

## Bioactivity of *Eucalyptus camaldulensis* essential oil against *Microcerotermes diversus* (Isoptera: Termitidae)

Elahe Shafiei Alavijeh<sup>1</sup>, Behzad Habibpour<sup>1\*</sup>, Saeid Moharramipour<sup>2</sup> and Arash Rasekh<sup>1</sup>

1. Department of Plant Protection, College of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

2. Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

**Abstract:** *Microcerotermes diversus* Silvestri (Isoptera: Termitidae) is the most economically destructive wood pest in structures in Khuzestan province (Iran). Chemicals such as essential oils and plant extracts that are compatible with the environment and have high potential to be used in integrated pest management programs are extremely important resources. This study evaluated contact and digestive toxicity of *Eucalyptus camaldulensis* Dehneh. (Myrtaceae) essential oil in no-choice and choice bioassays and feeding inhibition trials on *M. diversus*. Concentrations of the essential oil ranged from 0.3 to 1.6%. The results of the choice tests and feeding inhibition trial showed that the essential oil could act as a repellent at 0.7% concentration. Concentrations used in these tests resulted in mortality of termites, and a direct relationship between concentration and mortality was observed. The essential oil also increased the mortality of termites at concentrations higher than 0.7%. Termite feeding decreased with increase in concentration. Due to the ability of termites to choose the untreated filter-paper in the choice trial, values of LT and LC, were higher than in no-choice trials. The highest effects of Eucalyptus essential oil ( $\approx 100\%$  mortality) was obtained by the concentration 1.6%. Overall, this study reveals that Eucalyptus essential oil may be suggested as an effective toxicant with suitable contact and digestive toxicity on *M. diversus*.

**Keywords:** Eucalyptus essential oil; Contact and digestive toxicity; *Microcerotermes diversus*.

### Introduction

Termites can have devastating effects on agriculture, urban landscaping such as trees, and human habitations (Cowie *et al.*, 1989). Unfortunately, the wood used in buildings is the appetizer for termites and stimulates them to be attracted to residential and commercial buildings (Pearce, 1997). The most important species of termites in Khuzestan province (Iran) is *Microcerotermes diversus* Silvestri

(Isoptera: Termitidae) (Habibpour, 2006). This termite has a wide range of preferred foods and is able to create secondary community in walls, ceilings, and in trees. Therefore, its eradication and/or control is associated with many difficulties. For its control, the conventional chlorinated termiticides are used but have negative environmental impacts and threaten human health and wildlife. Application of these chemicals is currently limited, and some attempts have been made to introduce natural pesticides (Verma *et al.*, 2009). Many natural pesticides have anti-termite activities. Among these, essential oils may be considered as a

Handling Editor: Dr. Masumeh Ziaee

\* **Corresponding author**, e-mail: habibpour\_b@scu.ac.ir  
Received: 18 April 2013, Accepted: 16 September 2013

potential alternative in controlling termites. *Eucalyptus* spp. are likely to have promising essential oils with repellent and pesticide effects (Nerio *et al.*, 2010). Through an environmental and toxicological viewpoint, the use of *Eucalyptus* essential oil as a natural pesticide has a better effect than the irregular use of other pesticides, and thereby the problem of pest resistance can be overcome (Batish *et al.*, 2008). Among different components of *Eucalyptus* essential oil, the 1,8-cineol is the most important and plays an important role in its insecticidal activity (Duke, 2004). The review of termiticide mechanism of the leaf oil of *E. camaldulensis* against the *Coptotermes formosanus* Shiraki termite by Siramon *et al.*, (2009) reveals that this leaf oil not only causes mortality in the population of termites, but also inhibits acetylcholinesterase activity and affects the nervous system. The aims of this study were to investigate the effects of eucalyptus essential oil (*Eucalyptus camaldulensis* var. *camaldulensis* Dehneh.) in choice and no-choice trials, and to determine the contact and digestive toxicity against subterranean termite, *M. diversus*, as well as to achieve an effective control in the form of compounds compatible with environment.

## Materials and Methods

### Collection of termites

Among the most important and frequently used commercial wood in the Ahvaz region, *M. diversus* feeds most readily on beech (*Fagus orientalis* Lipsky) wood (Ekhtelat, 2009; Habibpour, 1994), so this wood was used to collect termites. First, beech blocks of 20 × 6 × 2 cm were prepared (Ekhtelat, 2009), then they were put in infested soils in Ahvaz (Khuzestan, Iran). After sampling, the termites were isolated by using a brush and were transferred into plastic containers containing filter paper (moistened with distilled water) to nourish the termites and to provide moisture. To relieve stress on the termites before biometrics trials, the plates were kept in the dark for 24 h at a

temperature of 28 ± 2 °C and relative humidity of 85 ± 5%. Only active and healthy termite workers were used in the trials.

