PRELIMINARY EVALUATION OF *OCIMUM SANCTUM* AS TOXICANT AND REPELLENT AGAINST TERMITE, *HETEROTERMES INDICOLA* (WASMANN) (ISOPTERA: RHINOTERMITIDAE)

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ABSTRACT: In the backdrop of recent revival of interests in developing plant based insecticides, the present study was carried out to evaluate the antitermitic properties in medicinal plant *Ocimum sanctum*. The toxic and repellent activity of crude extracts of inflorescence, leaf, root and stem of *Ocimum sanctum* in different solvents i.e., hexane, butanol, chloroform, methanol, ethyl acetate and water were studied against the termite species, *H. indicola*. The termite mortality was observed after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11th day of treatments. Results revealed that after eleven days of feeding, all extracts showed moderate toxic effect, however the maximum termite mortality (84.45 ± 27.21) was observed in the ethyl acetate leaves extract and minimum mortality (43.89 ± 39.97) was observed in stem extract of water. In each extract, mortality was significantly different from control. Also the leaves extract caused more mortality, suggesting the availability of high contents of toxic materials in leaves. All extract treated filter paper had a significantly repellent effect on *H. indicola*. It was also revealed that maximum repellency (29.1) was in methanol root extracts while water extracts showed minimum repellency (21.3).

Key words: Botanical, solvent, mortality, repellency, *H. indicola*

INTRODUCTION

During last 15 years, interest in plant insecticides has increased to look for substitutions for synthetic insecticides with those based on naturally occurring substances (Singh and Saratchandra, 2004). The use of organochlorine insecticides has been banned in developed countries and the alternative methods of pest control are being investigated (Klein and Dunkel, 2003). More than 1000 species of plants have been reported to have chemicals in leaves, stems, flowers, seeds and roots which have insecticidal property, only a few of them have been used for practical insect control on a commercial scale in the past (Shahid, 2003). The use of plants as repellents is very old but has not received the necessary attention for proper development. (Isman, 1997).

Ocimum sanctum is one of the most popular herbs grown in the world and is commonly called Tulsi. It is native to Asia (India, Pakistan, Iran, Thailand and other countries) and can be found growing wild in tropical and sub-tropical regions of the world. Because of its popularity, it is often referred to as the "queen of the herbs" (Bhatnagar *et al*., 1993).The leaves oil is reported to possess anti-bacterial properties and acts as an insecticide. It inhibits the in vitro growth of *Mycobacterium tuberculosis* and *Micrococcus pyogenes* var. *aureus*. It has marked repellent action and insecticidal activity against mosquitoes (Nadkarni, 1976).

In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the toxicant potential of the extracts from the medicinal plant against the termite species *H. indicola.* Although *O.sanctum* has been tried as medicinal plant but its antitermitic activity has not yet been tested so this would be the first study on the antitermitic activity of crude extract of different parts of *O. sanctum* against the termite species *H. indicola* (Wasmann).

MATERIALS AND METHODS

Individuals of *H. indicola* (Wasmann) were collected by bucket trap method placed in the lawn of the Lahore College for Women University, Lahore Pakistan.

The *O.sanctum* (Tulsi) plants were collected from the Botany Department, Government College Lahore University, Pakistan and were brought to Entomology Research laboratory, Lahore College of Women University, Pakistan. The inflorescences, leaves, stem, and root were separated from *O. sanctum* plant and allowed to air dry under shade and were chopped finely up to powder form with the help of mortar and pestle. Twenty gms of the powder sample of each part were

