EVALUATION OF ESSENTIAL OILS OF *Eucalyptus* spp. FOR THE CONTROL OF THE SUBTERRANEAN TERMITE *Coptotermes gestroi* (WASMAN)[1]

Talita Vieira Zampieri Mikola[2], Marcos Roberto Potenza[3*], Fabricio Caldeira Reis[4], Vanessa Coelho da Silva[5], Mario Eidi Sato[3] and Massako Nakaoka Sakita[6]

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[2] Instituto Biológico,Pós-Graduação em Sanidade Alimentar e Ambiental no Agronegócio, São Paulo, Brasil. E-mail: <tatamikola@gmail.com>.

[3] Instituto Biológico, São Paulo, Brasil. E-mail: <potenza@biologico.sp.gov.br> and <mesato@biologico.sp.gov.br>.

[4] Instituto de Pesquisas Energéticas e Nucleares, Pós-Graduação em Tecnologia Nuclear, São Paulo, Brasil. E-mail: <fabriciocaldeirareis@hotmail.com>.

[5] Centro Universitário São Camilo, Graduada em Ciências Biológicas, São Paulo, Brasil. E-mail: <vanessacoelho1@hotmail.com>.

[6] Instituto Florestal, São Paulo, Brasil. E-mail: <messako_nakaoka@yahoo.com.br>.

*Corresponding author.

ABSTRACT – The subterranean termite *Coptotermes gestroi* (Wasman) (Isoptera: Rhinotermitidae) is considered one of the main pest species in urban areas in the Southeast Region of Brazil. For the control of this pest, the use of chemical insecticides is recommended, but this method is problematic in urban areas because of the risks of intoxication in the population and environmental contamination along with difficulties in isolating the treated areas. Therefore, it is necessary to find alternative methods aimed at minimizing the undesirable effects on the human population and the environment caused by termite control measures. The objective of this research was to evaluate the toxicity of several essential oils of *Eucalyptus* (*E. camaldulensis*, *E. citriodora*, *E. tereticornis*, *E. pseudoglobulus,* and *E. maidenii*) to the termite *C. gestroi*, under laboratory conditions. The oils were applied on filter papers that were infested with *C. gestroi* immediately after the treatment. The most toxic oil to the termite was *E. citriodora*, with the lowest lethal concentration (LC_{50}: 0.63%) and the shortest lethal time (LT_{50}: <1 h at 10%, LT_{50}: 42.4 h at 1.25%). The least toxic oil was *E. pseudoglobulus*, with the highest lethal concentration (LC_{50}: 3.66%) and the longest lethal time (LT_{50}: 11.1 h at 10%; LT_{50}: 473 h at 1.25%). These results indicate the potential for use of eucalyptus essential oils, especially for *E. citriodora,* for the control of *C. gestroi*. This article also provides information on the yield from essential oil extraction for different eucalyptus species.

Key words: Isoptera; Plant extracts; *Eucalyptus citriodora*.

TOXICIDADE DE ÓLEOS ESSENCIAIS DE Eucalyptus spp. PARA O CONTROLE DO CUPIM SUBTERRÂNEO Coptotermes gestroi (WASMAN)

RESUMO – O cupim subterrâneo ***Coptotermes gestroi*** *(Wasman) (Isoptera: Rhinotermitidae) é considerada uma das principais espécies-praga, em ambiente urbano, na região Sudeste brasileira. Para o controle dessa praga recomenda-se o uso de inseticidas químicos, mas esse controle torna-se problemático em áreas urbanas, devido aos riscos de intoxicação da população, contaminação do ambiente e dificuldade de isolamento de áreas tratadas. Considerando-se essas dificuldades, há necessidade de se recorrer a métodos alternativos de controle com vistas a minimizar os possíveis efeitos indesejáveis do controle de cupins para a população e ao ambiente. O objetivo desta pesquisa foi avaliar a toxicidade de diversos óleos essenciais de* ***Eucalyptus*** *(****E. camaldulensis****,* ***E. citriodora****,* ***E. tereticornis****,* ***E. pseudoglobulus*** *e* ***E. maidenii****) ao cupim* ***C. gestroi****, em condições de laboratório. As aplicações dos óleos foram feitas sobre papel de filtro, infestadas em seguida com* ***C. gestroi****. O óleo mais tóxico ao cupim foi o de* ***E. citriodora****, apresentando a mais baixa concentração letal (CL_{50}: 0,63%) e o menor tempo letal (TL_{50}: <1 h a 10%; TL_{50}: 42,4 h a 1,25%). O óleo menos tóxico foi o de* ***E. pseudoglobulus****, com a mais alta concentração letal (CL_{50}: 3,66%) e o maior tempo letal (TL_{50}: 11,1 h a 10%; TL_{50}: 473 h a 1,25%). Os resultados indicam bom potencial de uso de óleos de eucalipto para o controle de* ***C. gestroi****, com destaque para* ***E. citriodora****. No presente artigo, também são apresentados dados de rendimento de extração de óleos essenciais, para as diferentes espécies de eucalipto.*

