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# Evaluation of Plant Extracts on Mortality and Tunneling Activities of Subterranean Termites in Pakistan

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## 1. Introduction

Plant extracts offer a vast, virtually untapped reservoir of chemical compounds with many potential uses. One of these uses is in agriculture to manage pests with less risk than with synthetic compounds that are toxicologically and environmentally undesirable. Increasing evolution of resistance in pest population further derives the need to search for new bioactive compounds with a wide range of new modes of action. Various experiments using plant extracts in human and animal health protection, agriculture and household pest management have been particularly promising (Pascual-Villalobos & Robledo, 1999; Scott et al., 2004). The apparent societal hope for using plant extracts in place of more traditional pesticides has also increased the attention paid to natural products in the past decade (Duke et al., 2003). Plant products have been exploited as insecticides, insect-repellents, antifeedants and insect growth and development regulators (Saxena, 1998). The deleterious effects of phytochemicals or crude plant extracts on insects are manifested in several ways, including suppression of calling behaviour (Khan & Saxena, 1986), growth retardation (Breuer & Schmidt, 1995), toxicity (Hiremath et al., 1997), oviposition deterrence (Zhao et al., 1998), feeding inhibition (Wheeler & Isman, 2001) and reduction of fecundity and fertility (Muthukrishnan & Pushpalatha, 2001).

Many plants have been recognized to have anti-termite activities (Sakasegawa et al., 2003, Park & Shin, 2005, Jembere et al., 2005, Cheng et al., 2007, Ding & Hu, 2010, Supriadi and Ismanto, 2010) or repellent to the termites i.e., *Eucalyptus globules*, lemon grass, *Eucalyptus citrodora*, cedar wood, clove bud and vetiver grass (Zhu et al., 2001a, b), *Taiwania cryptomerioides* Hayat (Chang et al., 2001), *Dodonaea viscosa* (Purple hop bush) a termite resistant shrub (Anonymous, 2001), *Ocimum basilicum* L., *Cymbopogon winterianus* Jowitt, *Cinnamomum camphora*, *Rosmarinus officinalis* (Sbeghen et al., 2002) and *Coleus ambionicus* (Singh et al., 2004) are less extensively studied against termites.

The extracts of plants having anti-termite properties and termite-resistant formulations have been prepared, reported and tested in the laboratory and fields. Substrates in these tests were soil, sand and filter paper. Mortality and inhibition of consumption of wood were indicators of toxic and feeding deterrent activity of these extracts. Those tested in laboratory were extracted in various organic solvents in addition to water. Alcoholic and phenolic compounds in extracts of *Juniperus procera* (Kinyanjui et al., 2000), pine resin and eight of its derivatives (Nunes et al., 2004), 2.0 % chloroform leaf extracts of *Polygonum hydropiper* L. and *Pogostemon parvillorus* against tea termite, *Odontotermes assamensis* Holm. with highest toxic activity (100% mortality) in the extract of *P. hydropiper* (Rahman et al., 2005) are some of the examples. Effects of hexane, ethanol, and petroleum ether extracts of the black pepper fruits, *Piper nigrum*, were studied on the dry-wood termite, *Cryptotermes brevis*. Hexane extract at 0.5% concentration induced 50% mortality, which dropped to 4.76 and 14.28% with ethanol and petroleum ether, respectively, 2 days post treatment (Moein & Farrag, 2000). The termite, *Coptotermes curvignathus*, workers responded differently to soils and pine blocks treated with varying concentrations of *Azadirachta excelsa* leaf extracts in acetone, hexane and methanol. The result showed that extracts from *A. excelsa* leaves had an inhibitory effect on subterranean termites, *C. curvignathus*. The soils treated with the extracts did pose a hindrance to the tunneling activities of the termites (Sajap and Aloysius, 2000). The chloroform extracts of the woods Ipe (*Tabebuia* sp.) and Itauba (*Mezilaurus* sp.) on the dry-wood termite, *Cryptotermes brevis*, applied at a rate of 0.1 g/mL to filter paper to feed the termites and later analyses of substrate consumption rates and mortality by the Kruskal-Wallis method indicated a statistically significant reduction of feeding rates and increased mortality after 30 days (Cabrera et al., 2001). Phytoextracts from *Adhatoda vasica*, *Cynodon dactylon*, *Pongamia pinnata*, *Rauvolfia serpentina*, *Cleistanthus collinus*, *Tamarindus indica* and *Eichhornia crassipes* controlled the termites, *Microcerotermes mycophagus* (Nilanjana & Chattopadhyay, 2003). The biological activity of extracts of Meliaceae in relation with *Heterotermes tenuis* was studied in the laboratory. The effect of aqueous extracts from *Melia azedarach*, *Trichilia pallida* and *Azadirachta indica* (neem) (1 and 5% w/v), neem oil (1 and 2% v/v), and Nimkol, obtained from neem leaves (0.5 and 1% a.i.), were measured for the survivability of the termites. Nimkol caused a significant mortality of *H. tenuis* after the third day of feeding (1% a.i.) (Castiglioni and Vendramim, 2003). Seed and leaf extract of *Polygonum hydropiper* and *Cannbis sativa* against *Heterotermes indicola* and *Coptotermes heimi* showed more toxicity of seed (52-64% and 70-74% mortality) than leaf extracts (28-54 % and 28-58%) in both species. Crude extracts of various reproductive and vegetative parts of *Calotropis procera* had toxic effects on *H. indicola* (Badshah et al., 2004). *Datura alba*, *D. stramonium* and *Calotropis procera* were the most effective against the termites with 62.5% protection (Bajwa & Rajpar, 2001; Ayodele & Oke, 2003). 5% Chloroform extract of *Lantana camara* var. aculeate at a concentration of 5% was found to be significantly effective against termite workers (Verma & Verma, 2006).

