

Potential use of essential oils to control the leaf-cutting ants; *Atta sexdens rubropilosa* and *Acromyrmex subterraneus molestans* (Hymenoptera: Formicidae)

Rafael C. Ribeiro¹ and Hany A. Fouad^{2*}

¹Departamento de Fitotecnia, Universidade Federal de Viçosa, 36570-900, Viçosa, Minas Gerais, Brazil

²Faculty of Agriculture, Plant Protection Department, Sohag University, 82786, Sohag, Egypt.

Abstract

The present study was developed in order to evaluate the effect of five essential oils on the workers of the leaf-cutting ants; *Atta sexdens rubropilosa* and *Acromyrmex subterraneus molestans* by contact with a treated surface and ingestion with a treated leaves. The essential oils of cinnamon, clove and mustard had generally more effective with 5, 10 and 15% concentrations after 24, 48, 72 and 96 h against workers of *A. sexdens rubropilosa* and *A. subterraneus molestans* in contact bioassay, but mustard was the most effective in ingestion bioassay on both species. On the other hand, there was no significant difference among the essential oils with 1% concentration and control after 24, 48 and 72 h of treatment in contact and ingestion bioassays against workers of *A. sexdens rubropilosa*. However, Andiroba oil had less efficiency values in all concentrations been used. Therefore, the essential oils of mustard, cinnamon and clove have contact and ingestion effects on workers of *A. sexdens rubropilosa* and *A. subterraneus molestans*, and may be promising on the leaf-cutting ant control.

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Introduction

Leaf-cutting ants comprise over 12,000 species, but the leaf-cutting ants of the genera *Atta* sp. Fabricius (Hymenoptera: Formicidae) and *Acromyrmex* sp. Mayr (Hymenoptera: Formicidae) (known as saúvas and quenquéns in Brazil, respectively) are among the best known species of the family Formicidae in the New World. These ants are considered the main agricultural and forest pest in countries such as Brazil, as they attack plants at any stage of their development, cutting their leaves, flowers, buds, and branches (Mariconi, 1981), which are then transported to the interior of their nests. These

nests composed of hundreds of interconnected underground chambers and trails opening out at the soil surface. The exterior of the nest usually exhibits a loose soil mound originating from the chambers formed by the nest (Gonçalves, 1984). Holes can be found in the loose soil mound or outside of it. The sprouting of seedlings in native forests can be prevented by high infestation of leaf-cutting ants (Perin and Guimarães, 2012). Leaf-cutting ants of the genera *Atta* and *Acromyrmex* are the main pests found in *Pinus* and *Eucalyptus* plantations (Zanetti *et al.*, 2003; Nickele *et al.*, 2009; Poderoso *et al.*, 2009). A single leaf-cutting ant colony per hectare of forest can reduce the annual tree growth by

*Corresponding author.

Hany A. Fouad

Faculty of Agriculture, Plant Protection Department, Sohag University, 82786, Sohag, Egypt.

