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Effect of Leaf and Seed Extracts of *Jatropha curcas* Linn. On Mortality and Tunneling of Subterranean Termites, *Odontotermes obesus* (Ramb.) (Termitidae: Isoptera)

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ABSTRACT

Leaf and seed extracts of *Jatropha curcas* Linn. in various polar and non polar solvents were used against workers and soldiers of subterranean termites, *Odontotermes obesus* (Ramb.) to determine the effect on mortality and tunneling behaviour. The extracted materials were added to soil in 1, 5 and 10% concentrations of the final yield. A control was also maintained with solvents alone. The studies showed that all extracts at 1, 5 and 10% concentrations had significantly low LT_{50} in hours than their respective control treatments. LT_{50}s among various polar and non polar solvents were statistically different ($P<0.05$). The reduction in tunnel length (mm) was observed in all extracts and was significantly different from their respective controls. Results are discussed in relation to possible differences of activities in various extracts towards mortality and tunneling of the termites.

INTRODUCTION

In the past, the control of termites has been totally based on chemicals especially synthetic insecticides such as persistent organo-chlorine (OC) and organophosphate (OP) insecticides (Anonymous, 2000; Venkateswara et al., 2005). The maximum residual effects as well as the development of insecticide resistance in target pests along with adverse effects on human health and concerns for environmental deterioration are some of disadvantages that hinder widespread use of pesticides (Coats, 1994). Replacement of synthetic by bio-rational insecticides is a universal acceptable and practical approach worldwide (Logan et al., 1990). In this regard, bioactive compounds of plant origin are considered as ecologically safe alternatives. The plant extracts with complex mixtures of such compounds have been investigated for their insecticidal, repellent, and anti-feedant properties (Zhu et al., 2001; Isman et al., 2006). The defense chemicals in plants offer such promise of developing them as insecticides that can be effective against insects including termites in which case these plant chemicals would replace the persistent synthetic insecticides (Ahmed and Qasim, 2011).

In recent years, the use of local botanicals has gained much importance mainly among the researchers because of their high bio-efficacy against termites. The Lemon grass (*Cymbopogon Citratus*), Cassia leaf (*Cinnamomum cassia*), Vetiver (*Vetiveria zizaniodes)* (Maistrello et al. 2001), Eucalyptus (*Eucalyptus citrodora*, *Eucalyptus globules*), Cedar wood *(Cedrus atlantica)*, Clove bud (*Syzgium aromaticum*) (Zhu et al. 2001), *Coleus amboinicus* (Singh et al., 2004), Isoborneol (Blaske et al., 2003) and *Calotropis procera* (Singh et al., 2002) are some of plants possessing anti-termite activities. The chemical compounds extracted in different organic solvents from these plants, when applied to termites. It caused not only mortality but also made changes in behavior of these insects. The differential anti-termite activities in various solvent extracts of the plants have described organic solvents being the mostly active in the experiments for the said purpose (Ogunsina et al., 2009; Upadhyay et al., 2010; Manzoor et al., 2011 a, b; Elango et al., 2012).

Jatropha curcas, a potential anti-feedant candidate, belongs to the family, Euphorbiaceae. The seed of this

plant is black and oval in shape is rich in fixed oil (Shukla et al., 1996). Both the plant and its seeds are toxic to animals and humans and are therefore used worldwide as hedges to protect agricultural fields (Islam et al., 2011). Jatropha oil is mostly used as a biofuel. The oil has also been used to produce soup, medicine and pesticides (Shanker and Dhyani, 2006). In addition, the Jatropha oil was tested in the protection of stored grain beetle, *Callosobruchus maculatus* (Boateng and Kusi, 2008). *J. curcas* leaf and seed extracts have been found toxic and repellent against the Philippine milk termite *Coptotermes vastator* and *Microcerotermes beesoni* (Singh and Sushilkumar, 2008; Acda, 2009). The present research work highlights an efficacy of *Jatropha curcas* leaf and seed extracts in various solvents against *Odontotermes obesus* (Ramb.) (Termitidae: Isoptera) under laboratory conditions.

MATERIALS AND METHODS

Collection of termites

The workers and soldiers of the termites were collected within the damaged canes from the sugarcane field and from the corrugated cardboard baits in PVC monitors installed in the field at various places around the campus and at Post Graduate Agriculture Research Station, University of Agriculture, Faisalabad (Ahmed et al., 2006). The termite samples were identified in the laboratory; and it was found that only one species, O*dontotermes obesus* (Ramb.) (Termitidae: Isoptera) was abundant and exclusively present at these places.

