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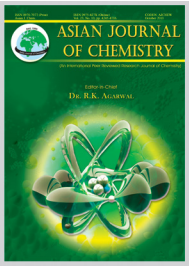

Chemical Analysis and Comparison of Antitermitic Activity of Essential Oils of Neem (*Azadirachta indica*), Vetiver (*Vetiveria zizanioides*) and Mint (*Mentha arvensis*) against *Heterotermes indicola* (Wasmann) from Pakistan

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In present study, three esential oils (neem, vetiver and mint) were analyzed by GC/MS spectroscopy and their physical parameters were also evaluated. The major components of the three oils were: β-terpineol, menthol, isomenthone, menthone, azulene, 5,6-azulenedimethanol, 1*H*-cycloprop[e]azulene, α-hexylcinnamaldehyde, dihydromyrcenol, ethyl palmitate, methyl(2E,6E)-farnesoate, *n*-eicosane and *n*-docosane. The bioactivities of these essential oils were tested for repellency, toxicity and fumigation against the most common termite species of Pakistan, *Heterotermes indicola*. Results exposed that vetiver oil was the most rerpellent and most toxic killing 60 % termites at concentration of 25 µg/µL while neem and mint oils showed 54 % and 56.66 % mortality rate, respectively at the same concentration. The attribute of vetiver oil as most effective repellent was due to its long lasting activity. For fumigation activity, the three essential oils were tested at concentrations of 0.03125, 0.0625, 0.125, 0.25 and 0.5 %. Out of the three tested oils, vetiver oil was the most valuable fumigent against *Heterotermes indicola*.

Key Words: Antitermitic activity, Neem, Vetiver, Mint, *Heterotermes indicola*.

INTRODUCTION

Throughout the world, scientists have diverted their attention to use essential oils as toxic, repellent and feeding deterrent against insects due to their environment friendly nature[1]. Several plants have been investigated to produce volatile compounds and used as alternatives to termiticides[2-7]. Odoriferous plants are main source of essential oils, which are used in perfume, food, medicines and as bio-insecticides[1,8,9]. Essential oils extracted from three coniferous trees showed 100 % antitermitic activity[10]. Bioactivity of the 8 oils (vetier grass, cassia leaf, clove bud, cedarwood, eucalptus globules, eucalyptus citrodora, lemon-grass and geranium) against formosanus subterranean termites has been evaluated. These oils proved environment friendly termiticides[1,3].

Neem oil, neem extracts and different neem products have been used and proved as effective pesticide exhibiting growth inhibition and antifeeding properties, against more than 200 species of the insects including different termite species. It detailed profile studies of oil and different solvent extracts has also been carried out by different scientists[11-15].

Vetiver grass is valued due to the presence of essential oil in its roots, which originate in the form of tufted fibrous mass. Vetiver oil comprises of more than 100 sesquiterpene type compounds and their derivatives[16]. Vetiver oil was evaluated as most effective, long lasting repellent among all the tested essential oils, with a repelling duration of 12-24 days against formosan subterranean termite, *Coptotermes formosanus*[1]. Termiticidal activities of the vetiver oil have successfully been proved in several researches[17-20].

The essential oil is extracted from several mint species, constitute different components, which possess insecticidal and antifumigant properties against insect pests[8]. Appel *et al.*[21] recorded an inverse relationship between LC50 values and increasing rate of mint oil while direct relationship was observed between the repellency and treatment per square centimeter of mint oil and exposure time.

Present study was carried out to test the potential of neem, vetiver and mint oil as repellent, feeding deterrent and fumigant against termites of Pakistan. These oils were analyzed by GC/MS in order to isolate and identify the specific termiticidal components.

EXPERIMENTAL

The neem (*A. indica*), vetiver (*V. zizanioides*) and mint (*M. arvensis*) were the experimental materials to be used in

this study. The extraction of oils was done by steam distillation (reverse dean-stark method). The percentage yield of essential oil was determined by:

% age yield of essential oil = weight of the essential oil obtained /weight of fresh plant material used × 100

Oils were also tested for physical parameters such as solubility, specific gravity, refractive index, acid value and ester value.