Tel: +(2093) 2280126

E-mail: haafouad@yahoo.com

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5% in *Eucalyptus* and by 10% in *Pinus* (Amante, 1967). The application of conventional insecticides, including cyfluthrin (pyrethroid), imidacloprid (neonicotinoid), furathiocarb (carbamate), sulfluramid (fluoroaliphatic sulfonamide), and fipronil (phenyl pyrazole) are the most common method used to control leaf-cutting ants in the forest areas (Boaretto and Forti, 1997; Rizental *et al.*, 2003; Zanetti, 2007). Due to the problems these products may cause to the environment and humans, their use has been restricted by governments and forest product certification bodies (FSC 2007; 2010), which have demanded and encouraged the development of alternative control strategies to these insecticides, such as the use of natural products, entomopathogenic fungi, and pheromones (Oliveira *et al.*, 2011). Recently, natural products can be used to control ant populations through several mechanisms. Baits with plant extracts were effective in the field as a control measure, stopping ant activity (Zanetti *et al.*, 2008). Some of these substances can act directly against the ant, leading to its death, such as citrus seed oils; *Citrus sinensis* (L.) Osbeck, *Citrus limon* (L.) Burm or *Citrus reticulata* Blanco (Rutaceae) (Fernandes *et al.*, 2002). Other Plants can be toxic to leaf-cutting ants, such as *Tithonia diversifolia* (Asteraceae), *Ricinus communis* (Euphorbiaceae), *Eucalyptus maculate* (Myrtaceae), *Hymenaea courbaril* (Fabaceae) (North *et al.*, 2000; Marinho *et al.*, 2005; Marinho *et al.*, 2008; Alonso *et al.*, 2013; Castaño-Quintana *et al.*, 2013) and/or to their fungus, such as *Piper piresii* (Piperaceae), *Simarouba versicolor* (Simaroubaceae), *Raulinoa echinata* *Coffea* spp. (Rutaceae) (Biavatti *et al.*, 2002; Pagnocca *et al.*, 2006; Peñaflo *et al.*, 2009). Therefore, in the present work, introduce a bioassay to evaluate the effect of essential oils from five aromatic plants of *Brassica juncea* L. (Brassicaceae), *Carapa guianensis* L. (Meliaceae), *Citrus sinensis* L. (Rutaceae), *Cinnamomum zeylanicum* L. (Lauraceae) and *Syzygium aromaticum* L. (Myrtaceae) on mortality of the leaf-cutting ant workers of *Atta sexdens rubropilosa* and *Acromyrmex subterraneus molestans* and we discuss the possible use of these oils for controlling leaf-cutting ants.

Materials and Methods

Insect species

