

Repellent, Antifeedant, and Toxic Activities of *Lantana camara* Leaf Extract Against *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

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ABSTRACT This study investigated biological activity of chloroform extract of dry *Lantana camara* ‘Mozelle’ leaves against the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), an important structural pest. Repellent activity was assessed using a paper-disc choice test and a sand arena choice test. Antifeedant and toxic properties were assessed using a no-choice paper test and a topical application method. In the choice tests, significantly fewer termites made contact with treated paper-discs at test concentrations ≥ 0.016 mg/cm² (equivalent to 0.0023 wt:wt) or tunneled into treated sand at test concentrations ≥ 0.125 mg/g, compared with control. In the no-choice tests, termite feeding activity was significantly reduced and termite mortality was greatly increased in treatments than control. Exposure to filter paper treated at 0.212 and 0.106 mg/cm² (equivalent to 0.03 and 0.015 wt:wt) resulted in >90% mortality and 78% reduction in feeding, and $\approx 52\%$ mortality and 40% reduction in feeding, respectively. Top-dorsal application led to >60% mortality at 4 μ g/termite. This study showed that the chloroform leaf extract of *L. camara* had excellent repellent and moderate toxic and antifeedant activities.

KEY WORDS botanical insecticide, subterranean termite, chloroform extract

Subterranean termites (Isoptera: Rhinotermitidae) forage for cellulosic food and frequently attack structural wood. In the United States, the Eastern Subterranean Termite, *Reticulitermes flavipes* (Kollar), is one of the most economically important termite species (Su 1996). Termite control has historically relied upon soil application of synthesized insecticides (Su and Scheffrahn 1998, Anonymous 2002).

Lantana camara L. (Lamiales: Verbenaceae) is the most cultivated species of the ≈ 150 species of the genus *Lantana*, with >650 varieties grown worldwide in many different forms and a multitude of colors. It is a fast-growing, low-maintenance plant with wide ecological tolerance. It can be an invasive and poisonous weed in tropical and subtropical environments, but also presents many positive attributes such as enriching soil, retaining humus, slowing soil erosion, and providing a nectar source for honey bees and butterflies (Day et al. 2003). Cultivated varieties are mostly noninvasive and have gained popularity as flowering plants in gardens and landscaping. This is reflected in an increasing geographic range in temperate climates (Day et al. 2003). This plant has been historically used in folk medicines for treating various human illnesses (Ghisalberti 2000, Sharma et al. 2007) and plant extracts have exhibited a broad range of biological activities (Sharma et al. 2007). Although the

extracts of its aerial parts have been reported to have insecticidal properties against various insect pests (Abdel-Hady et al. 2005, Innocent et al. 2008), there is little scientific investigation of the effects against termite (Verma and Verma 2006). Recently, two studies documented that the leaves of two *L. camara* cultivars, ‘Mozelle’ and ‘New Gold,’ fresh or dry, acted as an anti-termite barrier when incorporated into soil and effectively reduced termites (Rhinotermitidae) foraging activity (Ding and Hu 2010, Yuan and Hu 2011). We are interested in whether the leaf extract has termiticidal activities if used on termite food source and tunnel substance.

This study was conducted to assess the repellent, antifeedant, and toxic activities of *L. camara* Mozelle leaf extract on the workers of *R. flavipes*. We hypothesized that the extract would reduce termite tunneling activity, paper consumption, and survival.

Materials and Methods

Leaf Collection and Extract Preparation. Mature leaves of *L. camara* Mozelle were collected from pre-flowering living plants in the city of Auburn, AL, in June 2010. Leaf samples were oven-dried (637G Iso-temp, Fisher, Pittsburgh, PA) at 40°C for 3 d and afterwards ground using an electric stainless steel blender. The ground leaf material was sealed in glass jars at 4°C before extraction. Samples (100 g) of ground leaf material were placed into 1 liter Erlenmeyer flasks (VWR, Suwanee, GA) and extracted with chloroform (0.5 liter) three times at room tempera-

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ture. Extract times were 1, 3, and 5 d. Crude extracts were combined and filtered through filter paper (Fisherbrand P8). The filtrates were concentrated under reduced pressure 23 mmHg at 40°C in a rotary evaporator (Rotavapor R-210/R-215, BÜCHI Labortechnik AG, Flawil, Switzerland). The resulting residues were weighed and mixed with chloroform to obtain a 20 g/L stock solution. The stock solution was diluted to a series of concentrations (1.25, 2.5, 5, 10, and 20 g/L). These solutions were stored in air-tight glass bottles at 4°C before being used.

