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Potential of Antitermitic Activities of Eucalyptus Oil

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Abstract.- Laboratory bioassays on essential oil from Eucalyptus (*Eucalyptus citriodora*) was tested for its toxicity, repellency and fumigant activity against three economically important termite species: *Heterotermes indicola* (Wasmann), *Microtermes obesi* (Holmgren) and *Odontotermes obesus* (Rambur) of Pakistan. Termite toxicity was tested at different doses *i.e.* 1.25mg/600µl, 2.5 mg/600µl, 5 mg/600µl and 10 mg/s600µl of ethanol. 100% mortality was observed after nine days of exposure for *M. obesi* at all doses applied. For *H. indicola*, 100% mortality was not observed at the dose of 1.25mg/600µl at the end of test period (after 14 days). For *O. obesus* eucalyptus oil proved to be toxic and repellent within 24 hours of exposure at all the tested doses. Furthermore, it was also evident from the results that eucalyptus oil was highly repellent against termites. LC_{50} values for *H. indicola* and *M. obesi* were 3.715, and 7.15 respectively. So present study investigated the potential of eucalyptus oil from commonly used plants in Pakistan as an environmentally safe measure to control termites, the major urban pest of Pakistan.

Key words: *Heterotermes indicola, Microtermes obesi, Odontotermes obesus,* eucalyptus oil, toxicity, repellency

INTRODUCTION

Termites are economically important urban and agricultural pests of Pakistan. For controlling termites, insecticides derived from plants, which are environment friendly and least toxic method of pest control are used (Ring *et al*., 2000). The use of plants in pest management is not only useful for suppression of pest population but also helps to maintain the sound ecological balance. Many environmental problems such as development of resistance of pesticides in pests, resurgence of target and non-target pests, destruction of beneficial organisms may be reduced after proper use of the active ingredients present in the plants (Singh and Saratchandra, 2002).

Thus, the economically important termite species: *Heterotermes indicola* , *Microtermes obesi* and *Odontotermes obesus* are common pests of building structural wood in Pakistan, the essential oils are cheap source as a control measure to replace the insecticides. Laboratory trials were conducted to find out the toxic and repellent effects of eucalyptus oil as a potential toxicant and repellant to control termite.

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Eucalyptus (*Eucalyptus citriodora*) belongs to family Myrtaceae. The main chemical components of *E. citriodora* oil are 1, 8-cineol (65%), limonene (12 %). Eucalyptus oil is extracted from the fresh or partially dried leaves and young twigs. Eucalyptus oil has a history of wide application, as a pharmaceutical, antiseptic, repellent, flavoring, fragrance and industrial uses (Salari *et al*., 2006). The use of eucalyptus oil as a natural pesticide have immense significance in view of the environmental and toxicological implications of the indiscriminate use of synthetic pesticides and overcoming/reducing the problem of increasing pest resistance (Batish *et al*., 2000).

MATERIALS AND METHODS

Collection of termites

Individuals of *H. indicola* , *M. obesi* and *O. obesus* were collected by bucket trap method from infested areas. The traps were collected and transported to the laboratory, all the debris was removed and termites (workers and soldiers) were kept in plastic boxes with moist filter paper. Before exposure to essential oil, termites were kept in the insectariums at (26±2°C, 80% R.H) in constant darkness to acclimatize active termites. Only active and healthy termite workers (possible 6 and 7 instars) were used for the experiments.

Collection of plant materials and extraction of essential oil

Fresh leaves of Eucalyptus (*E. citriodora*) were collected from Jallo forest park, Lahore, brought to Entomology Research laboratory, Lahore College for Women University, Lahore Pakistan. Oil was extracted by steam distillation (Reverse Dean-Stark method)

Bioassays

Soil toxicity test

The experimental method of this bioassay is same as published by Kang *et al.* (1990). The essential oil serial dilutions of 1.25mg/600µl, 2.5mg/600µl, 5mg/600µl, and 10mg/600µl were made using ethanol solvent. Essential oil concentrations were topically applied to filter paper (Whattman No. 1). Filter paper samples treated with solvents only were used as control. Once the solvent was removed from the treated papers by air drying at room temperature (65% RH, 20°C), 10 active termites (workers) were put on the vicinity of each filter paper impregnated with the test material placed in a Petri dish. The test dishes with covers were then placed in a climatic room temperature maintained at 27°C, 80% RH with few drops of water were periodically dripped to the bottom edge of each Petri dish. The rate of mortality and the consumption percentage of filter paper were examined daily for 14 days. Dead termites were removed by forceps after every counting. Three replicates were made for each test sample and the data were averaged.