The bioassays were performed with workers of *Atta sexdens rubropilosa* and *Acromyrmex subterraneus molestans*. The workers were caught in colonies located around the Campus of the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais State, Brazil. Than these colonies were maintained at a temperature of $25 \pm 5^\circ\text{C}$, RH of $75 \pm 5\%$ in 24 h scotophase, and fed with leaves of different vegetables, changed daily, as well as a water supply (Della Lucia *et al.*, 1993). The volumes of the fungus gardens were at least three liters per colony.

Essential oils

The essential oils of andiroba (*C. guianensis*), sweet orange (*C. sinensis*), cinnamon (*C. zeylanicum*) and clove (*S. aromaticum*) were acquired companies Viessence Natural Products Trade Ltda. (Porto Alegre, Rio Grande do Sul, Brazil) and Ferquima Industria e Comercio Ltda. (Vargem Grande Paulista - Sao Paulo, Brazil), taken on an industrial scale and by hydrodistillation drag of water vapor (Dapkevicius *et al.*, 1998; Santos *et al.*, 2004.). The essential synthetic oil of mustard (*B. juncea*), use in the food industry, provided by the company Marie Fine Chemicals (Itaquaquecetuba, São Paulo, Brazil).

Contact with a treated surface

Using a precision microsyringe, 100 μL of either each oil solution at 1, 5, 10 and 15% or ethanol (control) was applied to the surface of a Petri dish (9 cm diameter x 2 cm height). Each dish was left without direct sunlight for 10 min, after which 10 individuals of *A. sexdens rubropilosa* or *A. subterraneus molestans* were placed in each one of five replicates. The dishes were closed with a glass cover and kept in a room chamber at $25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ RH and 12 h photoperiod (Bueno *et al.*, 1997). The mortality of adults was evaluated 24, 48, 72 and 96 h after starting the test.

Ingestion with a treated leaves

Four concentrations (1, 5, 10 and 15%) were prepared in ethanol, and ethanol was used to control. In each concentration, ten disks (10.75 cm²) of leaf were dipped for ten seconds, and left for ten min to dry under laboratory conditions. After this period, the leaves were transferred to Petri dish, which contained ten individual adults of *A. sexdens rubropilosa* or *A. subterraneus*

molestans. These adults were placed in five replicates and left to feeding on disks of plant leaves treated with oils for 24, 48, 72 and 96 h. After this period, the mortality of adults was calculated.