GC-MS analysis: Hewlett-Packard 5890 gas chromatograph, equipped with capillary column HP-5® (25 m × 0.22 mm ID and 0.25 m film thickness; an equivalent of SPB-5®), was combined with a Jeol, JMS-HX 110 mass spectrometer operating in EI mode with ion source at 250 ºC and electron energy at 70 eV. Helium was used as a carrier gas at a pressure of 1.4 kg/cm^2. The analyses were performed with an initial temperature of 60 ºC for 1 min, then ramped at a rate of 8 ºC/min to a final temperature of 240 ºC with holding time of 0.5 h. Injector with a splitting ratio of 1:30 was set to 250 ºC and FID (flame ionization detector) was set to 270 ºC. The sample was dissolved in chloroform. Injection volume was adjusted between 1.0-5.0 µL depending upon the detector response.

Various components were identified by their retention time and peak enhancement with standard samples in gas chromatographic mode and MS library search from the derived mass fragmentation pattern of various components of the essential oil.

The workers of *Heterotermes indicola* (Wasmann) were collected from the bucket traps, placed in botanical garden of the Lahore College of Women University Lahore, Pakistan. All debris was removed from the traps and termites (workers and soldiers) were kept in plastic boxes with moist filter paper. Before treatment with oils, termites were maintained in the laboratory at 28 ºC, 80 % relative humidity in constant darkness to remove injured or inactive termites. Only healthy termite individuals were used for the experimentation.

Repellency test: Repellency test was carried out after McDonald *et al*.[22] method with few modifications. Whatman filter paper No. 1 strips measuring 5 cm × 2.5 cm were treated with different conc. of plant oils (0.03125, 0.0625, 0.125, 0.25 and 0.5 %). Control treatment was given with distilled water only. After sufficient evaporation for 0.5 h at room temperature, treated filter paper strips were placed in the Petri plates pre marked as treated and untreated zones. At the centre of Petri plate, 3rd instar termites (7 workers and 3 soldiers) were released and Petri plates were placed in an incubator (26 ± 2 ºC, 85 % relative humidity). Each treatment was replicated three times. Termites settled on treated and untreated halves were counted after every 5 min for 1 h. Percentage repellency was calculated and data was processed and analyzed.

Toxicity test: Toxicity test method was as adopted by Highland and Wilson[23] with few modifications. Filter paper disks measuring 25 mm diameter were oven dried and weighed. The four concentrations (0, 1 5 and 25 µg/µL) of the test compounds were applied to disks. These disks were air dried to evaporate the solvent at room temperature and were reweighed. The treated disks were placed in the petri plates measuring 45 mm × 10 mm diameter over a thin layer of soil. Termites (45 workers and 5 soldiers) were then released into the Petri plate and placed in an incubator maintained at 28 ºC and 80 % relative humidity. After 7 days the filter papers were removed, cleaned from debris, oven dried and weighed to determine the consumption. Number of dead termites was also recorded to determine the percentage mortality. Each concentration of oil was repeated thrice to check accuracy.

Fumigation test: For fumigation test, modified AWPA E1-06 (2007) method was used to observe the fumigant activity of oils against termites. Modifications include the use of soil instead of sand and glass container was 87 mm diameter × 93 mm height instead of poplar wood specimens (25 mm × 25 mm × 5 mm diameter) were dipped in individual test oil (0.03125, 0.0625, 0.125, 0.25 and 0.5 %) for 15 sec. Treated specimens were air dried and then placed in an incubator at 25 ºC over night. This test was conducted in glass container (87 mm diameter × 93 mm height) with 20 g soil, 3 mL water, filter paper as food source and 25 termites (20 workers and 5 soldiers). Wood specimens were placed in the bottom of glass container. Containers were covered with lids and placed in incubator at 27 ºC and 85 % relative humidity and checked the termite mortality after 24 h for consecutive days. The percentage mortality rates were calculated.

