Journal of Bioanalysis & Biomedicine

Mehmood and Shahzadi, J Bioanal Biomed 2014, 6:1
DOI: 10.4172/1948-593X.1000100

Research Article Open Access

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

Ferhat Mehmood[1] and Phool Shahzadi[2]*

[1]*Department of Botany, Govt. MAO College Lahore, Pakistan*
[2]*Scientific Officer, PCSIR Labs Complex Ferozpur Road Lahore, Pakistan*

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 µl, 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]. *Boeninginghusenia 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 *Lasiusniger* (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

***Corresponding author:** Phool Shahzadi, Scientific Officer, PCSIR Labs Complex Ferozpur Road, Lahore, Pakistan, Tel: 924-299-230-688-263; Fax: 092-429-923-0705; E-mail: psk_a@yahoo.com

Received December 18, 2013; **Accepted** January 27, 2014; **Published** January 30, 2014

Citation: Mehmood F, Shahzadi P (2014) Insect Toxicity and Repellent Activity of Phytochemicals from "Flea killer, *Boenninghausenia albiflora*" against "Black Garden Ant, Lasius Niger" of Pakistan. J Bioanal Biomed 6: 005-008. doi:10.4172/1948-593X.1000100

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 µL volume was used. For making 10% stock solution 270 µL ethanol and 30 µL of essential oil was used, for 5% stock solution 285 µL ethanol and 15 µL oil was used. Similarly for making 1% stock solution, 297 µL ethanol and 3 µL 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 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].

Plant part	Wt. in Kg	Wt.of oil in g	Recovery in %	Color
***B. albiflora*stem**	1.6	0.5	0.031	Greenish pale clear
***B. albiflora*root**	1.5	0.4	0.026	Brownish pale
***B. albiflora* leaf**	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. albiflora*stem, BR= *B. albiflora*root
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 (%)
B. albiflora stem essential oil	1	0	0.5
	5	10	20.66
	10	20	43.3
B. albiflora root essential oil	1	0	0.5
	5	10	20.66
	10	20	43.3
B. albiflora 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.

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 µL. 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].

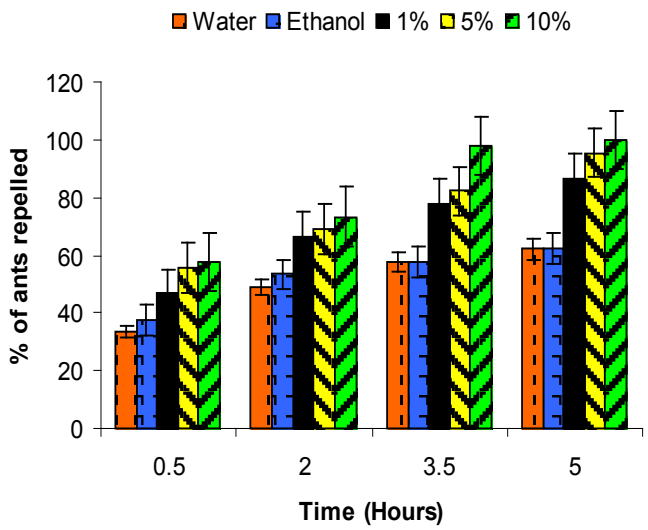

Figure 1: Time dependant repellent effect of different concentrations of roots, stem and leaves essential oil on adult *Lasiusniger* (Black ant) at various times (hours).

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

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.

