murraya koenigii* (0.5%) and *Lantana camara* (0.1%), respectively.

B. Antifungal bioassay of essential oils

The results shows that all six essential oils has significant antifungal effect (p<0.05) over mycelial growth of both test fungi. *Artemisia indica* oil has better effects over *Alternaria alternata* than *Aspergillus niger*. As, it completely inhibits the mycelial growth of *Alternaria alternata* at 80 µl/ml concentration (Fig. 1). Meanwhile, camphor oil has better effects over *Aspergillus niger* than *Alternaria alternata* because it completely inhibits the mycelial growth of *Aspergillus niger* (Fig. 1B). Similarly, tejpaat oil exhibited better effects over *Alternaria alternata* than *Aspergillus niger* (Fig. 1C).



(A)



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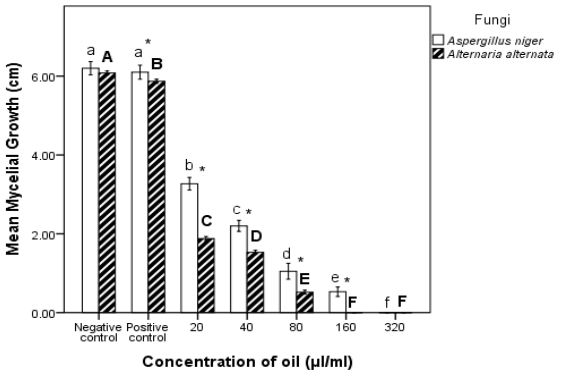

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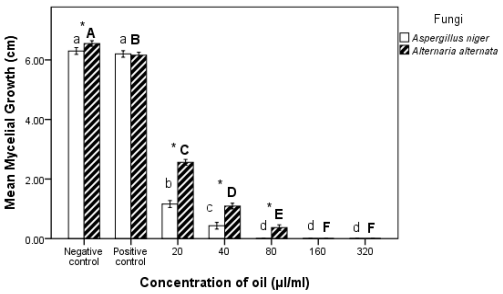

(D)

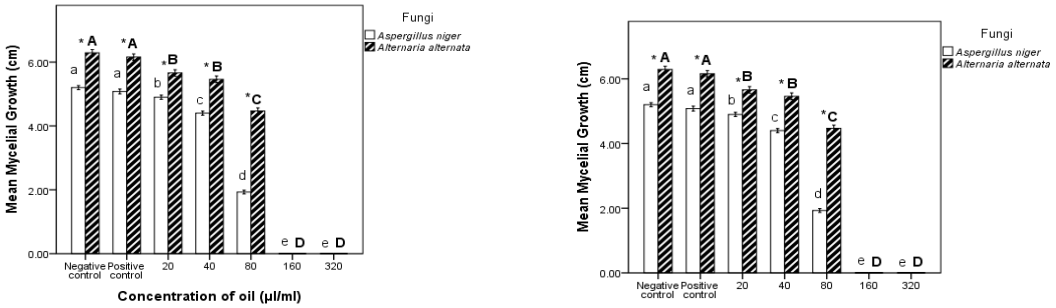

Fig. 1. (A). Antifungal effect of *Artemisia indica* against *Aspergillus niger* and *Alternaria alternata* (B). *Cinnamomum camphora* (C). *Cinnamomum tamala* (D). *Eucalyptus citriodora* (E). *Lantana camara* (F). *Murraya koenigii*. [The mean values sharing same alphabet are not significantly different."*" indicates the significant difference between the mycelial growth of two fungi at different concentration].

Eucalyptus citriodora oil has greater effects over *Aspergillus niger* than *Alternaria alternata* (Fig. 1D). Similarly, *Lantana camara* oil has better effects over *Alternaria alternata* than *Aspergillus niger.* As, it completely inhibits the mycelial growth of *Alternaria alternata* at 160 µl/ml concentration (Fig. 1E). *Murraya koenigii* oil has better effects over *Aspergillus niger.* But, exceptionally at 160 µl/ml concentration oil completely inhibits the mycelial growth of *Alternaria alternata* (Fig. 1F). Similarly, two way ANOVA results for both the test fungi illustrates that plant species (oil type), concentration of oil and their interaction all have significant effect on mycelial growth of the test fungi (Table 1 and 2).

Table 1. ANOVA for mycelial growth of *Aspergillus niger* at different oil concentrations.

Source	Sum of square	Degree of freedom (df)	Mean square	F	Sig.
Plant(Oil type)	143.222	5	28.644	4.200	0.005
Concentration of oil	1155.513	6	192.586	28.240	0.000
Interaction	204.590	30	6.820	873.915	0.000

Table 2: ANOVA for mycelial growth of *Alternaria alternata* at different oil concentrations.

