

# FUNGITOXICITY OF THE ESSENTIAL OILS OF SOME AROMATIC PLANTS OF MANANG AGAINST *ALTERNARIA BRASSICICOLA*.

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**Abstract:** Green plants have been recognized as the reservoir of potentially active chemical substances. They contain various active constituents with fungitoxic properties. Advantages of such substances are that they are biodegradable and nonpollutive as well as easily available. Plant essential oils have been reported to be effective toxicants against many pathogenic fungi in recent years.

Essential oils of *Thymus linearis*, *Artemisia gmelinii*, *A. dubia*, *Juniperus recurva*, *Nardostachys grandiflora* and *Zanthoxylum armatum* were assessed for their fungitoxicity against *Alternaria brassicicola* (causal agent of leafspot disease of cabbage). Fungitoxicity of all essential oils was assessed by poison food technique and the percentage of growth inhibition in different concentration was calculated. Essential oils affected the mycelial growth inhibition of the test fungus significantly ( $F = P < 0.05$ ). Further data analysis showed species level efficiency in order of *Nardostachys grandiflora* = *Thymus linearis* = *Artemisia dubia* > *A.gmelinii* > *Zanthoxylum armatum* (LSD = 5.56;  $P < 0.05$ ). Although all of the concentrations of E.Oils inhibited the mycelial growth of the test fungus significantly ( $P < 0.05$ ) the most effective was found to be 10  $\mu\text{l.ml}^{-1}$  (LSD = 6.0;  $P < 0.05$ ).

**Key words:** Essential oils; aromatic plants; fungitoxicity.

## INTRODUCTION

The Himalayan kingdom of Nepal is bestowed with a variety of habitats occupied with rich floristic wealth that contains a number (> 20%) of medicinal and aromatic plants (Malla, 1991; Manandhar and Aase, 2003 ;). Plant diversity serves the mankind as a renewable natural resource for a variety of biologically active chemicals. The volatile component of fresh leaves or other plant parts, isolated in the form of essential oils, are the chemical substances whose molecules possess a variety of properties i.e. bactericidal, fungitoxic, antiviral, anthelmintic, anticancer, sedative, and laxative, cardiotoxic diuretic and others. Active constituents of a medicinal plant (or its parts) have been found to be less phytotoxic, more systemic (Fawcett *et al.*, 1970), and easily biodegradable (Beye, 1998; Tripathi *et.al.*1980).

Plant essential oils (E.Oils) are the mixtures of terpenes, mainly the monoterpenes and sesquiterpenes, occurring in different tissues of a plant part i.e. flowers, leaves, root, bark, or seeds.

Cabbage (*Brassica oleraceae* var. *capitata* L.), a member of the family Brassicaceae, is a winter vegetable widely cultivated in cool, moist climate and heavy sandy loam soil. It contains large amounts of water (91%), proteins alongwith small amount of sugar, starch and mineral salts. The leaves are eaten as salads or cooked (Pandey *et al.*, 1993).

*Alternaria brassicicola* (Schw.) Wiltshire syn. *Alternaria*

*circinans* (Berk. and Curt.) Bolle. causes more severe disease than *Alternaria brassicae*. It is widespread and common in N. W. Europe, Great Britain, India, and Nepal (Ellis, 1971; Pawsey, 1989). In humid conditions, a wet rot develops and destroys whole of the bud-head and in dry conditions the fungus becomes established causing further damage (Coleno *et al.*, 1971).

The causal agent of the leaf spot disease of cabbage is known to be *Alternaria brassicicola*. The infected leaves appear dark brown to black, , zonate spots are circular (1 to 10 mm in diameter), in culture the colonies look amphigenous dark olivaceous brown. The mycelium is septate, hyphae branched, hyaline to brown, inter and intracellular. Conidiophores arise singly or in groups. The conidia are in chains, sometimes branched, acropleurogenous arising through small pores from the conidophore wall, straight, tapering towards the apex, the basal cell rounded, the beak present or absent, apical cell more or less rectangular, contain less than 6 transverse septa and few longitudinal septa. Conidia are constricted at the septa. (Ellis, 1971 and Singh, 1982). There are reports of the isolation of *Alternaria brassicicola* from different plant sources i.e. seeds of *Brassica* (Richardson, 1970), beetles (*Phyllotreta crucicola*) that fed on cabbage (Dillard *et al.* 1998), and the leaves of of *Phaseolus vulgaris* (Bera, 1983).

The present investigation aims to analyze the fungitoxic effects of some plant essential oils on *Alternaria brassicicola*, the causal agent of leafspot disease in *Brassica* sp.