RESULTS AND DISCUSSION

Physical parameters including colour, odor, yield, solubility, specific gravity, refractive index, acid values, ester values of vetiver (*V. zizanioides*), mint (*M. arvensis*) and neem (*A. indica*) oil are sumarized in Table-1.

TABLE-1
PHYSICO-CHEMICAL INVESTIGATION OF ESSENTIAL OIL OF VETIVER (*V. zizanioides*), MINT (*M. arvensis*) AND NEEM (*A. indica*)

Physical property	Vetiver oil	Mint oil	Neem oil
Colour	Golden yellow	Pale yellow	Dark brown
Odor	Smoky, earth fragrance	Sweet and pleasant	Specific repulsive garlic like odor
Yield	0.06 %	0.14 %	25 %
Solubility	Soluble in 70 % alcohol	Soluble in 70 % alcohol	Solubility in 100% alcohol
Specific gravity	0.98400-1.03500 (at 25 ºC)	0.89500- 0.89900 (at 25 ºC)	0.908- 0.934 (at 30 ºC)
Refractive index	1.520-1.530 (at 20 ºC)	1.44900- 1.46200 (at 20 ºC)	1.4750- 1.4810 (at 20 ºC)
Acid value	1.87	0.3-5	0.02
Ester value	112.0	210-255	185.75

β-Terpineol, menthol, isomenthone, menthone, azulene, 5,6-azulenedimethanol, ^{1}H-cycloprop[e]azulene, α-hexyl-cinnamaldehyde, dihydromyrcenol, ethyl palmitate, methyl-(2E,6E)-farnesoate, *n*-eicosane and *n*-docosane were the major components of the tested oils identified by GC-MS technique (Table-2).

Results of repellency test indicated that at 0.03125, 0.0625, 0.125, 0.25 and 0.5 % concentration of tested oil, neem oil was least repellent, mint oil showed intermediate repellency while vetiver oil was highly repellent (Fig. 1).

Table-3 reveals that vetiver oil is highly toxic causing 50 % mortality at 7.52 µg/µL concentration. While neem oil is least

TABLE-3
PERCENTAGE MORTALITY OF *Heterotermes indicola* (WASMANN) AT DIFFERENT CONCENTRATIONS OF VETIVER, MINT AND NEEM OIL. LC_{50} VALUES OF VETIVER, MINT AND NEEM OIL TO *Heterotermes indicola* (WASMANN) AFTER SEVEN DAYS OF TREATMENT

No. of observation	Treatment	Percentage mortality				LC_{50} (µg/µL)
		Control	1 µg/µL	5 µg/µL	25 µg/µL	
1	Vetiver oil	7.33^{a}±1.45	16.66^{a}±0.33	23.33^{a}±0.88	30.00^{a}±0.57	7.52
2	Mint oil	6.00^{b}±1.15	15.00^{b}±0.57	21.66^{b}±0.88	28.33^{b}±0.88	11.26
3	Neem oil	6.00^{b}±1.73	13.66^{c}±0.88	20.33^{c}±0.33	27.00^{c}±0.57	15.43

Means followed by similar letters in columns indicate non significant differences at the level of $p < 0.05$ according to Tukey's multiple comparison Test.

TABLE-4
MORTALITY RATE AND DURATION OF TEST ACCORDING TO FUMIGATION TEST OF ESSENTIAL OILS AGAINST *Heterotermes indicola* (WASMANN)

Essential oil	Minimum concentration (%)	Mortality rate (%)	Test duration	Maximum concentration (%)	Mortality rate (%)	Test duration
Vetiver oil	0.03125	100	9 days	0.5	100	5 days
Mint oil	0.03125	100	9 days	0.5	100	6 days
Neem oil	0.03125	100	9 days	0.5	100	6 days

toxic among the three tested oils causing 50 % mortality at 15.43 µg/µL concentration. The bioactivity of mint oil is intermediate showing 50 % mortality at 11.26 µg/µL.