Termites. Workers of *R. flavipes* were collected on the Auburn University campus from two colonies that were 1,115 m apart, using underground open-bottom bucket traps (Hu and Appel 2004). Termites were identified by using a key to the soldier caste (Scheffrahn and Su 1994) and vouchers previously identified using mtDNA COII gene (Foster et al. 2004). Termites were maintained in moistened corrugated cardboard rolls in an incubator at 25°C, 98% relative humidity (RH), in constant darkness and tested within 3 d of collection. Voucher specimens were preserved in 100% ethyl alcohol and deposited in the Auburn University Entomology Museum, AL.

Choice Tests. Two different choice tests were carried out, and both were completely randomized design. The first choice test was to determine the repellent activity of chloroform extract using a paper disc contact method modified from Bläske and Hertel (2001). Petri dishes (50 mm diameter × 9 mm height, Falcon, BD Biosciences, Franklin Lakes, NJ) were used as experimental units. Each unit contained two preweighed filter paper discs (Whatman No. 1; 7 mm diameter, 38.5 mm² area), one treated with 5 μl of a test solution (resulting in concentrations of 0.0163, 0.0325, 0.065, 0.13, and 0.26 mg/cm², corresponding to the five solution concentrations) and the other treated with 5 μl of solvent (acetone) as a control. Control units contained one untreated and one solvent treated disc. The discs were kept 10 h in a fume hood to allow solvent evaporation before being moistened with 8 μl distilled water per disc. The two paper discs were placed 1.5 cm apart from each other in the center of each unit. Thirty termites were introduced into each unit. For every treatment and control, six replicate units (three of each termite colony) were tested. The number of termites making contact with each paper disc was recorded at 5 min intervals during the first 30 min, then at 10 min intervals for the following 30 min, for a total of nine counts per paper disc. A digital camera (Cannon Power Shot SD450, Japan) was used to aid accurate counts. Paired *t*-tests ($P < 0.05$) were performed for the differences in the mean number of termites that contacted the treated and untreated paper discs within each treatment.

The second choice test assessed the repellent activity of chloroform extract using a sand arena design. The experimental units were two-section divided petri dishes (100 cm diameter × 15 cm height, BD Falcon). Each unit had one section filled with 30 g of autoclaved play sand (Bonsal American, Franklin, TN) treated with 3 ml of a test solution to achieve five concentra-

tions (0.125, 0.25, 0.5, 1.0, and 2.0 mg/g) and the other section filled with 30 g sand treated with 3 ml of acetone (control). Sand was press-leveled to the height of the partition. Control units contained acetone-treated sand in one section and untreated sand in the other. Experimental units were kept 24 h uncovered in a fume hood at room temperature to allow solvent evaporation. For each section, a preweighed filter paper (42.5 mm in diameter, four-fold) was buried into sand as a food source, and 3 ml of water was added to moisten the sand and paper. Groups of 100 termite workers were introduced to the center of each unit. Each treatment was replicated six times (three of each termite colony). The units were covered and sealed with Parafilm (American National Can, Chicago, IL), then placed in Plexiglas boxes (27 × 19 × 10 cm) to retain moisture. The boxes were kept in an incubator adjusted to 25 (±1) °C and 75 (±3) % RH in constant darkness, except for observation times. Tunnels made by termite were observed from the bottom of the units, copied by a scanner, and printed at actual size for measurements of total tunnel length with Scale Master Classic (V3.0 Digital Plan Measure) on day 0.5, 1, 3, and 7. All experimental units were dissected on day 7 to count live termites in each section. Filter papers were cleaned with distilled water, dried in a fume hood for 48 h at room temperature, and weighed to calculate paper consumption as the difference between the weight before and after the experiment. Tunnel length, paper consumption, and live termite distribution in each section of a unit within each treatment at each observation day were analyzed using a paired *t*-test ($P < 0.05$). The numbers of live termites in each unit were pooled and the mean survivor numbers were analyzed by analysis of variance (ANOVA), followed by Tukey's studentized range test to evaluate the difference of the treatments at the $P < 0.05$ level of significance (Statistix 9.0, Analytical Software 2008).