The extracts used in the field were mostly in the form of decoction with water and were used in the soil or poured into the termites' nest directly. Decoction of *Cassia fistula*, *Myrtus communis*, *Sapium sebiferum* and *Thevetia peruviana* at rate of 5% (5g: 100ml) provided significant protection against termites for three months in the field. Fermented extracts of *Tithonia diversifolia* and *Melia azedarach* controlled Isopteran insects when poured into their nest. The ash of *T. diversifolia*, *Cassia siamea* and *C. spectabilis* applied to affected trees provided protection from termites for up to 45 days. *Vernonia amygdalina* and *Agave sisalana*, not only controlled termites and but also contributed to soil fertility (Ghosh, 2009). Soil treated with 2% solution of

*Calotropis procera* L. and *Azadirachta indica* prevented damage to sugarcane setts by *Odontotermes obesus* (Rambur) controlled the termite (Deka & Singh, 2001; Singh et al., 2002). Several novel classes of termiticides have been isolated from plants and based on these natural products, more active analogs have been synthesized. Two sesquiterpenes (partheniol and argentone) and a triterpene (incanilin) from guayule resin showed different levels of antifeedant and toxic activity (Gutierrez et al., 1999). The effects of a commercial insecticide formulation (margosan-O) containing 0.3% Azadirachtin and 14% neem oil on orientation, tunneling, and feeding behaviour of the Formosan subterranean termite have been investigated (Grace & Yates, 1992). Sand treated with vetiver oil or nootkatone at 100 µg/g substrate were effective barriers to the termite, *Coptotermes formosanus* (Maistrello et al., 2001). Thiophenes from four *Echinops* species and columellarin and sesquiterpene lactone fraction from the heartwood of Australian white cypress (*Callitris glaucophylla*) showed anti-termitic activities against *C. formosanus* Shiraki (Watanabe et al., 2005; Fokialakis et al., 2006)). Vulgarone B (isolated from *Artemisia douglasiana* ), apiol (isolated from *Ligusticum hultenii*) and cnicin (isolated from *Centaurea maculosa*) exhibited significantly higher mortalities in Formosan subterranean termite (*C. formosanus*) than in untreated control in the laboratory bioassay (Meepagala et al., 2006). Oils extracted from plant parts have been applied in a number of situations to protect the substrate from termite infestation. The crude seed oil of *Piper guineense*, each at a 10% concentration at the rate of 18 litres ha<sup>-1</sup> significantly lowered damage by termites (*Microtermes* spp., *Macrotermes bellicosus* and *M. subhyalinus*) (Umeh & Ivbijaro, 1999). Neem seed oil inhibited growth of termite surface-tunnels (Yashroy & Gupta, 2000). For further references, annual meeting report of IRG can be consulted for efficacy of oils against termites. Many timbers contain chemicals or complex mixture of chemicals that repel or kill the termites or effect on gut flora in termites (Adams et al., 1988); among these are *Pometia pinnata*, *Homalium foetidum*, *Eucalyptus deglupta* and *Alstonia scholaris* (Rokova and Konabe., 1990). Relatively less mentioned other plants with termite control properties are presented below (Anonymous, 2001).

Species	Parts Used	Property
<i>Carya ovata</i>	Bark	Termiticidal
<i>Cedrela odorata</i>	Wood	Termiticidal
<i>Consolida regalis</i>	wood	Termiticidal
<i>Dodonaea viscosa</i>	Leaves, wood / pulp	Termiticidal
<i>Quercus prinus</i>	Bark	Termiticidal
<i>Hardwickia mannii</i>	Stem/ branches	Termiticidal
<i>Pinus strobus</i>	Bark	Termiticidal
<i>Samadera indica</i>	Leaves	Termiticidal
<i>Carica papaya</i>	Fruit, fresh leaves and roots	Insecticidal
<i>Grevillea robusta</i>	Leaves	Insecticidal
<i>Leucaena leucocephala</i>	Used as a leaf mulch	Repellent
<i>Commiphora Africana</i>	Gum/ resin	Repellent
<i>Cassia siamea</i>	Used as a leaf mulch	Repellent
<i>Hyptis spicigera</i>	Aerial parts	Repellent
<i>Ocimum canus</i>	Whole plant	Insecticidal, repellent

Source: HRD Publication UK

Many plant extracts have been found to alter the behaviour of termites. Chemicals showing antifeedant activities had also effect on tunneling of the termites (Ibrahim et al., 2004; Mao & Henderson, 2007).