Repellency test

The testing procedure for repellency was modified from Lewis *et al.* (1978). A Petri dish (5cm in diam and 1 cm high) was used as for toxicity. One half of the bottom of each Petri dish was covered with 2 grams of untreated soil and filter paper, the other half 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 on the termites. The temperature was maintained at 26.5±2°C and 80% RH. The number of termites on either the treated or untreated soil or filter paper were 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.

Tunneling bioassays

To evaluate the effect of different essential oils on the tunneling behavior of termite species *H. indicola*, *M. obesi* and *O. obesus*, the assay apparatus was designed 5 grams of soil moistened with 1ml distilled water was placed in the vials No.1 and No. 3 and in the middle vial No. 2, 10 grams of soil was mixed with 2ml distilled water. Soil was treated with eucalyptus oil (placed in vial 2) in its lowest 1.25 and highest doses of 10mg/600µl each. Soil was treated 24hrs prior to experiment and was evaporated in uncovered vials for 24hrs to allow the solvent to evaporate. 100 termites (90% workers) were placed in 1st vial so that they may travel to treated zone and travel through the whole apparatus. There were three replicates and in each control no treatment was applied. Tunneling activity was recorded daily.

Inhalation test

1 cm² of filter paper was treated with 10 µl of crude essential oil and with the most active diluted essential oils. These treated filter papers were placed on the lid of the cylindrical glass containers in order to avoid contact with the termites. 30 termite workers were added to the test device and the tests were performed in triplicate. Based on the mortality rate, the oils were classified as toxic without contact or not (Fig. 1)

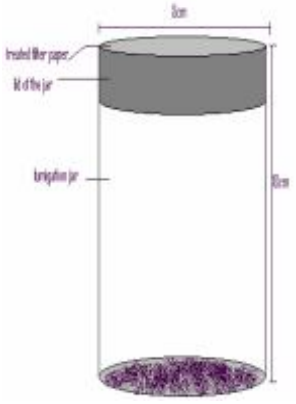

Fig 1. Inhalation test apparatus.

Feeding deterrence tests

Test containers of 4 x 5 cm diam. size were used for this test. Hundred termites (90 workers, 10 soldiers) and 50 grams soil mixed with 10 ml distilled water were kept in each container. Test blocks of popular were treated with different doses *i.e.* 1.25, 2.5, 5 and 10 mg/µl of six essential oils. Termites were exposed to top layer of soil in a no-choice feeding test. After soaking blocks were dried for 24 hrs prior to test. Blocks were weighed prior and after the experiment. Difference in initial and final over dried weight of the poplar wood blocks among treatment were tested by ANOVA.

Data analysis

The percentage mortality was corrected by using Abbott's formula, Consumption was calculated as the difference between the initial weight of filter paper and final weight after testing. Tukey's standardized range test was used to compare the difference between treatments for different species. Regression analysis was obtained according to Busvine (1971) and Finey (1964). Difference in initial and final over dried weight of the poplar wood blocks among treatment were tested by ANOVA.