taken and kept separately in two portions of 10 gm each. 200 ml of CH_3OH was taken in a 250ml flask , 10 gm of the root sample was added to it and remaining 10 gm in the other flask in the same way. The same procedure was repeated for the samples of stem, leaf and inflorescence as done in the previous step. Corks were tightly fixed on all the flasks and then all these flasks were kept in a shaker at 30°C at 122rpm for 24 hrs. Two portions were obtained by filtration, The filtrate was preserved as such and labeled as 1st day filtrate. The residues of all the samples were again soaked in 200ml of CH_30H in flasks separately and again placed in shaker at 30°C and 122rpm for 24hrs. After 24hrs, again the flasks were removed from shaker and refiltered. The residue was discarded while the filtrate was added to the previous filtrate so that both 1st day and 2nd day filtrates were collected and preserved. In the next step, 10ml of each of the filtrates was preserved in covered test tube as methanolic extract samples for further steps. The methanolic filtrate was evaporated in this step by using rotary evaporator. The filtrate was poured in the rotary flask and then attached with the rotary. Then vacuum of 500mmHg was established for sometime, raised it up to 650mmHg and then left .The constant rotation of the flask resulted in the evaporation of CH_30H which was collected in collecting flask. As the filtrate was evaporated, the more was added .The evaporation of filtrate resulted in the form of dried residues which sticked to the walls of the rotary flask. All the filtrates of root, stem, leaf and inflorescence were evaporated separately. The residue present in the flask was completely dry. All the dried residues were dissolved in 200ml distilled water and if it was not dissolved completely then added 20ml of CH_3OH. The same procedure was repeated with all the filtrates after treating through rotary. 10ml of all the filtrates were preserved as water extract sample, for further steps and labeled it as water extract before partitioning.

The remaining water extract was treated for partitioning with hexane, chloroform, ethyl acetate and butanol. First of all, the water extract was taken in a separating flask and then added 75ml of hexane to the separating flask but in three batches of 25 each .Hexane formed a separate layer above water from where it was collected in separate 75ml bottle that was also done in three batches. After hexane, the water extract was treated with chloroform 75ml in the three batches as above. Chloroform formed layer below the water extract due to its high density, it was also collected in separate 75ml bottle in three batches. After chloroform, it was treated with ethyl acetate, 75ml same in three batches. Ethyl acetate did not separate early so it was placed over night while adding the whole 75ml at the same time. In the next day, the layer of Ethyl acetate was collected in separate 75ml bottle. After ethyl acetate, the water extract was treated with 75ml butanol in three batches as previous. The layer of butanol was also collected in 75 ml bottle. At the end, water extract was also preserved in separate bottle and labeled it as water extract after partition. All these extracts were preserved for further process of bioassay applications.

Bioassays: The method used for testing toxicity of extract was forced feeding as adopted by Smith (1979).The soil used for the laboratory experiments was sandy loamy, free of contamination. The soil was sieved through a (10×18) mesh screen and was oven-dried at 100 ^{0}C for 24 hours. The required number of Petri dishes were washed and sterilized at 100 ^{0}C for 24 hours prior to the experiment. One ml of hot agar solution (1.5g agar in 100ml H_2O) was spread evenly in the bottom of each Petri dish and was allowed to cool. This agar layer provided adequate moisture for the termites and helped to hold sand in place that was layered above it. Two grams of untreated soil was spread evenly at the bottom of each Petri dish (45mm x15mm) so that soil covered the base of the Petri dish. Filter paper was cut according to the size of Petri dish and placed on the soil. Filter paper and soil were treated with specific amount (0.5ml) of plant extracts with the help of pipette and spread evenly throughout the Petri dish .Then filter papers were air dried for few minutes. Three replicates of each extract including controls were prepared. Finally, 10 termites were released in each Petri dish. The termite mortality was observed daily up to 11 days and dead individuals were removed from each petri dish by forceps and the number of dead termites was recorded.

Repellency Test: Dosage and treatment of the soil and filter paper were the same as those for the soil toxicity test. The only difference was that one half of the bottom of each Petri dish (90mmx15mm) was covered with 2 grams of untreated soil and filter paper and the other with treated soil and filter paper. Then termites were placed in the center of each dish. The dishes were kept in darkness so as to minimize the effect of light randomly on the termites. The temperature was maintained at 25-26^{0}C.The number of termites on either the treated or untreated soil and filter paper was recorded at fifteen minute intervals for each Petri dish. Ten observations were taken. A treatment concentration was considered as repellent when 21 or more of the termites (sum of three replicates) were observed on untreated soil.