Extraction method

For crude plant extracts different non polar to polar solvent such as petroleum ether, N-hexane, chloroform, methanol, ethanol, acetone and water were used.

Preparation of leaves for extraction process

Leaves of these plants 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 reduced to a powder form by grinding in an electrical grinder (Monilex Australia Pvt. Ltd) for 45 seconds.

Crude leaf extracts

One hundred gram (100 g) of the leaf powder from each of these plants 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 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.

Crude seed extracts

Seeds of above mentioned plants were purchased from the local market. These seeds were washed with distilled water, air dried in a room for two weeks. Rest of the procedure was same as described in case of leaf extracts.

Soil for Bioassay

The soil for carrying out bioassay was collected from University of Agriculture, Faisalabad and its physical properties composition was determined. Such soil was used in all bioassays. There was no application of pesticides of any sort in this soil. The soil was sieved 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.

Antitermitic bioassays using leaf and seed extracts were performed in Petri dishes of 10 cm diameter × 1.5 cm in height containing 20 g sifted sterilized soil and strips of sugarcane (1.5 cm × 6 cm) to keep the termites alive. 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. Twenty grams of sifted 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. A new batch of the termite workers and soldiers was taken every time and the experiment was repeated thrice, thus making three groups of termite individuals. Kaplan Meiyer Survival test was used to obtain LT_{50} of different plants extracts at various concentrations in different treatments (Minitab 15). The LT_{50}s obtained in each extract were separated by small letters to show differences among them. Hours in results are represented as hrs.

Effect of plant extracts on galleries formation

Members of the Family Termitidae make galleries during foraging. This shows the activity of termites in the soil. The termites started making tunnel along the bottom of each Petri dish around the sugarcane strip. Termites' response towards galleries formation for each extract at each concentration after 5, 10 and 15 hours were determined by plotting the tunnels on the cellophane paper and was measured in mm with the help of planimeter. However, length of tunnel at 15 hr was used for statistical analysis. The mean tunnel

lengths in various treatments of solvents and concentrations were compared by ANOVA using MSTATC though Least Significant Difference (LSD) test.

RESULTS

Mortality of *Odontotermes obesus* by leaf and seed extracts

The difference among three groups of the exposed to 0, 1, 5, and 10% concentrations of *J. curcas* leaf extract in different solvents, was non-significant ($P \geq 0.05$) (data not shown). LT_{50} values in different concentrations of the crude leaf extracts in different solvents were significantly less than control values. The lowest LT_{50} value was observed in acetone extract (112.37 hrs) and was non significantly different from N-hexane (120.35 hrs), ether (124.21 hrs), water (127.79 hrs), chloroform (129.32 hrs) at 10 % concentration and methanol (132.59 hrs) and water extract (136.07 hrs) at 5% concentration (Table 1).

LT_{50} values in case of seed extracts with different solvents represented same picture as that of leaf extract. The lowest LT_{50} value was observed in N-hexane extract (118.15 hrs at 10% concentration) which had non significant difference with acetone (124.92 hrs), chloroform (129.73), ether (130.27 hrs), water (130.00 hrs), methanol (131.49 hrs) at their respective 10% concentration and with acetone (141.63 hrs), ether (142.27 hrs), N-hexane (138.88 hrs) and water extracts (136.07) at 5% concentration (Table 2).

Tunneling activities of *Odontotermes obesus*

Leaf extracts in different solvents

The tunnel length formed by *O. obesus* at 0%, 1%, 5%, 10% concentrations of leaf extracts of *J. curcas* in different solvents is given in Table 3. Maximum tunnel length (127.00 mm) occurred at 1% of ethanol extract which had non-significant difference with petroleum ether, chloroform, and water at 1% concentration but significantly different from all solvents at 5% and 10% concentrations. Minimum tunnel length (45.67 mm) was formed in acetone extract at 10% concentration and had non-significant difference with all solvents at 10% but was significant difference from ethanol, petroleum ether, acetone, N-hexane, chloroform, and water at 5% and all solvents at 1% concentration. Control treatments of all solvents had significantly different from three concentrations of leaf extracts of *J. curcas* (Table 3).