No-Choice Tests (Antifeedant and Toxic Effects). A filter paper bioassay in no-choice format was carried out to determine the antifeeding and toxic effects of the leaf extract. The test was conducted in petri dishes (50 mm diameter × 9 mm height), using a completely randomized design. Treatment filter papers (42.5 mm in diameter, Whatman, Schleicher & Schuell, England) were uniformly wet with 0.15 ml of test solutions to obtain five concentrations (0.013, 0.026, 0.0525, 0.105, and 0.21 mg/cm²). Control papers were treated with solvent. The filter papers were left to dry in a fume hood for 24 h at laboratory temperature, reweighed, and moistened with 0.2 ml of distilled water. Groups of 100 termite workers were introduced to the test units and allowed to have direct and continuous contact with the treated or control filter papers. Each treatment was replicated six times (each termite colony triplication). The units were sealed with Parafilm (American National Can), placed in Plexiglas boxes (27 × 19 × 10 cm) to retain moisture and kept in an incubator adjusted to 25 (±1)°C and 75 (±3) % RH in constant darkness, except during observation to count the number of live termites at 2-d

intervals. The test was terminated on day 20. Unconsumed filter papers were dried under room temperature for 2 d, cleaned with a soft brush, and weighed. Filter paper consumption was calculated as the difference between the weights before and after the test. Live termite data were first calculated as mortality (%), and then transformed by the arcsine of the square root for normality. Termite mortality and filter paper consumption were analyzed using one-way ANOVA followed by Tukey honestly significant difference (HSD) for mean comparisons (Statistix 9.0, Analytical Software 2008).

Toxicity of the extract was further evaluated using topical application method and a completely randomized design. A 0.2 μl droplet of each test solution was applied to the dorsal thorax of each termite, using a Hamilton PB 600 repeating dispenser equipped with a 10 μl micro-syringe (Hamilton, Reno, NV) to achieve five doses (0.25, 0.5, 1.0, 2.0, and 4.0 μg per termite, corresponding to extract concentrations). For each treatment and control, six replicates (three replicates of each termite colony) were assayed. Acetone (0.2 μl) was used as a control. Test termites were kept in petri dishes (50 mm diameter, 9 mm height) with a moist filter paper (42.5 mm in diameter) serving as both food and a means to maintain moisture at 25°C. Termite mortality was recorded each day for the first 6 d, then at 4 d intervals from 7 to 30 d at a fixed time, by counting live termites and removing dead termites from the petri dishes. Data were transformed by the arcsine of the square root for normality and subjected to a one-way ANOVA, followed by Tukey HSD multiple comparisons for independent samples to determine significant differences in average mortality (Statistix 9.0, Analytical Software 2008).

Results

Choice Tests. The paper disc choice test showed strong repellent activity of the test extract at all five concentrations (Fig. 1). Significantly lower number of termites ($t > 4.61$; $df = 53$; $P < 0.001$) made contact with extract-treated paper discs compared with solvent-treated disc. Although the lowest number of contacting termites was observed in the highest extract treatment, there was no statistically significant difference between concentrations. In controls, the numbers of termites making contact with untreated and solvent-treated discs were not different ($t < 1.01$; $df = 53$; $P > 0.5$) during the 60-min test period.

The sand arena choice test confirmed repellent activity (Table 1). Termites tunneled significantly longer ($t > 5.27$; $df = 5$; $P < 0.01$) into the solvent-treated control section than into the extract-treated section at the five test concentrations, whereas the tunnel lengths in the two sections of control units were almost equal ($P > 0.5$). At day 7, when the test was terminated, significantly more termites stayed in solvent-control sections compared with extract-treated sections ($t > 8.42$; $df = 5$; $P < 0.01$), whereas termites presented in almost equal numbers ($P < 0.01$) in the two sections of control units. The concentration effect