Statistical analysis

The experiment was developed in, entirely, randomized design with six treatments and five replications. Data were corrected and the efficiency of control of essential oils was evaluated with the Abbott correction (1925). Data of oil efficiency were submitted to the analysis of variance (ANOVA). The means compared by Duncan's Multiple Range Test at 5% probability.

Results and Discussion

The tests to check the insecticide with essential oils of andiroba, cinnamon, clove, mustard and sweet orange on the leaf-cutting ant workers of *A. sexdens rubropilosa* and *A. subterraneus molestans* showed significant difference among them in the survival of ants (Tables 1, 2, 3 and 4). The efficiency was increased gradually in all the essential oils with increasing the days of exposure. Data in contact bioassay (Tables 1 and 2) showed that the essential oils of cinnamon, clove and mustard had generally more effective with 5, 10 and 15% concentrations after 24, 48, 72 and 96 h against workers of *A. sexdens rubropilosa* and *A. subterraneus molestans*. However, Andiroba oil had less efficiency values in all concentrations been used. The rest of essential oils had a moderate toxicity action. A non significant difference showed among the essential oils with 1% concentration and control after 24, 48 and 72 h of treatment in contact and ingestion bioassays against workers of *A. sexdens rubropilosa* (Tables 1 and 3). The efficiency of the tested essential oils was recorded in ingestion bioassay after 24, 48, 72 and 96 h from the beginning of treatment (Tables 3 and 4). The essential oils from all five medical plants increased the mortality of the leaf-cutting ant workers of *A. sexdens rubropilosa* and *A. subterraneus molestans*. Mustard oil revealed the highest residual toxicity effect on the leaf-cutting ant workers of *A. sexdens rubropilosa* and *A. subterraneus molestans*. On the other hand, the lowest effect was recorded in case of cinnamon and clove on *A. sexdens rubropilosa*, and in case of andiroba oil on *A. subterraneus molestans*. The Most strategies for controlling leaf-

cutting ants are based on killing them by contact poisons, but it is usually not enough to control populations in all area. Efficient control involves exterminating the whole colony, not only some individuals. Currently, the most appropriate method for controlling leaf-cutting ants is the use of toxic baits, which may effect by contact and ingestion, because they are incorporated into the colony feeding cycle and the insecticide acts through ingestion (Loeck and Nakano, 1984). The tested essential oils that made the contact and ingestion effectives on the leaf-cutting ants may have qualitative and quantitative differences in the chemical composition (Akhtar *et al.*, 2003). This composition may vary with chemotypes of plants, place and harvest time (Hudaib *et al.*, 2002; Formisano *et al.*, 2013.). Generally, the workers of *A. sexdens rubropilosa* and *A. subterraneus molestans* presented lower resistance to the mustard oil in contact and ingestion bioassays. The stimulating effect of synthetic oil of mustard in control and ingestion tests, is due to the allyl isothiocyanate (ITCA), the major component (90%) of this oil and highly volatile with 3.4 times vapor density greater than the air (Demirel *et al.*, 2009). This compound has fast steaming effect against stored grain pests (Wu *et al.*, 2009; Santos *et al.*, 2011) and nematodes (Oliveira *et al.*, 2011) and its high volatility and rapid decomposition can impact on the workers of *A. sexdens rubropilosa* and *A. subterraneus molestans* after 24, 48, 72 and 96 h of exposure. On the other hand, andiroba oil showed lower effect on the tested ant workers. In other study, andiroba oil (Morini *et al.*, 1997) and citrus seed oil (Fernandes *et al.*, 2002) present toxic effects to grass-cutting ants. Essential oil of cinnamon tree (*C. zeylanicum*) has been showed toxic effectives on adults of *Bruchidius incarnatus*, *Sitophilus granarius*, *S. zeamais*, *R. dominica* and *T. castaneum* (Salvadores *et al.*, 2007; Shayesteh and Ashouri, 2010; Fouad, 2013). Essential oil from *Cinnamomum osmophloeum* Kaneh leaves was toxic to red imported fire ants in open and closed exposure trials (Cheng *et al.*, 2008). The mortality of the red imported fire ant (*Solenopsis invicta*) treated with cinnamon soil at depths of 5 - 10 cm, which was high (Huang *et al.*, 2015). As well as, cinnamaldehyde isolated from cinnamon oil was considered contact toxicity to both *T. castaneum* and *S. zeamais* (Huang and Ho, 1998). The clove oil and powder (*S. aromaticum*) has toxic activities on several insect-pests such as; *B. incarnatus*, *R. dominica*, *S. invicta*, *S. oryzae* and *T. castaneum* (Sighamony *et al.*, 1986; Zeng *et al.*, 2010; Fouad, 2013; Kafle and Jen Shih, 2013), with the main chemical components of clove essential oil are