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## METHODOLOGY

### Source of E.Oils:

Both freshly extracted E.Oils in the laboratory of the Central Dept. of Botany (Tribhuvan University) and supplied by HPPCL (Herb Production and Processing Company Ltd., Koteshwar, Kathmandu) were used in the experiments. Shade-dried plants (*Thymus linearis*, *Artemisia dubia* and *Artemisia gmelinii*) were shredded to expose the oil glands, and subjected to hydrodistillation for 6-8 hrs in Clevenger's apparatus at lower water temperature (15 to 20°C). Essential oils were collected and dried over anhydrous sodium sulphate and stored at <5°C. Essential oils of *Nardostachys grandiflora*, *Zanthoxylum armatum* and *Juniperus recurva* were kindly supplied by HPPCL.

### Test Fungus and the growth condition:

*Alternaria brassicicola* was used as the test fungus species with source in the infected leaves of cabbage ( Hemmi and Issigami, 1953). Using standard pathological techniques, it was isolated, reconstituted and used in the experiments. Potato Dextrose Agar (PDA) medium was used for isolation of the test fungus and poisoned food method (Grover and Moore, 1962) to study fugitoxicity.

### Inoculum -Disc:

Seven days old culture of the test fungus was used to prepare inoculum discs (4 mm diameter). A single disc was aseptically placed upside down in the center of each petriplate containing E.Oil loaded PDA, so as to establish a direct contact with the medium,

### Experimental:

Acetone (80%) was used as the solvent to prepare

different E.Oil concentrations of (10 µl.ml<sup>-1</sup>, 5 µl.ml<sup>-1</sup>, 2.5 µl.ml<sup>-1</sup>, 1.250 µl.ml<sup>-1</sup> and 0.625 µl.ml<sup>-1</sup>) for use in experiments (Rao *et. al*, 1994) to assess fungitoxicity following poisoned food technique (Grover and Moore, 1962). A volume of 0.5ml of each coccentration of E.Oil was aseptically poured into the petriplate followed by the addition of melted PDA (9.5ml). The petriplate was kept swirling while adding PDA so as to get a thorough mixing of the contents. E.Oil was, however, replaced by an equal amount of acetone only in the control set.

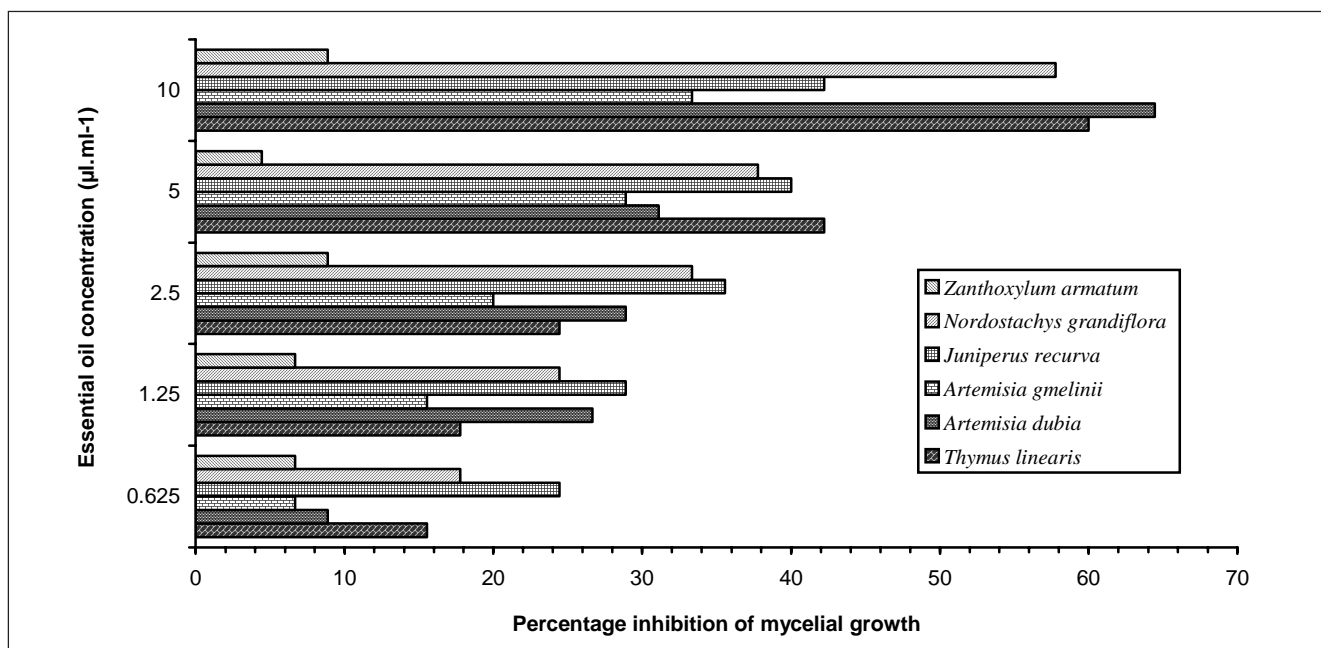
After the media solidified one inoculum disc of *Alternaria brassicicola* mycelium, the test fungus, was aseptically placed / inoculated to each petriplate and incubated at 25+2°C. The average diameter of fungal colonies was measured on the 7<sup>th</sup> day after inoculation and percentage mycelial growth inhibition was calculated (1979; Rao *et. al.*, 1994).