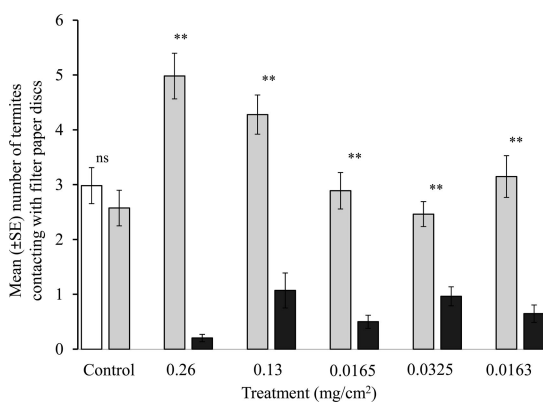


Fig. 1. Repellency of chloroform extract of *Lantana camara* 'Mozelle' leaves on *Reticulitermes flavipes* workers during a 60-min paper disc choice test (nine counts per disc; $N = 648$). Groups of 30 termites were introduced into choice-test units. White bar: untreated filter paper disc; gray bar: solvent-treated filter paper disc; black bar: extract-treated filter paper disc. ** indicates significant difference and "ns" indicates no significant difference between the two paper discs by Paired t -tests at $P \leq 0.05$.

on repellency was significant. Higher concentrations (0.5 mg/g and higher) completely repelled termites, no tunnel was observed and no termite present; lower concentrations (0.125 and 0.25 mg/g) showed short tunnels and a few termites. Paper consumption occurred in control sections but not in treatment sections, even at the two low concentrations where a few termites were present. None of the treatments led to termite mortality greater than control ($F = 0.48$; $df = 6, 14$; $P = 0.81$).

Antifeedant and Toxic Effects. All of the test concentrations significantly reduced feeding by *R. flavipes* as compared with control ($F = 32.78$; $df = 6, 35$; $P < 0.05$) in the filter paper no-choice test (Fig. 2). The antifeedant effect was more or less concentration dependent. Exposure to the treated filter papers also resulted in greater mortality of termites compared with control ($F > 2.57$; $df = 6, 35$; $P < 0.05$) and termite mortality increased as concentration and exposure time increased (Fig. 3). The concentrations of 0.212 and 0.106 mg/cm² resulted in termite mortality of 97.3 ± 0.61 and $52 \pm 9.8\%$, respectively. The filter paper weighed $\approx 7.1 \pm 0.34$ mg/cm², thus the concentrations of 0.212 and 0.106 mg/cm² were equivalent to 0.03 and 0.015 wt.wt.

Toxicity. The results of topical application of test solutions are presented in Fig. 4. Although the differences in termite mortality between some treatments and control after 18 d were statistically significant ($F > 19.8$; $df = 6, 35$; $P < 0.05$), treatment of 4 μg /termite was the dose that achieved mortality $>60\%$, and the toxic effect was maximized around 22 d.

Discussion

Many plants possess some forms of biological activity against different insects and other organisms

Table 1. Effects of chloroform extract of *Lantana camara* 'Mezelle' leaves against workers of *Reticulitermes flavipes* in a choice tunnel arena test (mean \pm SE)