RESULTS

Soil toxicity test

From this laboratory bioassay results it is evident that after day 1 of treatment, the percentage mortality for *H. indicola* was 0.00%, 10.00%, 16.66%, 23.33% and 0.00% at doses of 1.25mg/600µl, 2.5mg/600µl, 5mg/600µl, 10mg/ 600µl and control, respectively. During 9th day of treatment, all workers of *H. indicola* died giving 100% mortality at all doses tested with the exception of dose 1.25mg/600µl, but in control no natural termite mortality was observed after 14 days of test period. Analysis of variance revealed that different concentrations of eucalyptus oil tested against *H. indicola* were significantly different among all treatments (F = 3.400, df = 4, 20; p=0.0282). *M. obesi* , after day 1 of treatment, the percentage mortality was recorded 6.66%, 16.66%, 23.33%, 30.00% and 0.00% at doses of 1.25mg/ 600µl, 2.5mg/600µl, 5mg/600µl, 10mg/600µl and control respectively. During 9th day of treatment, all workers of *M. obesi* died giving 100% mortality at all tested doses. Increasing mortality was observed on all termite species with increasing dose and number of days. No termite mortality was observed at control (untreated) after 14 days of test period. Analysis of variance revealed that different concentrations of eucalyptus oil tested against *H. indicola* were significantly different (F=4.031, df= 4, 20; p=0.0148). Regarding toxicity of eucalyptus oil against *O. obesus*, it caused 100% mortality at all tested doses after one day (Table I). The LC_{50} values of eucalyptus oil against *H. indicola, M. obesi* were 7.58 and 3.71, respectively. Regression lines showing the LC_{50} of eucalyptus oil after 5 days of treatment for *H. indicola* and *M. obesi* exposed to 4 different concentrations are shown in Figure 2.

Table I.- Anti-termitic activities of eucalyptus oil against three species of termites at a dosage of 10mg/600µl of ethanol.

Termite species	Termite mortality (%)					
	1d	3d	5d	7d	9d	11d
M. obesi	6.30± 4.67 [a]	12.00± 5.19 [a]	20.01± 1.73 [a]	24.99± 4.58 [a]	30.0± 0.0 [a]	--
H.indicola	6.99± 1.73 [a]	9.99 [b] ±1.73	16.98 [b] ±1.73	21.99 [a] ±1.73	30.0± 0.0 [a]	--
O.obesus	30.0± 0.0 [b]	-	-	-	-	-
Control	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [c]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0±0.0

Numbers followed by similar letters within columns are not significantly different at the level of P<0.05 according to tukey's studentized range test. Mean (n= 3) using 30 termites per replicate.

As discussed in materials and methods when tunneling behavior of *H. indicola , M. obesi* and *O. obesus* was observed through the apparatus designed, results revealed that termites did not penetrate through the treated vial with the eucalyptus oil neither after 1 day nor after 14 days of treatment. Greater termite mortality was observed at dose of 2.5mg/600µl, 5mg/600µl, 10mg/600µl but lower mortality was observed at dose of 1.25mg/600µl. When behavior of termites was observed, it was seen that all the workers gathered at the top of apparatus and become sluggish to moribund rather than penetration. In solvent control, no termite mortality was recorded and primary,

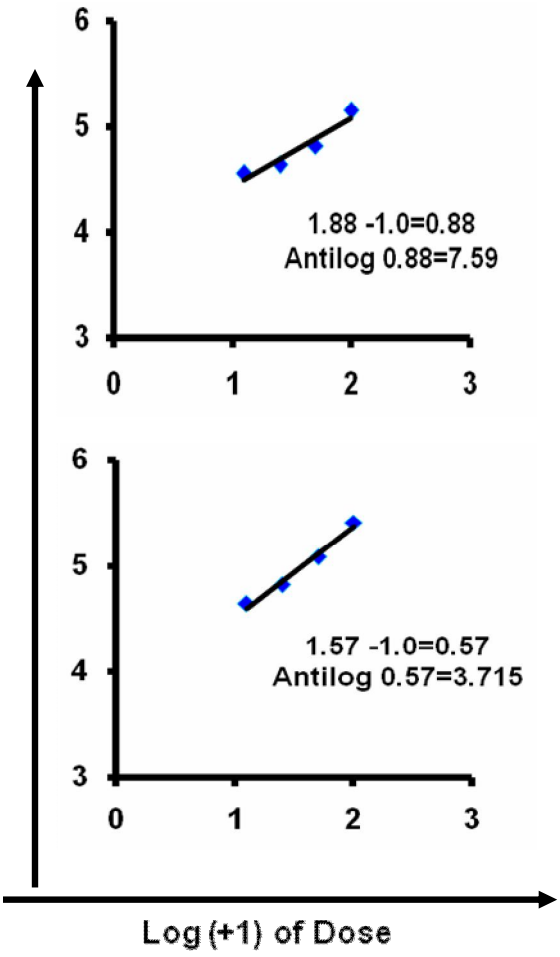

Fig. 2. Regression lines showing the LC_{50} of eucalyptus oil after 5 days of treatment for A), *H. indicolal* B), *M. obesi* exposed to 4 different concentrations.

secondary and tertiary tunneling was evident. Repellency results also showed that this oil had excellent repellent properties even at a lower dose of 1.25mg/600µl and should be considered as potential termiticide (Fig. 3). Inhalation study also revealed that despite termites were not in contact with the treated material but all termites died within 2-4 days of exposure.

