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Efficacy of *Moringa oleifera* against *Odontotermes obesus* (Ramb.) (Termitidae: Isoptera)

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Abstract
The present study was undertaken to test the efficacy of leaf and seed extracts of *Moringa oleifera* Linn. In various polar and non-polar solvents on the mortality of workers and soldiers of subterranean termites, *Odontotermes obesus* (Ramb.). The sterilized soil was mixed with extracted materials of *Moringa oleifera* at 1, 5 and 10% concentrations along with a control which had solvents alone. The results revealed decrease in LT_{50} (hours) values with the increase in concentrations. Minimum LT_{50} values (108.87 ± 3.05 to 143.93 ± 3.70 hours) were recorded in N-hexane leaf extracts at all concentrations. While in seed extract at 1% concentration, minimum LT_{50} value was recorded in chloroform (181.87 ± 9.13). The LT_{50} values at 5% and 10% concentrations were minimum in ether (165.98 ± 11.3) and n-hexane (151.10 ± 3.50) solvents.

Keywords: *Moringa oleifera; Odontotermes obesus,* Seed extract; LT_{50}

1. Introduction
Moringa oleifera Linn is distributed in many tropical countries including Pakistan, India, Philippines, Central America, North and South America, and the Caribbean Islands [1, 2]. Its different parts are nutritious and used as food [3]. The leaf and seed extracts of this plant has shown antispasmodic, antiulcer and hepatoprotective, antibacterial and antifungal, antitumor and anticancer activities [2]. The different doses of aqueous seed extract of *M. oleifera* had significant effect on the activities of some internal organs (heart, liver and kidney) and tissue enzymes of male albino rats [4]. The ethanolic extracts of *Moringa oleifera* and *Vitex negundo* had anti-anthelmintic activity against Indian earthworm *Pheritima posthuma* as dose dependent paralysis and death by both plant extracts but *M. oleifera* showed more activity as compared to *V. negundo* [5].
The insecticide properties of *M. oleifera* has recently been observed. A water-soluble lectin from *M. oleifera* seeds had negative effect on development and survival of *Aedes aegypti* larvae [6]. The effect of four *M. oleifera* powders caused mortality of adults and reduced emergence of maize weevil (*Sitophilus zeamais*) on stored wheat grains, however, extent of toxicity was not good enough as protectants atsaid concentrations as compared to *Alstonia boonei* [7]. In another study Ferreira [8] assessed the toxic effects of crude water extract of *M. oleifera* seeds on eggs and 3rd instar larvae of *A. aegypti* and laboratory animals (*Daphnia magna*, mice and rats). The effect of flower extract on gut trypsin and whole-larval acetylcholinesterase has also been shown by [9].
Insecticide property of extracts of *M. oleifera* has been determined mainly for repellency test for mosquitoes; however, a study had shown its termiticide activity as well. Paiva *et al.* [10] investigated the effects of crude and purified preparations containing lectins from *M. oleifera* seeds on *Nasutitermes corniger* workers and soldiers at concentrations of 1.0 and 1.5 mg ml^{-1}. These compounds disturb organization, structure, and maintenance of termite colonies.
The present studies report effect leaf and seed extracts of *M. oleifera* in different solvents along with a control of respective solvent on mortality of *Odontotermes obesus* (Ramb.) (Termitidae: Isopetra) in the laboratory.

2. Materials and Methods
2.1 Collection of *Odontotermes obesus*
The workers and soldiers of *O. obesus* were collected by using underground monitoring traps (Sornnuwat *et al.* [11] buried at the agricultural fields at various places in University of Agriculture, Faisalabad, Pakistan.

The infested traps were brought to laboratory and termites were separated by using the methodology of Gay *et al*. [12]

2.2 Preparation of leaves for Extraction process

Leaves of *M. oleifera* plant were collected from Botanical Garden, University of Agriculture, Faisalabad, where no pesticides or any other chemical was applied on them. The leaves were taken from periphery of plants and washed with distilled water, air dried in a room for two weeks ensuring sufficient air flow to avoid damping. The dried leaves were ground to a powder form by grinding in an electrical grinder (Monilex Australia Pvt. Ltd) for 45 seconds.

2.3 Crude leaf and seed extracts

For crude leaf and seed extracts different non polar to polar solvent such as petroleum ether, n-hexane, chloroform, methanol, ethanol, acetone and water were used and the extracts were prepared by following the methodology of Nisar *et al*. [13]. One hundred gram (100 g) of the leaf powder from each of these parts was extracted in 200 ml of the solvent in a ratio of 1:2 (w/v). The plant material was soaked in each solvent for 24 hours and then shaken in an electrical shaker for 72 hours. The supernatant was filtered with two layers of What-man Filter Paper No. 42. The above procedure was repeated thrice to obtain maximum amount of the extract. All the filtrates were pooled and evaporated under vacuum in a rotary evaporator. The crude extracts were weighed to measure the yield and then used in a desired concentration for the bioassay.Seeds of *M. oleifera* were obtained from fruits and were processed as in case of leaf extract.