Paired-choice Treatments (mg/g)	Tunnel length (cm) into the two choice sections of a petri dish at different days ^a				Filter paper consumption (mg) ^a	Live termites at day 7	
	0.5	1	3	7		No. in each section ^a	Total no. ^b
Untreated	32.4 \pm 1.6a	33.8 \pm 1.0a	45.7 \pm 0.7a	59.0 \pm 3.4a	16.7 \pm 5.3a	41.7 \pm 22.2a	92.0 \pm 2.6a
Solvent-treated	3.7 \pm 3.7b	12.4 \pm 6.2a	34.3 \pm 3.0a	56.4 \pm 4.8a	28.6 \pm 2.8a	50.3 \pm 19.7a	
0.125	3.4 \pm 1.4a	4.8 \pm 1.8b	5.9 \pm 1.9b	6.2 \pm 2.2b	0 \pm 0b	10.3 \pm 5.4b	92.7 \pm 1.9a
Solvent-treated	6.9 \pm 3.7a	15.6 \pm 0.7a	28.5 \pm 1.4a	57.0 \pm 1.2a	37.1 \pm 2.1a	71.3 \pm 23.2a	
0.25	2.2 \pm 2.2b	2.6 \pm 2.6b	3.9 \pm 3.9b	6.1 \pm 6.1b	0 \pm 0b	8.0 \pm 8.0b	91.7 \pm 1.5a
Solvent-treated	17.7 \pm 3.1a	19.8 \pm 1.4a	28.8 \pm 2.4a	75.3 \pm 2.4a	30.7 \pm 3.9a	83.7 \pm 8.0a	
0.5	0.0 \pm 0.0b	0.0 \pm 0.0b	0.0 \pm 0.0b	0.0 \pm 0.0b	0 \pm 0b	0.0 \pm 0.0b	94.3 \pm 0.7a
Solvent-treated	17.0 \pm 1.8a	23.4 \pm 1.1a	27.0 \pm 1.6a	58.7 \pm 1.3a	38.6 \pm 1.0a	94.3 \pm 0.7a	
1.0	0.0 \pm 0.0b	0.0 \pm 0.0b	0.0 \pm 0.0b	0.0 \pm 0.0b	0 \pm 0b	0.0 \pm 0.0b	92.7 \pm 1.2a
Solvent-treated	26.3 \pm 1.0a	37.5 \pm 2.1a	52.9 \pm 3.8a	74.5 \pm 4.9a	38.8 \pm 2.4a	92.7 \pm 1.2a	
2.0	0.0 \pm 0.0b	0.0 \pm 0.0b	0.0 \pm 0.0b	0.0 \pm 0.0b	0 \pm 0b	0.0 \pm 0.0b	94.0 \pm 0.6a
Solvent-treated	39.4 \pm 4.0a	42.9 \pm 3.8a	51.8 \pm 3.9a	84.0 \pm 7.0a	27.7 \pm 2.1a	94.0 \pm 0.6a	

^a Means followed by the same letter are not significantly different ($P > 0.05$; df = 5; Pair *t*-test) between the two choice of a unit.

^b Means followed by the same letter in the column are not significant difference ($F = 0.48$; df = 6, 14; $P = 0.81$).

(Jacobson 1989). *L. camara* is a versatile plant and has been cultured as a popular ornamental plant, an important medicinal plant, a renewable natural source of green mulch, or an additive for improving soil and crop production (Raju 2000). Further, *L. camara* has shown a broad range of pharmacological and biological activities, including antimicrobial, antipyretic, antiphlogistic, antimutagenic, alleopathic, antioxidant, antiproliferative, bactericidal, fungicidal, nematocidal, and others (Ghisalberti 2000, Ganjewala et al. 2009, Sousa and Costa 2012).

A wide variety of extracts/essential oils of *Lantana* and their constituents possess varying degrees of pest-controlling properties (reviewed by Sousa and Costa 2012). Documented insecticidal properties include antifeeding, antiovipositing, larvicidal, repellent, and toxic effects against a wide range of pest insects (Saxena et al. 1992, Fatope et al. 2002, Ogendo et al. 2004,

Verma and Verma 2006, Innocent et al. 2008). The methanolic leaf extract at 5% concentration resulted in >50% reduction in aphid establishment (Sharma and Mehta 2009). One application of the chloroform flower extract provided 100% protection for 2 h and up to 75.8% protection at 7 h against *Ae. spp.* mosquito bites (Dua et al. 2003). The hexane leaf extract inhibited egg-hatching and the hexane, acetone, and water extracts caused significant mortality in the first instar larvae of potato tuber moth *Phthorimaea operculella* Zeller at 10% concentrations (Iannacone and Lamas 2003). The petroleum ether and methanol extracts of aerial part displayed toxic effect on bean weevil *Callosobruchus chinensis* L. (Dixit et al. 1992). The essential oils of *L. camara* showed LD₅₀ values of 0.05–0.06 mg/cm² and KDT₅₀ values of 12–20 min on 0.208 mg/cm² impregnated paper against mosquito vectors (Dua et al. 2010) and a LC₅₀ value of 0.22 mg/cm² against rice weevil *Sitophilus oryzae* and flour beetle *Tribolium castaneum* (Mohamed and Abdelgaleil 2008). Essential oils of leaves and flowers led to 80 and 100% mortality in third instar larvae of house fly *Musca domestica*, respectively (Abdel-Hady et al. 2005).