Source	Sum of square	Degree of freedom (df)	Mean square	F	Sig.
Plant(Oil type)	105.550	5	21.110	3.979	0.007
Concentration of oil	1198.935	6	199.823	37.664	0.000
Interaction	159.160	30	5.305	685.613	0.000

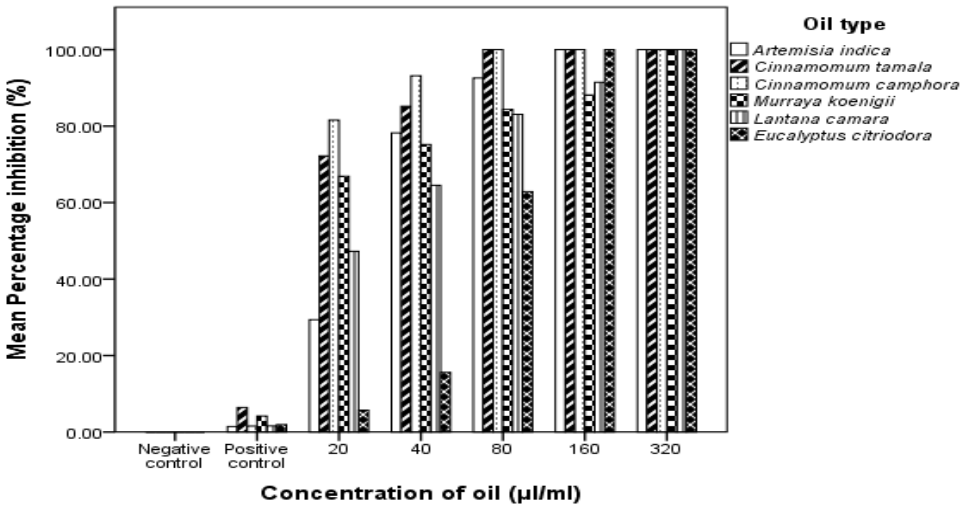

Fig. 2. Fungi toxicities of different essential oils at different concentrations against *Aspergillus niger.*

Among all six essential oils,*Cinnamomum camphora* showed the most effective antifungal activity against *Aspergillus niger*. The presence of camphor as main component (68%) and linalool, the second most important (9%) may be the reason for their effective antifungal activity (Frizzo*et al*., 2000; Chen *et al*., 2013). At 20 µl/ml concentration, *C. camphora* showed the highest inhibition (81.58%), followed by *C. tamala* (72.2%), *Murraya koenigii* (66.87%), *Lantana camara* (47.25%), *Artemisia indica* (29.35%) and *Eucalyptus citriodora* (5.7%) respectively. Similarly, at 40 µl/ml concentration, *C. camphora* showed the highest inhibition (93.17%), followed by *C. Tamala* (85.21%), *Artemisia indica* (78.22%), *Murraya koenigii* (75.2%), *Lantana camara* (64.51%), and *Eucalyptus ciriodora* (15.57%) respectively. Meanwhile, at 80 µl/ml oilconcentration, *C. camphora* and *C. tamala* 100% inhibition of mycelial growth followed by *Artemisia indica* (92.58%), *Murraya koenigii* (84.37%), *Lantana camara* (83.06%) and *Eucalyptus citriodora* (62.88%) respectively. At 320 µl/ml oil concentration rest of the oils also showed 100% inhibition of the mycelialcolony (Fig. 2).