2.4 Soil for Bioassay

The soil was sifted through a 30-mesh screen and moisture was determined with the help of a moisture meter. Soil was sterilized in a vacuum oven. Moisture of the soil in the field was also determined in order to obtain uniformity in the moisture content. No-choice bioassays using leaf and seed extracts were performed in Petri dishes of 10 cm diameter × 1.5 cm in height containing twenty grams of the sifted sterilized soil and strips of sugarcane (1.5 cm × 6 cm). Every treatment with 1%, 5% and 10% concentration of extracts and control (without extract) were repeated thrice with a different set of termite workers and soldiers. The soil in Petri dish having sugarcane strip were wetted/ mixed with plant extract concentrations. One hundred active workers and 10 soldiers were released in the Petri dishes having treated and untreated soil placed in a growth chamber under controlled conditions of 28±2°C and 80% ± 5% humidity. Data for mortality were recorded after an interval of 2 hours up to 12 hours and then after every 12 hours until mortality of 100 workers and 10 soldiers was occurred. Kaplan Meiyer Survival test was used to obtain LT_{50} of different extracts at various concentrations in different treatments.

4. Results

4.1 Mortality of *Odontotermes obesus* by leaf extracts of *Moringa oleifera*

The LT_{50}values were decreased with the increase in concentrations of the leaf extract of all polar and non-polar solvents. The LT_{50} values were less as compared to control. Among the seven solvents, minimum LT_{50} were recorded in N-hexane with LT_{50} (hours) values of 143.93 ± 3.70, 126.13 ± 3.59 and 108.87 ± 3.05 at 1, 5, 10% concentrations, respectively. However, maximum LT_{50} values were recorded in water solvent at 1% (159.70 ± 7.34), acetone at 5% (138 ± 3.35) and ethanol at 10% (128.45 ± 5.69) (Table 1).

4.2 Mortality of *Odontotermes obesus* by seed extracts of *Moringa oleifera*

The results revealed reduction is LT_{50} values with the increase in concentrations of the leaf extract of all solvents. The LT_{50} values were less as compared to control. At 1% concentration, minimum LT_{50} value was recorded in chloroform (181.87 ± 9.13). However, maximum LT_{50} values at 5% and 10% concentrations were observed in ether (165.98 ± 11.3) and n-hexane (151.10 ± 3.50). However, maximum LT_{50} values were recorded in acetone with LT_{50} values of 228.75 ± 3.01), 208.43 ± 3.27 and 198.88 ± 2.24 at 1, 5 and 10% concentrations, respectively (Table 2).

5. Discussion

The termite control in many countries is still done by the use of conventional insecticides which causes serious harmful effects on the environment and public health [14]. Botanical insecticides can be one of the best alternative of synthetic insecticides for termite control in urban environment with least harmful effects on the people and other non- target organisms. In the current study, the efficacy of *M. oleifera* extracts in various solvents was tested against an important fungus-growing termite, *O. obesus*. Idoko *et al*. [15] reported maximum efficacy of *M. oleifera* against Dry Wood Termite (*Cryptotermes cavifrons*). Ojo *et al*. [16] studied the efficacy of *M. oleifera* against stored grain pest, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) and reported that increase in mortality of *C. maculatus* with the increase in concentration. Murslain *et al*. [17] also observed the maximum mortality of *Meloidogyne javanica* juveniles when treated with *M. oleifera*. The results in the current study revealed that leaf extracts produce the mortality of *O. obesus* earlier than seed extracts (Table 1, 2). The minimum LT_{50} values were recorded in 10% concentrations of irrespective of extract and solvent type.

Table 1: Comparison of LT_{50} (hours) values in different concentration of leaf extracts of *Moringa oleifera.*

Treatments	Concentrations			
	1%	5%	10%	Control
Methanol	145.25 ±5.38	126.53 ± 7.26	111.22 ± 5.25	253.35 ± 4.11
Ethanol	158.92 ± 4.94	132.21 ± 3.34	128.45 ± 5.69	250.76 ± 3.43
Acetone	150.36 ± 3.68	138.00 ± 3.35	116.23 ± 7.13	262.17 ± 7.44
Ether	156.17 ± 3.67	134.25 ± 2.46	109.85 ± 2.08	266.51 ± 4.24
N-hexane	143.93 ± 3.70	126.13 ± 3.59	108.87 ± 3.05	250.75 ± 9.33
Chloroform	149.99 ± 9.24	134.14 ± 5.12	119.94 ± 3.12	256.66 ± 3.68
Water	159.70 ± 7.34	134.13 ± 1.93	120.36 ± 3.34	266.51 ± 4.24

Table 2: Comparison of LT_{50} (hours) values in different concentrations of seed extracts of *Moringa oleifera.*

Treatments	Concentrations			
	1%	5%	10%	Control
Methanol	216.22 ± 3.81	200.3 ± 6.13	169.86 ± 5.87	286.06 ± 6.41
Ethanol	211.89 ± 4.12	194.19 ± 7.04	178.47 ± 3.58	275.16 ± 1.9
Acetone	228.75 ± 3.01	208.43 ± 3.27	198.88 ± 2.24	323.83 ± 6.88
Ether	182.82 ± 2.89	165.98 ± 11.3	151.59 ± 5.15	267.88 ± 14.5
N-hexane	193.28 ± 5.83	177.9 ± 5.15	151.1 ± 3.5	251.22 ± 10.7
Chloroform	181.87 ± 9.13	169.87 ± 7.57	169.22 ± 9.91	244.84 ± 4.11
Water	206.72 ± 9.0	190.25 ± 3.58	177.04 ± 2.35	314.18 ± 5.48

6. Conclusion

The results suggested that for leaf extract, n-hexane is the best solvent based on minimum mortalities recorded at the three concentrations used in the experiment. At 10% concentration, seed extracts in n-hexane also gave minimum mortality showing the best solvent for *M. oleifera* to manage the infestation of *O. obesus*.

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8. References

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