With respect to termiticidal effects, the only study was reported recently by Verma and Verma (2006). Using *Microcerotermes beesoni* Snyder (an arboreal termite species) and seven different extracting solvents, they tested the leaf extracts of *L. camara* variety *aculeate* in India. They found that only 5% chloroform extract (treated filter paper) exhibited excellent termite mortality (68.7% at 48 h). They also found that the 5% chloroform extract showed a LD₅₀ (5.0 μ g/insect) that is comparable to the LD₅₀ (=4.8 μ g/insect) of 0.5% chlorpyrifos. Chlorpyrifos is a moderately toxic synthesized organophosphate insecticide that inhibits acetylcholinesterase. Our study corroborates the termiticidal effect of chloroform leaf extract, but a comparison of the toxicity of the chloroform extract between the results from our study and Verma and Verma (2006) is impossible. Their report was so preliminary and provided no information about

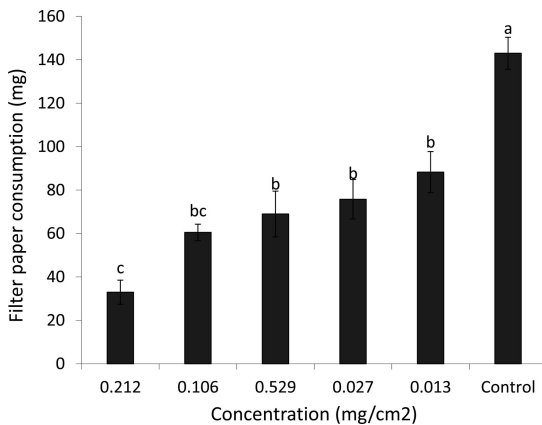


Fig. 2. Antifeedent effect of chloroform extract of *L. camara* Mozelle leaves on *Reticulitermes flavipes* workers. Groups of 100 workers were exposed to the filter paper treated at different concentrations. The bars represent the means (\pm SE) of filter paper consumption (mg) at different treatment concentrations in 20-d no-choice test.

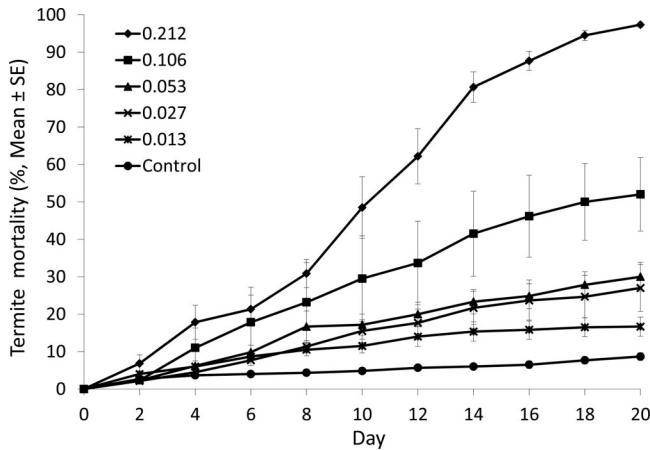


Fig. 3. Toxic effect of chloroform extract of *Lantana camara* Mozelle leaves on *Reticulitermes flavipes* workers by ingestion and tarsal/ventral contact. Groups of 100 termites were exposed to filter paper treated with chloroform extract at different concentrations (mg/cm^2). Lines represent the means ($\pm\text{SE}$) of termite mortality at different treatments and different days in 20-d no-choice test.

what amount of the 5% extract was applied to what areas (or weight) of filter paper, and whether the treated paper was consumed or not. These missing data are required for dose/concentration determination and LD_{50} calculation. Moreover, Verma and Verma (2006) used a different *L. camara* cultivar and tested a different termite species than ours. Species-specific sensitivity toward insecticides and repellency has been frequently reported (Smythe and Carter 1970, Heupel 2002). Ding and Hu (2010) and Yuan and Hu (2011) reported repellent activity of *L. camara* leaves (fresh or dry). Leaves incorporated into soil acted as an anti-termite barrier and effectively reduced tunneling activity of Rhinotermitidae termites. The current study confirms the repellent and toxic activities of *L. camara* leaves, and reports the anti-feedant property of *L. camara* against termites.

