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TERMITICIDE ACTIVITY OF SEED EXTRACT/OILS FROM *SCHINUS MOLLE* LINN. AGAINST SUBTERRANEAN TERMITES UNDER LABORATORY CONDITIONS

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ABSTRACT

Antitermitic activities of seed extract/oils were evaluated using termites mortality, repellency and reduction in tunnel length as endpoint of extract/oil toxicities. Seed extract and oil diluted in acetone for applying on filter paper or mixing with soil proved best and yielded maximum mortality and repelled maximum number of termites and shortest tunnels were made by termites' workers. Use of oil / extract as termiticide has been discussed.

Keywords: oil, seed, cold-pressed, termites, toxicity, false pepper.

INTRODUCTION

Plants possessing resistance against termites contain compounds which may be unique to certain plants and absent in others. These compounds may have many functions in addition to direct toxicity. Compounds extracted using various solvents differentiate their nature and stability (Ragon *et al*., 2008; Schultz *et al*., 2008).

The uses of plant extracts have been practiced since long to control pests and pathogens. Extracts from various parts of plants are rich in tannins, phenols, alkaloids and flavonoids etc. which are toxic to wood degrading organisms. There are few innovations that have been developed to use natural products to treat wood against insects and fungal attack. Many of these compounds are safe, non harmful to man but still effective against pests (Bacci *et al*., 2015).

A number of constituents of seed and leaf extracts have shown antitermitic activities (Ahmed *et al*., 2011). Essential oils among these constituents form an important group of the chemicals which are grouped into different categories such as toxic, repellent and antifeedant etc. Non edible seedcakes (jatropha, karanja, neem and mahua) and their crude active compounds (phorbol, karanjin, saponins and azadirachtin) against *Odontotermes obesus* Ramb. (Sharma *et al*., 2011), repellent effects of *Annona* crude seed extract on *Coptotermes gestroi* Wasmann (Acda, 2014), essential oil of *Alpinia galanga* (L.) against *Coptotermes gestroi* (Wasmann) and *Coptotermes curvignathus* (Holmgren) (Abdullah *et al*., 2015); anti-symbionts activity of neem, jojoba, linseed, eucalyptus and jatropha oils in *Zootermopsis augusticollis* (Fatima and Morrell, 2015) are some of examples which have recently been reported.

The overall objective of this study was to assess the use of natural extractives from seeds of *Schinus molle* L. as termiticides. Medicinal properties of *S. molle* have been recognized in treatment of various ailments due to toxic action of essential oils from leaves and fruits of this plant (Martins *et al.,* 2014). Microencapsulation of *S. molle* leaves essential oil possesses insecticidal properties on *Haematobia irritans* (Lopez *et al.,* 2014). The repellent activity rates of essential oils and leaf extract from *S. molle* against the oriental cockroach (*Blatta orientalis*) repelled the adults of *B. orientalis* in varying degrees. At all doses, both leaf essential oil and DEET were found to be equally effective, while fruit oils and leaf extract were not as effective as the leaf essential oil was found more effective than the leaf extract (Deveci *et al.,* 2010). Toxicity and repellency of *S. molle* against *Tribolium castaneum* in stored wheat grain was less than other plants tested in an experiment (Padin *et al.,* 2013). *S. molle* plant parts proved to have larvicidal and pupicidal activity against immature *Cx. quinquefasciatus* (Girmay *et al.,* 2014).

MATERIALS AND METHODS

Seeds of *S. molle* were isolated from ripe fruits by drying them at room temperature during months of April and May 2015 in Termite Research Laboratory, Department of Entomology, University of Agriculture, Faisalabad.

These ripe fruits were collected along road side wild plants in Islamabad, Pakistan. These seeds were then placed in air-tight containers to prevent aerial oxidation. Two types of extracts from seeds were prepared as follows:

Crude seed extracts: 250 g of seeds were pulverized in an electric small spice grinder and then poured the crushed material in solvents (methanol, ethanol, chloroform, acetone) in 1:2 ratio. Mixtures were shaken manually at regular intervals and filtered through two layers of cheese cloth. Each of these mixtures was then kept in brown colored bottles separately in refrigerator for the use in bioassay.