### Preparation of Essential Oils

*E. camaldulensis*, is commonly known as River Red Gum. The leaves of older trees were used for extracting essential oil to apply in the experiments (Nuttal *et al.*, 2006). The leaves of *E. camaldulensis* were collected in June and July of 2011, from the trees on the campus of the Faculty of Agriculture in Shahid Chamran University of Ahvaz (Khuzestan province, Iran), and placed in the shade to dry at room temperature after being washed. The essential oil was extracted from the crushed leaves by hydro-distillation method using Clevenger apparatus and then kept in glass containers covered with aluminum foil in the refrigerator at a temperature of 4 °C. After preliminary trials, the effective concentrations were determined by using a logarithmic relationship (Fisher and Yates, 1963). Concentrations applied for the trials were 0.3, 0.4, 0.5, 0.7, 0.9, 1.2 and 1.6%. Methanol was used as the solvent in these trials.

### No-Choice Trial

The aim of this experiment was to investigate the effect of the oil on starved termites in non-choice conditions. For this experiment, concentrations of eucalyptus oil (0.3, 0.4, 0.5, 0.7, 0.9, 1.2 and 1.6%) were injected onto respective Whatman filter papers (9 cm diameter), which were then transferred into the outdoors for half an hour to evaporate the solvent. Methanol was used as a solvent control. After drying and weighing each filter paper, they were put in petri dishes, then 0.7 ml of distilled water was added to the filter paper and 50 termite workers were put in each Petri dish (9 cm diameter and 1 cm height). Then the trial units were placed in the dark at 28 ± 2 °C and relative humidity of 85 ± 5%. There were four replicates for each treatment. The cover of Petri dishes were opened daily to detect the dead insects by brush touching. Mortality was recorded for 14

days. After this time, the filter papers were dried and weighed.

### Choice Trial

The aim of this trial was to determine the tendency of termites to move away from essential oil-impregnated paper and to determine the contact toxicity and repellency effects. In this method, Whatman filter papers (No. 1) were cut in two semi circles such that when they were placed in the Petri dishes (9 cm in diameter), 1.5 cm space was created between them. Half of the papers were treated with 0.5 ml of the selected concentrations (0.3, 0.4, 0.5, 0.7, 0.9, 1.2 and 1.6%) of the oil; the other half of the papers were treated with methanol and then placed in the outdoors for half an hour to be dried. After removing from the oven, the filter papers were weighed and both sides of the papers were put in the Petri dishes. Then both sides were wetted with 0.4 ml distilled water. Then, 50 healthy worker termites were left between the two halves of the papers with 1.5 cm space between them and four replications were used in each treatment. After transferring the Petri dishes to the incubator ( $28 \pm 2$  °C,  $85 \pm 5\%$  RH), termite mortality was recorded for 14 days and the dead bodies were removed from the trial dishes every day. After 14 days, the filter papers were weighed after drying. It should be mentioned that the same petri dishes were used for different time intervals. The percentage repellency (PR) values were computed using the formula of Liu *et al.*, (2006):  $R = (C - E) / T \times 100$

### Feeding Inhibition Trial

To perform this trial, 4 g of sand with 2 g of vermiculite (2: 1 ratio) were weighed and placed inside each Petri dish. Whatman filter papers were cut to the dimensions of  $2 \times 2$  cm and treated with 0.1 ml of the respective concentrations (0.3, 0.4, 0.5, 0.7, 0.9, 1.2, and 1.6%) of the oil. After drying and weighing, the papers were wetted with 0.1 ml distilled water and placed in the Petri dishes. The vermiculite was added to maintain water in the sand. The control

consisted of filter paper treated with methanol. To preserve humidity in the Petri dishes, 2 ml of distilled water was added to the bed soil, and then 50 worker termites were placed in each trial dish. Four replications were used for each treatment. The Petri dishes were transferred to a dark incubator ( $28 \pm 2$  °C,  $85 \pm 5\%$  RH) and the mortality was recorded daily for 14 days. After 14 days, the filter papers were weighed after drying.

### Statistical analysis

The percentage of termite mortality was determined and then corrected using Abbott's formula (Abbott, 1925). The lethal concentration ( $LC_{90}$  and  $LC_{50}$ ) and mortality time ( $LT_{50}$  and  $LT_{90}$ ) were determined by probit analysis using the SAS software (9.1). Analysis of variance (one-way ANOVA) was used to compare the effect of the concentrations on termites, and LSD test at 5% level were also used to compare the means (SAS Institute, 2003).

### Results

#### No-Choice Trial

Average percent worker mortality in no-choice trial was statistically significant. Termite mortality increased ( $F = 37.32$ ,  $df = 7$ ,  $P < 0.01$ ) at EO concentration of  $\geq 0.9\%$  (1.2% and 1.6%). At these concentrations, significant difference was observed (Table 1). The termite feeding trial showed significant differences between the concentrations, and feeding decreased with increasing concentration ( $F = 22.87$ ,  $df = 7$ ,  $P < 0.01$ ) (Table 1). The average percent of feeding reduction, compared to the control, increased with a concentration increase, but no significant difference was observed among them at the three higher concentrations of eucalyptus essential oil ( $F = 27.82$ ,  $df = 6$ ,  $P < 0.01$ ) (Table 1). The  $LT_{50}$  and  $LT_{90}$  values were calculated at different concentrations by using probit analysis. The results showed that  $LT_{50}$  and  $LT_{90}$  values decreased due to the

immediate effect of the essential oil at high concentrations. Due to the rapid toxic effect of the oil at 1.6%, LT values were not calculated (Table 4). LC<sub>50</sub> and LC<sub>90</sub> values after 14 days were also calculated (Table 3).