From the feeding deterrence test when tested on popular blocks (treated with different essential oils) it was evident that significantly less or no feeding occurred on wooden blocks (treated) when placed in soil with all treatments of essential oils. A significant weight loss was observed in control treatments.

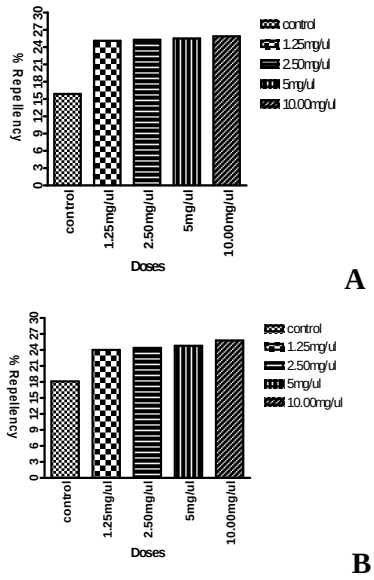

Fig. 3. Repellency (%) of *H. indicola* (A) *M. obesi* (B) exposed to different concentrations of eucalyptus oil.

DISCUSSION

Public awareness and resulting environmental agencies rulings have led to the removal of some chlorinated, organophosphorous and carbamate insecticides from the market. It is because of growing concern for clear environmental and insect populations that are resistant to conventional chemicals (Singh and Saratchandra, 2005). New generation Chemicals like thiodan, cypermethrin, imidacloprid, fipronil, carbosulfan and triazophos are being recommended (Kumawat, 2001; Rana *et al*., 2001). Increasing reliance on conventional pesticides to combat intricate pest problems during the past four decades paid rich dividends in terms of enhanced productivity and suppression of diseases but proved extremely risky to environment and health and safety issues. Repeated application of synthetic organic pesticides resulted into pest resistance and out break (Bultman *et al*., 1979).

Researchers in different parts of the world have been using plants in controlling pests including termites, because previous studies revealed that the different plants extracts are eco-friendly and can play more role in the control of termites. Our knowledge of the biology and economic significance of the termites damaging wood in the

building is poor. On the other hand, quite extensive work has been completed in different parts of the world to determine the natural termite resistance wood (timber).

In view of the recently increased interest in developing plant origin insecticides as an alternative to conventional insecticide, this study was undertaken to assess the toxicant potential of eucalyptus oil against the economically important termite species in Pakistan. Although essential oils have been tried as mosquito repellent, antifungal and antimicrobial agents but there are few studies on the antitermitic activity of essential oil in Pakistan (Akhtar and Jabeen, 1981). So keeping in view the significance of essential oils, the present study was carried on the antitermitic activity of essential oils against the termite species *H. indicola*, *M. obesi* and *O. obesus*. When termite species *O. obesus* was tested to evaluate its toxicity, repellency, tunneling ability, feeding deterrence test and inhalation test (fumigation). All the termite workers were found to be dead within 8 hours of treatment showing highly repellent and toxic effects of eucalyptus oil. When all the three termite species were compared for toxicity, oil was more toxic for *O. obesus*. When *H. indicola* and *M. obesi* were compared for toxicity there were no significant ($P<0.05$) differences in toxicity for all the tested doses of oil. The difference in mortality between these species may be due to difference in their nest building nature. Overall eucalyptus oil appeared to be best at dose of 10mg/600µl of ethanol.

CONCLUSIONS

From these laboratory bioassay results it was concluded that eucalyptus oil in ethanol solvent have excellent termiticidal properties, showing 100% mortality after 14 days of the treatment against *Heterotermes indicola*, *Microtermes obesi* and *Odontotermes obesus*. In future, further investigations can enhance our results by isolating individual components by either HPLC separation or GC-MS identification methods, and eucalyptus oil would prove to be economic and ecofriendly biopesticide.

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