

# Analysis of Insect toxicity and repellent activity of Phytochemicals from “*Skimmia laureola*, Nair” against “Black garden ant, *Lasius niger*” of Pakistan

Ferhat Mehmood<sup>1\*</sup>, Zaheer-ud-Din Khan<sup>1</sup>, Farkhanda Manzoor<sup>2</sup> and Muhammad Jamil<sup>3</sup>

<sup>1</sup>Department of Botany, GC University, Lahore, Pakistan

<sup>2</sup>Department of Zoology, Lahore College for Women University, Lahore, Pakistan

<sup>3</sup>Outpatient laboratory Mayo Hospital, Lahore, Pakistan

**Abstract:** A study was conducted to evaluate the toxicity and repellency of essential oils from root, stem and leaves of Nazar panra, *Skimmia laureola* (DC.) Zucc. Ex Walp. of family (Sapindales: Rutaceae) ver. Nair of Pakistan. The oils were tested at three concentrations i.e. 1, 5 and 10%. Black garden ant, *Lasius niger* L. (Hymenoptera: Formicidae) of Pakistan were selected and exposed to essential oils at room temperature. All essential oils showed Insecticidal activity with  $LC_{50}=10.15$ , while dose dependant effect was significant with  $R^2=0.98$ . It can be concluded that the three Essential oils in this study have both Insecticidal as well as repellent effect.

**Keywords:** Insecticidal activity, essential oil (EO), Nair,

## INTRODUCTION

Rutaceae, commonly known as Rue or Citrus family is represented in Pakistan by 11 genera and 27 species, many of which have been naturalized here, being cultivated and hybridized for edible, medicinal and ornamental purposes. Most species are aromatic with glands on the leaves. *Skimmia laureola* (*S. laureola*) locally name as Nair is a significant member of this family. In Pakistan, it is common in the Hazara region, Murree Hills, Kashmir, upper Swat and Shangla (Hamayun, 2007 and Nadkarni, 1976). Curative values of the plant have been renowned in various cultures of the world. It has been used for the treatment of cold, fever and headache. Smoke of leaves and twig smoke are used as evil spirit repellent (Hamayun, 2007). In the form of smoke, dry leaves have been in use for nasal tract clarity. Moreover, leaves of the plant have been suggested for numerous purposes together with relieve coughs (Baart *et al.*, 2004), insecticides, and pesticides (Qureshi *et al.*, 2009). Leaves are commercially harvested and used in foodstuff as flavoring agent, in traditional medicinal and cultural practices, being made into garlands and considered sacred (Shandesh *et al.*, 2006). Essential oils extracted from the plant showed profound anthelmintic activity (Mehmood *et al.*, 2011). The presence of essential oils in members of family Rutaceae with diverse activities claimed and the increasing demand for natural sources of insecticides encouraged us to undertake a comprehensive study of the Insecticidal and repellent activities of the essential oils for first time in Pakistan.

\*Corresponding author: e-mail: ferhatmehmood786@yahoo.com

## MATERIALS AND METHODS

The plant material was collected from natural habitat of Abbotabad, identified at Botany department GC University, Lahore.

### *Extraction of essential oils and their analysis*

The plant parts were separated and subjected to hydro-distillation for about 4h. The essential oils obtained thus were dried over anhydrous sodium sulphate and stored in dark colored glass bottle at temperature of about 4°C. Chemical composition was determined through GC-MS. GC-MS analyses were performed using DB-5MS column in triplicate with a blank run after every analysis on a Shimadzu GCMS-QP2010A system, and EI mode (70eV) equipped with injector at 250°C. The data thus obtained was processed using Shimadzu Lab Solution GCMS Post run Analysis software. Comparing the mass fragmentation pattern of the reported data and NIST 147 and NIST 27 libraries, the components of essential oils were identified.

### *Collection of ants*

The ants were collected from Lawns of Lahore College for Women University by putting corrugated cardboard (with syrup) in ground traps, then traps were brought back to Entomological Research Laboratory, ants were separated and identified with the help of key.