The foregoing examples are just crust of the copious literature available on this aspect of termite management and control. Previously we have demonstrated some of the above mentioned properties of many extracts of plants, shrubs and trees in our laboratory and have found significant results in controlling termites in the field (Ahmed et al., 2005, 2006, 2007). In order to find out inexpensive alternate to synthetic insecticides, anti termite properties from plants will continue to expand base of the effective molecules to be developed to go well with the ecology of termites.

2. Materials and methods

2.1 Collection of termites

The assorted workers of the termite species, *Microtermes obesi* Holm., in the later instars were collected, within the damaged canes from sugarcane fields and from the corrugated cardboard baits in PVC monitors installed in the fields at different places at the Experimental Area, Department of Agri-Entomology, University of Agriculture, Faisalabad.

2.2 Following plants were selected to obtain their leaf extracts

Botanical name	Family	Common name
<i>Adhatoda vasica</i> (Nees)	Acanthaceae	Malabar nut
<i>Dodonaea viscosa</i> (Linn.) Jacq	Sapindaceae	Hopbush
<i>Thevitia peruviana</i> (Pers) Merr	Apocynaceae	Yellow oleander
<i>Nerium odorum</i> Soland	Apocynaceae	Indian oleander
<i>Salvadora oleiodes</i> Decne	Salvadoraceae	Vann
<i>Alstonia scholaris</i> (R. BR.)	Apocynaceae	Devil tree
<i>Delphinium ajacis</i> Linn.	Ranunculaceae	Larkspur
<i>Papaver somniferum</i> Linn.	Papaveraceae	Garden poppy
<i>Lucaena leucocephala</i> (Lam.) DeWit	Mimosaceae.	Iple iple
<i>Grevilla robusta</i> A.Cunn.Ex.R.Br	Proteaceae	Silky oak
<i>Tephrosia purpurea</i> Linn.	Fabaceae	Wild indigo
<i>Nerium oleander</i> Linn.	Apocynaceae	Rose bay
<i>Jatropha integerrima</i> Jacq.	Euphorbiaceae	Peregrina

2.3 Extraction method

2.3.1 Preparation of leaves for extraction process

Fresh fully developed leaves in the season from middle portion of the plants from Botanical Garden as well as from areas within campus, University of Agriculture, Faisalabad, Pakistan, were collected and these plants were never exposed to pesticides. These leaves were washed with tap water and then air dried in a laboratory for 2 weeks ensuring sufficient air flow to avoid damping. The room-dried leaves were reduced to a powder form by grinding with an electric grinder running at a speed of 6000 rpm for 50-60 sec.



### **2.3.2 Crude methanolic extract of leaves**

One hundred gram (100 g) of powder from each of the plants was extracted in 200 ml of 80% methanol in the ratio of 1:2 (w/v) by following method of extraction (Sadek, 2003). It was kept for 72 hours at room temperature and shaken at intervals to get a better extraction. Thereafter, the extract was filtered through Whatman filter No. 42. After filtering, the methanol was removed at 60°C using rotary evaporator, to obtain solid extract, dried in vacuum desiccator. The final yield of dry material was used to prepare percent solution of crude extract with 2% methanol.

### **2.3.3 Aqueous extracts of leaves**

To get the aqueous extracts, above procedure was followed except powder was extracted in distilled water. The filtrates were stored in a refrigerator at 5°C for subsequent use in bioassays.

## **2.4 Bioassay**

### **2.4.1 Soil preparation for bioassay**

The soil used in bioassays was sandy clay loam (52.6% sand, 24.8% silt and 20.6% clay). There had been no known applications of agro-chemicals in this soil for the control of termites. The soil was sieved through a 30-mesh screen and moisture was determined with the help of a moisture meter. Water was added in this soil to simulate 50% of water holding capacity, to avoid mortality of termites due to dehydration during assays.

### **2.4.2 Bioassays by mixing leaf extract in the soil**

Antitermitic sugarcane strip bioassays (ASSB) using different leaf extracts were done in Petri dishes (95 × 15 mm) containing 20 g sifted sterilized soil and strips of sugarcane (1.5 cm × 6 cm) to keep the termites alive. Every treatment with 10%, 20% and 40% of extracts and control (without extract) were repeated thrice in Completely Randomized Design (CRD). 20 g of sifted soil in Petri dish having sugarcane strip was wetted/ mixed with respective concentration of the extract. 50 active workers and 5 soldiers were released in the Petri dishes having treated and untreated soil.

### **2.4.3 Filter paper bioassay**

Whatman filter paper No. 42, 9 cm in diameter was treated with 10, 20 and 40% concentrations of leaf extracts at the rate of 31 µl/cm<sup>2</sup> and placed in Petri dishes (95 × 15 mm). 50 workers and 5 soldiers of were released in the Petri dishes having treated and untreated filter paper.

The Petri dishes having filter paper and/or soil bioassay were placed in growth chamber under controlled conditions of 28±2°C and 80%±5 humidity. Data for mortality were recorded after every 2 hours up to 12 hours, and then after every 12 hours until all workers and soldiers died. Each treatment was repeated three times.

## **2.5 Formation of Galleries (FG)**

Members of 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. Termite's response towards galleries formation for each plant extract at each concentration after 5, 10 and 15 hours was determined by plotting the tunnels

on the cellophane paper and measured the length in mm<sup>2</sup> with the help of planimeter. The values were correlated with the chemical concentrations. Tunnelling activities were analyzed by Factorial Analysis (CRD).

