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Toxic potential of *Melia azedarach* leaves extract against *Odontotermes obesus* and *Microtermes obesi*

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Key words: *Melia azedarach, Odontotermes obesus, Microtermes obesi,* biochemical components, termiticidal.

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Abstract

The termites are small insects (order: Isoptera, class: Insecta), world widely distributed but their infestation cause a loss of billions of dollar each year in a single country. The two termite species *Microtermes obesi* and *Odontotermes obesus are* commonly found in Pakistan and for the present study, were collected from the locality of Quaid-i-Azam University Islamabad in the month of June 2013. The extracts in water and methanol solvent with varying concentrations (100ppm, 200ppm, 300ppm) of *Melia azedarach* were tested against both termite species selected. 40 termite workers were placed in each petri dish having filter paper soaked in every concentration of extract taken in each solvent. Mortality of termites and LC_{50} and LC_{90} was calculated after each 24, 48 and 72 hrs of the experiment compared with day zero and found to be 0.002 and 0.007 % in water solvent and 0.0003 and 0.0004% after 72 hrs in methanol solvent against *O. obesus* respectively. Similarly LC_{50} and LC_{90} value against *M. obesi* after 72 hrs were found to be 0.001 and 0.009% in water solvent and 0.002 and 0.008% in methanol solvent. The results were analyzed by using one way ANOVA and Tukey test. Total carbohydrate (mg/g), lipid mg/dl (triglyceride, cholesterol and high density lipid) and Protein (mg/g) contents of dead termites were estimated by Lowry's method, phenol sulphuric acid method and by biochemistry analyzer. Carbohydrate, lipid contents were decreased and protein contents of both the termite species was increased as compared to control. The change in these biochemical components may be due to the insecticidal stress caused by these extract which lowered the feeding, proper digestion of food and metabolism. The *M. azedarach* leaves extract were found to be toxic for both termite species. The active component can be characterized and isolated by GCMS for commercial use.

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Introduction

Billions of dollars are spent annually throughout the world to control and prevent termite infestation and renovation processes (Su *et al.*, 1987; Su and Scheffrahn, 1990; Ahmed *et al.*, 2007). A total of 2,600 known termite species exist, out of which only 40 species has been identified in the United States and 53 in Pakistan. Out of these 11 species were found to cause damage (Kambhampati and Eggleton, 2000; Iqbal and Saeed, 2013).

In Pakistan agricultural crops are mostly attacked by fungus growing termites, *Microtermes* and *Odontotermes* species. *Microtermes obesi* and *Odontotermes obesus* cause severe damage to the green foliage crops in different areas of Punjab including Lahore, Qadeerpur and Gojra. Major damage was found to be caused by *M. obesi* to sugarcane fields (Akhtar and Shahid, 1993). Wheat and sunflower are more badly affected by *O. obesus*. The Indian white termite, *O. obesus* (Rambur) has wide range of distribution in central Asia which results into major economic losses i.e. damage wooden cabinets, fuel wood, floor timber and railway tracks (Akhtar and Anwar, 1975).

Synthetic pesticides which are used for the control of insects and pests, they directly target the human health causing serious disorders such as immune system deformities, cancer and birth defects and are more expensive than natural products (Nigam and Bhatt, 2001; Bounias, 2003). Many attempts have been made in field and laboratory to exploit antitermite activities of plants extracts. Some plant species were used in past to explore their anti-termite activities, insecticidal properties and anti-feedant activities and contain certain chemicals that reduce termite growth or kill them (Adams *et al.*, 1988), such as *Taiwania cryptomerioides* (Chang *et al.*, 2001), *Eucalyptus globulus* (Zhu *et al.*, 2001), *Calotropis procera* (Shing *et al.*, 2002), *Tabebina guaycan, Lysitoma seemnii, Diospyros sylvatica, Pseudotusuga menziesii,* (Ganapaty *et al.*, 2004), *Coleus amboinicus* (Singh *et al.*, 2004), *Curcuma aromatica* and *Euphorbia kansuii* (Shi *et al.*, 2008). These extracts reduced termites feeding habit and decrease their survival rate. The aim of present study was to formulate non-toxic bio pesticide of *M. azedarach* crude leaves extracts to control highly infestation causing termites *O. obesus* and *M. obesi.*

Materials and methods

Termite collection

Two termite species *M. obesi* and *O. obesus* were collected from the locality of Quaid-i-Azam University in the month of June 2013 by using a collection trap unit as described by Sornnuwat *et al.*, (1996) with some modifications and identified with the help of taxonomic keys (Akhtar, 1983).