Table 1. Efficiency (mean±standard error) for *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) workers after 24, 48, 72 and 96 h of contact with 1, 5, 10 and 15% essential oils of andiroba, cinnamon, clove, mustard and sweet orange.

Conc.	Time	Efficiency (%) ^a					Control	F	P value
		<i>B. juncea</i> (Mustard)	<i>C. guianensis</i> (Andiroba)	<i>C. sinensis</i> (Sweet orange)	<i>C. zeylanicum</i> (Cinnamon)	<i>S. aromaticum</i> (Clove)			
1 %	24 h.	1.78±3.92	-2.22±2.22	-0.22± 3.34	-0.22±3.34	-2.22±2.22	2.00±2.00	0.39	0.85*
	48 h.	9.00±9.27	-5.00±5.00	-3.00±5.83	9.00±9.27	9.00±9.27	4.00±4.00	0.74	0.60*
	72 h.	8.61±8.61	-16.67±3.40	-5.56±5.56	8.61±11.20	-5.28±5.28	14.00±2.45	2.33	0.07*
	96 h.	21.11±15.18 a	-19.72±3.24 b	-7.22±6.32 ab	22.22±17.45 a	-5.00±6.37 ab	18.00±2.00 a	3.02	0.03
Mean	10.12±8.50 a	-10.90±2.94 b	-4.0±5.66 ab	9.90±9.32 a	-0.87±3.51 ab	9.50±2.15 a	2.19	0.0893	
5 %	24 h.	68.44±12.3 a	-0.22±3.34 b	-2.22±2.22 b	87.56±5.99 a	83.56±5.20 a	2.00±2.00 b	49.75	<0.0001
	48 h.	87.00±4.36 a	-0.50±3.57 b	-2.50±2.50 b	93.00±4.90 a	91.50±2.18 a	4.00±4.00 b	177.45	<0.0001
	72 h.	92.50±5.00 a	-7.22±4.59 c	-9.44±2.38 c	100.00±0.00 a	95.28±2.90 a	14.00±2.45 b	261.51	<0.0001
	96 h.	100.0±0.0 a	17.78±15.98 b	27.50±12.75 b	100.0±0.0 a	95.00±3.06 a	18.00±2.00 b	25.13	<0.0001
Mean	86.89±5.24 a	2.46±5.90 b	3.33±4.02 b	95.19±2.52 a	91.33±2.32 a	9.50±2.15 b	141.41	<0.0001	
10 %	24 h.	85.11±9.40 a	4.22±2.59 b	10.67±6.09 b	74.00±16.61a	81.78±3.63 a	2.00±2.00 b	23.89	<0.0001
	48 h.	100.0±0.0 a	22.50±11.12 bc	38.00±15.30 b	82.00±18.00 a	96.00±4.00 a	4.00±4.00 c	14.05	<0.0001
	72 h.	100.0±0.0 a	24.72±15.15 b	42.00±15.94 b	88.00±12.00 a	100.0±0.0 a	14.00±2.45 b	14.50	<0.0001
	96 h.	100.0±0.0 a	25.00±14.25 b	31.94±17.57 b	95.56±4.44 a	100.0±0.0 a	18.00±2.00 b	18.42	<0.0001
Mean	96.28±2.35 a	19.11±10.46 b	28.29±13.63 b	84.56±12.99 a	94.44±1.81 a	9.50±2.15 b	20.62	<0.0001	
15 %	24 h.	100.0±0.0 a	6.22±2.55 c	70.00±16.73 b	100.0±0.0 a	100.0±0.0 a	2.00±2.00 c	45.86	<0.0001
	48 h.	100.0±0.0 a	26.50±11.17 c	72.00±16.55 b	100.0±0.0 a	100.0±0.0 a	4.00±4.00 c	25.75	<0.0001
	72 h.	100.0±0.0 a	31.94±17.02 b	72.78±16.30 b	100.0±0.0 a	100.0±0.0 a	14.00±2.45 c	15.58	<0.0001
	96 h.	100.0±0.0 a	36.94±14.38 b	74.72±16.56 a	100.0±0.0 a	100.0±0.0 a	18.00±2.00 b	16.08	<0.0001
Mean	100.0±0.0 a	25.40±10.72 c	72.38±16.48 b	100.0±0.0 a	100.0±0.0 a	9.50±2.15 c	25.54	<0.0001	