2.6 Statistical analysis

LT<sub>50</sub>s in soil and filter paper bioassay was determined using Kaplan Meier Survival Test. In all tests, values of LT<sub>50</sub>s among replication was non significant and were thus taken as mean LT<sub>50</sub>. Tunnelling activities analyzed by Two way ANOVA, however, data are represented as mean activity of all time intervals at each concentration. The difference among concentrations was determined by Duncan Multiple Range Test (DMR) at p<0.05.

3. Results

The activity of methanolic and aqueous extracts of *D. viscosa*, *A. vasica*, *N. odorum*, *T. peruviana*, *D. ajacis*, *S. oleiodes* and *A. scholaris*, applied on filter paper and / or mixed into soil at various concentrations is expressed as LT<sub>50</sub> (hours) and is shown in Tables 1 to 4. Extracts have exhibited the activity of causing morality in the termites, *M. obesi*, and were significantly different from control treatments. The activities were concentration dependent and LT<sub>50</sub> decreased with increase in concentrations. Methanolic extract of *D. viscosa* at 40% (27.5 hours), *D. ajacis* at 10% (47.0 hours) and *N. odorum* at 20% (34.3 hours) concentrations were effective, being shown as lowest LT<sub>50</sub>s on filter paper (Table 1).

Concentrations	LT <sub>50</sub> (hours)						
(%)	<i>D. viscosa</i>	<i>A. vasica</i>	<i>N. odorum</i>	<i>T. peruviana</i>	<i>D. ajacis</i>	<i>S. oleiodes</i>	<i>A. scholaris</i>
0	291. 5	282. 8	278. 9	281.0	278. 8	263.0	269. 2
10	52.3	63.5	57.6	67.7	47.0	64.8	64.3
20	34.8	42.6	34.3	41.7	36.4	48.2	47.5
40	27.5	29.9	27.2	35.0	32.5	39.4	37.4

Table 1. LT<sub>50</sub> values with methanolic extracts of *D. viscosa*, *A. vasica*, *N. odorum*, *T. peruviana*, *D. ajacis*, *S. oleiodes*, *A. scholaris* leaves at different concentrations against *M. obesi* in Petri dishes having treated filter paper.

Concentrations	LT <sub>50</sub> (hours)						
(%)	<i>D. viscosa</i>	<i>A. vasica</i>	<i>N. odorum</i>	<i>T. peruviana</i>	<i>D. ajacis</i>	<i>S. oleiodes</i>	<i>A. scholaris</i>
0	308. 4	309. 3	282. 5	300.0	292.0	314. 8	257. 3
10	67.5	66.7	71.1	111.6	77.3	98.0	107.5
20	37.9	45.6	50.4	65.1	52.8	74.5	74.1
40	35.2	40.9	35.8	39.8	39.6	40.5	55.8

Table 2. LT<sub>50</sub> values with methanolic extracts of *D. viscosa*, *A. vasica*, *N. odorum*, *T. peruviana*, *D. ajacis*, *S. oleiodes*, *A. scholaris* leaves at different concentrations against *M. obesi* in Petri dishes having treated soil.

*A. vasica* at 10% (66.7 hours) and *D. viscosa* at 20 and 40% concentrations (37.9 and 35.2 hours, respectively) had lowest LT<sub>50</sub> when these were mixed in the soil (Table 2). Aqueous

extract of *N. odorum* had lowest LT<sub>50</sub>s (48.9, 33.9 and 27.0 hours) at 10, 20 and 40% concentrations on filter paper (Table 3). ). Aqueous extract of *D. ajacis* at 10 and 20% and *A. vasica* at 40% concentrations (36.0 hours) had lowest LT<sub>50</sub>s (114.2 and 72.1 hours) when mixed in the soil (Table 4).

Concentrations	LT <sub>50</sub> (hours)						
(%)	<i>D. viscosa</i>	<i>A. vasica</i>	<i>N. odorum</i>	<i>T. peruviana</i>	<i>D. ajacis</i>	<i>S. oleiodes</i>	<i>A. scholaris</i>
0	332.2	287.8	293.4	287.5	320.3	293.6	320.5
10	71.5	69.6	48.9	53.9	70.7	89.2	111.2
20	55.7	40.9	33.9	37.9	44.2	69.8	78.7
40	41.1	33.0	27.0	28.6	37.7	59.3	56.6

Table 3. LT<sub>50</sub> values with aqueous extracts of *D. viscosa*, *A. vasica*, *N. odorum*, *T. peruviana*, *D. ajacis*, *S. oleiodes*, *A. scholaris* leaves at different concentrations against *M. obesi* in Petri dishes having treated filter paper.