Collection of Plants

The leaves of *M. azedarach* were collected from the locality of Quaid-i-Azam University, Islamabad, Pakistan and identified by using key (Nasir and Ali, 1977).

Extraction method

Leaves of *M. azedarach* were splashed with water to remove the accompanying organisms and attached salts. Leaves were dried in oven at 37°C and crumpled with the aid of electric grinder. 30g grinded leaves (40 passed and 60 mesh retained) were extracted in 300ml of two different solvents i.e. methanol and water in a Soxhelt extraction apparatus. The dried residues were collected by evaporating the solvent with the help of rotary vacuum evaporator stored in a refrigerator for making stock solution.

Antitermite Assay

The no choice feeding method described by Kang *et al.* (1990) with some modifications was carried out to find out anti termite efficacy of extracts. Different concentrations like100 ppm, 200 ppm and 300 ppm were prepared and 1.5 ml of each solution was applied on filter paper (Whatman No. 1) dried the filter paper and 40 termite workers were put in the each petri dish and forced them to feed on extracts impregnated filter papers for humidity and water source a cotton plug soaked in water was placed with termites. Control experiment was set up in which the filter

paper was dipped in solvents only. Few drops of water were dropped daily on cotton plugs keeping constant humidity. Experiment was conducted in triplicate for each sample concentration along with the set of control and percent mortality was counted after 24, 48 and 72 hours.

% mortality = ODP÷TP×100.

Biochemical assay

After the exposure of termites to plant extract the dead termites were removed, washed with saline solution, dried and measured with electrical balance. Sucrose solution (0.25 molar) was used to homogenize samples with the help of dounce homogenizer. The homogenate was centrifuged at 13000 rpm for 15-20 minutes. The supernatant was collected and stored at 20°C for biochemical analysis. Protein contents of termites were evaluated by using Lowry's method. The standard was Bovine serum albumin (BSA). For estimation of carbohydrate content Phenol Sulphuric acid method was used. The standard here used was Glucose solution. Triglyceride (TG), cholesterol, and high density lipid (HDL) contents were estimated through biochemistry analyzer.

Statistical analysis

Mortality ratio percentage of termites was calculated and analyzed by using one way Anova and Tukey test. Values of $P<0.05$ were considered significant statistically. LC_{50} and LC_{90} were calculated by using Probit analysis (Finney, 1971).

Results

Effect of M. azedarach leaves extract on O. obesus in water solvent

Water soluble leaves extract of *M. azedarach* showed 7.50±0.57, 18.33±0.88, 10.20±1.76 percent mortality by feeding on 100 ppm, 200 ppm and 300 ppm respectively on first day of experiment where as in control no significant mortality was observed i.e. 3.33±0.33 (Figure 1.1). LC_{50} value was 5.64% and value of LC_{90} was 28.7% (Table 1). On 2nd day of experiment percent mortality rate on same concentration (100 ppm, 200 ppm and 300 ppm) was 50±0.24, 69.16±1.20 and 73.33±1.86 while in control observed mortality was as 4.16±0.33 which was non-significant (Figure 1.1). LC_{50} value was 0.009% and value of LC_{90} was 0.084% (Table 1). Maximum mortality rate was observed on 3rd day of experiment at all above mentioned concentrations which was 93.33±1.76, 96.66±0.33, 99.16±0.33 and non-significant mortality rate of control was 7.5±0.58 observed (Figure 1.1). LC_{50} value was 0.002 and value of LC_{90} was 0.007% (Table 1).

