

Insect Toxicity and Repellent Activity of Phytochemicals from “Flea killer, *Boenninghausenia albiflora*” against “Black Garden Ant, *Lasius Niger*” of Pakistan

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Abstract

A study was conducted to evaluate the insecticidal activity of essential oils obtained from root, stem and leaves of *Boenninghausenia albiflora* (Sapindales: Rutaceae) against Black garden ant *Lasius Niger* L. (Hymenoptera: Formicidae). The major compounds in these essential oils were identified using gas chromatography-mass spectrometry and their insecticidal activity was tested at three concentrations i.e. 1, 5 and 10% in ethanol. All essential oils showed similar insecticidal and repellent activity at each concentration but significantly different at $p \leq 0.05$ from controls with $LC_{50} = 12.35 \mu\text{l}$, while dose dependent effect was significant with $R^2 = 0.803$. 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); *Boenninghausenia albiflora*

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 in different areas, being cultivated and hybridized for edible, medicinal and ornamental purposes. Most species are frequently aromatic with glands on the leaves or thorns [1]. *Boenninghausenia albiflora* is an important genus of this family with medicinal history. The leaves are pounded and applied to cuts and wounds as a styptic and to speed the healing process. They are crushed and placed in the nostrils in the treatment of malaria. The external application of the leaves is also used in the treatment of scabies. The leaf juice is dropped into wounds in order to kill microorganisms [2]. Applied externally to the forehead, the juice is said to relieve headaches. A tea of the roots of *B. albiflora* is used in the treatment of malaria while its leaves had toxicological properties against mosquito vectors of malaria, filariasis and dengue [3]. Similarly essential oils from root of *B. albiflora* showed maximum positive results regarding Irritant Contact Dermatitis (ICD) [4]. *B. albiflora* was also found to be active against *Spodoptera litura* pest [5]. The ethnobotanical properties, presence of essential oils 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 from *B. albiflora* using *Lasius niger* (Black garden ant). They can infest buildings, particularly foundation forms in contact with soil, becoming a minor nuisance [6,7].

Material and Methods

The plant material was collected from natural habitat of Abbottabad, 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 4 h. The essential oils obtained thus were dried over anhydrous sodium sulphate and stored in dark colored glass bottle at temperature of about 4°C.

Gadget used for GC-MS analyses was Shimadzu GCMS-QP2010A system in EI mode (70eV), which was equipped with injector at

250°C, using DB-5MS column. Each sample was injected at 250°C with a split ratio of 50/50. Volume of injected sample was 1 μl and electronic pressure programming was employed for the maintenance of a constant flow (0.67 ml/min) of the Helium carrier gas. The oven temperature was programmed from 100°C (4 min) to 250°C at a rate of 2°C/min and held at this temperature for 2 min. For the purposes of scanning the mass spectrometer was set with range 40 amu to 600 amu with ion source temperature 200°C and interface temperature 250°C. Samples analyzed were in triplicate manner and a blank was run after every sample analysis. Data obtained thus was further processed with the help of software named “Shimadzu Lab Solution GCMS Postrun Analysis”. Retention indices were also calculated for identification of chemical constituents.

Collection of ants

The ants were collected from Lawns of Lahore College for Women University, Lahore, 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 under supervision of zoologist at department of Zoology, Lahore College for Women University, Lahore. The ants were immediately subjected to the tests.

Experimental setup

An experimental setup was designed to check repellency as well as insecticidal activity. For this purpose three plastic bottles were taken

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and interconnected by making holes at bottom level (two opposite holes in the central bottle and one each in the side bottles) using 10 cm long typhon pipe. Central bottle would serve as experimental while the side bottles as controls. The side bottles would provide an opportunity to the ants to flee freely under the effect of essential oil. This experiment was conducted with three different stock concentrations viz. 10%, 5 % and 1%. For each test 300 μ L volume was used. For making 10% stock solution 270 μ L ethanol and 30 μ L of essential oil was used, for 5% stock solution 285 μ L ethanol and 15 μ L oil was used. Similarly for making 1% stock solution, 297 μ L ethanol and 3 μ L oil was used. Here ethanol was used as solvent for making stock concentrations.