### *Experimental setup*

Laboratory bioassays were conducted to test the repellent and insecticidal effect of above-mentioned Essential oils. The method was as adopted by Grace *et al* 1992 with some modifications. For this purpose, three plastic cylinders were taken and holes were made in them with an iron rod. These plastic containers were connected by

means of 10 cm long Typhon tube. Three different concentrations (1, 5 and 10 %) of oil were prepared in ethanol as solvent. About 3 cm round filter paper (Whatmann no#1) disks were taken. For each experimental unit three filter paper discs were taken and soaked with water, E. oil and (solvent) ethanol. All three filter paper discs were air dried at room temperature prior to exposing to ants. In experimental setup, central container served as experimental unit (with oil treated filter paper disc) while container on the sides served as control units i.e. water control and solvent control. Containers on sides provided an opportunity to the insects to migrate freely, depending upon their response to the repellent effect of oil in the treatment bottle. Ants were placed in the central container that already contained oil treated filter paper disc. All bottles were closed with lids and covered the whole experimental setup with black cloth for the maximum activity of ants. Each essential oil concentration was tested for repellency and toxicity. Readings were taken after every 0.5h for 5h and recorded for further usage to draw conclusions. At the end of experiment, number of dead insects was recorded to estimate the toxicity of the oil. Criteria considered for death was loss of any motility in the insect, observed under dissecting microscope. The dead insects were kept in the separate bottle on filter paper, treated with distilled water for 24h to ensure death. For control units blank control and ethanol control units were used. Three replicates of each concentration of all essential oils were tested. At the end of experiment average number of ants was calculated for each concentration of essential oil used. Various test statistics were applied to draw inference.

### STATISTICAL ANALYSIS

The percentage mortality rates were corrected by using Abbott's formula and data were analyzed using a one-way ANOVA Test using Excel and SPSS 13.0 (statistical software) on the data.

### RESULTS

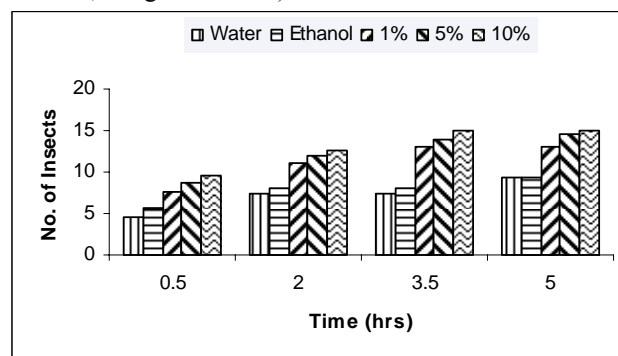
#### *Yield and physical characteristics of essential oils*

Yield and Physical characteristics of Essential oils and variety of classes of compounds were detected in all essential oils are given in table 1 and 2 respectively.

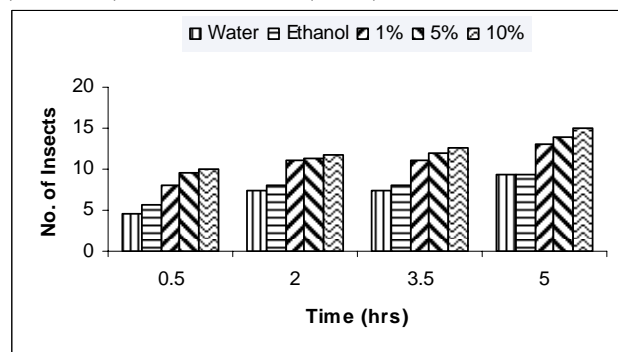
#### *Chemical composition*

According to phytochemical analysis Monoterpene alcohol, Monoterpene, Ester, Acyclic ester peroxide and Hydrocarbons dominated in the essential oils. Similarly table 2 shows top three chemical components in essential oils from different parts in percentage. In detail, essential oils from *Skimmia laureola* leaves had D-Limonene 32.32%, Linalool acetate 23.53%,  $\alpha$ - Terpineol 16.68%. Essential oils from *S. laureola* root had 1,3-

cycloheptadiene 36.86%, Linalyl isobutarate 19.78%, Nerolacetate 19.06% while in *S. laureola* stem Beta Linalool 43.61%,  $\alpha$  terpineol 16.22%, Solanone 12.68%. Gertsch *et al.* 2002 found Caryophyllene to cause spiciness of black pepper. It is a dietary cannabinoid having psychomodulatory effect. *In vitro* studies show anti-proliferative effects of beta-elemene in various cancer cells through cell-cycle arrest and induction of apoptosis (González *et al.* 2002; Yao *et al.* 2008; Li *et al.* 2005; Li *et al.* 2010). It also enhanced the activity of cisplatin against prostate cancer cells. The parenteral form of beta-elemene isolated from *Rhizoma zedoariae*, a type of ginger, has been studied in Asia and is reported to relieve pain, decrease the side effects of chemotherapy, and increase the quality of life in cancer patients. However, human trials conducted so far are of poor quality (Chen *et al.* 2010). The presence of Ketones in EO from leaves make it best suitable insecticide (Yu *et al.* 2011; Peng *et al.* 2006).