Table 1. Toxicity value of aqueous and methanolic crude leaves extract of *M. azedarach* against *O. obesus* and *M. obesi.*

Plant	solvent	Species	Time (days)	LC_{50} %	LC_{90} %
M. azedarach	Water	*O. obesus*	1	5.64	28.7
			2	0.009	0.08
			3	0.002	0.007
		M. obesi	1	0.049	0.515
			2	0.017	0.206
			3	0.001	0.009
	Methanol	*O. obesus*	1	0.31	17.01
			2	0.006	0.064
			3	0.0003	0.004
		M. obesi	1	0.049	1.385
			2	0.009	0.068
			3	0.002	0.008

Effect of M. azedarach leaves extract on M. obesi in water solvent

Water soluble leaves extract of *M. azedarach* showed 20.83±1.20, 25.83±1.45 and 42.5±1.73 percent mortality by feeding on 100 ppm, 200 ppm and 300 ppm respectively on first day of experiment where as in control no significant mortality was observed i.e. 4.16±0.88 (Figure 1). LC_{50} value was 0.049% and value of LC_{90} was 0.515% (Table 1). On 2nd day of experiment percent mortality rate on same concentration (100 ppm, 200 ppm and 300 ppm) was 41.66±1.20, 45.83±0.88 and 65.83±0.4 while in control observed mortality was as 9.16±0.88 which was not significant (Figure 1.1). LC_{50} value was 0.017% and value of LC_{90} was 0.206% (Table 1). Maximum mortality rate was observed on 3rd day of experiment at all above mentioned concentrations which was 91.66±1.02, 93.33±0.88 and 99.16±0.33 and mortality rate of control was 12.50±0.57 which was not significant (Figure 1). LC_{50} value was 0.001% and value of LC_{90} was 0.009% (Table 1).

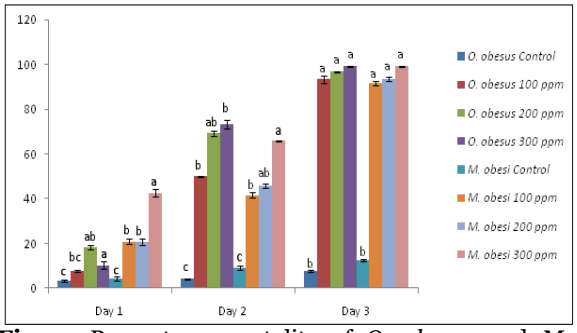

Fig. 1. Percentage mortality of *O. obesus* and *M. obesi* treated with different concentrations *M. azedarach* leaves extract in water solvent. Columns with the same letter at specific time interval are not significantly different (p<0.005) by Tukey test.

Effect of M. azedarach leaves extract on O. obesus in methanol solvent

Leaves extract of *M. azedarach* in methanol solvent showed 13.33±1.20, 18.33±1.0 and 22.5±1.53 percent mortality by feeding on 100 ppm, 200 ppm and 300 ppm respectively on first day of experiment where as in control no significant mortality was observed i.e. 3.33±0.88 (Figure 2). LC_{50} value was 0.31% and value of LC_{90} was 17.01% (Table 1). On 2nd day of experiment percent mortality rate on same concentration (100 ppm, 200 ppm and 300 ppm) was 59.16±1.02, 71.66±1.20 and 80.88±1.76 while in control observed mortality was as 5.83±0.33 which was non-significant (Figure 1.2). LC_{50} value was 0.006% and value of LC_{90} was 0.064% (Table 1). Maximum mortality rate was observed on 3rd day of experiment at all concentrations which was 95.83±1.50, 97.5±1.53 and 99.16±1.20 and mortality rate of control was 8.33±0.57 which was non-significant (Figure 2). LC_{50} value was 0.0003% and value of LC_{90} was 0.004% (Table 1).

