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Research Paper

Termiticidal and repellency efficacy of botanicals against *Odontotermes obesus*

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Abstract

The repellency effect of *Tagetes erecta* and *Citrus sinensis* oil was investigated against termite *(Odontotermes obesus).* A repellency apparatus was used to conduct the experiments and movement of termites was observed for two and half hours after 15 min interval.The marigold oil repelled maximum 82% termite while orange oil 76% at 6.30 µl/cm^2 concentration. However, constant repellency was recorded in last half hour. The present study suggested that marigold and citrus oils have termite repellent potency and can be proved as better, safe and non-toxic green termite control measure. The active components of these oils can also be isolated and tested for the enhanced activity of plant based termiticides. Marigold and citrus oils can also be formulated and commercialized after field testing.

Keywords: *Citrus sinensis,* Green-pesticides, Repellency, *Tageteserecta,* Termite.

Introduction

The termites, one of the most devastating insects, severely damage agricultural crops and urban infrastructure. They reach to the damaging site (indoor wooden structure) through narrow channels on the wall. It is very difficult to locate their mound as theyalso live in colonies as a nest under the soil and hence the eradication of termites is a challenge to human. Termites damage almost all major crops like wheat, maize, sugarcane, cotton, groundnut, pulses etc. Economic lossdue to termites is estimated several millions of rupees every year. In India, the total yield loss has been estimated about 10-25% from most fields and forest crops and about 15-25% from maize alone[1,2].

Extensive use of conventional chemical pesticides causes resistance in the termites and toxicity to plants and environment. It risks upon human and animal health resulting into various modern diseases (cancer, parkinson's, paralysis, abnormal infants and birth rate, increasing pregnancy risks, low weight child birth, improper memory and mental development, abnormal reflexes, mental & emotional problems). In the view of deleterious effects of chemical pesticides, the plant based pesticides have emerged as safe, effective and eco-friendly alternate in the form of green technology. Plants contain several bioactive compounds like terpenoids, alkaloids, glycosides, phenols, tannins and flavonoids etc. in their leaves, stem, bark, seed and oil. These natural bioactive compounds are reported to have potent anti-termite property. Among botanical pesticides, essential oils may be considered as most efficient alternative in controlling termites[3]. Bultman et al (1979) tested 42 tropical African woods and suggested that insecticidal and termiticidal activity of essential oils may be due to containing volatile compounds majorly[4]. Anti-feeding action of ferruginol, manool, and nezukol compounds isolated from Bald cypress, *Taxodiumdistichum* L. wood against *C. formosanus* was reported by Scheffrahn et al. (1988)[5]. Toxicity of essential oil components chamaecynone, an acetylenicterpenoidfrom *Chamaecy parispisidera*, 7-methyljuglone, a naphthoquinone from *Diospyrosvirginiana* and torreyalfrom *Torreyanucifera* was also tested against termites [6-8]. Nagnan and Clement (1990) studied the topical toxicity of geranyllinalool to termites [9].

Repellent activity of termites from essential oils of Cedarwood, *Litseacubeba* and *Cinnamomum* spp. was also confirmed by Adams (1991), Lin and Yin (1995a) and Lin and Yin (1995b) respectively [10-12]. Cornelius et al (1997) and Sharma et al (1994) reported that the terpenoids (citronellal, citral, gernaniol, limonene, piperitone, cedrol,ferruginol, manool, nezukol and eugenol etc.) in essential oils are theresponsible compounds for the repellent activity of the Formosan subterranean termite[13,14]. Recently, Nootkatone (sesquiterpene) and other terpenoids in vetiver oil have been found to be highly efficient repellents and toxicants to Formosan subterranean termites, fire ants, ticks and cockroaches[15].