**Fig. 1:** Time dependant repellent effect of different concentrations of leaves essential oil on adult *Lasius niger* (Black ant) at various times (hours)

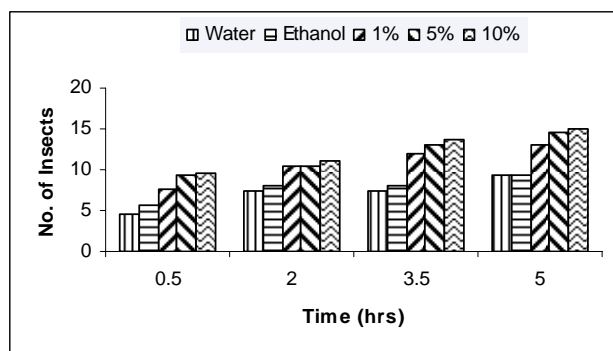


**Fig. 2:** Time dependant repellent effect of different concentrations of stem essential oil on adult *Lasius niger* (Black ant) at various times (hours)

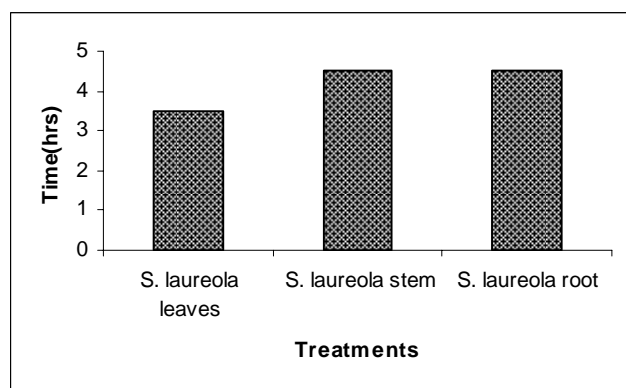
#### **Toxicity and repellent activity of different concentrations of essential oils**

Essential oil of each plant part included in present study obtained, after applying One way ANOVA and LSD test showed to possess Insecticidal activity as well as repellent effect against *Lasius niger* (Black ant). The essential oils showed varied repellent effect. The essential oil from

leaves took least time to repel all the insects, while essential oils from stem and roots exhibited similar effect but significantly different from the controls. Essential oil of each plant part included in present study showed toxicity as well as repellent effect against *Lasius niger* (black ant) figs. 1, 2 and 3. The data of regression model with R<sup>2</sup> value, i.e., 0.984 revealed the dose dependant effect of the essential oils. Table 3 shows percentage values of dead and sluggish insects indicating the effective ness of the essential oils. Each concentration of all essential oils exhibited similar results in this respect, but percentage of insects rendered sluggish by the essential oils is higher than the dead. Affected insects were kept under observation and then viewed under the dissecting microscope. It was noted that none of the dead insect could revive from the dead status even after 24 hours of post treatment time period. Similarly the sluggish condition of the insects worsened after 24 hours. All essential oils of *S. laureola* had LC<sub>50</sub> =10.15μL. The excellent repellent effect of Essential oils is indicative of their promising commercial prospects as insect repellent if prepared in some formulation. Their repellent effect can further be tested by experimentation on non-human mammals prior to be recommended for human use.



**Fig. 3:** Time dependant repellent effect of different concentrations of roots essential oil on adult *Lasius niger* (Black ant) at various times (hours)



**Fig. 4:** Profile showing time in hrs taken by different Essential oils to repel all insects adult *Lasius niger* (Black ant) from the experimental bottle