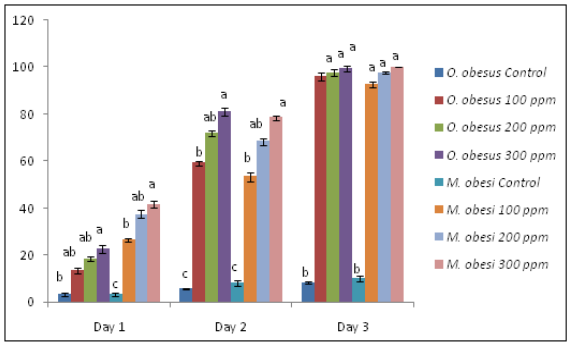

Fig. 2. Percentage mortality of *O. obesus* and *M. obesi* treated with different concentrations *M. azedarach* leaves extract in methanol solvent. Columns with the same letter at specific time interval are not significantly different (p<0.05) by Tukey test.

Effect of M. azedarach leaves extract on M. obesi in methanol solvent

Leaves extract of *M. azedarach* in methanol solvent showed 26.5±0.88, 37.33±1.73 and 41.66±1.45 percent mortality by feeding on 100 ppm, 200 ppm and 300 ppm respectively on first day of experiment where as in control no significant mortality was observed i.e. 3.33±0.88 (Figure 2). LC_{50} value was 0.049% and value of LC_{90} was 1.38% (Table 1). On 2nd day of experiment percent mortality rate on same concentration (100 ppm, 200 ppm and 300 ppm) was 53.33±1.85, 68.33±1.45 and 78.33±0.85 while in control observed mortality was as 8.33±1.20 which is non-significant (Figure 2). LC_{50} value was 0.008% and value of LC_{90} was 0.068% (Table 1). Maximum mortality rate was observed on 3rd day of experiment at all above mentioned concentrations which was 92.5±1.15, 97.5±0.57 and 100±0.00 respectively and a non-significant mortality rate of control was observed i.e. 10.00±1.15.

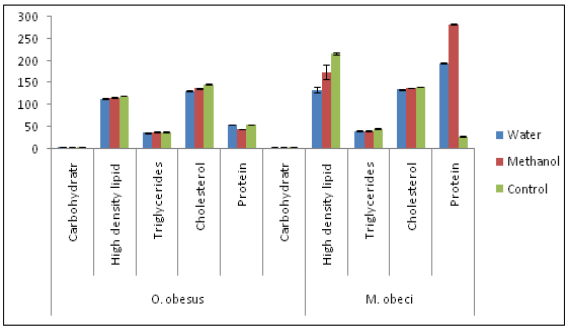

Fig. 3. Comparison of Carbohydrate, lipid and protein content of *O. obesus* and *M. obesi* in *M. azedarach* leaves extract in methanol and water (solvent) at 300 ppm.

(Figure 2). LC_{50} value was 0.002% and value of LC_{90} was 0.008% (Table 1).

Biochemical test

After the treatment of both termite species with *M. azedarach* leaves extract in methanol and water (solvent) at 300 ppm, protein level of *O. obesus* was 44.36±0.39 and 55.08±0.33 which was lowered from control 54.61±0.05 in methanol and higher in water solvent. Similarly protein level of *M. obesi* was 283.22±0.21 and 194.33±0.52 which was also less as compared to control 286.21±1.04 (Figure 1.3). Carbohydrate level of *O. obesus* and *M. obesi* in *M. azedarach* in methanol and water (solvent) at 300 ppm was 2.243±0.64 and 4.839±0.03 and 3.47±0.61 and 3.15±0.85 respectively which was also lowered from control 4.83±0.06 and 4.82±0.05 (Figure 3).

Similarly cholesterol level of *O. obesus* in *M. azedarach* leaves extract in methanol and water (solvent) at 300 ppm was 137.50±1.52 and131.20±0.44 lowered from control 145.60±1.27. In case of *M. obesi* cholesterol level was 137.80±0.40 and 135.10±1.42 which was also lowered from control 140.60±0.75 (Figure 3). Level of triglyceride of *O. obesus* was 38.06±0.92 and 36.71±1.33 lowered from control 39.48±0.54. Similarly TG level of *M. obesi* was 40.9±1.03 and40.14±1.28 lowered from control 45.57±0.49 (Figure 1.3). HDL level of *O. obesus* in *M. azedarach* leaves extract in methanol and water (solvent) at 300 ppm was 16.85±1.34 and 113.55±1.45 which was lowered from control 119.57±0.48. The HDL level of *M. obesi* was 174.41±15.84 and133.97±7.26 lowered from control 215.87±2.61 (Figure 3).