$$\text{Percentage mycelial growth Inhibition} = \frac{g_c - g_t}{g_c} \times 100$$

Where,  $g_c$  = Growth of mycelial colony after incubation period in control set subtracting the diameter of inoculum disc;  $g_t$  = Growth of mycelial colony after incubation period in treatment set subtracting the diameter of inoculum disc.

### Expt. Layout and Analysis of the Data

- E.Oils of 6 Plant Species X 6 Concentrations ie 36 treatment combinations (inclusive of the control).
- Experiments were carried out three times. Each of the experimental unit was replicated thrice and an average value was obtained for Analysis of variance (ANOVA).
- Least Significant Difference (LSD): Analysis for LSD was carried out to locate the significance whether of



**Fig.1:** Antifungal activities of the essential oils of different plant species against *Alternaria brassicicola*. The effect was significant ( $F=P<0.05$ )

plant species or the concentrations used. Error Mean Square (EMS) of the ANOVA and value of "t" for error df at 0.05 P was used to calculate the LSD for the significant cases. Marginal Means were, however, used to elaborate the efficiency of a particular effect.

## RESULTS AND DISCUSSION

### Assessment of fungitoxicity:

Fungitoxicity of each of the essential oils was assessed using its different concentrations against *Alternaria brassicicola* by poisoned food technique. As shown in Fig.1 the results were obtained in terms of percent inhibition of the mycelial growth over the control according to the module already explained above (methodology). Usual ANOVA was carried out to obtain the degree of significance.. The associated analysis for LSD was also carried out to locate the significance for both the plant species and the concentrations of the E.Oils that were used in the present investigation.

### Comparative fungitoxicities:

As shown in fig.1 a significant difference among the plant species was found evident for the fungitoxicity of their E.Oils (P<0.05). The plant species were selected from the aromatic herbaceous plant population of the high Himalayas of Nepal with habitats in Manang from Pisang (28°36'8"N and 84°8'7"E with masl 3,050m) to Khangsar (28° 40' 1" N and 83°58'3" E with masl 3,800m).

Different essential oils showed differential fungitoxicities. Percentage of inhibition of mycelial growth varied with different oil concentrations. Percentage inhibitions of mycelial growth of *Alternaria brassicicola* were found 60.00%, 42.22%, 24.44%, 17.77% and 15.55% by *Thymus linearis*; 64.44%, 31.11%, 28.88%, 26.66% and 8.88% by *Artemisia dubia*; 33.33%, 28.88%, 20.00%,

15.55% and 6.66% by *Artemisia gmelinii* ; 42.22% 40.00%, 35.55%, 28.88% and 24.44% by *Juniperus recurva*; 57.77%, 37.77%, 33.33%, 24.44% and 17.77% by *Nardostachys grandiflora* and 8.88%, 4.44% 8.88%, 6.66% and 6.66% by *Zanthoxylum armatum* at 10 µl.ml<sup>-1</sup>, 5 µl.ml<sup>-1</sup>, 2.5 µl.ml<sup>-1</sup>, 1.25 µl.ml<sup>-1</sup> and 0.625 µl.ml<sup>-1</sup> essential oil concentrations respectively