Concentrations	LT <sub>50</sub> (hours)						
(%)	<i>D. viscosa</i>	<i>A. vasica</i>	<i>N. odorum</i>	<i>T. peruviana</i>	<i>D. ajacis</i>	<i>S. oleiodes</i>	<i>A. scholaris</i>
0	328.9	293.5	325.0	353.1	290.7	351.3	308.6
10	115.8	101.8	119.1	148.7	114.2	139.1	136.4
20	68.8	78.2	85.1	92.7	72.1	98.3	85.0
40	46.2	36.0	60.9	61.0	50.3	63.3	62.6

Table 4. LT<sub>50</sub> values with aqueous extract of *D. viscosa*, *A. vasica*, *N. odorum*, *T. peruviana*, *D. ajacis*, *S. oleiodes*, *A. scholaris* leaves at different concentrations against *M. obesi* in Petri dishes having treated soil.

The methanolic extract of *P. somniferum* at 10 and 20% and *G. robusta* at 40% concentrations had lowest LT<sub>50</sub>s (26.5, 18.6 and 12.5 hours, respectively) (Fig. 1a) on filter paper, whereas *G. robusta* had lowest LT<sub>50</sub>s at all concentration when mixed in the soil (Fig. 1b). Aqueous extract of *P. somniferum* at 10% (42.1 hours) and of *T. purpurea* at 20 and 40% concentrations (33.3 and 22.7 hours, respectively) had lowest LT<sub>50</sub>s on filter paper (Fig. 2 a). In soil, *G. robusta* at 10% (75.5 hours) and of *P. somniferum* at 20 and 40% concentrations (44.3 and 21.4 hours, respectively) yielded lowest LT<sub>50</sub>s (Fig. 2b). Two types of plant extracts have shown 40 to >80% % of LT<sub>50</sub> on filter paper and in the soil when compared with control treatments.

In other studies, leaf extracts of *Jatropha integerrima*, *N. oleander* and *Lucaena leucocephala* in acetone, methanol, petroleum ether and aqueous solvents showed activity in terms of mortality of termite workers at different concentrations when mixed in the soil in Petri dishes and is represented in Tables 5-7. *J. integerrima* was the most effective among three plants and showed lowest LT<sub>50</sub> at 10% (9.72 hours) in acetone and then in petroleum ether at 20 and 40% concentrations (6.53 and 4.99 hours, respectively). Lowest LT<sub>50</sub> of two other plants was shown in acetone extract at 40% concentration (47.8 hours). It is interesting to note that *N. oleander* has shown less activity than *N. odorum*, in addition to acetone and petroleum ether, in methanol and aqueous extracts as well.



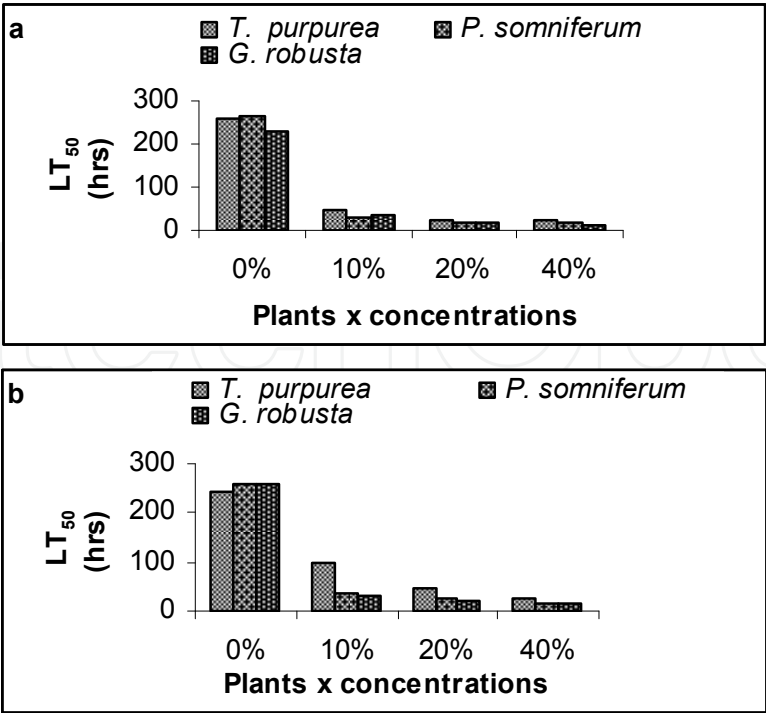


Fig. 1.  $LT_{50}$  values with methanolic extract of *Tephrosia purpurea*, *Papaver somniferum*, *Grevilla robusta* leaves at different concentrations against *M. obesi* on (a) filter paper (b) mixed in the soil.