Palavras-Chave: Isoptera; Extrato de plantas; ***Eucalyptus citriodora****.*

1. INTRODUCTION

Termites belong to the order Isoptera and are considered eusocial insects, because of their characteristics such as care of offspring, presence of reproductive and sterile castes, and overlapping of generations (ZORZENON; POTENZA, 1998; COSTA-LEONARDO, 2002). Basically, termites feed on cellulosic and lignocellulosic materials, which are found in a variety of forms, such as live or dead wood, grasses, roots, seeds, manure of herbivorous animals, humus, manufactured products, and some products of animal origin such as leather and wool (BERTI FILHO, 1993; ZORZENON; POTENZA, 1998; FONTES; ARAÚJO, 1999; DONOVAN et al., 2001; BIGNELL; ROISIN, 2010; FERREIRA et al., 2011; JACOBS, 2014).

Termites are considered to be very important organisms for the maintenance of decomposition processes and for carbon and nutrient cycling, mainly owing to the biomass of their populations and the variety of their feeding habits (BANDEIRA; VASCONCELLOS, 2002). Despite the benefits provided by these organisms in the ecosystem, termites are better known as pasture and wood pests and for causing the reduction in the planting area by the presence of their nests (termite mounds) on the surface of the soil (RESENDE et al., 2007; FERREIRA et al., 2011).

There are currently 2,882 termite species described, and the neotropical region comprises 562 species (CONSTANTINO, 2012), distributed in seven different families: Hodotermitidae, Kalotermitidae, Mastotermitidae, Rhinotermitidae, Serritermitidae (with occurrence only in Brazil), Termitidae, and Termopsidae (BERTI FILHO, 1993; CONSTANTINO, 2012).

Despite the large number of species known, the proportion of species considered as pests is relatively small. Among the genera that cause more problems, *Coptotermes* and *Heterotermes*, known as subterranean termites, and *Cryptotermes*, known as dry-wood termite, stand out. In these genera, *Coptotermes gestroi* (Wasman) and *Cryptotermes brevis* (Walker) are those that cause the greatest losses, and *C. gestroi* is considered the main pest species in the Southeast Region of Brazil (ZORZENON; POTENZA, 1998).

Souza et al. (2009) evaluated the susceptibility of wood of five forest trees to the action of the subterranean termite, *C. gestroi*. As evidence, wood stakes of the following species were used: pine (*Pinus* sp.) (Pinaceae), chestnut (*Bertholletia excelsa*) (Lecythidaceae), cabbage angelin (*Andira inermis*) (Leguminosae, Papilionoideae), masaranduba (*Manilkara huberi*) (Sapotaceae), and pink lapacho (*Tabebuia avellanedae*) (Bignoniaceae). The results indicated that pine, chestnut, and angelin stakes were more susceptible to attack by the pest. The woods more susceptible to attack by *C. gestroi* were of moderate to light density.

Lelis (1994) estimated a loss of US$ 3.5 billion caused by termites when surveying 240 buildings in the city of São Paulo from 1973 to 1993. Serpa (1986) verified the presence of termites of the genus *Cryptotermes* destroying sacred works, painting frames, altars, beams, rafters, slats, and components of the woodwork of roofs of the historic city of Olinda, in Pernambuco (SILVA et al., 2004).

In an urban setting, termites are not problems unique to buildings. The occurrence of a large number of tree species infested by termites in the cities is also noticeable and the insects may infest buildings through underground dispersal or by flocks occurring during mating seasons. Urban trees represent important foci of reinfestations in treated buildings, because these trees serve as shelters for these termites while also being victims of severe termite attacks that cause great damage and may result in plant death (FONTES; BERTI FILHO, 1998).

For the control of these pests, insecticides (WILKEN, 1992; RESENDE et al., 1995; PETERSON, 2010) are usually used and this control method becomes problematic in urban areas due to the risks of intoxication to the population, contamination of open areas, and difficulty in isolating treated areas.

In this aspect, botanical insecticides represent an alternative to synthetic insecticides. Several studies are being carried out to evaluate the effects of natural products, in particular plant essential oils and their components, on termites (RAINA et al., 2007). Park and Shin (2005) reported the effect of essential oils with fumigant activity on *Reticulitermes speratus* (Kolbe). In addition, several other studies have also observed mortality of termites after their exposure to plant essential oils (ZHU et al., 2001, 2003; CHANG; CHENG, 2002; CHAUHAN; RAINA, 2006; MEEPAGALA et al., 2006; REGNAULT-ROGER, 2012; ALMEIDA et al., 2015).