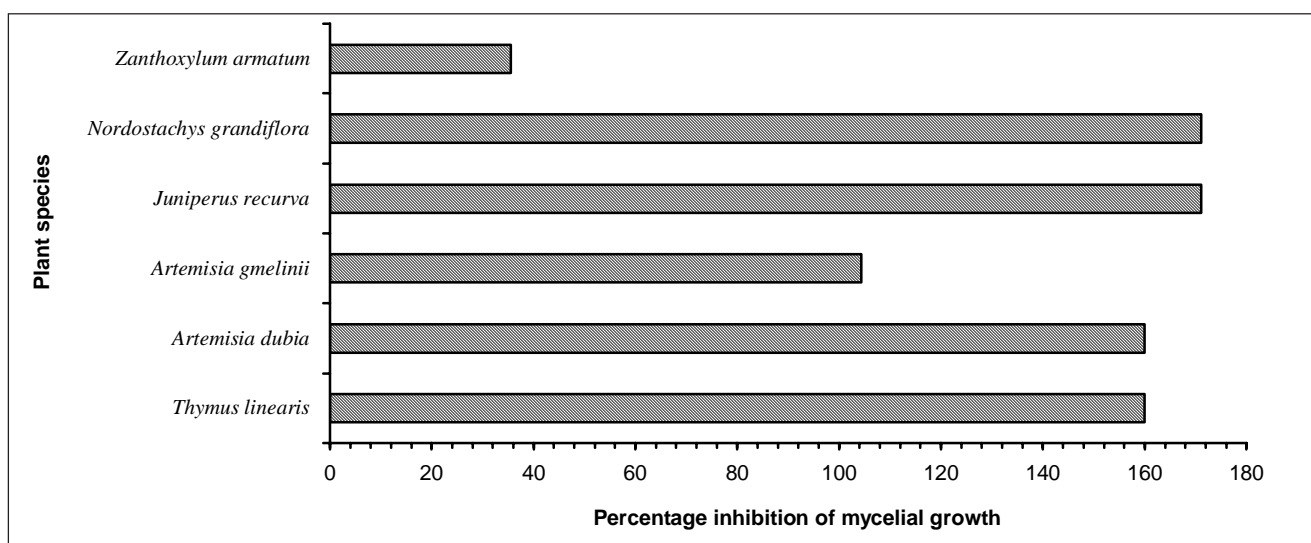
The plant species showed variable effects among themselves (Fig. 2.). Relative variation in the content of active chemical in the E.Oil was speculated to be one of the evident reasons. It provides ample opportunity to extend the the present investigation involving active principle of the E.Oils in relation to species of the plants.

Saxena *et al.* (1983), Singh *et al.* (1983), Chandra *et al.* (1982), Dwivedi *et al.* (1990), Mishra and Dubey (1990), Rao and Srivastava (1994), Dubey *et al.* (1983) Singh *et al.* (1983) and Renu *et al.* (1980) also studied the fungitoxicities of essential oils of different plant species against different fungal species and assessed fungitoxicities in terms of percentage of mycelial growth inhibition but none of them carried out the study about the fungitoxicity of essential oils against *Alternaria brassicicola*.

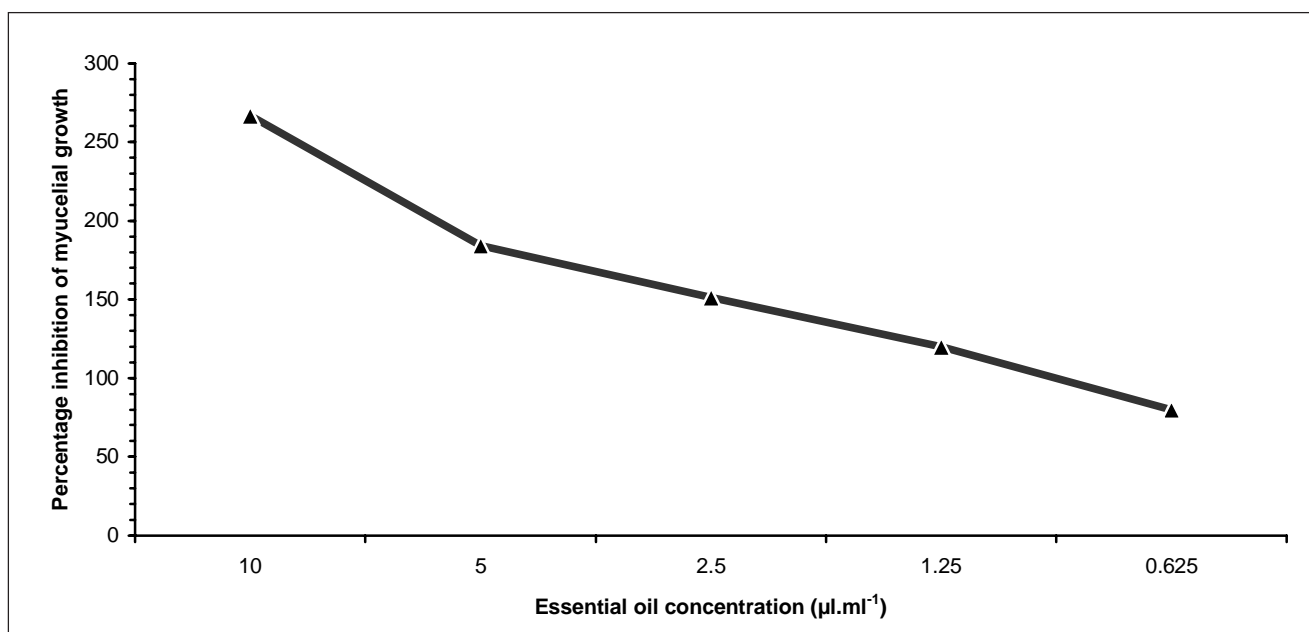
Although all of the concentrations of E.Oils inhibited the mycelial growth of the test fungus significantly (P<0.05) the most effective was found to be 10 µl.ml<sup>-1</sup> (LSD = 6.0; P<0.05). *Artemisia dubia* showed the highest inhibition (64.44%) followed by *Thymus linearis* (60.00%), *Nardostachys grandiflora* (57.77%),