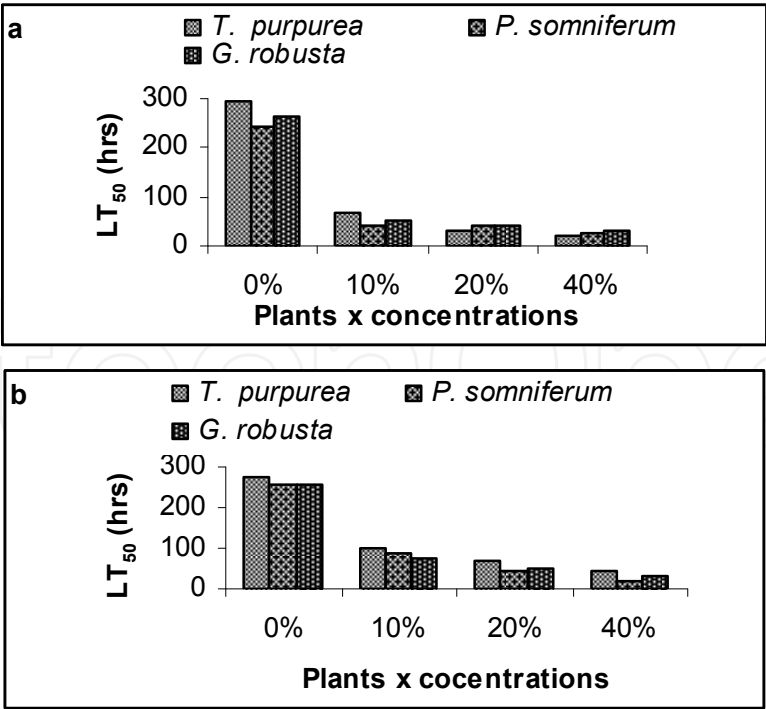


Fig. 2.  $LT_{50}$  values with aqueous extract of *Tephrosia purpurea*, *Papaver somniferum*, *Grevilla robusta* leaves at different concentrations against *M. obesi* in Petri dishes having treated (a) filter paper (b) soil.

Concentrations	LT <sub>50</sub> (hours)			
(%)	acetone	methanol	aqueous	petroleum ether
0	396.9	483.5	457.4	483.5
10	13.8	9.7	79.6	108.6
20	8.6	5.4	17.9	6.5
40	6.7	4.5	15.9	4.9

Table 5. LT<sub>50</sub> values of various extract of *Jatropha integerrima* at different concentrations against *M. obesi* in Petri dishes having treated soil.

Concentrations	LT <sub>50</sub> (hours)			
(%)	acetone	methanol	aqueous	petroleum ether
0	154.0	174.8	162.2	177.5
10	123.1	165.2	128.2	143.2
20	85.8	130.0	127.0	142.0
40	85.6	116.2	94.9	138.7

Table 6. LT<sub>50</sub> values of various extract of *Nerium oleander* at different concentrations against *M. obesi* in Petri dishes having treated soil.

Concentrations	LT <sub>50</sub> (hours)			
(%)	acetone	methanol	aqueous	petroleum ether
0	149.0	167.4	182.0	176.9
10	84.5	152.0	133.7	147.8
20	64.6	146.0	125.7	147.5
40	47.8	135.7	99.0	133.0

Table 7. LT<sub>50</sub> values of various extract of *Leucaena leucocephala* at different concentrations against *M. obesi* in Petri dishes having treated soil.

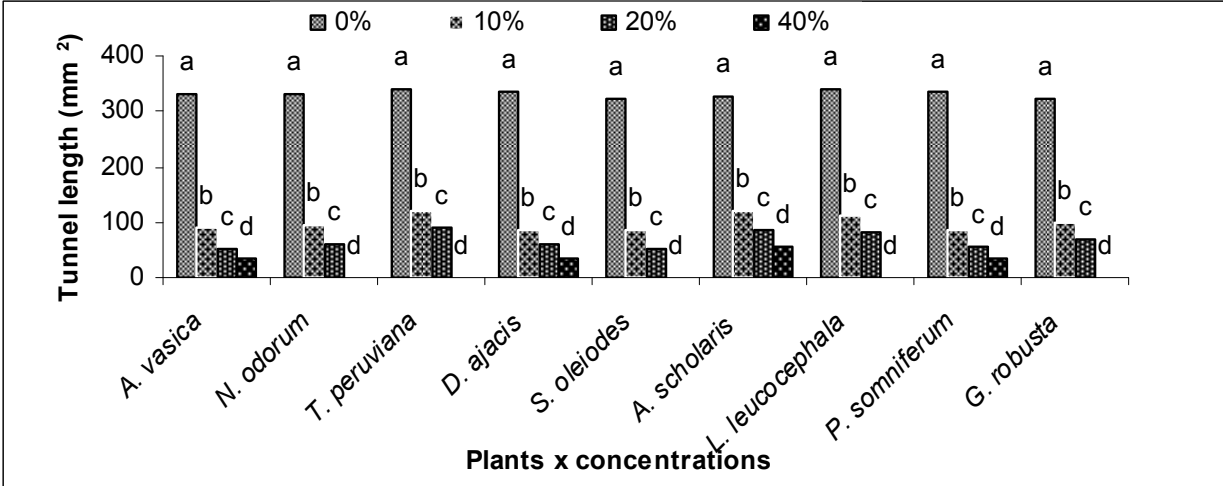


Fig. 3. Comparison of tunnel length at different concentrations of leaf methanol extracts of various plants.