Statistical Analysis: The percentage mortality rates were corrected by using Abbott's formula and data were analyzed using a one way ANOVA technique using Graph pad Prism Version 4.00.

RESULTS AND DISCUSSION

The mortality of termites, *H. indicola* (Wasmann), exposed to inflorescences, leaves, root and

stem extract of *O. sanctum* in different solvents i.e. hexane, butanol, chloroform, methanol, ethyl acetate and water in 11 days were recorded. Results indicate that hexane leaves extract were more effective on first day of treatment against *H. indicola* as it caused 16.34% mortality compared to inflorescence (10.00%), root (0.00%), stem (0.00%) and water (0.00%). Analysis of variances revealed significant differences in % mortality in hexane extract of inflorescences, leaves, root, and stem (F., 3.427; d.f., 4:25 P < 0.023). Similarly, butanol leaves extracts were more effective on first day of treatment as it caused 20% mortality compared to inflorescences (6.60%). There were also significant differences in % mortality in butanol extract of inflorescences, leaves, root, and stem (F., 3.663; d.f., 4:25 P < 0. 0176).

As far as antitermitic activity of Chloroform extracts are concerned, it was seen that the inflorescence extracts caused 10.00% mortality on first day of treatment compared to 6.60% mortality in leaves and stem extracts. Analysis of variances revealed significant differences in chloroform extract of inflorescences, leaves, root, and stem (F., 3.072; d.f., 4:25 P < 0. 0346). Percentage mortality of *H. indicola* (Wasmann) in methanol extracts of *O. sanctum* was 20.00% on 1st day of treatment, compared to 16.60% mortality of inflorescence extracts and 100% mortality was observed on 5th day of treatment. Analysis of variances revealed significant differences in % mortality in methanol extract of inflorescences, leaves, root, and stem (F., 3.863; d.f., 4:25 P < 0. 01415). Regarding ethyl acetate extracts, it was observed that the leaves extract of *O. sanctum* caused 40.00% mortality compared to 23.34% in inflorescence and 3.34% in root and stem extracts on 1st day of treatment. Analysis of variance that there were significant differences in % mortality in ethyl acetate extract of inflorescences, leaves, root, and stem (F., 5.965; d.f., 4:25 P < 0. .0016). In water solvent also leaves extract was more effective on first day of treatment as it caused more mortality (10.00%) compared to inflorescence (6.60%), roots (0.00%) and stem (0.00%). Leaves and inflorescence extracts showed 100% mortality on 9th day of treatment while root and stem extracts showed 100% mortality on 11th day of treatment. Analysis of variances revealed significant differences in % mortality in water extract of inflorescences, leaves, root, and stem (F., 2.908; d.f., 4:25 P < 0. 041905).

Table 1 reveals the comparison of the termite percentage mortality in crude extracts of inflorescence, leaves, roots and stems in hexane, butanol, chloroform, methanol, ethyl acetate and water. The inflorescences leaves, root and stem extract in different solvents were not significantly different (P>0.05) from each other but differ significantly (P<0.05) from percentage mortality in control treatment. However, maximum mortality (84.45 ± 27.21) was recorded for leaves extracts in ethyl acetate. Table 2 show results of repellency of *O. sanctum* extract, maximum repellency (25.1) was recorded in ethyl acetate inflorescences extracts while water extracts of inflorescences showed minimum repellency (20.3).