^a Abbott (1925). Means ± standard error followed by the same letter per row do not differ by the F test (P = 0.05).

Table 2. Efficiency (mean±standard error) for *Acromyrmex subterraneus molestans* (Hymenoptera: Formicidae) workers after 24, 48, 72 and 96 h of contact with 1, 5, 10 and 15% essential oils of andiroba, cinnamon, clove, mustard and sweet orange.

Conc.	Time	Efficiency (%) ^a					Control	F	P value
		<i>B. juncea</i> (Mustard)	<i>C. guianensis</i> (Andiroba)	<i>C. sinensis</i> (Sweet orange)	<i>C. zeylanicum</i> (Cinnamon)	<i>S. aromaticum</i> (Clove)			
1 %	24 h.	0.0±0.0 b	4.0±2.45 b	0.0±0.0 b	0.0±0.0 b	14.0±7.48 a	0.0±0.0 b	3.06	0.028
	48 h.	0.0±0.0 c	10.0±7.75 bc	4.0±2.45 c	24.0±2.45 b	48.0±13.19 a	0.0±0.0 c	8.60	<0.0001
	72 h.	-2.22±2.22 c	10.89±8.61 bc	2.0±2.0 bc	24.22±3.81 b	55.56±15.84 a	2.0±2.0 bc	8.39	0.0001
	96 h.	2.22±2.22 b	10.89±8.61 b	2.0±2.0 b	24.22±3.81 b	59.78±17.51 a	2.0±2.0 b	7.61	0.0002
Mean	0.0±0.0 b	8.94±6.73 b	2.00±1.46 b	18.11±12.97 b	44.33±2.33 a	1.00±1.0 b	7.87	0.0002	
5 %	24 h.	100.0±0.0 a	2.0±2.0 c	4.0±2.45 c	54.0±7.48 b	90.0±6.32 a	0.0±0.0 c	120.22	<0.0001
	48 h.	100.0±0.0 a	2.0±2.0 c	4.0±2.45 c	60.0±9.49 b	98.0±2.0 a	0.0±0.0 c	133.94	<0.0001
	72 h.	100.0±0.0 a	-0.22±3.34 b	5.78±4.22 b	100.0±0.0 a	100.0±0.0 a	2.0±2.0 b	519.19	<0.0001
	96 h.	100.0±0.0 a	-0.22±3.34 b	5.78±4.22 b	100.0±0.0 a	100.0±0.0 a	2.0±2.0 b	519.19	<0.0001
Mean	100.0±0.0 a	0.89±2.52 c	4.89±2.84 c	78.50±4.23 b	97.0 ±2.00 a	1.00±1.0 c	396.37	<0.0001	
10 %	24 h.	100.0±0.0 a	0.0±0.0 d	24.0±7.48 c	66.0±17.20 b	90.0±4.47 a	0.0±0.0 d	32.15	<0.0001
	48 h.	100.0±0.0 a	2.0±2.0 c	32.0±6.63 b	82.0±15.62 a	100.0±0.0 a	0.0±0.0 c	45.65	<0.0001
	72 h.	100.0±0.0 a	0.0±0.0 c	50.89±12.1 b	95.55±4.44 a	100.0±0.0 a	2.0±2.0 c	81.05	<0.0001
	96 h.	100.0±0.0 a	4.00±4.0 c	55.11±10.25 b	100.0±0.0 a	100.0±0.0 a	2.0±2.0 c	108.70	<0.0001
Mean	100.0±0.0 a	1.50±1.00 c	40.50±7.30 b	97.50±1.11 a	85.89±9.21 a	1.00±1.0 c	91.34	<0.0001	
15 %	24 h.	100.0±0.0 a	6.0±6.0 c	40.0±10.49 b	98.0±2.0 a	96.0±4.0 a	0.0±0.0 c	80.89	<0.0001
	48 h.	100.0±0.0 a	18.0±8.60 c	68.0±15.94 b	100.0±0.0 a	100.0±0.0 a	0.0±0.0 c	37.01	<0.0001
	72 h.	100.0±0.0 a	31.78±14.79 c	68.44±17.42 b	100.0±0.0 a	100.0±0.0 a	2.0±2.0 d	19.92	<0.0001
	96 h.	100.0±0.0 a	39.78±15.35 c	68.44±17.42 b	100.0±0.0 a	100.0±0.0 a	2.0±2.0 d	18.17	<0.0001
Mean	100.0±0.0 a	23.89±9.85 c	61.22±15.19 b	99.50±0.50 a	99.00±1.0 a	1.00±1.0 d	34.05	<0.0001	

^a Abbott (1925). Means ± standard error followed by the same letter per row do not differ by the F test (P = 0.05).

Table 3. Efficiency (mean±standard error) for *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) workers after 24, 48, 72 and 96 h of feeding on leaves with 1, 5, 10 and 15% essential oils of andiroba, cinnamon, clove, mustard and sweet orange.