The objective of this work was to evaluate the essential oils of several *Eucalyptus* species (*E. camaldulensis* Derth, *E. citriodora* Hook, *E. tereticornis* Smith, *E. pseudoglobulus* (Naudin ex Maiden), and *E. maidenii* F. Muell for the control of the termite *C. gestroi*. There is no information currently in literature on the susceptibility of *C. gestroi* to eucalyptus essential oils.

2. MATERIALAND METHODS

To prepare the essential oils, leaves of *E. citriodora*, *E. tereticornis,* and *E. camaldulensis* were collected at the Experimental Station of Luiz Antonio, and those of *E. maidenii* and *E. pseudoglobulus* were collected at the Experimental Station of Itapeva, both belonging to Instituto Florestal (Table 1). The oils were extracted at the Laboratory of Phytochemistry of the Section of Wood and Forest Products of Instituto Florestal. A high pruning shear was used to collect enough material for extraction. For distillation of essential oils, the leaves were dried in the shade, at room temperature and in a ventilated place, processed in a knife and hammer mill, and subjected to hydrodistillation in a Clevenger apparatus modified by Wasicky (1963). The material was subjected to extraction for 4 hours. The percentage by weight of the material used was calculated by measuring the volume of the essential oils obtained.

In order to obtain the *C. gestroi* workers used in this study, corrugated cardboard traps adapted from Camargo-Dietrich and Costa-Leonardo (2003) were installed in the facilities of Instituto Biológico, in São Paulo, SP (State of São Paulo). Traps were prepared using PET bottle (Poly Ethylene Terephthalate) with a size of 15 cm in height x 8.5 cm in diameter. Inside the bottles, a roll of corrugated paperboard (2.0 m long x 11 cm wide) was placed. The traps were buried with the opening facing down.

To perform the toxicological tests, the method of filter paper impregnated with the products was used, as described by Takematsu (1983). The essential oils were diluted to 10.0%, 5.0%, 2.5%, and 1.25%. An amount of 0.5 ml of the product was applied to the filter paper (7 cm in diameter). A control, without oil application, was also prepared.

The experiment was conducted in a completely randomized design, with five replicates. Each plot consisted of 20 *C. gestroi* workers placed on a treated filter paper disk in a Petri dish. The insects were kept in an incubator chamber at 25 ± 2 °C and 70 ± 10% relative humidity. The evaluations were performed after 1, 2, 3, 4, 24, 48, and 72 hours after termite exposure to eucalyptus essential oils. Tests in which the termite mortalities in the control were equal to or greater than 10% were not considered in this study.

The lethal concentrations (LC_{50}) of the essential oils were estimated by Probit analysis (FINNEY, 1971), using the POLO PLUS program (LEORA SOFTWARE, 2003). Lethal times (LT_{50}) were also estimated for concentrations of 1.25% and 10%, using the same statistical program.

3. RESULTS

The extraction yield of the essential oils is shown in Table 2. The eucalyptus species that presented the highest essential oil content in leaves was *E. pseudoglobulus* (3.72%), followed by *E. maidenii* (2.72%).

All tested oils caused 100% mortality for *C. gestroi* at the concentration of 10%. At 5% concentration, only the oils of *E. citriodora*, *E. tereticornis,* and *E. maidenii* caused mortality rates equal to or greater than 80%. At the concentration of 1.25%, only *E. citriodora* oil caused mortality equal to or above 80% (Figure 1).

3.1 Estimates of lethal concentrations

The mortality data of *C. gestroi*, for the different essential oils tested, fitted the Probit model (X^2 not significant, $p > 0.05$). The oil of *E. citriodora* presented the lowest median lethal concentration (LC_{50}: 0.63%), being significantly lower than the other essential oils, based on the criterion of non-overlapping of the LC_{50} confidence intervals at 95% probability (Table 3).

The other essential oils tested (*E. camaldulensis*, *E. tereticornis*, *E. pseudoglobulus,* and *E. maidenii*) presented LC_{50} values equal to or above 2.95%, and there were no statistical differences among these treatments (Table 3).

The highest LC_{50} was observed for *E. pseudoglobulus* oil (LC_{50}: 3.66%), which was 5.8 times higher than the LC_{50} estimated for *E. citriodora*.