Moreover, Elango et al (2012) investigated efficacy of solvent extracts of 8 medicinal plants including *Tageteserecta* against Formosan subterranean termite, *Coptotermesformosanus*[16]. All the crude extracts showed anti-termitic activity in a dose-dependent manner and exhibited a significant activity after 24 h and 48 h of exposure suggesting them to be novel, safe and renewable source of natural wood preservatives andtermiticides.A field study was conducted by Aschalew et al (2008) to evaluate the efficacy of eleven pesticidal plants against termites (*Microtermesadschaggae*) on hot pepper during2001, 2003 and 2004 cropping seasons[17]. They recorded lower percentage of damaged plants (25.5 %), higher stand count at harvest (36.0) and higher amounts of dry pod yield (170 kg/ha) with *Tagetesminuta*. Similar study was conducted on hot pepper by Ahmed and Abraham (2014) during 2009, 2010 and 2011 cropping seasons[18]. They obtained moderate results of lower percentage of damaged plants (16.94 %), higher stand count at harvest (37.67) and higher amounts of dry pod yield (2.50 qt/ha) with *T. minuta* leaf powder. Aschalew et al. (2005) also reported that *C. macrostachyus* and *T. minuta* have repellent properties against termites while *D. stramonium, F. vasta, A. indica* leaves and *Chenopodium*sp. had shown insecticidal effects [19].Raina et al (2007) used citrus peel and orange oil extract to control Formosan subterranean termite, *Coptotermesformosanus* Shiraki in US[20]. They reported that *d*-limonene (~92% constituent in orange oil extract) was the responsible compound for termite mortality.

Jatrophacurcas is a multipurpose, drought resistant, perennial plant. It is found to possess insecticidal, molluscicidal, and fungicidal properties[21-23]. Different parts of *J.curcas* have many therapeutic values and are used for several purposes[24]. Verma et al (2013) reported termiticidal activity of solvent extracts of Jatropha root, stem and bark[25]. They also determined phyto-components of *J. curcas* root and found thatterpenoids are the responsible compounds for anti-termite activity. Antifeedant, anti-tunneling, repellent and termiticidal activity of *J. curcas* oil against *Coptotermesvastator* was studied by Acda et al (2009)[26]. Sharma et al (2011) investigated termiticidal potency of water extracts of non-edible oil seed cake of *J. curcas*and its crude active component phorbol ester[27]. They observed 100 % termite mortality with seed cake aqueous extract and phorbol esterin 1 week and 12 h respectively. Ede and Demissie (2013) studied biocide potential of Jatropha oil against termite (*Odontotermesobesus*)[28]. They obtained 100 % termite mortality with Jatropha seed oil (10 %) in 48 h. A wood protection study was conducted by Singh and Kumar (2008) using Jatropha oil and its toxic fraction against *Microcerotermesbeesoni*[29]. Minimum weight loss (18.77%) was recorded inJatropha oil treated wood which was enhanced up to 10.48 % by its toxic fraction.In this paper leaf, root and bark of *J. curcas* and oils of *Tageteserecta* and *Citrus sinensis* were tested against termite (*Odontotermesobesus*). The biocidal activity of *J. curcas* extract and repellent activity of Tagetes and Citrus oil were studied for termite control.

Materials and Methods

Collection of termites

Wooden blocks (20 X 4 X 2 cm) of kail wood were inserted into soil of termite infected area and made it wet to maintain the moisture. After 10 days the wooden blocks were infested with termites and were collected into plastic containers carefully with brush. Termites were kept in dark for 24 h at 28 ± 2 °C temperature and 85 ± 5% relative humidity before experiments. Adult, healthy and active worker termites were separated and used for bioassay.

Termiticidal bioassay

No-choice bioassay (Kang et al., 1990) was performed to evaluate the termiticidal activity of various parts of Jatropha extracts[30]. The tests were done in petri dish (inner diameter 4.8 cm) filled with 1 g sand. 0.5 g/ml concentration of butanol, ether, hexane and methanol extracts of leaves, roots and bark of *J. curcas* were prepared by diluting each extract with their respective solvents. Filter paper discs (diameter 4.5 cm) were treated with one ml of each extract. Discs were air dried and kept in petri dishes. Control set contains only solvent. Ten adult workers were then released into the centre of the

petri dishes and kept in BOD incubator (28 ± 2°C and 80 ± 10% RH). Three replicates of each treatment were made. Mortality of termites was observed after regular time interval for 48 hours. Dead termites were removed after every counting to avoid fungi or other infestations. Mortality data was analysed and percentage mortality was determined.