Round filter paper disks of 3cm diameter were used. For each experimental unit three filter paper discs were taken. One soaked with water, second with required oil and the third with (solvent) ethanol. All three filter paper discs were air dried at room temperature prior to exposure to ants. In experimental setup, central bottle served as experimental unit while bottles on the peripheral sides served as control units i.e. water control and solvent control. Solvent treated and water treated filter paper discs were placed in their respective side bottles. Fifteen ants were placed in the central bottle 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. Repellency rate for each oil was checked after every 30 minutes. Experiment was conducted for 5 hours. In this way about 6 readings were recorded. In the last number of dead and sluggish insects were recorded to estimate the insecticidal activity of each oil. Criteria considered for death was loss of any motility in the insect while that for sluggish was restricted movement, under dissecting microscope. The dead insects were kept in the separate bottle with distilled water treated filter paper for 24 hrs to ensure death. For control units, blank control and solvent control tests were conducted. In case of blank control, central bottle contained distilled water soaked filter paper disks, while solvent control test contained ethanol solvent filter paper disk in the central bottle. For each oil and controls, data was compiled by counting number of ants in all bottles separately. Three replicates performed for each concentration of oils.

Statistical analysis

The percent mortality rates were corrected by using Abbott's formula [8]. After checking the assumptions of normality of the data one-way ANOVA test and LSD were applied using Excel and SPSS 13.0 statistical software. LC_{50} was determined using Probit-Regression test.

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 Tables 1 and 2, respectively.

Chemical composition

According to phytochemical analysis Sesquiterpene, Monoterpene, Oxygenated Bi cyclic Sesquiterpene, Ketone and Ester dominated in the essential oils. Table 3 shows top three chemical components in essential oils from different parts in percentage. Gertsch et al. [9] 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 [9-12]. It also enhanced the

Plant part	Wt. in Kg	Wt.of oil in g	Recovery in %	Color
<i>B. albiflorastem</i>	1.6	0.5	0.031	Greenish pale clear
<i>B. albifloraroot</i>	1.5	0.4	0.026	Brownish pale
<i>B. albiflora leaf</i>	1.5	0.6	0.04	Yellowish clear

Table 1: Profile of plant parts and essential oils.

Types of compounds	Percent AUC*			RI
	BL	BS	BR	
(+)-3-Carene	19.97	-----	-----	1001
Caryophyllene oxide	11.61	-----	-----	1573
α -Bourbonene	9.27	-----	-----	1374
[-]-Spathulenol	-----	47.78	42.89	1575
Globulol	-----	15.02	-----	1576
β -Humulene	-----	9.04	-----	1440
Gamma Elemnene	-----	-----	12.54	1430
Viridiflorol	-----	-----	10.9	1590

BL=*B. albiflora* leaves, BS= *B. albiflorastem*, BR= *B. albifloraroot*
AUC* = Area under the curve, RI =retention index

Table 2: Profile of top tree chemical compounds by GC-MS analysis of essential oils of leaves, stem and roots.

Treatments	Oil (%)	Dead (%)	Sluggish (%)
<i>B. albiflora</i> stem essential oil	1	0	0.5
	5	10	20.66
	10	20	43.3
<i>B. albiflora</i> root essential oil	1	0	0.5
	5	10	20.66
	10	20	43.3
<i>B. albiflora</i> leaf essential oil	1	0	0.5
	5	10	20.66
	10	20	43.3
Water(Control)		0	0
Ethanol(Control)		0	0

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

activity of cisplatin against prostate cancer cells. The parenteral form of beta-elemene isolated from *Rhizomazedoariae*, 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 [13].The presence of Ketones in EO from leaves make it best suitable insecticide [14,15].

Insecticidal and Repellent activity of different concentrations of essential oils

Essential oil of each plant included in present study obtained, after applying one way ANOVA and LSD test showed to possess insecticidal activity as well as repellent effect against *Lasiusniger* (Black garden ant). Although repellent effect of each concentration of essential oils was similar but significantly different from that of controls, Figure 1. The data of regression model with R^2 value, i.e., 0.803, revealed the dose dependent effect of essential oils. Table 2 shows percentage values of dead and sluggish insects indicating the effectiveness of the essential oils. Each concentration of all essential oils exhibited similar results in this respect, but the 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 *B. albiflora* had $LC_{50} = 12.35 \mu\text{L}$. A comparison of repellent effect revealed that the essential oils from *B. albiflora* root and stem took least time, i.e. 3.5 hrs for 100% repellency while essential oil from leaves took maximum time i.e. 4 hrs in this respect Figure 2.