### Choice Trial

Comparing the mean percentage mortality in the choice trial showed that 1.6% concentration had significant difference with other concentrations. In this experiment, termite mortality increased with increasing oil concentration, and significant differences were observed among different concentrations of essential oil at 5% level using LSD ( $F = 27.22$ ,  $df = 7$ ,  $P < 0.01$ ) (Table 2). Mortality trends in the choice trial showed that the termites avoided the treated filter paper, and mortality at concentrations of 0.3% to 0.9% increased gradually from 6 to 34%. But at 1.2%, the maximum mortality was 76%, and at concentration of 1.6%, fumigation effect was observed and caused 100% mortality after 4 days of exposure. Changes in the mean values of feeding in the untreated filter paper treatment were significant, and a significant difference was also obtained among the concentrations. With increasing concentrations up to 0.7%, increase in feeding rate was observed, while for the other three concentrations, due to mortality, feeding rate decreased (Table 2). The decreased feeding related to the treated paper in the choice trial, indicates a reduction in feeding with an increase in the concentration of the essential oil. However, there were no significant differences among the concentrations, and average comparisons of the untreated papers were not significant either ( $F = 4.38$ ,  $df = 6$ ,  $P = 0.5944$ ) (Table 2). Comparisons of total feeding were significant, suggesting that feeding decreased with increasing concentrations of *Eucalyptus* essential oil ( $F = 5.18$ ,  $df = 7$ ,  $P < 0.01$ ) (Table 2). In the choice trial, due to the possibility of selection by the termites (the existence of the non-treated filter paper), the LC<sub>50</sub> and LC<sub>90</sub> values (Table 3) were higher

than the no-choice trial. Also due to termite mortality after 4 days at a concentration of 1.6%, the LT<sub>50</sub> and LT<sub>90</sub> values were calculated (Table 4). In this experiment, owing to the low percentage mortality in the ranges of 0.3% to 0.7%, the LT values could not be calculated. LT<sub>50</sub> changes at various concentrations of the essential oil in the choice trial showed a downward trend. This reflected the lower time required for 50% termite mortality with increasing eucalyptus concentration.

Comparing the percentage repellency in the choice trial showed significant differences among the concentrations ( $F = 11.37$ ,  $df = 6$ ,  $P < 0.01$ ) (Table 6). In 1.6% concentration all of termites died therefore the related data were omitted. The highest repellency was also obtained in 0.7% concentration.

### Feeding Inhibition Trial

The mortality of the termites was statistically significant. But due to low mortality and close similarity of the averages in this trial, no significant differences among different concentrations of essential oil were obtained ( $F = 5.36$ ,  $df = 7$ ,  $P = 0.0009$ ) (Table 6). The mortality trend indicated that termites avoided the filter papers treated with essential oils. In the study of the termites fed with filter paper impregnated with different concentrations of eucalyptus essential oil, the comparison of feeding percentage ( $F = 1.88$ ,  $df = 7$ ,  $P = 0.1175$ ) as well as the reduction of feeding as compared with control ( $F = 1.87$ ,  $df = 6$ ,  $P = 0.1339$ ) were not statistically different (Table 6). But in the comparison of the mean concentrations and the study of feeding by LSD trial at the 5% level on the three feeding levels, a meaningful difference was observed among the concentrations. In this experiment, due to the low average percentage of mortality (< 30%), and high calculated LT values (more than 14 days), and meaningless probit analysis for LC, the LC and LT values are not reported.

**Table 1** Comparison of the mean mortality and mean of feeding (%) by *M. diversus* at different concentrations of eucalyptus essential oil in no-choice trial.

Concentration (%)	Mortality $\pm$ SE (%)	Feeding $\pm$ SE (%)	Decreased feeding compared to control $\pm$ SE (%)
0.3	13.5 $\pm$ 0.0001 <sup>c</sup>	3.14 $\pm$ 0.15 <sup>a</sup>	3.066 $\pm$ 4.31 <sup>d</sup>
0.4	24.5 $\pm$ 0.0003 <sup>c</sup>	2.47 $\pm$ 0.21 <sup>b</sup>	26.06 $\pm$ 5.16 <sup>c</sup>
0.5	59.5 $\pm$ 0.0003 <sup>c</sup>	1.98 $\pm$ 0.16 <sup>c</sup>	39.52 $\pm$ 4.78 <sup>b</sup>
0.7	94 $\pm$ 0.0071 <sup>b</sup>	1.93 $\pm$ 0.021 <sup>cd</sup>	40.37 $\pm$ 1.37 <sup>b</sup>
0.9	99 $\pm$ 0.0023 <sup>b</sup>	1.48 $\pm$ 0.08 <sup>d</sup>	56.89 $\pm$ 2.43 <sup>a</sup>
1.2	100 $\pm$ 0.012 <sup>b</sup>	1.40 $\pm$ 0.022 <sup>de</sup>	54.34 $\pm$ 1.07 <sup>a</sup>
1.6	100 $\pm$ 0 <sup>a</sup>	0.68 $\pm$ 0.16 <sup>de</sup>	54.003 $\pm$ 4.1 <sup>a</sup>

\*Means followed by the same letter in a column are not significantly different at 5% probability level (LSD).