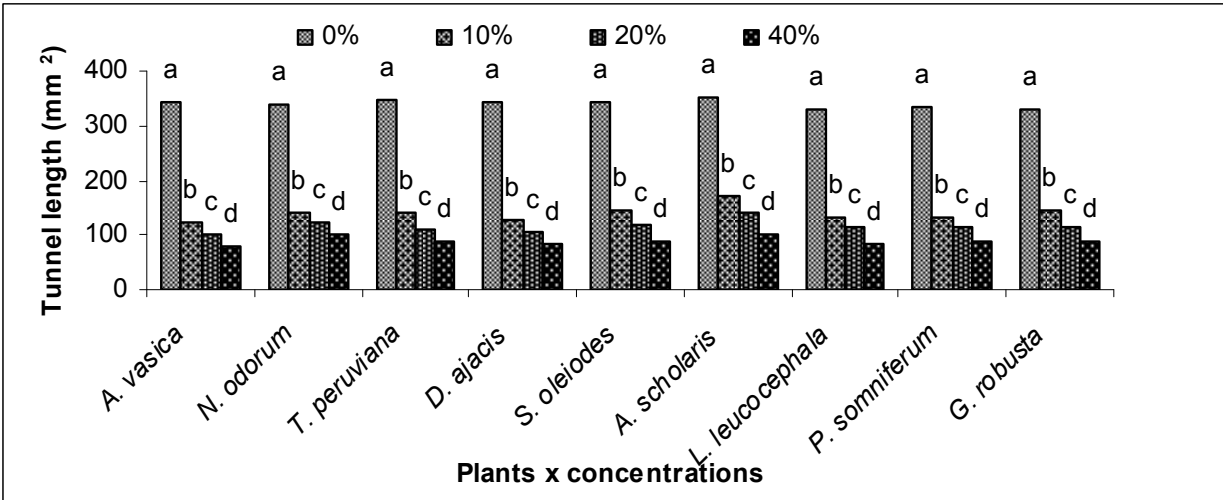


Fig. 4. Comparison of tunnel length at different concentrations of leaf aqueous extracts of various plants.

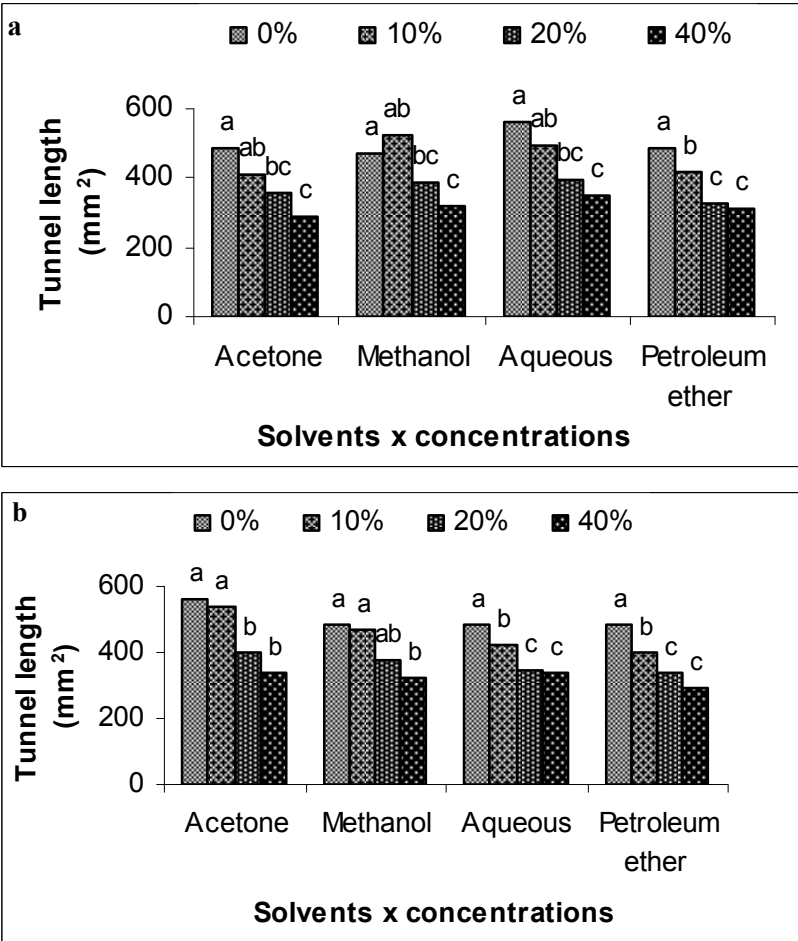


Fig. 5. Comparison of termites' tunnel length in leaf extracts in various solvents of (a) *Nerium oleander* (b) *Lucaena leucocephala*.

Various concentrations had significant difference among them with respect to the tunnelling (tunnel length) by the termites when mixed with the soil at various time intervals (5-15

minutes). After 15 minutes, it was difficult to draw the tunnel length on the paper, however, data are shown as mean tunnel length of the time intervals but not of the concentrations. *A. vasica*, *N. odorum*, *S. oleiodes*, *T. purpurea* and *G. robusta* leaf extracts in methanol at 40% did not show any tunnelling, nevertheless, termites had mined in the aqueous extracts of the same plants at 40% concentration (Figs. 3 & 4). In contrast to *N. odorum*, other species of the same plant *N. oleander* could not prevent tunnel formation as in case of former species, but tunnel length at 40% concentrations had significant difference than that at 10 and 20% concentrations depending upon type of solvent (Fig. 5 a & b).