## DISCUSSION

Essential oils from different parts of some plant species of family Rutaceae were evaluated for their repellent and Insecticidal activity in this study. All essential oils showed appreciable repellent as well as Insecticidal activities. Garden ant, *Lasius niger*, adults were used as test insect. EO from leaves of *S. laureola* showed highest repellent effect which is in agreement with the findings of Manandhar 2002, who found both *S. laureola* and *B. albiflora* leaf products to be good insect repellent. While the least repellent activity was exhibited by Essential oils from stem and root EO. It seems that due to aromatic nature and strong odor Essential oils, had exhibited repellent effect more than the Insecticidal activity. The vaporized Essential oils got easy entrance through the tracheal system of the insects, thus choking them and causing irritation at the same time, hence compelled the insect to flee from the treatment bottle having EO, towards the control bottles (having filter paper discs treated with either water or ethanol in them). Another fact was observed that repellent and Insecticidal effect increased with increase in the concentration of the Essential oils indicating the dose dependant effect of the oils. This is evident from figs. 1, 2 and 3. EO from leaves of *S. laureola* showed highest repellent effect at 10% concentration as compared to that at 1%. Similarly highest mortality rate was seen in 10% concentration oils than of 1% concentration this is comparable to the Insecticidal activity of extracts of *M. paniculata* observed by Mollah and Islam (2008) and Rani and Devanand (2011) Zhang *et al.*, (2010); Dube *et al.*, (1990) and Sisko *et al.*, (2007). The Essential oils from *S. laureola* leavestook least time, i.e. 3.5hrs for 100% repellency while Essential oils took maximum time i.e. 4hrs in this respect from stem and root fig. 4. The excellent repellent effect of Essential oils is indicative of their promising commercial prospects as insect repellent if prepared in some formulation. Their repellent effect can further be tested by experimentation on non-human mammals prior to be recommended for human use. Insecticidal activity, although, less than the repellent effect but very encouraging. Data indicates that higher concentrations will be effective for improved Insecticidal effect. High percentage of mortality of insects at 10% concentration was from all Essential oils. Another unignorable observation was percentage of insects rendered sluggish by the Essential oils. This percentage remained higher than that of dead percentage. This is definitely indicative of the fact that in still higher concentrations, the Essential oils included in this study can have increased Insecticidal activity. It is noteworthy that none of the dead insect could revive from the dead status even after 24 hours of post treatment time period. Similarly the sluggish condition of the insects worsened after 24 hours, kept under observations and then viewed under the dissecting

**Table 1:** Profile of plant parts and essential oils

Plant Name	<i>S. laureola</i> (Stem)	<i>S. laureola</i> (Root)	<i>S. laureola</i> (Leaves)
Weight of specimen (Kg)	1.56	1.765	2.20
Weight of oil (g)	1.50	0.05	70.3
Percent recover	0.01	0.003	3.19
Color of oil	Clear light pale	Very light yellow	Clear greenish oil

**Table 2:** Profile of top tree chemical compounds in each essential oil in percentage.

Types of compound	SL	SS	SR
β-Linalool	32.32	-----	-----
Linalool acetate	23.53	-----	-----
α- Terpineol	16.68	-----	-----
Beta Linalool	-----	43.61	-----
α terpineol	-----	16.22	-----
Solanone	-----	12.68	-----
1,3-cycloheptadiene	-----	-----	36.86
Linalyl isobutarate	-----	-----	19.78
Nerolacetate	-----	-----	19.06

SL= *S. laureola* leaves, SS= *S. laureola* stem, SR= *S. laureola* root

**Table 3:** Profile showing no. of dead and sluggish insects after 5 hours of exposure to different essential oils on adult *Lasius niger* (Black ant) in comparison with Ethanol and water as control

Treatments	Oil (%)	Dead (%)	Sluggish (%)
<i>S.laureola</i> stem essential oil.	1	13.33	20
	5	20	33.33
	10	33.33	53.3
<i>S.laureola</i> leaves essential oil.	1	13.33	26.66
	5	20	40
	10	33.33	50
<i>S.laureola</i> roots essential oil.	1	13.33	13.33
	5	20	26.66
	10	33.33	40
Water (control)		0.00	0.00
Solvent/ethanol (control)		0.00	0.00

microscope. Maximum number of insects became sluggish by 10% of all Essential oils.

## REFERENCES

Baart JLG, Baart-Bremer EL and Sagar MZ (2004). Names of plants in Kalam Kohistani (Pakistan). Work Papers of the Summer Institute of Linguistics, University of North Dakota Session[C]: 48.

Chen W, Lu Y and Wu J (2010). Beta-elemene inhibits melanoma growth and metastasis via suppressing vascular endothelial growth factor-mediated angiogenesis. *Canc. Chemo. and Pharmacol.*, **67**(4): 799-808.