**Table 2** Comparison of the mean mortality and mean of feeding (%) by *M. diversus* in different concentrations of eucalyptus essential oil in choice trial.

Concentration (%)	Mortality $\pm$ SE (%)	Feeding of untreated part $\pm$ SE (%)	Feeding of treated part $\pm$ SE (%)	Total feeding $\pm$ SE (%)
0.3	12.5 $\pm$ 0.0791 <sup>b</sup>	4.535 $\pm$ 0.643 <sup>a</sup>	2.033 $\pm$ 0.183 <sup>a</sup>	3.262 $\pm$ 0.355 <sup>a</sup>
0.4	6 $\pm$ 0.0771 <sup>b</sup>	4.648 $\pm$ 0.851 <sup>b</sup>	2.261 $\pm$ 0.097 <sup>a</sup>	3.563 $\pm$ 0.346 <sup>a</sup>
0.5	16 $\pm$ 0.112 <sup>b</sup>	4.332 $\pm$ 0.208 <sup>c</sup>	1.999 $\pm$ 0.250 <sup>a</sup>	3.181 $\pm$ 0.202 <sup>a</sup>
0.7	16 $\pm$ 0.050 <sup>b</sup>	5.241 $\pm$ 0.394 <sup>cd</sup>	1.528 $\pm$ 0.164 <sup>a</sup>	3.354 $\pm$ 0.243 <sup>a</sup>
0.9	34 $\pm$ 0.0318 <sup>b</sup>	2.379 $\pm$ 0.481 <sup>c</sup>	1.254 $\pm$ 0.289 <sup>a</sup>	1.82 $\pm$ 0.267 <sup>b</sup>
1.2	76 $\pm$ 0.549 <sup>b</sup>	1.236 $\pm$ 0.035 <sup>de</sup>	1.196 $\pm$ 0.357 <sup>a</sup>	1.216 $\pm$ 0.186 <sup>bc</sup>
1.6	100 $\pm$ 3.071 <sup>a</sup>	0.404 $\pm$ 0.089 <sup>de</sup>	0.978 $\pm$ 0.209 <sup>a</sup>	0.690 $\pm$ 0.0789 <sup>c</sup>

\*Means followed by the same letter in a column are not significantly at 5% probability level (LSD).

**Table 3** Values of LC<sub>50</sub> and LC<sub>90</sub> (%) in all of eucalyptus essential oil concentrations after 14 days.

Experiment	n	Slope $\pm$ SE	$\chi^2$	df	p-value	LC <sub>50</sub> (95% confidence limits)	LC <sub>90</sub> (95% confidence limits)
No-choice trial	50	4.32 $\pm$ 0.57	25.075	7	0.403	0.681 (0.617-0.801)	1.029 (0.951-1.101)
Choice trial	50	5.173 $\pm$ 1.044	13.409	7	0.367	1.029 (0.951-1.101)	1.343 (1.247-1.52)

**Table 4** Values of LT<sub>50</sub> and LT<sub>90</sub> in different treatments of eucalyptus essential oil.

Experiment	Concentration (%)	n	Slope ± SE	χ <sup>2</sup>	df	p-value	LT <sub>50</sub> (days) (95% confidence limits)	LT <sub>90</sub> (days) (95% confidence limits)
No-choice trial	0.5	50	1.75 ± 0.09	6.02	7	0.124	10.18 (9.47-11.04)	54.92 (44.54-71.12)
	0.7	50	2.36 ± 0.15	7.02	7	0.127	0.39 (0.32-0.45)	0.94 (0.88-1.002)
	0.9	50	13.90 ± 0.30	7.90	7	0.103	0.37 (0.07-0.57)	0.84 (0.52-1.003)
	1.2	50	3.64 ± 1.11	8.14	7	0.146	0.27 (0.20-0.33)	0.60 (0.55-0.68)
Choice trial	0.9	50	0.755 ± 0.087	10.47	7	0.395	10.18(9.47-11.04)	54.92 (44.54-71.12)
	1.2	50	1.445 ± 0.176	5.77	7	0.215	3.20 (2.34-3.98)	24.66 (17.43-43.39)
	1.6	50	3.593 ± 0.280	6.40	7	0.262	0.94 (0.82-1.041)	2.13 (1.96-2.35)

**Table 5** Percent repellency (mean% ± SE) of the essential oils from eucalyptus essential oil on *M. diversus* using choice trial.