Phytochemical studies have isolated and identified various constituents and secondary metabolites with

varying structure patterns from different parts of species of the genus *Lantana*. Qualitative chemical analyses of various crude extracts and essential oils have identified the presence of ≈ 138 compounds belonging mainly to terpenoids, phenylpropanoids, flavonoids, furanonaphthoquinones, oridoid glycosides, and steroids (reviewed by Sousa and Costa 2012). Terpenoids, phenylpropanoids, and flavonoids are considered the main class components with relevant biological activities (Ghisalbert 2000). With respect to insecticidal properties, although substantial research was carried out on crude extracts/essential oils, few, however, attempted to identify/characterize individual chemicals' bioactivities. Innocent et al. (2008) compared the activity of crude leaf extract, fractions, blends, and pure compounds against African malaria vector *Anopheles gambiae* s.s. larvae. They found the crude extract and fractions exhibited different level of larvicidal activity with subtraction of some fractions

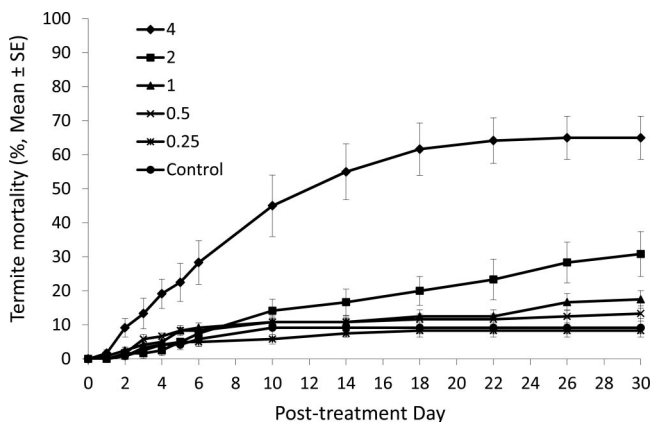


Fig. 4. Toxic effect of chloroform extract of *Lantana camara* Mozelle leaves on *Reticulitermes flavipes* workers by dorsal contact. Lines represent the means ($\pm\text{SE}$) of termite mortality in 30-d test. Groups of six termites received a $0.2 \mu\text{l}$ -droplet of the test solution applied to dorsal thorax to achieve doses of $0.25\text{--}4.0 \mu\text{g}/\text{termite}$.

resulting in activity enhancement. The active fractions contained furanonaphthaquinones regionisomers and camaric acid (29) as active principles. However, they also found that the betulinic acid (65) obtained from the least active fraction exhibited a similar level of the larvicidal effect as the crude extract. Verma and Verma (2006) reported that the termite-active chloroform leaf extract of *L. camara* variety *aculeate* contained significantly more triterpenoids than other non-termite-active solvent extracts, indicating that the effectiveness of a plant extract is largely dependent upon the type of solvent used and is also concentration dependent (Verma and Verma 2006, Ganjewala et al. 2009). Seventy-four triterpenoids have been isolated from different *Lantana* species (Sousa and Costa 2012) and lantadenes are considered the main bioactive constituents (Sharma et al. 2007). Different lantadenes vary little in their physicochemical properties but the difference in structure plays an important role in their bioactivity. Up to date, no work has been conducted to understand the insecticidal mechanisms of the actions of these chemical constituents, probably because of the difficulty in their isolation and cost-effective issue (Sharma and Sharma 2006).

In summary, chloroform extract of *L. camara* Mozelle leaves exhibited strong repellency, moderate reduction in feeding and toxicity against workers of *R. flavipes*. The repellent effect was demonstrated by the significant numbers of termites that were averse to making contact with treated paper discs or tunnel into treated sand under choice conditions. The antifeedant effect was evidenced by the significantly low feeding of treated paper under no-choice conditions. Termites would not feed on treated paper if given choice of untreated paper, but would taste treated paper when given no choice. As a result, it is difficult, if not impossible, to differentiate between or recognize repellent and antifeedant properties if a choice behavior test is used. In the sand arena choice test, termites consumed paper buried in untreated sand but not paper in treated sand. This result can be explained as either a repellent response of termites or an antifeedant activity of test extract that inhibited food uptake. Termite mortality is an expression of toxic effect. Extract concentration of 0.212 mg/cm² or higher caused mortality >90% by ingestion and contact, or a dose of 4 µg/termite led to mortality >60% by contact, implying a greater toxicity by ingestion than by contact. These results indicate that a behavioral approach, rather than toxicity test, is appropriate when investigating plant-derived extracts or essential oils because they may have diverse bioactivities.

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