Repellency bioassay

A repellency apparatus was used to conduct the repellency experiments of termites. This apparatus is made up of glass, having a large dish of 133 mm inner diameter and 41 mm height. The glass dish is connected with 8 glass jars (inner diameter 38 mm and height 60 mm) through 8 glass tubes of length 25 mm and inner diameter 5 mm. 5 grams of sand was spread at the base of the glass dish in the apparatus and made it wet. Tissue paper treated with orange oil and marigold oil (6.28µl/cm^2) was kept in the centre of the glass dish. 50 termites (*Odontotermesobesus*) were transferred into the apparatus from the opening in the centre of glass dish. Termites started moving from centre to all the directions in the glass jars of the apparatus. This repellency movement of termites was observed for two and half hours and percentage repellency of termites was calculated. After two and half hours remaining termites died.

Results and Discussion

Figure 1, 2 and 3 depicts the mortality percentage occurred with butanol, ether, hexane and methanol extracts of *J. curcas*root, leaf and bark respectively. Methanolic extract of *J. curcas* root (0.5 g/ml) showed maximum termite mortality (100 %) in 48 hours while 67 %, 60 % and 47 % mortality were observed with hexane, ether and butanolic extract respectively. Furthermore, maximum 70 % termite mortality was achieved with ether extract of Jatropha leaf in 48 h at the same concentration. Successively, decreased termitemortality i.e. 67 %, 60 % and 37 % was recorded with butanolic, hexane and ether extract of leaf. In case of bark, maximum 63 % termite mortality was observed with hexane extract after 48 h while methanolic and butanolic extract showed moderate (50 % and 43 %) mortality. Ether extract of Jatropha bark conferred only 40 % mortality in the same time duration. The control imparted maximum 10 % termite mortality in 48 h. The work has already been published in International Journal of Ecology and Environmental Sciences[25].