Oil from seed extracts: For the oil extraction from the seeds, the method of Nyakudya *et al.*, 2013) was followed. 250 g of crushed seeds were put into a conical flask with 750 mL of each solvent, methanol, ethanol, chloroform and acetone (Sigma Aldrich). These flasks were sealed at the top and wrapped with aluminum foil and placed in shaker for five days continuously. Mixtures in each solvent were then passed through two layers of cheese cloth. All solvents were allowed to evaporate in a fume chamber and resultant oil contents were collected and placed in vials and kept in refrigerator for the use in bioassay. The yield of the extracted oil (%) was expressed on a dry matter basis.

Cold pressed oil: 2 kg of seeds were cold pressed in local pressing machine in oil extraction market in Faisalabad and 200 mL of oil was obtained. This oil was not further modified and used as such**.**

Collection of termites: Termite's workers along with soldiers were collected from the corrugated cardboard in PVC monitors installed at different places at the Post Agricultural Research Station, Jhang Road, University of Agriculture, Faisalabad.

Mortality bioassay in treated soil: The soil used in bioassay was that used by termites to make galleries on corrugated cardboard. The collected soil was brought to laboratory in zipped plastic bags and kept in refrigerator until used for bioassay. The soil was sieved through a 30 mesh screen and was moistened to an extent to simulate field condition with a concentration of extract in a quantity of 20 g. A group of 100 termite workers with 10 soldiers were released in Petri dish of 9 cm diameter having treated soil with oil/extract in different solvents. Each oil solvent combination was replicated thrice with a new set of termite workers and soldiers. Petri dishes with this experimental set up were put in an incubator at 28±2°C. A control treatment having solvent only was run parallel. Data for mortality was recorded after 7 days of exposure to soil treated with oil in different solvents.

Repellency test: Whatman's filter paper No. 42 of 9 cm in diameter was divided into two parts. One half was treated with each concentration of oil/extract in different solvents and other half was treated with respective solvent only. Both halves of filter paper were placed in a glass Petri dish and 100 termite workers with 10 soldiers were placed at the center of each Petri dish. The Petri dishes were then placed in an incubator at 28°C for 60min. After every 10min, the numbers of termites on each filter paper were counted. Oil/extract combination was replicated thrice with a new set of termite worker and soldiers.

Formation of Galleries (FG): 20 g soil in Petri dish of 9cm diameter was divided into two halves by keeping a filter paper in between. One half was treated with oil/extract in different solvents while other half was treated with respective solvent only. 100 termites with 10 soldiers were released in Petri dishes in each half and were placed in an incubator at 28±2^{o}C. The galleries formation in each half was measured after 60 minutes. Each treatment was repeated with a new set of termite workers and soldiers.

Statistical analysis: The experiments were set according to Completely Randomized Design (CRD). The comparison of mortality in soil and filter paper bioassay, repellency and difference in gallery formation were determined using Tukey's test. For the analysis of data Minitab 16 software was used and data was analyzed by General Linear Model.

RESULTS

Acetone diluted oil/extract from seeds of *S. molle* caused high mortality (84.33 to 96.33%) and was significantly different from ethanol and chloroform dilution. Percent mortalities in oil from seed (93.33) and cold pressed oil (96.33) diluted in acetone were non-significantly different from cold pressed oil (92.33) in methanol. Similarly percent mortality in seed extract in acetone (84.33) was non-significantly different from oil from seed in methanol (85). Percent mortalities in chloroform and ethanol diluted cold pressed oil and oil from seeds, respectively were non-significantly different (74.33 and 74.67). Likewise, oil from seed in chloroform (64.33) and seed extract in ethanol (64.67) respectively were non-significantly different from each other (Table 1).

Table 1.Comparison of termites' mortality (%) after 7 days exposure to oil/extract from *S. molle* under laboratory conditions.

Treatments	Solvents			
	Methanol	Ethanol	Chloroform	Acetone
Seed extract	78.00 ± 0.58cd	64.67 ± 1.20e	57.67 ± 0.88 f	84.33 ± 0.67b
Oil from seed	85.00 ± 1.00b	74.67 ± 0.67cd	64.33 ± 0.67e	93.33 ± 0.88 a
Cold pressed oil	92.33 ± 1.20a	80.33 ± 0.88bc	74.33 ± 1.45d	96.33 ± 2.03a
Control	18.67 ± 1.20gh	17.67 ± 1.45gh	13.33 ± 1.20h	12.00 ± 1.00g

Means sharing same letters in rows and columns are not significantly different from each other at p=0.05.