Conc.	Time	Efficiency (%) ^a					Control	F	P value
		<i>B. juncea</i> (Mustard)	<i>C. guianensis</i> (Andiroba)	<i>C. sinensis</i> (Sweet orange)	<i>C. zeylanicum</i> (Cinnamon)	<i>S. aromaticum</i> (Clove)			
1 %	24 h.	-5.22±5.97	3.28±7.83	-4.72±2.90	-2.22±2.22	4.28±4.41	6.00±4.00	0.98	0.45
	48 h.	12.78±10.42	20.78±18.41	12.78±10.42	10.78±5.72	46.72±10.28	10.00±4.47	1.68	0.18
	72 h.	33.28±12.68	37.72±16.07	20.28±10.06	29.22±6.99	54.72±11.95	10.00±4.47	1.92	0.13
	96 h.	44.72±8.74 ab	55.17±12.40 a	25.00±8.06 bc	40.44±6.43 ab	64.17±8.23 a	10.00±4.47 c	5.55	0.002
Mean	21.39±6.96 ab	29.24±13.15ab	13.33±6.81 b	42.47±8.29 a	19.56±4.74 ab	9.00±4.00 b	2.31	0.076	
5 %	24 h.	94.00±4.00 a	4.72±2.90 b	6.22±4.06 b	12.22±5.82 b	17.72±6.16 b	6.00±4.00 b	56.77	<0.0001
	48 h.	98.00±2.00 a	31.39±7.09 c	22.72±5.35 cd	61.56±11.56 b	43.17±7.42 bc	10.00±4.47 d	20.56	<0.0001
	72 h.	98.00±2.00 a	53.83±7.37 cd	41.17±9.07 d	76.78±8.52 b	65.11±4.18 bc	10.00±4.47 e	22.11	<0.0001
	96 h.	98.00±2.00 a	71.78±9.59 ab	66.61±15.90 b	85.50±6.73 ab	87.50±5.81 ab	10.00±4.47 c	13.25	<0.0001
Mean	97.00±2.42 a	40.43±6.66cd	34.18±7.94 d	53.37±4.50 bc	59.01±7.49 b	9.00±4.00 e	25.16	<0.0001	
10 %	24 h.	100.0±0.0 a	4.00±2.45 b	37.50±18.60 b	16.00±8.12 b	27.94±16.04 b	6.00±4.00 b	11.08	<0.0001
	48 h.	100.0±0.0 a	22.72±11.06 c	58.00±15.30 b	64.00±6.87 b	54.00±6.00 b	10.00±4.47 c	13.37	<0.0001
	72 h.	100.0±0.0 a	50.89±8.30 c	80.00±9.49 b	78.00±3.74 b	68.00±3.74 bc	10.00±4.47 d	28.14	<0.0001
	96 h.	100.0±0.0 a	77.78±5.08 b	84.50±7.68 b	91.50±3.84 ab	84.00±4.85 b	10.00±4.47 c	44.45	<0.0001
Mean	100.0±0.0 a	38.85±5.23 c	62.75±12.50 b	56.15±6.40 bc	60.46±3.67 b	9.00±4.00 d	21.21	<0.0001	
15 %	24 h.	100.0±0.0 a	1.78±3.92 d	71.00±16.00 b	37.39±9.73 c	25.39±5.52 cd	6.00±4.00 d	21.53	<0.0001
	48 h.	100.0±0.0 a	32.89±7.17 d	82.50±10.90 ab	53.89±5.39 c	65.28±8.14 bc	10.00±4.47 e	22.55	<0.0001
	72 h.	100.0±0.0 a	62.06±5.48 c	91.50±5.89 a	71.33±1.62 bc	76.28±5.44 b	10.00±4.47 d	51.84	<0.0001
	96 h.	100.0±0.0 a	82.78±3.56 c	96.00±4.00 ab	82.78±3.56 c	89.00±3.22 bc	10.00±4.47 d	93.59	<0.0001
Mean	100.0±0.0 a	44.87±3.24 d	85.25±8.64 b	63.99±4.65 c	61.35±3.72 c	9.00±4.00 e	44.61	<0.0001	

^a Abbott (1925). Means ± standard error followed by the same letter per row do not differ by the F test (P = 0.05).

Table 4. Efficiency (mean±standard error) for *Acromyrmex subterraneus molestans* (Hymenoptera: Formicidae) workers after 24, 48, 72 and 96 h of feeding on leaves with 1, 5, 10 and 15% essential oils of andiroba, cinnamon, clove, mustard and sweet orange.