Discussion

In Pakistan many entomologists and agronomists used plants' extracts in different solvents as termiticidal. Whereas the loss of wood, live plantation and agriculture fields due to highly termite infestation in urban and rural areas increasing day by day especially by *Odontotermes obesus* and *Microtermes obesi.* Many plants showed the significant resulted as Ahmed *et al* 2007 reported extracts of leaves, seeds and wood of *Withania somnifera, Croton tiglium Hygrophila auriculata*, *Adhatoda vasica, Salvadora oleiodes, Grevillae robusta* and *Tephrosia purpurea* against the foraging and tunneling behavior of *Microtermes obesi.* The extracts of leaves of *M. azedarach* are antifeeding, deterrent and modify the growth patterns of insects. Crude acetone extract and oil of ripe fruit of *M. azedarach* exhibited highly signigicant insecticidal activity against the second and fourth instar larvae of spodoptera littoralis (Boisd) (Lepidotera: Noctuidae) (Farag *et al.*, 2011).

The persistent use of synthetic insecticides producing environmental contamination which leads to increased resistant capacity and the extinction of non-target organisms. So it has been resulted in need to find out plant derived compounds as a substitute for termite control (Rupal *et al.*, 2011).

C. decidua extracts and its mixture with other compounds have been evaluated by Upadhyah *et al.* (2010) against Indian white termite *O. obesus*. This plant shows very strong termicidal activity. And when different components were used to prepare mixtures with *C. deciduas* and applied to termites their mortality rate was significantly increased and higher mortality was observed ($p<0.05$ and 0.01). Abbas *et al.* (2011) in Pakistan reported antitermitic activity and phytochemical analysis of fifteen medicinal plants in which *Melia azadirachta resulted significant eradication of termites at concentration of 10% in ethanol extracts.*

In the present study crude leaves extracts of M. azedarach in water and methanol solvent used against *O. obesus* and *M. obesi* at 100ppm, 200ppm and 300ppm respectively. Significant mortality was observed at 300ppm in both termite species on 3rd day of experiment. The toxicity data of these two solvents extracts tested showed that methanol extracts were found to have more toxicity (LC_{50} 0.003) against *O. obesus* and (LC_{50} 0.002%) against *M. obesi* at 3rd day of experiment whereas in water solvent against *O. obesus* LC_{50} was 0.002% and LC_{50} 0.001% against *M. obesi* on 3rd day.

Biochemical analysis of dead termites (treated with 300 ppm in methanol and water solvent) resulted in a significant decrease in glucose level of *O. obesus* and *M. obesi* as compared to control. This decrease in glucose level as compared to control is due to insecticidal stress activated by these extracts and decrease in foraging behavior and incomplete utilization of food material (Sharma *et al.*, 2011).

Similarly protein level in both termite species is was increased as compared to control and increase in this protein content is due to extracts having insecticidal interference of protein production hormone (Sharma *et al.*, 2009) and degradation of body wall content. Lipid level (Cholesterol, HDL, TG) of both termite species was also considerably less. This decrease in lipid profile displays a negative effect of extracts on lipid amount. It may be due to change in energy absorption, peroxidation and insecticidal stress (Sake *et al.*, 2006). These results are in accordance with Lohar *et al.* (1993) who reported diminution in oocyte heamolymph and fat bodies of *Tenebriomolitor* after exposing to malathion.

Conclusion

The present study revealed the antitermitic activity of *M. azedarach* crude leaves extracts in water and methanol have the possibilities to cultivate new and non-toxic products for the control of *O. obesus* and *M. obesi* infestation. *M. azedarach* is most common, accessible and affordable in Pakistan. Therefore, the *M. azedarach* could be a useful substitute for synthetic insecticide controlling termite infestation. And it can also be used as wood preservative to protect wood, furnishers, agriculture crops and living vegetation.

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