Concentration (%)	Repellency ± SE (%)
0	8.5 ± 3.095 <sup>c</sup>
0.3	12.5 ± 3.201 <sup>dc</sup>
0.4	28 ± 6.164 <sup>d</sup>
0.5	50 ± 5.715 <sup>ab</sup>
0.7	57 ± 4.654 <sup>a</sup>
0.9	40 ± 4.242 <sup>bc</sup>
1.2	17 ± 9.255 <sup>de</sup>

**The effect of eucalyptus essential oil on behavior of *M. diversus***

The remarkable point about termite behavioral reaction after exposure to essential oil used in different concentrations, in the first day, was that the termites became lethargic.

The fumigation effect of the oil at high concentrations caused 100% mortality in all of the experiments, including the no-choice and choice

tests, in the first 24 hours. At lower concentrations of the oil, the effect of fumigation decreased, and termite mortality was the result of contact and ingestion. Morphological changes in the termites appeared over time, showing a shrinking and compression of the abdomen without any change in color (Fig. 1). Another contact effect was the appearance of burnt antennae and legs (Fig. 2). These changes resulted from a drying of these parts and separation of them from the termites' body, and thus increased their mortality.

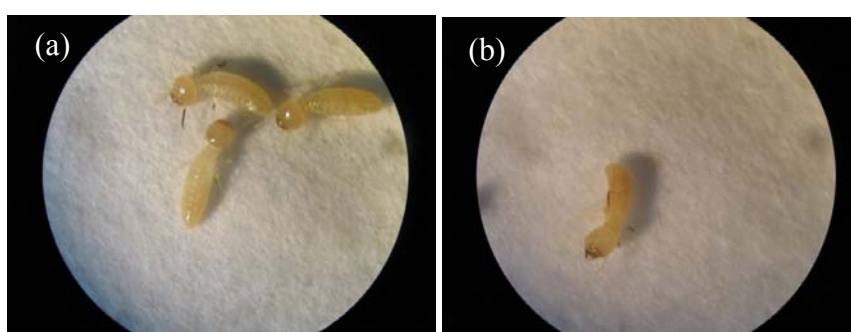
**Discussion**

A comparison of the mortality of worker termites showed that mortality increased with increasing concentration of eucalyptus oil. According to Siramon *et al.*,’s results (2009), high feeding levels in a toxicity trial could be attributed to effect of eucalyptus oil on the nervous system of termites. While the results of non-choice experiment (at the same conditions but for 14 days) showed that in lower concentrations, the feeding of termites was increased because they survived longer than the termites that were exposed to higher concentrations.

**Table 6** Comparison of the mean mortality and mean of feeding (%) by *M. diversus* at different concentrations of eucalyptus essential oil in feeding inhibition trial.

Concentration (%)	Mortality $\pm$ SE (%)	Feeding $\pm$ SE (%)	Decreased feeding compared to control $\pm$ SE (%)
0.3	19.0 $\pm$ 0.0618 <sup>a</sup>	21.770 $\pm$ 2.945 <sup>a</sup>	12.860 $\pm$ 12.436 <sup>ab</sup>
0.4	20.0 $\pm$ 0.1773 <sup>a</sup>	20.52 $\pm$ 2.024 <sup>a</sup>	21.259 $\pm$ 7.726 <sup>ab</sup>
0.5	20.5 $\pm$ 0.0449 <sup>a</sup>	20.38 $\pm$ 1.986 <sup>a</sup>	21.299 $\pm$ 8.922 <sup>ab</sup>
0.7	19.5 $\pm$ 0.1283 <sup>a</sup>	18.86 $\pm$ 3.33 <sup>b</sup>	23.884 $\pm$ 12.674 <sup>ab</sup>
0.9	20.5 $\pm$ 0.1025 <sup>a</sup>	16.67 $\pm$ 0.460 <sup>b</sup>	35.433 $\pm$ 2.589 <sup>a</sup>
1.2	23.5 $\pm$ 0.1316 <sup>a</sup>	16.65 $\pm$ 0.908 <sup>b</sup>	33.070 $\pm$ 3.218 <sup>a</sup>
1.6	19.5 $\pm$ 0.0736 <sup>a</sup>	16.30 $\pm$ 1.975 <sup>b</sup>	37.007 $\pm$ 6.653 <sup>a</sup>

\*Means followed by the same letter in a column are not significantly at 5% probability level (LSD).

**Figure 1** A photograph of dead termites exposed to eucalyptus essential oil.**Figure 2** Morphological changes appearing in termites upon exposure to eucalyptus essential oil. (a) burnt antennae, (b) burnt legs.

As the results show, at the concentrations of 1.2 and 1.6%, essential oil acted as a fumigant and as a contact toxin. At a concentration of 0.9%, the oil acted as a fumigant, contact and stomach toxin, and at the remaining

concentrations it was effective as a contact and digestive toxin. Average mortality during 14 days, despite being statistically significant, was very low in all of the applied concentrations. Lack of significant differences in feeding

inhibition trial and the aggregation of termites at a corner away from the filter paper showed that eucalyptus essential oil was a feeding deterrent.