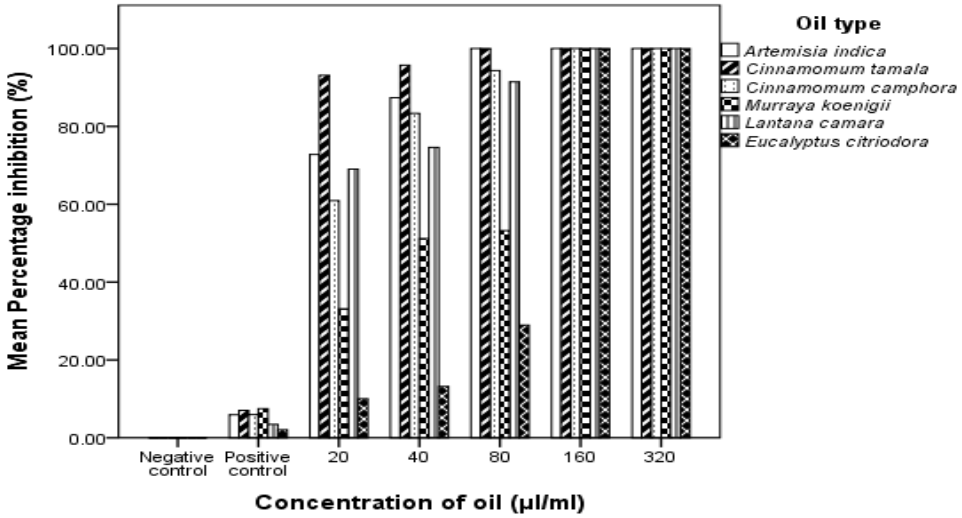

Fig. 3. Fungi toxicities of different essential oils at different concentrations against *Alternari aalternata.*

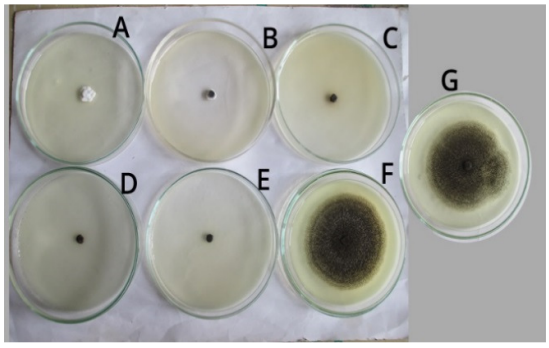

Fig. 4. Antifungal activity of *Cinnamomum camphora* against *Aspergillus niger.*

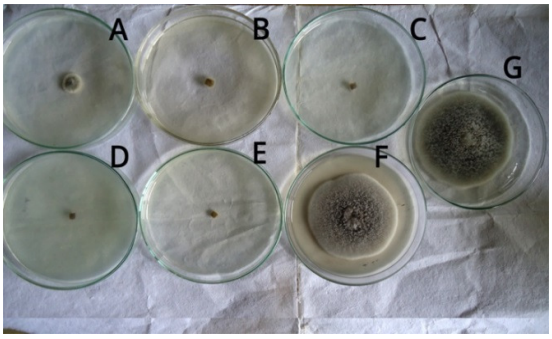

Fig. 5. Antifungal activity of *Cinnamomum tamala* against *Alternaria alternata.*

Cinnamomum tamala showed best activity whereas *Eucalyptus citriodora*showed least antifungal effect in controlling *Alternaria alternata* among all six oils (Fig. 3). It contains eugenol, cinnamaldehyde, cinnamyl alcohol, cinnamylacetate and cinnamic acid and many other responsible for the observed antifungal properties (Pandey *et al*., 2012). At 20µl/ml oil concentration, *C. tamala* showed highest inhibition (93.11%) followed by *Artemisia indica* (72.8%), *Lantana camara* (69.02%), *C. camphora* (60.91%), *Murraya koenigii* (33.16%) and *Eucalyptus citriodora* (10.01%) respectively. *C. tamala* showed highest inhibition (95.71%) followed by *Artemisia indica* (87.36%), *C. camphora* (83.35%), *Lantana camara* (74.62%), *Murraya koenigii* (51.19%) and *Eucalyptus citriodora* (13.19%) at 40 µl/ml oil concentration respectively. Similarly, *C. tamala* and *Artemisia indica* showed the highest inhibition (100%) followed by *C. camphora* (94.35%), *Lantana camara* (91.43%), *Murraya koenigii* (53.23%) and *Eucalyptus citriodora* (28.93%) at 80 µl/ml oil concentration respectively. And, at 160 µl/ml oil concentration, rest of the oils exhibited 100% inhibition of mycelial growth of *Alternaria alternata.*

The difference in fungi toxicity at same concentration in different essential oils may be due to different chemical composition of the oils (Singh *et al.*1983).

CONCLUSION

This study concludes that six different EOs extracted from six different plants can be promising in management of post-harvest diseases of grapes especially in controlling two rots fungi namely *Aspergillus niger* and *Alternaria alternata*. The oil of *Cinnamomum camphora* and *Cinnamomum tamala* showed the most effective antifungal activity against *Aspergillus niger* and *Alternaria alternata* respectively. The results suggest their possible use as an alternative or complements to synthetic compounds. Further studies on isolation and characterization of the active (antifungal) compound are needed.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Ganesh Neupane, Dr. Chandra Prasad Pokhrel and Mr. Sujan Balami CDB, T.U., for their valuable support. Comments provided by Dr. Jay Kant Raut, Senior Scientist, NAST have helped for improvement of the manuscript.

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