Table 2. Percent repelled termites at 24 hours exposure to oil/extract from *S. molle* under laboratory conditions.

Treatments	Solvents			
	Methanol	Ethanol	Chloroform	Acetone
Seed extract	63.33 ± 2.85e	75.67 ± 0.67cd	52.00 ± 0.58f	78.00 ± 0.58bcd
Oil from seed	75.33 ± 0.67cd	81.67 ± 3.84abc	72.33 ± 0.67d	82.33 ± 1.20abc
Cold pressed oil	80.67 ± 0.33abc	84.67 ± 0.33ab	77.67 ± 0.88bcd	87.00 ± 0.58a

Means sharing same letters in rows and columns are not significantly different from each other at p=0.05.

Table 3. Length of termites' galleries in soil treated with oil/extract from *S. molle* under laboratory conditions.

Treatments	Solvents			
	Methanol	Ethanol	Chloroform	Acetone
Seed extract	31.33 ± 0.44cde	34.00 ± 0.58cd	42.00 ± 0.58b	26.17 ± 0.44ef
Oil from seed	26.00 ± 0.58ef	27.67 ± 0.89def	38.17 ± 0.44bc	21.50 ± 0.29fg
Cold pressed oil	20.83 ± 0.44fg	23.83 ± 0.73fg	35.33 ± 1.20bc	17.17 ± 0.44g
Control	83.00 ± 2.08a	81.67 ± 3.18a	80.67 ± 2.91a	77.67 ± 1.76a

Means sharing same letters in rows and columns are not significantly different from each other at p=0.05.

Termites repelled in the presence of seed extract, seed oil and cold pressed oil are shown in Table 2. Highest numbers of repelled termites were found in cold pressed oil (87.00%) followed by ethanol diluted cold pressed oil and both have non-significant difference between each other. Chloroform diluted oil/extract repelled less number of termites as compared to other solvents. Seed extract had lowest repellency in chloroform dilution (52%) (Table 2). Correspondingly, tunnels length were shortest in acetone dilution of each oil/extract source and chloroform and ethanol dilution behaved similarly as in case of mortality and repellency (Table 3).

DISCUSSION

Oil from *Schinus molle* showed antitermitic activity by exhibiting mortality when termites were exposed to either seed extract or only oil from the seeds. These oil sources were also found to repel termites and limit their activities to portion of filter paper which had no oil treatment. Shortest galleries were made in oil diluted in acetone and especially in free oil from seeds. These properties have also been reported from other pepper species. Negro pepper, *Xylopia aethiopica* (Elhassan *et al.*, 2010) and West African black pepper (WABP), *Piper guineense* (Ajayi and Olonisakin, 2011; Abdullah *et al.*,2015).

Oils from plant parts generally contain a blend of volatile substances that affect insects by bringing irreversible changes in their behavior which ultimately lead to death of the insects. Various components in oils may have functions of toxicity, repellency and inhibit movement at later stage of exposure to oil for a longer period of time. The oil of black pepper consists of monoterpenes (51.13%) and sesquiterpene (39.71%) both as hydrocarbon and oxygenated forms (Elhassan *et al.*, 2010). More so, *Piper guineense* has been found to contain isobutylamides, a plant secondary compounds that act as neurotoxins in insects. Germacrene D, betacaryophyllene and delta-cadinene and alphacadinol from oils of leaf of *S. molle* has shown repellent activity against cattle ticks, aphids and some other arthropods (Deveci *et al.*, 2010). Repellent action of these oils are well established on many insect species, however, toxicity of oils is mostly followed in fumigant action in many insects which were exposed to oils. Subterranean termites forage in soil and take organic matter in soil in their guts and possibly oil interact with various parts in gut or their symbionts. Soil applications require a large volume of oils which depend upon type of soil. The best way to repel them by use bait woods treated with oils.

S. molle is grown as avenue tree in Pakistan and has no food or spicy value. Under cultivation it can be grown on very dry sites with a rainfall of as low as 250 mm/yr. This plant has shown termiticidal activity in laboratory conditions and deserves further investigations.

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