In the choice trials, feeding in the treated area showed no significant differences, but average feeding decreased with increasing concentration. In examining the total amount of feeding, reduced feeding was observed with increasing concentration, as was seen in the no-choice trial. In this experiment, despite deterrent effects of eucalyptus oil at 0.7% concentration and subsequent reduction of this effect at lower concentrations (that probably occurred due to oil leaking from the treated part to the untreated part, and also from the fumigation effect of the oil at high concentrations), 100% mortality was observed at the end of the trial at high concentrations. Due to the ability of termites to choose the non-treated filter paper, the LC<sub>50</sub> and LC<sub>90</sub> values, as well as the LT<sub>50</sub> and LT<sub>90</sub> values, were higher than the no-choice tests. Also, repellency effects of eucalyptus essential oil were demonstrated in olfactometry trials (Shafiei Alavije *et al.*, 2013).

Ohmura *et al.*, (2000) observed anti-feedant effects of flavonoids and related compounds against the subterranean termite, *C. formosanus*, and toxic effects against the termite *Cryptotermes Brevis* Walker in choice and non-choice trials. The results of this study were consistent with their results. In a study conducted by Blaske and Hertel (2001), in choice trials with herbal extracts, a significant repulsion was found in groups of *Reticulitermes santonensis* (Feytaud), *Reticulitermes virginicus* (Bank) and *C. formosanus*. No-choice trials documented the contact and fumigation toxicity of these extracts. Zhu *et al.*, (2003) evaluated the repellent properties of 12 vetiver grass (*Vetiveria zizanioides* (L.) Roberty) plants against termites *C. formosanus*, and the results showed that most of these compounds had repellent properties to termites. Badshah *et al.*, (2004) demonstrated the toxic effects of *Calotropis procera* (Aiton) extracts against two species of termites, *Heterotermes indicola* (Wasmann) and *C. heim* (Wasmann). In

studying the toxic effects of different concentrations of seed powder extract of Birbira (*Milletia ferrugina* (Hochst.) Baker) against various classes of mature termite *Macrotermes subhyalinus* (Rambur) and *Macrotermes herus* (Sjostedt) and *Pachnoda interrupta* (Olivier) in non-choice trials, Jembere *et al.*, (2005) reported increasing mortality of termites with increasing concentration of the extracts, and their achievements were in conformity with the results of this study. Watanabe *et al.*, (2005) documented the repellent effects of sesquiterpene compounds obtained from wood *Callitris glaucophylla* Joy Tompson as a factor against subterranean termite *C. formosanus* in two choice experiments. Verma and Verma (2006) reported toxicity of *Lantana camara* extracts against termite workers *Microcerotermes beasoni* Synder. Taylor *et al.*, (2006) demonstrated the toxic effects of plant extracts taken from different parts of *Thuja plicata* Donn ex D. Don and *Chamaecyparis nootkatensis* (D. Don), to protect wood from attack by termites (*C. formosanus*) and brown rot fungi (*Postia placenta* (Fr.) M. Larsen and Lombard). Morimoto *et al.*, (2006) demonstrated the anti-feeding effects of Pterocarpan compounds and Pterocarpol in *Pterocarpus macrocarpus* Kruz against *Spodoptera littoralis* (Boisduval), and against the subterranean termite *Reticulitermes speratus* Kolbe. Ahmed *et al.*, (2007) demonstrated repellency effects of plant extracts, *Withania somnifera* (L.) Dunal, *Croton tiglium* L. and *Hygrophila auriculata* (Schum.) Hiene Syn. against termite *Odontotermes obesus* (Rambur). Barbarinde and George (2008) proved acute toxicity and repellency of different compounds of plants such as *Tithonia diversifolia* (Hemsl.) A. Gray and *Xylopiya aethiopica* (Dunal) A. Rich. against *Nasutitermes* species. Repellency and toxic effects of extracts obtained from leaves, fruits and bark of castor plant, *Ricinus communis* L. on the termite *Macrotermes natalensis* (Haviland), were demonstrated by Barbarinde *et al.*, (2008). In a study conducted



by Siramon *et al.*, (2009), oil of Eucalyptus leaves showed good termiticidal activity against the termite *C. formosanus* in both contact and non-contact assays. On the other hand, in non-choice and choice trials conducted by Nakayama and Osbrink (2010), using wood blocks treated with Indian walnut extract, *Aleurites moluccana* (L.), it was found that the treated wooden blocks were eaten less than the control blocks, which agrees with the results we obtained here.

Scientists from different parts of the world in their studies are using plants and their by-products to control pests, including termites, because plants are eco-friendly and can play a larger role in the control of termites. Due to increasing interest in the development of plant materials as an alternative to chemical pesticides, this study examined the effect of Eucalyptus essential oil against *Microcerotermes diversus* species. Although essential oils have been identified as repellents for mosquitoes and as anti-microbial and fungicidal agents, their termiticidal activity has not been investigated in Iran. This study has revealed the contact, digestive and fumigation toxicity of eucalyptus oil against *M. diversus*. Essential oils and their major constituents, especially monoterpenoids, are the most suitable alternatives to pesticides. Consequently, by further investigation in future and by separating the dominant components of Eucalyptus essential oil, an economical and eco-friendly pesticide can be developed for controlling termites.