Use of plant products as insect-control agents can be traced back at least as far as the Romans who used species such as white hellebore (*Veratrum album*) and black hellebore (*V. nigrum*). Essential oils & plant extracts are still an important natural resource of pesticides/ insecticides: (Raguraman and Singh, 1997; Gbolade, 2001) or larvicides: (Jacobson, 1983; Adebayo *et al*, 1999; Murty and Jamil, 1987) or insect repellents (Sadik, 1973; Thorsell *et al.* 1998; Oyedele *et al,* 2000). Hostettman (1989). Similarly, plants with pest control properties have been studied by Rosenthal and Janzen, 1979. Grainge and Ahmad (1988) have reported that plants are composed of compounds which have usually been regarded as a part of the plants defense against plant feeding insects. Blaske and Hertel (2001) have studied the repellant and toxic effects of four plant extracts on Subterranean Termites (*Isoptera:Rhinotermitidae*).

So we can conclude our study that *O. sanctum* extract in ethyl acetate could be used as a potential natural termiticide. The whole plant showed repellent activity against *H. indicola*. Such extracts with multiple antitermitic properties may be given priority in future test to isolate antitermitic constituents and to determine their mode of action against termites. However, the results of our study revealed that *O. sanctum* leaf extract have remarkable toxic effect against notorious termites species *H. indicola*. So different parts are arranged for toxicity in following order of preference leaf > inflorescence > root > stem.

Table 1: Effect of different extracts of *O. sanctum* on the Percentage Mortality of *H. indicola* (Wasmann) (X ± SD)

Extracts Solvents	Inflorescences	Leaf	Root	Stem	Control
Hexane	60.0 ± 39.5 a	70.56 ± 36.9b	49.96 ± 39.71c	48.83 ± 41.33d	1.113 ± 1.725e
Butanol	62.21± 38.82a	72.78 ± 37.8b	52.70 ± 39.55c	48.81 ± 40.06d	1.668 ± 2.725e
Chloroform	56.66 ± 39.91a	60.55 ± 39.14b	45.44 ± 38.67c	35.76 ± 29.87d	1.113 ± 1.725e
Methanol	62.21 ± 39.33a	73.89 ± 36.9b	49.37 ± 38.23c	46.00 ± 38.43d	1.668 ± 2.725e
Ethyl acetate	73.89 ± 35.55a	84.45 ± 27.21b	63.33 ± 41.31c	60.56 ± 40.79d	0.5567 ±1.364e
Water	53.88 ± 37.64a	61.67 ± 37.64b	48.89 ± 40.15c	43.89 ± 39.97d	0.00 ± 0.00e

Means marked by the same letter within a row are not significantly different at 0.05 level determined by Tukeys studentized range test. .

The activity of crude plant extracts against termites is often attributed to the complex mixture of active compounds, so this preliminary screening is a good form of evaluation of the potential termiticideal activity of *O.sanctum* used for this purpose

Table 2: Repellency Test for Inflorescence, Leaves, Root and Stem Extract of *Ocimum sanctum* in different Solvents against *H. indicola* (Wasmann)

Extracts	Solvent	No. of Termites on untreated soil and filter paper
Inflorescence	Hexane	21.4 Repellent
	Butanol	23.4 Repellent
	Chloroform	24.4 Repellent
	Methanol	22.2 Repellent
	Ethyl acetate	25.1 Repellent
	Water	20.3 Non- Repellent
Leaf	Hexane	24.4 Repellent
	Butanol	21.6 Repellent
	Chloroform	28.3 Repellent
	Methanol	22.8 Repellent
	Ethyl acetate	25.8 Repellent
	Water	20.6 Non- Repellent
Root	Hexane	26.4 Repellent
	Butanol	25.9 Repellent
	Chloroform	22.3 Repellent
	Methanol	29.1 Repellent
	Ethyl acetate	25.2 Repellent
	Water	20.8 Repellent
Stem	Hexane	28.00 Repellent
	Butanol	28.7 Repellent
	Chloroform	24.9 Repellent
	Methanol	25.9 Repellent
	Ethyl acetate	25.3 Repellent
	Water	20.4 Non-Repellent

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