Conc.	Time	Efficiency (%) ^a					Control	F	P value
		<i>B. juncea</i> (Mustard)	<i>C. guianensis</i> (Andiroba)	<i>C. sinensis</i> (Sweet orange)	<i>C. zeylanicum</i> (Cinnamon)	<i>S. aromaticum</i> (Clove)			
1 %	24 h.	85.0±16.0 a	12.0±4.90 b	4.0±2.45 b	4.0±2.45 b	8.0±5.83 b	0.0±0.0 b	19.16	<0.0001
	48 h.	88.00±12.0 a	35.78±5.76 c	61.66±6.40 b	51.67±8.50 bc	30.72±8.72 cd	10.0±3.16 d	11.70	<0.0001
	72 h.	97.50±2.50 a	47.50±6.37 c	79.16±7.35 ab	72.22±9.75 b	69.17±10.87 b	16.0±2.45 d	15.08	<0.0001
	96 h.	100.0±0.0 a	58.57±7.28 c	92.50±7.50 ab	77.14±9.69 bc	78.21±9.18 bc	28.0±2.0 d	83.39	<0.0001
Mean	92.73±7.62 a	38.46±4.57 c	59.31±4.74 b	46.25±4.99 bc	51.52±6.72 bc	13.50±1.27 d	23.29	<0.0001	
5 %	24 h.	100.0±0.0 a	12.0±4.90 bc	8.0±3.74 c	10.0±4.47 bc	22.0±6.33 b	0.0±0.0 c	81.66	<0.0001
	48 h.	100.0±0.0 a	33.50±7.13 cd	54.28±11.82 bc	59.28±11.59 bc	68.72±10.85 b	10.0±3.16 d	12.51	<0.0001
	72 h.	100.0±0.0 a	44.44±10.82 b	80.28±10.21 a	73.61±12.30 a	76.39±10.79 a	16.0±2.45 c	10.87	<0.0001
	96 h.	100.0±0.0 a	57.5±10.36 b	100.0±0.0 a	100.0±0.0 a	97.50±2.50 a	28.0±2.0 c	499.34	<0.0001
Mean	100.0±0.0 a	36.86±6.41 c	60.63±5.13 b	66.15±7.15 b	60.72±5.76 b	13.50±1.27 d	33.30	<0.0001	
10 %	24 h.	100.0±0.0 a	16.8.12 b	14.0±4.0 b	16.0±4.0 b	12.0±3.74 bc	0.0±0.0 c	71.69	<0.0001
	48 h.	100.0±0.0 a	46.67±6.24 b	64.78±5.97 b	43.56±14.52 b	52.61±11.91 b	10.0±3.16 c	11.88	<0.0001
	72 h.	100.0±0.0 a	62.50±10.6 c	90.83±4.20 ab	85.28±6.16 bc	77.78±7.38 bc	16.0±2.45 d	30.23	<0.0001
	96 h.	100.0±0.0 a	91.43±5.71 b	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	28.0±2.0 c	1296.0	<0.0001
Mean	100.0±0.0 a	53.37±5.80 c	67.40±2.65 b	60.39±3.99 bc	60.60±3.75 bc	13.50±1.27 d	64.03	<0.0001	
15 %	24 h.	100.0±0.0 a	6.0±4.0 b	30.0±11.40 b	30.0±15.49 b	28.0±11.57 b	0.0±0.0 b	14.65	<0.0001
	48 h.	100.0±0.0 a	37.50±9.84 cd	72.50±12.61 ab	55.39±15.33 bc	82.56±7.74 ab	10.0±3.16 d	11.32	<0.0001
	72 h.	100.0±0.0 a	65.0±9.35 b	100.0±0.0 a	80.56±11.54 ab	90.56±5.80 a	16.0±0.0 c	23.52	<0.0001
	96 h.	100.0±0.0 a	94.29±3.50 b	100.0±0.0 a	100.0±0.0 a	100.0±0.0 a	28.0±2.0 c	1296.0	<0.0001
Mean	100.0±0.0 a	50.70±4.60 c	75.62±5.31 b	66.49±7.38 bc	75.28±5.89 b	13.50±1.27 d	36.37	<0.0001	

^aAbbott (1925). Means ± standard error followed by the same letter per row do not differ by the F test (P = 0.05).

phenylpropanoids such as eugenol that is a phenolic compound with diverse biological activity (Huang *et al.*, 2002). According to data presented, the essential oils of mustard, cinnamon and clove can be used in integrated pest management by reducing a population of *A. sexdens rubropilosa* and *A. subterraneus molestans* to replace conventional synthetic insecticides.

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