### Acknowledgments

We thank Drs Chris Peterson and Brad Kard (USA) for reviewing earlier drafts of this paper. This research was supported by Shahid Chamran University of Ahvaz, Iran.

### References

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.
- Ahmed, S., Riaz, M. A., Malik, A. H. and Shahid, M. 2007. Effect of seed extracts of *Withania somnifera*, *Croton tiglium* and *Hygrophila auriculata* on behavior and physiology of *Odontotermes obesus* (Isoptera, Termitidae). *Biologia*, 62 (6): 880-885.
- Babarinde, S. A. and George, O. A. 2008. Acute toxicity and repellency different mixtures of *Tithonia diversifolia* and *Xylopiya aethiopica* Nasutitermes species. *Journal of Entomological Research*, 32 (3): 229-232.
- Babarinde, S. A., Pitan, O.O.R. and Iyiola, F. A. 2008. A prescreen of termiticidal potentials of aerial part of castor *Ricinus communis* (Euophorbiaceae). *Journal of Entomology*, 5 (4): 218-223.
- Badshah, H., Farmanullah, S. Z. S. and Shakur, M. 2004. Toxic effects of ak (*Calotropis procera*) plant extracts against termites (*Heterotermes indicola* and *Coptotermes heimi*), Isoptera: Rhinotermitidae. *Pakistan Journal of Biological Sciences*, 7 (9): 1603-1606.
- Batish, D. R., Singh, H. P., Kohli, R.K. and Kaur, S. 2008. Eucalyptus essential oil as a natural pesticide. *Forest Ecology and Management*, 256: 2166-2174.
- Blaske, V. U. and Hertel, H. 2001. Repellent and toxic effects of plant extracts on subterranean termites (Isoptera: Rhinotermitidae). *Journal of Economic Entomology*, 94: 1200-1207.
- Cowie, R. H., Logan, J. W. and Wood, T. G. 1989. Termite (Isoptera) damage and control in tropical forestry with special reference to Africa and Indo-Malaysia: a review. *Bulletin of Entomological Research*, 79: 173-184.
- Duke, J. A. 2004. Dr. Duke's Phytochemical and Ethnobotanical databases. Available online at <http://www.ars-grin.gov/duke/> (accessed on 9 June, 2008).
- Ekhtelat, M. 2009. Investigation on feeding behavior and estimating foraging population of *Microcerotermes diversus* (Silvestri) (Isoptera: Rhinotermitidae). M. S. thesis, College of Agriculture, Shahid-Chamran University of Ahvaz, Iran. 120 pp.
- Fisher, R. A., and Yates, F. 1963. *Statistical tables for biological, agricultural and medical research*. 6<sup>th</sup> Ed. Oliver and Boyd, Edinburgh and London. 146 pp.