#### 4. Discussion

Treatment of soil with natural/synthetic compounds to control the termites is common method. Insecticides have been used to form barrier in soil against subterranean termites to prevent their tunnelling and reaching to food sources. Chlorpyrifos, bifenthrin, fipronil and many others have been extensively used for this type of barrier against termites (Su et al., 1997). Criteria used to evaluate potential soil termiticides have been termites' ability to tunnel through treated soil and toxicity of material (plant extracts) in laboratory experiments (Grace et al., 1993; Su et al., 1993).

The results exhibited herein are mostly the confirmation of the results obtained elsewhere for the medicinal plant extracts having anti-termite properties and termite-resistant formulations (Singh et al., 2001; Ding & Hu, 2010). *Dodonaea viscosa* (Purple hop bush) has been reported as a termite resistant shrub (Anonymous, 2001), but bioassay of its extracts with termites has been investigated for the first time in this report. The extracts from *Adhatoda vasica* and *Nerium oleander* are some of the above mentioned plants which have been tested for same purpose (Nilanjana & Chattopadhyay, 2003).

The results revealed two important aspect of toxicological inference (i)  $LT_{50}$  irrespective of medium for feeding and movement was almost equal and non-significant depending upon fiducial limits (ii)  $LT_{50}$  of methanol extract was shorter than aqueous extract. Many studies have shown activity of plant extracts when applied on filter paper and / or mixed in soil to determine mortality (Blaske & Hertel, 2001; Blaske et al., 2003; Jembere et al., 2005) and concluded that plant extracts have the potential for under- and above-ground application for the termite control.

The present results showed that the tunnelling activities are the function of time and concentration. All concentrations of aqueous and methanolic leaf extracts have less tunnelling activities of *M. obesi* as compared to control. Means of the tunnelling activities of *M. obesi* in methanolic extract treated soil were less than the means of the tunnelling activities in aqueous extract treated soil. There was no tunnelling in leaf methanol extracts of *A. vasica*, *N. odorum*, *S. oleiodes*, *T. purpurea* and *G. robusta* at their 40% concentrations. These results are confirmation of earlier reports mentioned elsewhere depending upon species and kind of plant parts being studied. It is evident from these results that extracts did pose a hindrance to tunnelling activities of the termites (Sajap & Aloysius, 2000; Maistrello et al., 2001; Peterson & Ems-Wilson, 2003; Mao & Henderson, 2007), but the termites, however, may become insensitive towards the extracts upon longer period of exposure and this period depends upon termites species.

The extracts usually oils have been reported for toxicity and tunnelling inhibition studies, however, extracts in water and organic solvents have also yielded results for the above properties for termites' control. It has been summarized from various studies that extracts

having both properties of being toxic and inhibit tunnelling is good for various types of application and can be used in baiting and media application. There is no denying that potential application against termites would require large volume of plant materials, thus a number of plants should be studied to use alternatively. The water extract may be used for delivery into the soil to directly kill termites, or a paint-on material which may prevent termites from infesting wood. Inhibition of tunnelling may be exploited in a number of situations in agricultural ecosystem where seed or plant parts may be prevented from access of termites. One such example is setts protection from plant extract against termites where significant germination of setts of sugarcane in plots treated with plant extracts than in control plots (Ahmed et al. 2005; 2007).

## 5. Conclusions

1. Laboratory bioassays with a range of plant extracts in particular indicated the potential of some of them as termiticides.
2. Plant extracts used in the present studies had repellency for termites as these checked the tunnelling activities of termites in the soil, and may be used to keep the termites away from plants and ultimately, saving the plants from damage.
3. Leaf extracts of *D. viscosa*, *D. ajacis* and *N. oleander* can be the good candidates for further process of isolation, and characterization of active compounds in the extracts.

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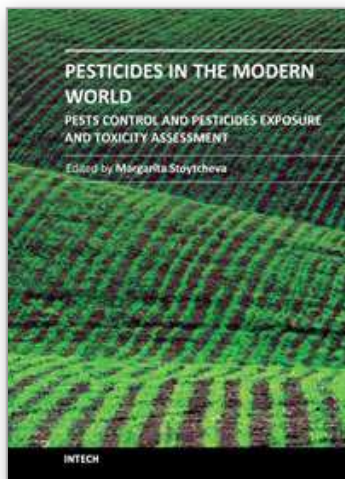
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## **Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment**

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The present book is a collection of selected original research articles and reviews providing adequate and up-to-date information related to pesticides control, assessment, and toxicity. The first section covers a large spectrum of issues associated with the ecological, molecular, and biotechnological approaches to the understanding of the biological control, the mechanism of the biocontrol agents action, and the related effects. Second section provides recent information on biomarkers currently used to evaluate pesticide exposure, effects, and genetic susceptibility of a number of organisms. Some antioxidant enzymes and vitamins as biochemical markers for pesticide toxicity are examined. The inhibition of the cholinesterases as a specific biomarker for organophosphate and carbamate pesticides is commented, too. The third book section addresses to a variety of pesticides toxic effects and related issues including: the molecular mechanisms involved in pesticides-induced toxicity, fish histopathological, physiological, and DNA changes provoked by pesticides exposure, anticoagulant rodenticides mode of action, the potential of the cholinesterase inhibiting organophosphorus and carbamate pesticides, the effects of pesticides on bumblebee, spiders and scorpions, the metabolic fate of the pesticide-derived aromatic amines, etc.

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