Discussion

Essential oils from different parts of *B. albiflora* 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 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. The mechanism of action of the action of terpenoid in essential oils is not fully understood but may be involved in membrane disorder by the lipophilic compounds [16,17]. Different compounds/active principles of essential oils may have different targets to exert toxicity and repellency on insects. The known target sites on parasites are solely proteins and include ion channels, enzymes, structural proteins, transport molecules, etc. [18-22]. Sonia et al determined effect

of *Anthumgraveolens* L. extract on biochemical and histopathological alteration of deltamethrin in rats [23]. Essential oils from roots and stem of *B. albiflora* showed highest repellent effect, while the least repellent activity was exhibited by essential oils from leaves. More repellent effect than the insecticidal activity seems to be related to aromatic nature and strong odor of essential oils. Resultantly 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 with essential oils, towards the control bottles (having filter paper discs treated with either water or ethanol in them). Similarly highest mortality rate was seen in 10% concentration oils is comparable to the insecticidal activity of extracts of *M. paniculata* [24-27].

Conclusion

It can be concluded that essential oils from *B. albiflora* can be used as insecticidal as well as repellent in place of the synthetic insecticides. Moreover 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 as insect repellent.

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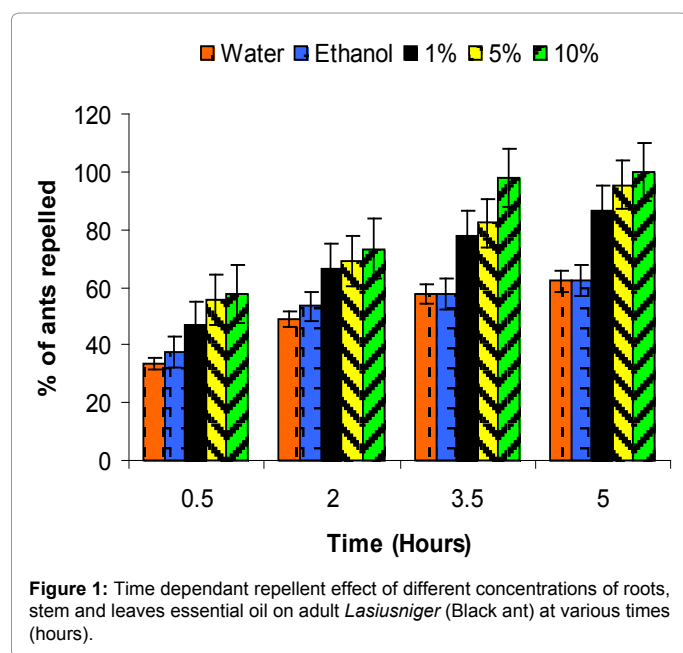


Figure 1: Time dependant repellent effect of different concentrations of roots, stem and leaves essential oil on adult *Lasiusniger* (Black ant) at various times (hours).

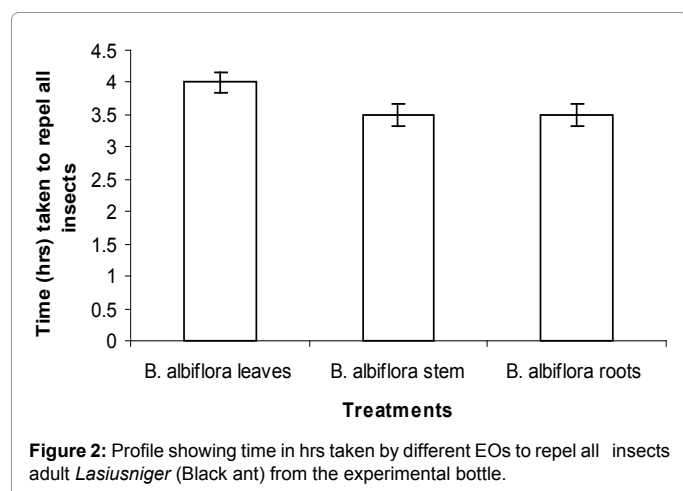


Figure 2: Profile showing time in hrs taken by different EOs to repel all insects adult *Lasiusniger* (Black ant) from the experimental bottle.

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