Dubey S, Kumar A and Tripathi SC (1990). Antifungal and insect-repellent activity of essential oil of *Zanthoxylum alatum*. *Annals of Botany*, **4**(5): 457-459.

Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ, Altmann KH, Karsak M and Zimmer A (2008). Beta-caryophyllene is a dietary cannabinoid. *Proc. Natl. Acad. Sci. USA*, **105**(26): 9099-9104.

González de CN, Sánchez F, Quintero A, Usubillaga A. (2002). Chemotaxonomic value of essential oil compounds in *Citrus* species. *Acta. Horticulturae.*, **576**: 49.

Grace JK, Goodell BS, Jones WE, Chandhoke V and Jellison J (1992). Evidence for inhibition of termite (Isoptera: Rhinotermitidae) feeding by extra cellular metabolites of a wood decay fungus. *Proc. Hawaiian Entomol. Soc.*, **31**: 249-252.

Hamayun M (2007) Traditional uses of some medicinal plants of Swat Valley, Pakistan. *Ind. J. Trad. Know.*, **6**(4): 636-641.

Li QQ, Wang G, Huang F, Banda M and Reed EJ (2010). Synergistic effects of Endostar combined with β-

- elemene on malignant ascites in a mouse model. *J. Pharm. Pharmacol.*, **62**(8): 1018-1027.
- Li X, Wang G and Zhao J (2005). Antiproliferative effect of beta-elemene in chemo resistant ovarian carcinoma cells is mediated through arrest of the cell cycle at the G2-M phase. *Cell Mol. Life Sci.* **62**(7-8): 894-904.
- Manandhar NP (2002). Plants and people of Nepal. *Timber Press Inc.*, USA. Pp. 122-129.
- Mehmood F, Qasim M, Khan Z, Iqbal N, Mehmood S, Lateef M (2011). *In vitro* evaluation of anthelmintic activity of essential oils from different parts of *Skimmia laureola* (DC.) Zucc. ex Walp., var. *Nair*. *Pak. J. Bot.*, **43**(6): 2915-2918.
- Mollah V, Islam W. 2008. Toxicity of *Murraya paniculata* (L.) Jack leaf-derived materials against *Callosobruchus maculatus* (F.) (coleoptera: Bruchidae). *Pak. Entomol.*, **30**(1): 61-64.
- Nadkarni KM (1976). Indian Materia Medica. Bombay: Popular Prakashan Private Limited (Popular Press); p. 1142.
- Peng X, Zhao Y and Liang X (2006). Assessing the quality of RCTs on the effect of beta-elemene, one ingredient of a Chinese herb, against malignant tumors. *Contemp. Clinic. Trials*, **27**(1): 70-82.
- Qureshi RA, Ghufuran MA, Gilani S, Yousaf Z, Abbas G and Batool A (2009). Indigenous medicinal plants used by local women in southern Himalayan regions of Pakistan. *Pak. J. Bot.*, **41**(1): 19-25.
- Rani PU (2011). Devan and P efficiency of different plant foliar extracts on grain protection and seed germination in maize. *Res. J. Seed Sci.*, **4**: 1-17.
- Shandesh B, Ram C and Robin T (2006). Ethnomedicinal plants used by the people of Manang district, central Nepal. *J. Ethnobiol. Ethnomed.*, **2**: 41.
- Siskos EP, Konstantopoulou MA, Mazomenos BE and Jervis M (2007). Insecticidal activity of *Citrus aurantium* fruit, leaf and shoot extracts against adult olive fruit flies (Diptera: Tephritidae). *J. Econom. Entomol.*, **100**(4): 1215-1220.
- Yao Y, Ding QX and Jia YC (2008). Anti-tumor effect of beta-elemene in glioblastoma cells depends on p38 MAPK activation. *Cancer Letters*, **264**(1): 127-134.
- Yu Z, Wang R and Xu L (2011). Beta-Elemene piperazine derivatives induce apoptosis in human leukemia cells through downregulation of c-FLIP and generation of ROS. *PloS One*, **6**(1): e15843.
- Zhang Y, Peng YH, Zeng DQ, Chen FF, Qin QH and Huang Y (2010). Insecticidal activity of essential oil from *Zanthoxylum armatum* fructification against two mosquito species. *Guihaia*, **2**: 26-28.