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ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS AGAINST *GLOMERELLA CINGULATA* (STON.) SPAULD. & H. SCHRENK

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ABSTRACT
Medicinal and aromatic plant possesses essential oils which may provide possible alternatives to the chemical controls agents. The essential oils from *Artemisia indica, Eucalyptus globulus, Juniperus recurva, Mentha arvensis, Thymus linearis* and *Zanthoxylum armatum* were assessed *in-vitro* for activity against *Glomerella cingulata*, the causal agent of fruit rot of apple. Pathogenecity test was confirmed by inoculating the pathogen into the healthy fruit. Fungi toxicity was assessed by poisoned food technique using different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%) and the assessment was carried out in terms of percentage of mycelia growth inhibition of the tested fungus. The extracts of all the plants were found to be efficient in inhibiting the mycelia growth. The extract of *Thymus linearis* and *Mentha arvensis* completely inhibited the mycelia growth of the fungus at the concentration of 40%; followed by *Zanthoxylum armatum* at 60%; *Eucalyptus globulus* at 70%; *Juniperus recurva* at 80%; and *Artemisia indica* at 90%. The results showed that the plants harboured the fungi toxic principle that inhibited the mycelial growth. *Thymus linearis* and *Mentha arvensis* were found to be more effective in its fungi toxic properties. We recommend the use of plant essential oils as a new promising choice to harmful antifungal treatments which will permit for a safe and environmentally sound and scientifically acceptable management practices for controlling several fungal diseases in Nepal.

KEYWORDS: Antifungal properties, essential oils, fruit, medicinal plants, Nepal

1. INTRODUCTION
Glomerella cingulata is a plant pathogenic fungus that causes disease on many different hosts including apple bitter rot. Bitter rot occurs mainly on the harvesting fruit. The fungus first appears as a small circular brown spots and afterward the lesions enlarge and become saucer shaped. These lesions increase in size and turn dark brown to black within a few days. Spores are produced within the lesions appearing at first as blister and then as distinctive circles in shades of pink or orange. A cross section of the fruit shows the rotting tissue as a V shaped from the skin to the core (see figure I & II).

The key technique to manage bitter rot and other post harvest disease is based on application of synthetic chemical products but, these-days consumers' demand less use of synthetic chemicals.[1] The appearance of artificial antimicrobials in the mid of the last century lead to lack of interest in plants as a natural source for antimicrobial drugs.[2] But, in the recent years the condition has modified and the field of bio-prospecting research has increased.[3-9]

Application of essential oil has long been considered a very promising scheme for controlling postharvest disease. The production of essential oils by plants has been considered to be principally a defence means against pathogens and pests and definitely most of the oils have been shown to possess antimicrobial and antifungal properties.

A review of literatures reveals that there are many essential oils which acquire antifungal activity.[10-22] Essential oils and their elements are expanding interest because of their comparatively safe status, wide acceptance by consumers and their exploitation for potential multi-purpose functional uses.[19-20]

Essential oils are made up of diverse volatile compounds and the composition varies between species.[21] It appears that the antifungal and antimicrobial effects are the outcomes of many compounds acting synergistically.[22] These oils are one of the most promising groups of natural compounds for the development of safer antifungal agents.[13,18]

A scientific and systematic investigation on antifungal properties of the essential oil of the above selected six plants namely: *Artemisia indica*, *Eucalyptus globulus*, *Juniperus recurva*, *Mentha arvensis*, *Thymus linearis* and *Zanthoxylum armatum* is lacking. We aimed to examine the inhibitory effects of essential oils extracted from six plants against the fungus *Glomerella cingulata* and to evaluate the potential application of essential oils to control the fungus of apple fruit.

2. MATERIALS AND METHODS

2. 1 Extraction of essential oil

The sample of *Thymus linearis* and *Artemisia indica* was collected from Manang and Kathmandu districts of Nepal respectively. The essential oils of *Juniperus recurva*, *Zanthoxylum armatum*, *Mentha arvensis* and *Eucalyptus globulus* were obtained from the Herbs Production and Processing Company Limited (HPPCL), Koteshwor, Kathmandu, Nepal. About 50 grams of shade dried plant samples of *Thymus linearis* and *Artemisia indica* are surface sterilized with 0.1% of mercuric chloride followed by proper washing in distilled water.

The sample was then pulverized. The hydro-distillation of plant sample was carried out for 6-8 hours in Clevenger's apparatus in 500 ml water in the Central Department of Botany laboratory of the Tribhuvan University, Kathmandu, Nepal. The volatile fractions condensed after hydro-distillation exhibited two distinct layers a supper aromatic layer of oil and a lower colourless aqueous layer.

The aromatic layer was collected and dehydrated over anhydrous sodium sulphate and stored at 10^0C following.[23-24] Essential oils were diluted into different concentration (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%) with 80% acetone and then the different concentration of each oil was labelled and stored at 10^0C.

2.2 Test fungus

Glomerella cingulata was isolated from the infected apple fruit using standard pathological techniques. The media used was Potato Dextrose Agar (PDA) which was prepared using standard method. The pure culture of the test fungus was maintained. The assessment of fungitoxicity was done by poisoned food technique.[23]

2.3 Preparation of inoculums disc

The culture of *Glomerella cingulata* was obtained from the infected apple. Some piece of fungal colony was transferred aseptically on a petri plates containing PDA. It was incubated in inverted position in an incubator at ± 25^0C for one week. The pure culture of *Glomerella cingulata* was preserved by sub culturing in several slants and plates containing PDA. For testing the antifungal activity, seven days old culture of the test fungus was used for the preparation of inoculums disc of 4 mm in diameter.