References

1. Duke JA, Ayensa ES (1985) Medical Plants of China. Reference publications, Inc, Algonac, U.S.A.
2. Manandhar NP (2002) Plants and people of Nepal. Timber Press Inc., USA 122-129.
3. Alam MF, Safhi MM, Chopra AK, Dua VK (2011) Toxicological properties of several medicinal plants from the Himalayas (India) against vectors of malaria, filariasis and dengue. Trop Biomed 28: 343-350.
4. Mehmood F, Khan Z (2012) Determination of skin irritancy by essential oils from some members of Family Rutaceae of Pakistan.BIOLOGIA (PAKISTAN) 58: 161-166.
5. Negi DS, Kumar A, Negi N, Tamta ML (2012) Himalayan Plant Species as Pesticidal Agents. Mini-Reviews in Organic Chemistry 9: 143-150.
6. Klotz JH (2010). Urban Pest Management of Ants in California. UCANR Publications, Division of Agriculture and Natural Resources, University of California, USA 54-55.
7. Antonelli A (2007) "Extension Bulletin 1382: Moisture Ants". Washington State University.
8. Abbott WA (1925) Method for computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.
9. Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, et al. (2008) Beta-caryophyllene is a dietary cannabinoid. ProcNatlAcadSci U S A 105: 9099-9104.
10. González de CN, Sánchez F, Quintero A, Usubillaga A (2002) Chemotaxonomic value of essential oil compounds in Citrus species. ActaHorticulturae 576: 49-51.
11. Yao YQ, Ding X, Jia YC, Huang CX, Wang YZ, et al. (2008) Anti-tumor effect of beta-elemene in glioblastoma cells depends on p38 MAPK activation. Cancer Lett 264: 127-134.
12. Li X, Wang G, Zhao J, Ding H, Cunningham C, et al. (2005) Antiproliferative effect of beta-elemene in chemoresistant ovarian carcinoma cells is mediated through arrest of the cell cycle at the G2-M phase. Cell Mol Life Sci 62: 894-904.
13. Li QQ, Wang G, Huang F, Banda M, Reed E (2010) Antineoplastic effect of beta-elemene on prostate cancer cells and other types of solid tumour cells. J Pharm Pharmacol 62: 1018-1027.
14. Chen W, Lu Y, Wu J, Gao M, Wang A, et al. (2011) Beta-elemene inhibits melanoma growth and metastasis via suppressing vascular endothelial growth factor-mediated angiogenesis. Cancer Chemother Pharmacol 67: 799-808.

15. Yu Z, Wang R, Xu L, Xie S, Dong J, et al. (2011) Î²-Elemene piperazine derivatives induce apoptosis in human leukemia cells through downregulation of c-FLIP and generation of ROS. PLoS One 6: e15843.

16. Peng X, Zhao Y, Liang X, Wu L, Cui S, et al. (2006) Assessing the quality of RCTs on the effect of beta-elemene, one ingredient of a Chinese herb, against malignant tumors. Contemp Clin Trials 27: 70-82.

17. Lacey E (1988) The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. Int J Parasitol 18: 885-936.

18. Geary TG, Klein RD, Vanover L, Bowman JW, Thompson DP (1992) The nervous systems of helminths as targets for drugs. J Parasitol 78: 215-230.

19. Martin RJ (1993) Neuromuscular transmission in nematode parasites and antinematodal drug action. Pharmacol Ther 58: 13-50.

20. Köhler P (2001) The biochemical basis of anthelmintic action and resistance. Int J Parasitol 31: 336-345.

21. Mottier L, Alvarez L, Ceballos L, Lanusse C (2006) Drug transport mechanisms in helminth parasites: passive diffusion of benzimidazoleanthelmintics. Exp Parasitol 113: 49-57.

22. Mollah V, Islam W (2008) Toxicity of Murrayapaniculata (L.) Jack leaf-derived materials against Callosobruchusmaculatus (F.) (coleoptera: bruchidae). Pak Entomol 30: 61-64.

23. Somia I, El-Maghraby, Hamdy A. Taha, Nabila S H (2010) Effect of AnthumGraveolens L. Extract on Biochemical and Histopathological Alteration of Deltamethrin in Rats. J Bioanal Biomed 2: 008-012.

24. Rani PU, Devanand P (2011) Efficiency of different plant foliar extracts on grain protection and seed germination in maize. Res J Seed Sci 4: 1-17.

25. Zhang Y, Peng YH, Zeng DQ, Chen FF, Qin QH, et al. (2010) Insecticidal activity of essential oil from Zanthoxylumarmatum fructification against two mosquito species. Guihaia 2: 26-28.

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

27. Siskos EP, Konstantopoulou MA, Mazomenos BE, Jervis M (2007) Insecticidal activity of Citrus aurantium fruit, leaf, and shoot extracts against adult olive fruit flies (Diptera: Tephritidae). J Econ Entomol 100: 1215-1220.