- Habibpour, B. 2006. Laboratory and field evaluation of bait-toxicants for suppression of subterranean termite populations in Ahvaz. Ph. D. thesis, College of Agriculture, Shahid-Chamran University of Ahvaz, Iran. 150 pp.
- Habibpour, B. 1994. Termites (Isoptera) fauna, economic importance and their biology in Khuzestan, (Iran). M.S. thesis, College of Agriculture, Shahid-Chamran University of Ahvaz, Iran. 120 pp.
- Jembere, B., Getahun, D., Negash, M. and Seyoum, E. 2005. Toxicity of birbira (*Milletia ferruginea*) seed crude extracts to some insect pests as compared to other botanical and synthetic insecticides. 11<sup>th</sup> NAPRECA (Natural Products and Drug Discovery) Symposium Book of Proceeding, Asntanarivo, Madagascar, pp. 88-96.
- Liu, C. H., Mishra, A. K., Tan, R. X., Tang, C., Yang, H. and Shen, Y. F. 2006. Repellent and insecticidal activities of essential oils from *Artemisia princeps* and *Cinnamomum camphora* and their effect on seed germination of wheat and broad bean. *Bioresource Technology*, 97: 1969-1973.
- Morimoto, M., Fukumto, H., Hiratani, M., Chavasiri, W. and Komai, K. 2006. Insect antifeedants, pterocarpan and pterocarpol, in heartwood of *Pterocarpus macrocarpus* Kruz. *Bioscience Biotechnology and Biochemistry* 70: 1864-1868.
- Nakayama, F. S. and Osbrink, W. L. 2010. Evaluation of kukui oil (*Aleurites moluccana*) for controlling termites. *Industrial Crops and Products*, 31 (2): 312-315.
- Nuttal, L., Butler, M., Gartlan, C. and Avington, A. 2006. *Eucalyptus camaldulensis* var. *camaldulensis*, River Red Gum. The name of publisher and the place are unacknowledged, 1-5 pp.
- Nerio, L.S., Olivero-Verbel, J. and Stashenko, E. 2010. Repellent activity of essential oils: a review. *Bioresource Technology*, 101: 372-378.
- Ohmura, W. D. S., Aoyama, M. and Ohara, S. 2000. Antifeedant activity of flavonoids and related compounds against the subterranean termite *Coptotermes formosanus* Shiraki. *Journal of Wood Science*, 46 (2): 149-153.
- Pearce, M. J. 1997. Termites: Biology and Pest Management. CAB International, UK, 172 pp.
- SAS Institute. 2003. SAS users guide: statistics, version 9.1. SAS Institute, Cary, NC.
- Siramon, P., Ohtani, Y. and Ichiura, H. 2009. Biological performance of *Eucalyptus camaldulensis* leaf oils from Thailand against the subterranean termite *Coptotermes formosanus* Shiraki. *Japan Wood Research*, 55: 41-46.
- Shafiei Alavije, E., Habibpour, B., Moharramipour, S. and Rasekh, A. 2013. The investigation into repellency effects of eucalyptus (*Eucalyptus camaldulensis*) essential oil on the termite *Microcerotermes diversus*. *Journal of Plant Protection*, 26 (4): 408-415.
- Taylor, A. M., Gartner, B. L., Morrell, J. J. and Tsunoda, K. 2006. Effects of heartwood extractive fractions of *Thuja plicata* and *Chamaecyparis nootkatensis* on wood degradation by termites or fungi. *Japan Wood Research*, 52 (2): 147-153.
- Verma, M., Sharma, S. and Prasad, R. 2009. Biological alternatives for termite control: a review. *International Biodeterioration and Biodegradation*, 63: 959-972.
- Verma, R. K. and Verma, S. K. 2006. Phytochemical and termicidal study of *Lantana camara* var. *aculeata* leaves. *Fitoterapia* 77 (6): 466-468.
- Watanabe, Y., Mihara, R., Mitsunaga, T. and Yoshimura, T. 2005. Termite repellent sesquiterpenoids from *Callitris glaucophylla* heartwood. *Journal of Wood Science*, 51: 514-519.
- Zhu, B. C. R., Henderson, G., Adams, R. P., Mao, L. and laine, R. A. 2003. Repellency of vetiver oils from different biogenetic and geographical origins a gainst formosan subterranean termites (Isoptera: Rhinotermitidae). *Sociobiology*, 42 (3): 623-638.

## اثر زیستی اسانس اکالیپتوس *Eucalyptus camaldulensis* علیه موربانه *Microcerotermes diversus* (Isoptera: Termitidae)

الهه شفیعی علویجه<sup>۱</sup>، بهزاد حبیب پور<sup>۱\*</sup>، سعید محرمی پور<sup>۲</sup> و آرش راسخ<sup>۱</sup>

۱- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه شهید چمران اهواز، اهواز، ایران.

۲- گروه حشره‌شناسی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران.

\* پست الکترونیکی نویسنده مسئول مکاتبه: [habibpour\\_b@scu.ac.ir](mailto:habibpour_b@scu.ac.ir)

دریافت: ۲۹ فروردین ۱۳۹۲؛ پذیرش: ۲۵ شهریور ۱۳۹۲

**چکیده:** موربانه *Microcerotermes diversus* (Isoptera: Termitidae) مهم‌ترین آفت اقتصادی مخرب ساختمان‌ها در استان خوزستان است. استفاده از مواد سازگار با محیط‌زیست با پتانسیل کاربرد در برنامه مدیریت تلفیقی آفات مثل اسانس‌ها و عصاره‌های گیاهی دارای اهمیت بسیاری هستند. در این تحقیق اثر دورکنندگی، سمیت تماسی و گوارشی اسانس اکالیپتوس در آزمون‌های انتخابی و غیرانتخابی و بازدارندگی تغذیه‌ای روی موربانه *M. diversus* مورد بررسی قرار گرفت. حدود غلظتی اسانس اکالیپتوس استفاده شده در آزمون‌های انجام شده ۰/۳ تا ۱/۶٪ (گرم بر میلی‌لیتر) تعیین شد. نتایج آزمون انتخابی و بازدارندگی تغذیه‌ای نشان داد که اسانس مذکور در غلظت‌های به کار برده شده به‌عنوان دورکننده عمل کرد. همچنین در غلظت‌های بالاتر از ۰/۷٪ مرگ و میر موربانه‌ها افزایش یافت. تغذیه موربانه‌ها با افزایش غلظت اسانس کاهش یافت. همچنین به سبب قدرت انتخاب موربانه‌ها در آزمون انتخابی، مقادیر LC و LT در مقایسه با آزمون غیرانتخابی، بالاتر بودند. بالاترین اثر اسانس اکالیپتوس (مرگ و میر حدود ۱۰۰٪) در غلظت ۱/۶٪ ثبت شد. در مجموع، این بررسی نشان می‌دهد که اسانس اکالیپتوس را می‌توان به‌عنوان یک ترکیب مناسب با سمیت تماسی و گوارشی علیه موربانه *M. diversus* پیشنهاد کرد.

**واژگان کلیدی:** اسانس اکالیپتوس، سمیت تماسی و گوارشی، *Microcerotermes diversus*