2.4 Antifungal assay

The toxicity of the oil against the *Glomerella cingulata* was assessed by using the poisoned food technique adopted by.[23] The preliminary antifungal action of oils was determined. About 0.5 ml of each oil concentration was aseptically poured into the petriplate followed by the addition of 9.5 ml of melted PDA and was swirled gently to achieve thorough mixing of the contents. In control set, no extract was used. After the solidification of the media, one inoculums disc of the test fungus was aseptically inoculated upside down at the centre of the petriplate and incubated at 25 ± 2^0C for seven days. All experiment was revised thrice. The average diameter of fungal colonies was measured on the 7^{th} day of incubation and percentage of mycelial growth inhibition was calculated by.[23]

$$\text{Mycelial growth inhibition (\%)} = \frac{g_c - g_t}{gc} \times 100$$

Where,

g_c = growth of mycelial colony in control set after incubation period subtracting the diameter of inoculums disc.

g_t = growth of mycelial colony in treatment set after incubation period subtracting the diameter of inoculums disc.

3. RESULTS AND DISCUSSION

The antifungal activity of six plants essential oils is shown in (Table 1) and there the mycelia growth inhibition is given in percentage. All the oils tested exhibited different degrees of antifungal activity against *Glomerella cingulata*.

Among the tested plants, *Thymus linearis* and *Mentha arvensis* noticeably inhibited the fungus growth even at a low concentration of 40%; followed by *Zanthoxylum armatum* at 60%; *Eucalyptus globulus* at 70%; *Juniperus recurva* at 80%; and *Artemisia indica* at 90% (see Table 1 and Plate I-IV for detail).

The inhibition was found to be 73 to 100% by the extracts of *Thymus linearis*; 60 to 100% by *Mentha arvensis*; 36 to 100% by *Juniperus recurva*; 24 to 100% by *Artemisia indica*; 12 to 100% by *Zanthoxylum armatum*; and 48 to 100% by *Eucalyptus globulus*. The results showed that the essential oils contain volatile substances which indirectly influence the growth of *Glomerella cingulata* and significantly prevent its growth.

Table 1: Mycelial growth inhibition (in %) by six plant essential oils at different concentrations

SN	Concentrations in (%)	Mycelial growth inhibition (in %)					
		Thymus linearis	*Mentha arvensis*	*Juniperus recurva*	*Artemisia indica*	*Zanthoxylum armatum*	*Eucalyptus globulus*
1	10	73	60	36	24	12	48
2	20	80	80	53	30	31	60
3	30	90	85	65	36	53	70
4	40	100	100	82	54	80	80
5	50	100	100	85	63	90	90
6	60	100	100	87	75	95	95
7	70	100	100	97	85	100	100
8	80	100	100	100	90	100	100
9	90	100	100	100	100	100	100
10	100	100	100	100	100	100	100

The inhibition of the mycelia growth of the tested fungus was variable for each plant extracts. The extracts of *Thymus linearis* and *Mentha arvensis* were found to be most effective for the control of tested fungus. Relative variation in the content of the active chemical constituents in the essential oils was speculated to be the most probable reason for the inhibition of the mycelial growth at different concentrations.

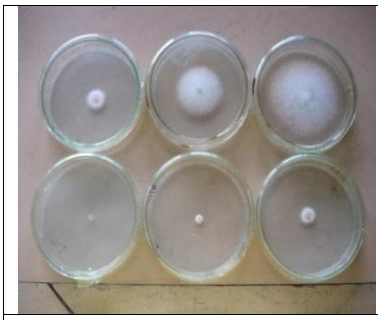

Plate I: Antifungal activity of *Thymus linearis*

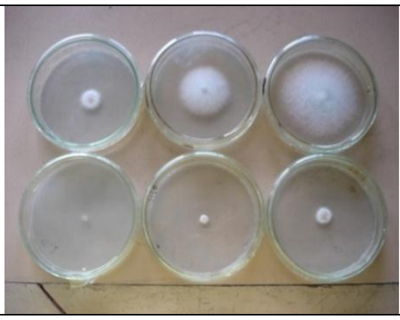

Plate II: Antifungal activity of *Mentha arvensis*

Figure I: Conidia and conidiophores of *Glomerella cingulata*

Plate III: Antifungal activity of *Juniperus recurva*

Plate IV: Antifungal activity of *Zanthoxylum armatum*

Figure II. Bitter Rot of Apple

There are various methods to extract essential oils but the most popular method is the steam distillation in which water is heated to produce steam that carries the most volatile chemicals of the plant materials with it. The steam is further cooled in a condenser and the resulting distillate is collected. The oil normally float on top of the Hydrosol (the distillate water component) and may be separated off.[11,25]

To reduce the utilization of synthetic chemicals, extensive research has been made over the past two decades. Such researches made possible exploitation of plant compounds as natural commercial products that are safe for humans and also to the environment.[26] Though, wild medicinal and aromatic plants essential oils have been reported to be effective against a wide range of microorganisms *in-vitro*[27], but failed to inhibit *in-vivo* pathogen growth[28] and to control diseases under field trials.

It has been realized that i*n-vitro* screening of plant extracts is the first steps in identifying plants with potential application in agriculture, but *in-vivo* confirmation of this potential is essential in the search for plant derived preparations with potentials to be commercialized.[29]

4. CONCLUSION

The costs of research and development for botanical fungicides are less compared to that of chemical fungicides. The results also supported a concept that plant essential oils have a role as pharmaceuticals and can be used as a prospective source of sustainable environmental sound botanical fungicides. Essentials oils tested against *Glomerella cingulata* showed promising visions for the research scientists towards the consumption of natural plants/oils in post harvest disease control.

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