Seed extracts in different solvents

The tunnel length formed by *O. obesus* at 0%, 1%, 5%, 10% concentrations of seed extracts of *J. curcas* in different solvents is shown in Table.4. Maximum tunnel length (139.67 mm) took place at 1% of water extract which had non-significant difference with ethanol but

Table 1: Comparison of LT_{50} values (hrs) of different concentrations of leaf extracts of *J. curcas* in different solvents

Treatment	Concentrations			
	1%	5%	10%	Control (0%)
Methanol	149.40 ± 3.37b-e	132.59 ± 3.68d-g	127.23 ± 3.69efg	243.63 ± 3.42a
Ethanol	160.87 ± 3.19b	143.82 ± 3.36b-f	136.45 ± 2.15c-f	242.89 ± 5.28a
Acetone	150.11 ± 3.90b-e	139.94 ± 1.94b-f	112.37 ± 5.31g	262.18 ± 4.02a
Ether	157.87 ± 5.60bc	140.07 ± 5.28b-f	124.21 ± 5.37fg	239.94 ± 5.07a
N-hexane	153.28 ± 5.38bcd	138.88 ± 4.13b-f	120.35 ± 3.59fg	253.98 ± 7.40a
Chloroform	161.32 ± 5.85b	143.82 ± 3.36b-f	129.32 ± 2.08efg	256.66 ± 3.68a
Water	157.19 ± 3.78bc	136.07 ± 1.93c-g	127.79 ± 2.20efg	260.60 ± 4.25a

Means sharing similar letter in a row or in a column are statistically non-significant ($P>0.05$). Small letters represent comparison among interaction means.

Table 2: Comparison of LT_{50} (hrs) values of different concentrations of seed extracts of *J. curcas* in different solvents

Treatment	Concentrations			
	1%	5%	10%	Control (0%)
Methanol	155.24 ± 3.38cde	139.68 ± 2.08d-i	131.49 ± 1.92f-i	245.61 ± 4.12ab
Ethanol	168.90 ± 1.74c	149.64 ± 3.37c-g	140.31 ± 2.31d-i	246.83 ± 1.83ab
Acetone	154.01 ± 1.72c-f	141.63 ± 1.83d-i	124.92 ± 3.66hi	260.21 ± 4.46ab
Ether	159.81 ± 7.43cd	142.27 ± 3.70d-i	130.27 ± 1.93ghi	239.94 ± 5.07b
N-hexane	145.50 ± 2.08d-h	138.88 ± 4.13d-i	118.15 ± 5.16i	257.93 ± 11.2ab
Chloroform	169.12 ± 1.95c	147.70 ± 5.14c-h	129.73 ± 2.08ghi	250.76 ± 3.43ab
Water	159.14 ± 1.84cd	136.07 ± 1.93e-i	130.00 ± 2.20ghi	264.54 ± 5.50a

Means sharing similar letter in a row or in a column are statistically non-significant ($P>0.05$). Small letters represent comparison among interaction means.

Table 3: Comparison of tunneling length (mm) at different concentration of Leaf extracts of *J. curcas* in various solvents

Treatments	Concentrations				Means
	1%	5%	10%	Control (0%)	
Methanol	95.33 d-g	67.00 h-k	46.00 k	301.33 a	127.42 b
Ethanol	127.00 c	93.67 d-g	53.33 jk	285.67 a	139.92 ab
Ether	108.67 cde	85.67 fgh	48.33 jk	281.00 a	130.92 ab
Acetone	87.00 e-h	70.33 ghi	45.67 k	254.00 b	114.25 c
N-hexane	100.67 def	77.33 ghi	46.33 k	237.67 b	115.50 c
Chloroform	111.00 cd	84.67 fgh	49.00 jk	242.67 b	121.83 bc
Water	111.00 cd	89.33 d-h	57.67 ijk	242.67 b	125.17 bc
Means	105.81 b	81.14 c	49.48 d	263.57 a	

Means sharing the same letters in columns/ rows are not significantly different at $P<0.05$.

Table 4: Comparison of tunneling length (mm) at different concentrations of seed extracts of *J. curcas* in various solvents

Treatments	Concentrations				Means
	1%	5%	10%	Control (0%)	
Methanol	104.33 gh	72.00 k-n	57.00 nop	306.67 ab	135.00 bcd
Ethanol	130.00 ef	95.00 g-j	56.33 nop	289.67 bcd	142.75 ab
Ether	110.00 fg	82.00 i-l	43.33 p	286.00 cd	130.50 cd
Acetone	97.33 g-j	73.33 k-n	49.33 op	283.33 d	125.83 d
N-hexane	99.33 ghi	78.00 j-m	44.67 p	300.00 bcd	130.50 cd
Chloroform	110.00 g	75.33 k-n	59.00 m-p	316.33 a	140.17 abc
Water	139.67 e	89.33 h-k	64.33 l-o	303.67 abc	149.25 a
Means	113.05 b	80.71 c	53.43 d	297.95 a	