TABLE-2
MAJOR COMPONENTS OF THREE OILS IDENTIFIED BY GC-MS ANALYSIS

S. no.	Components
1	β-Terpineol
2	Menthol
3	Isomenthone,
4	Menthone
5	Azulene
6	5,6-Azulenedimethanol
7	1H-Cycloprop[e]azulene
8	α-Hexylcinnamaldehyde
9	Dihydromyrcenol
10	Ethyl palmitate
11	Methyl(2E,6E)-farnesoate
12	*n*-Eicosane
13	*n*-Docosane

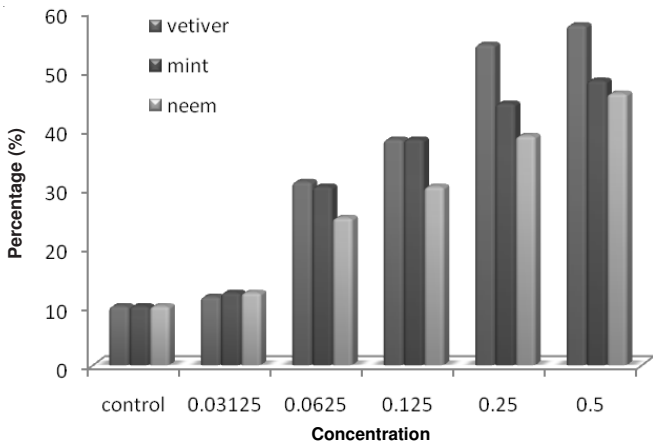

Fig. 1. Comparison of percentage repellency at different concentration of Vetiver, Mint and Neem oil

Results obtained with the threshold of neem (*Azadirachta indica*), vetiver (*Vetiveria zizanioides*) and mint (*Mentha arvensis*) oils acted by fumigation between 1-9 days on *Heterotermes indicola* (Wasmann) revealed that even though termites were not in contact with the oils but all died between 1-9 days of treatments. It is also evident from the table that vetiver oil proved to be highly repellent and toxic while neem oil showed minimum repellency and toxicity. The repellent and toxic effect of mint is in between the both vetiver and neem oils (Table-4).

Previous studies have also proved vetiver oil as repellent and toxic to termites[22]. Zhu *et al.*,[1] indicated the toxic and repellent activity of vetiver oil against *Coptotermes formosanus* (Shiraki). The present study supports the previous work in this regard. Long lasting activity of vetiver oil proved it to be a strong termiticide.

Strong repellency of termites by neem oil was observed in this study, which is in agreement with Deka and Singh[12] who reported the reduction in termites in sugarcane field when treated with neem oil and several other authors reported the same repellent activity of subterranean termites against neem oil and other neem products[23]. Mortality in *Heterotermes indicola* (Wasmann) was assessed under the effect of neem oil. Significant mortality occurred when treated with high level of neem oil. Same results were reported by Grace and Yates[24] while working on *Coptotermes formosanus* (Shiraki).

The results of repellency and toxicity of mint oil to *Heterotermes indicola* (Wasmann) are related to the work of Appel *et al.*,[21]. Repellency increased with the increasing dose of mint oil in the specific time period. The strong repellency potential of the mint oil is due to prescence of menthone, which is in strong agreement with present findings.

The potential of fumigation and toxicity of mint oil against *Heterotermes indicola* was indicated in this study and the results coincide with the findings of Duarte *et al.*[25] in which fumigant activity and insecticidal properties of essential oil of *Mentha arvensis* (mint) were tested against insects.

The bioactivity of essential oils is dependent on their chemical composition. Monoterpene hydrocarbons are less toxic against workers of termites in contrast to oxygenated monoterpenes.

In the present study, the most active essential oil *i.e.*, vetiver oil contained phenolic components but due to the

synergistic effects of phenolic compounds with other components, it is not necessary that an essential oil having phenol is more active in repelling termites.

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