*Juniperus recurva* (42.22%) *Artemisia gemlinii* (33.33%) and *Zanthoxylum armatum* (8.88%) at 10 µl.ml<sup>-1</sup> essential oil concentration respectively.

*Thymus linearis* showed the highest inhibition (42.22%) followed by *Juniperus recurva* (40.00%), *Nardostachys*



**Fig.2:** Variations among plant species, ie the source E.Oil for the efficiency of mycelial growth inhibition of *Alternaria brassicicola*. The effects were found significant ((LSD = 5.56; P<0.05). Based on the data of marginal means (ANOVA) the relative efficiency of plant species followed the sequence of *Juniperus recurva* = *Nardostachys grandiflora* = *Thymus linearis* = *Artemisia dubia* > *A.gmelinii* > *Zanthoxylum armatum* (LSD = 5.56; P<0.05).



**Fig. 3:** All of the concentrations affected inhibition of mycelial growth significantly ( $P < 0.05$ ), the most effective was found to be  $10 \mu\text{l.ml}^{-1}$  (LSD = 6.0;  $P < 0.05$ ). The data of marginal means have been plotted against percent inhibition (ANOVA).

*grandiflora* (37.77%), *Artemisia dubia* (31.11%), *Artemisia gmelinii* (28.88%) and *Zanthoxylum armatum* (4.44%) at  $5 \mu\text{l.ml}^{-1}$  essential oil concentration respectively.

*Juniperus recurva* showed the highest inhibition (35.55%) followed by *Nardostachys grandiflora* (33.33%), *Artemisia dubia* (28.88%), *Thymus linearis* (24.44%), *Artemisia gmelinii* (20.00%) and *Zanthoxylum armatum* (8.88%) at  $2.5 \mu\text{l.ml}^{-1}$  essential oil concentration respectively.

*Juniperus recurva* showed the highest inhibition (28.88%) followed by *Artemisia dubia* (26.66%), *Nardostachys grandiflora* (24.44%), *Thymus linearis* (17.77%), *Artemisia gmelinii* (15.55%) and *Zanthoxylum armatum* (6.66%) at  $1.25 \mu\text{l.ml}^{-1}$  essential oil concentration respectively. *Juniperus recurva* showed the highest inhibition (24.44%) followed by *Nardostachys grandiflora* (17.77%), *Thymus linearis* (15.55%), *Artemisia dubia* (8.88%), *Artemisia gmelinii* (6.66%) and *Zanthoxylum armatum* (6.66%) at  $0.625 \mu\text{l.ml}^{-1}$  concentration respectively.

Essential oil of each of the plant species showed significant fungitoxic effects at all concentrations viz.  $10 \mu\text{l.ml}^{-1}$ ,  $5 \mu\text{l.ml}^{-1}$ ,  $2.5 \mu\text{l.ml}^{-1}$ ,  $1.25 \mu\text{l.ml}^{-1}$  and  $0.625 \mu\text{l.ml}^{-1}$ . The effects were, however, linear with increasing concentration with the highest effect at  $10 \mu\text{l.ml}^{-1}$  ( Fig.3).

## CONCLUSION

Present study highlights that *Thymus linearis*, *Artemisia dubia*, *Artemisia gmelinii*, *Juniperus recurva*, *Nardostachys grandiflora* and *Zanthoxylum armatum* harbour the chemical components that inhibit the mycelial growth of the test fungus *Alternaria brassicicola*. The

fungitoxicity was found to be variable with different plant essential oils. However, the E.Oils of those test plant species could be recommended for cabbage (*Brassica oleracea* vr. *capitata*) protection against *Alternaria brassicicola*. The present work is to be extended further involving more analytical processes, test plants and the fungal pathogens to yield results useful on economic fronts.

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