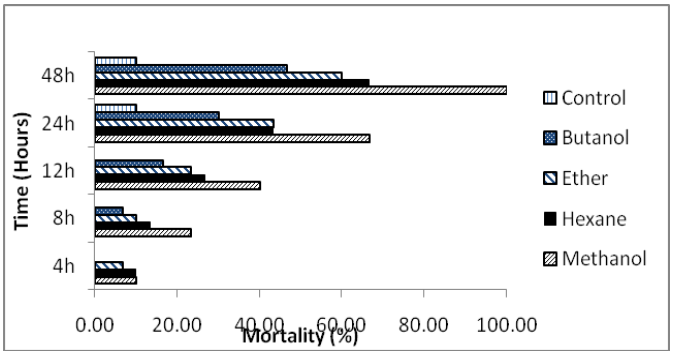

Figure 1: Effect of *J.curcas* root extracts on termite mortality

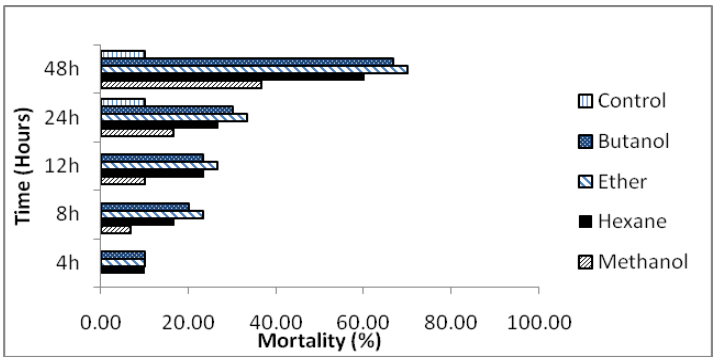

Figure 2: Effect of *J. curcas* leaf extracts on termite mortality

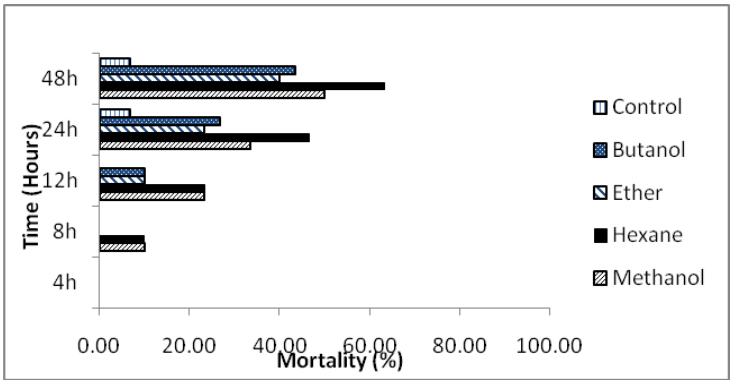

Figure 3: Effect of *J. curcas* bark extracts on termite mortality

Figure 4 shows the Lethal Time (LT) 50 of termites for methanol, hexane, ether and butanol extract of Jatropharoot, leaf and barkfor 48 h with concentration 0.5 g/ml at 95% confidence limits.Methanolic root extract exhibited minimum LT 50 (17.194 h) while leaf extract of the same solvent showed maximum LT 50 (56.755 h) value. The lower value of lethal time represents the higher efficacy of the extract and vice-versa hence the root extract of methanol was found to be most effective against termites.

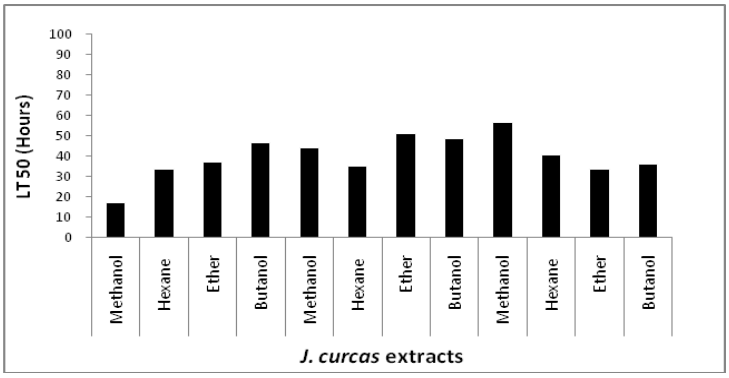

Figure 4: LT 50 response of solvent extracts of *J. curcas*against termite

Percentage repellency of termites by marigold and orange oil are shown in figure 5 and 6 respectively. The results revealed that marigold oil showed 82 % termite repellency while orange oil showed 76 % at 6.30 µl/cm^2 concentration after two and half hour. Tagetes oil repelled termites by 40, 54, 66, 78, 78, and 82 % after every 15 min for 1.30 h respectively. From 1.30 h to 2.30 h,constant repellency (82 %) was observed for termite by marigold oil. Similarly, citrus oil showed 30, 44, 52, 59, 63 and 68 % mortality after every 15 min successively for the same time. 72 % termite repellency was recorded after 1.45 h.However, orange oil showed no changes in repellent effect (76 %) in between 2 to 2.30 h.