Means sharing the same letters in columns/ rows are not significantly different at $P<0.05$.

significantly different with methanol, petroleum ether, acetone, N-hexane, chloroform extracts and with all the solvents at 5% and 10% concentrations, respectively. The minimum tunnel length (43.33 mm) occurred in ether extract at 10% concentration and had non-significant difference with methanol, ethanol, acetone, N-hexane, chloroform extracts at 10% but was significantly different from water at 10% and with all the solvents at 1% and 5% concentrations. Tunneling length decreased with the increase in concentrations. Control treatments (0%) of all solvents were significantly different from three concentrations of seed extracts of *J. curcas*.

DISCUSSION

Mortality time (LT_{50}) and reduction in tunnel length at highest concentration tested (10%) of *J. curcas* leaf and seed extracts in organic solvents and water had non significant difference among them (Tables 1-3).

The highest toxic activity (100%) was found in the 2.0% chloroform extracts of *P. hydropiper* against tea termites, *O. assamensis* (Rahman et al., 2005). While a highest mortality rate was recorded with 5 % chloroform extract of *Delonix regia* (80 % at 48 hrs), with *Samanea saman, Cassia siamea, Pithecellobium dulce, Eucalyptus camaldulensis, Polyalthia longifolia* 75, 75, 55, 50 and 45 % mortality occurred respectively (Rupal et al., 2011). Contrary to our results, the maximum termite (*Heterotermes indicola*) mortality (84.45%) was observed in the ethyl acetate leaf extract and the minimum mortality (43.89%) was observed in stem extract, in water, of *Ocimum sanctum* (Manzoor et al., 2011a). Plant extracts of *Curcuma longa*, *Melia azadarach* and *Nerium indicum* in ethanol solvent have termiticidal properties against *H. indicola* (Manzoor et al., 2011b). The toxic fraction in any solvent depends upon plant and termites species, type of the test, solvents' polarity, yield and partition (Isman et al., 2006).

The water extract of plants has also been found effective as termtiticide in the filter paper bioassays and extract of *Milletia ferruginea* caused higher toxicity to all the castes of alates of the *Macrotermes* termites in which 93 to 100% mortality was recorded at all concentration levels (Bekele et al., 2005).

The insecticidal activity of seed oil of *J. curcas* has been found due to the presence of several sterols and terpene alchohols (Adebowale and Adedire, 2006). The maximum wood protection against *O. obesus* and *Microcerotermes beesoni* termites by *J. curcas* oil and its toxic fraction were obtained at their highest concentration i.e., 20% (Singh and Sushilkumar, 2008). In the present studies, all the leaf and seed extracts showed termiticide activity in comparison to their respective solvent controls, and water extract as well, lowest LT_{50} being in N-hexane extract.

It is very unlikely that both the hexane and water extracts (for example) would contain the same metabolites. Particularly when considering the LT_{50} values all of the extracts are significantly more active than the control. This could lead to an assumption that there is same general metabolites present in all extracts that could be having an effect.

These results have also indicated that all extracts posed hindrance in making extensive galleries in soil treated with plant extracts. The chemicals showing antifeedant activities had also effect on tunneling activities of the termites. Inhibition of tunneling may be exploited in a number of situations in agricultural ecosystem where seed and plant parts may be prevented from access of the termites (Ahmed et al., 2007). Seed and leaf extracts of *Withania somnifera*, *Croton tiglium* and *Hygrophila auriculata* had shorter tunneling area in seed extracts of plants as compared to leaf extracts on numerical terms (Ahmed et al., 2006). The efficacy of *Capparis deciduas* and its combinatorial mixtures against Indian white termite *Odontotermes obesus* was studied by Upadhyay et al. (2010) and they found that all the treatments had successfully controlled the ascending and descending movements of the termites and prohibited the tunnel formation by the workers. Cessation of tunneling in the soil treated with catnip (*Nepeta Cataria*) (Peterson and Ems-Wilson, 2003), *Azadirachta excelsa* (Sajap and Aloysius, 2000) *Ocimum canun, Ocimum gratissimum, Zanthoxylum xanthoxyloides, Sporobolus pyramidalis* and *Allium sativum* (Owusu et al., 2008) and *J. curcas* (Acda, 2009) was observed against the termites.

Based on results of present studies, it can be concluded that organic solvent or aqueous extracts of leaf and seed of *J. curcas* can be used for isolating the toxic active fraction which have exhibited not only toxic action in terms of mortality of termites but also impeded tunneling behaviour.

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