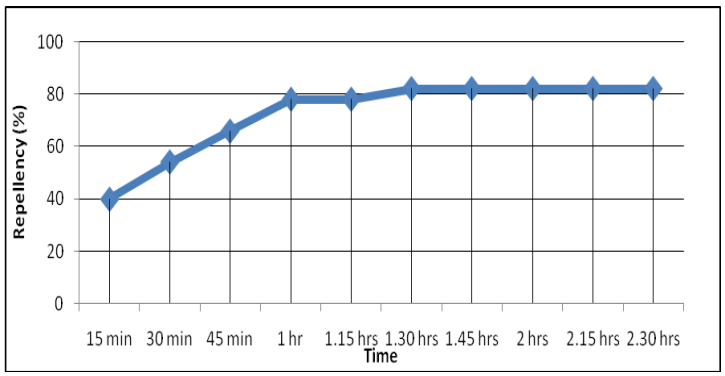

Figure 5: Percentage repellency of termite by marigold oil

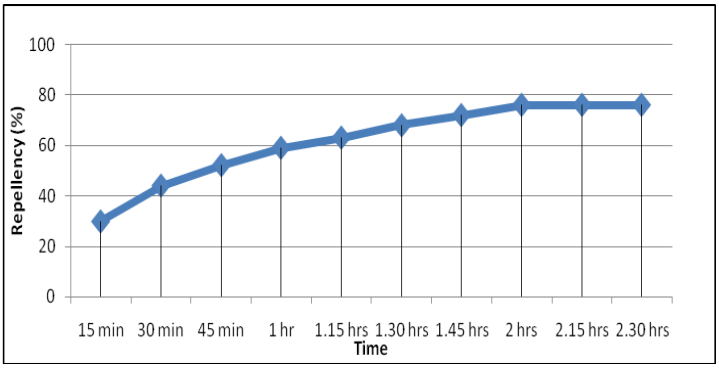

Figure 6: Percentage repellency of termite by orange oil

Overall, marigold oil showed better termite repellency (82%) than orange oil (76%) at the same concentration 6.30 µl/cm^2. Our results pertaining to termite repellency of Tagetes and Citrus oil are supported by many other workers who have also reported the insecticidal efficacy of these oils against other insects.Bioefficacy of three citrus essential oils against the dengue vector *Aedesalbopictus* was done by Giatropoulos et al (2012)[31]. They evaluated LC(50) values ranging from 25.03 to 37.03 mg l(-1) against mosquito larvae. They also tested the citrus oil components and found to be more effective than citrus oil. In repellent bioassays, lemon essential oil, S-(-)-limonene, citral (mixture of neral\geranial) and (+)-β-pinene were the most effective compounds compared to other citrus essential oils and components against adult mosquitoes. Repellent bioassays revealed that limonenes and β-pinenes were the responsible compounds for repellent activity.In another study, repellant activity of *Citrus hystrix, Curcuma longa, Cymbopogonwinterianus* and *Ocimum americanum* oils was investigated by Tawatsin (2001)[32]. 100% protection was estimated for 8 h, 3 h, 1.5 h and 2.5 h against *An. stephensi, Ae. aegypti, C. quinquefasciatus* and *An. dirus* respectively.Emeasor and Okorie (2008) used sweet orange (*Citrus sinensis*) rind powder and oil for controlling maize weevil (*Sitophiluszeamais*)[33]. They confirmed no significant mortality effect of orange rind powder on adult *S. zeamais* resulting in severe damage of the grains and weight loss while rind oil showed significant reduction of grain damage protecting grain damage. Thus they inferred that *C. sinensis* rind oil, (not the powder) can be used for the storage of maize grains. Abdullahi et al (2011) tested citrus peel powder against stored products infesting pest *Triboliumcastaneum* (Coleoptera: Tenebionidae)[34]. The result oftheir study indicated that the highest dose of the citrus peel powders (8g) recorded themaximum mortality of the insect after every 24hours interval of exposure.

Insecticidal activity of tagetes oil was also tested by several workers. Garcia et al (2012) studied the bioactivity (acaricidal effect) of *Tagetesminuta* against *Rhipicephalusmicroplus, Rhipicephalussanguineus, Amblyommacajennense* and *Argasminiatus*[35]. They observed over 95% efficacy against all four species of ticks ata concentration of 20% though adult immersion test (AIT) and the larval packet test (LPT).The repellency of essential oils of Myrtle (*Myrtuscommunis*) and Marigold (*Calendula officinalis*) was compared with DEET (N, N-diethyl-m toluamide) against *Anopheles stephensi* by Tavassoli et al (2011)[36]. The protection time of marigold and myrtle (50%) were found to be 2.15 and 4.36 hours respectively compared to 6.23 hours for DEET (25%).Similarly, Weaver et al (1994) studied insecticidal activity of floral, foliar and root extracts of *Tagetesminuta* (Asterales: Asteraceae) against adult Mexican bean weevils (Coleoptera: Bruchidae)[37]. Floral and foliar extracts were found to be more effective than root extract. Males were observed to be more susceptible than females. Bhatt (2013) analysed the larvicidal activity of essential oils of *Cymbopogonflexeous* (Lemon grass) and *Tageteserecta* (Marigold) against *Aedesaegypti* larvae[38]. LC50 values of *Cymbopogonflexeous* were documented to be 136.8, 52.736 and 24.056 ppm and for *Tageteserecta* 81.765, 48.951 and 17.729 ppm after 12, 24 and 48 h of exposure.The results and supporting studies suggested that orange oil and tagetes oil have potent insecticidal activity and could be used for termite management.

Conclusion

The present study revealed the repellent activity of *Tagetuserecta* and *Citrus sinensis* oil against termites. Tagetes oil showed better termite repellency than citrus oil. Consequently, in further investigationseco-friendly termiticidal formulations can be developed using these oils separately as

well as in combinations which can be cost effective and non-toxic to environment and human health. The active components of these oils can also be isolated and used for the enhanced activity of plant based termiticides.

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