3.2 Estimation of lethal times

The oil that provided the fastest control of *C. gestroi* was of *E. citriodora*, with an estimated mean

Table 1 – *Eucalyptus* species (family: Myrtaceae): common name, collecting place, geographical coordinates, and plant parts used in the study.
***Tabela 1** – Espécies de **Eucalyptus** (família: Myrtaceae): nome vulgar, local de coleta, coordenadas geográficas, partes das plantas utilizadas no estudo.*

Species	Common name	Place of collection	Geographical coordinates	Part used
E. citriodora	Lemon-scented Gum	Exp. Sta. of Luiz Antonio	21º31’ to 21º 41’ S47º40’ to 47º51’W	Leaves
E. maidenii	Maiden’s Gum	Exp. Sta. of Itapeva	24º02’ to 24º06’ S49º03’ to 49º08’ W	Leaves
E. pseudoglobulus	Blue Gum	Exp. Sta. of Itapeva	24º02’ to 24º06’ S49º03’ to 49º08’ W	Leaves
E. tereticornis	Forest Red Gum	Exp. Sta. of Luiz Antonio	21º31’ to 21º 41’ S47º40’ to 47º51’W	Leaves
E. camaldulensis	River Red Gum	Exp. Sta. of Luiz Antonio	21º31’ to 21º 41’ S47º40’ to 47º51’W	Leaves

Table 2 – Output of essential oils in different *Eucalyptus* species.
***Tabela 2** – Rendimento de óleos essenciais em diferentes espécies de **Eucalyptus**.*

Species	Amount used(in g of leaves)	Oil yield(in mL)	Oil yield (%)
E. citriodora	503.21	6.66	1.32
E. maidenii	330.40	9.00	2.72
E. pseudoglobulus	295.40	11.00	3.72
E. tereticornis	577.52	7.00	1.21
E. camaldulensis	473.92	5.00	1.05

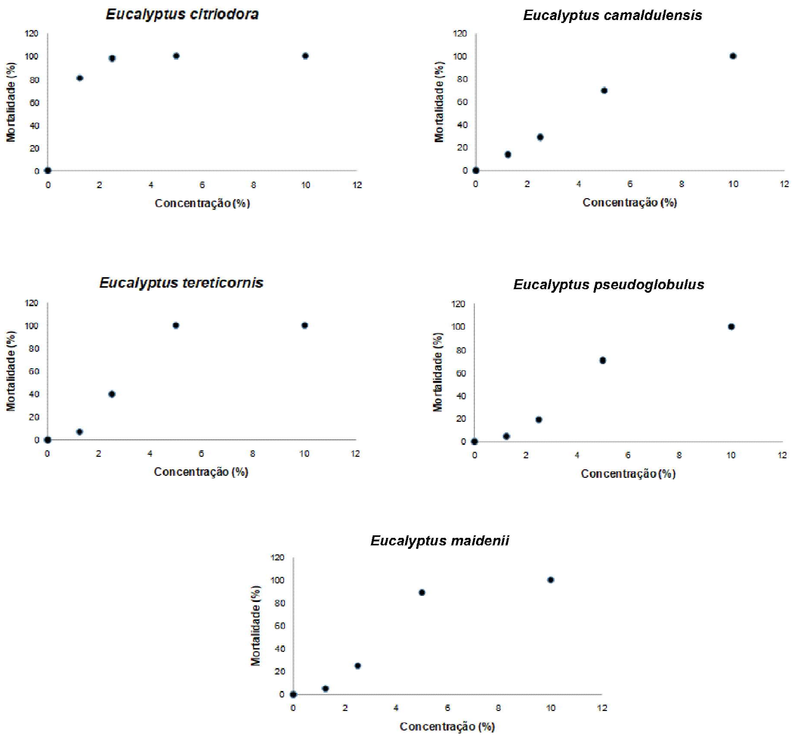

Figure 1 – Toxicity of different eucalyptus essential oils to *Coptotermes gestroi*: percentage of mortality at 72 hours after the initial exposition of the termites to the oils.
***Figura 1** – Toxicidade de diferentes óleos essenciais de eucalipto a **Coptotermes gestroi**: porcentagens de mortalidade 72 horas após o início da exposição dos cupins aos óleos.*

Table 3 – Lethal concentrations (%) of eucalyptus essential oils for *Coptotermes gestroi*, at 72 hours after the initial exposure of the termites to the oils: botanical species, total number of termites tested (*n*), median lethal concentration (LC_{50}), slope and standard error, chi-square (X^2), and degree of freedom (d.f.).
***Tabela 3** – Concentrações letais (%) de óleos essenciais de eucalipto para **Coptotermes gestroi**, 72 horas após o início da exposição dos cupins aos óleos: espécie botânica, número total de cupins testados, concentração letal média (CL_{50}), coeficiente angular e erro padrão da média, qui-quadrado (X^2), grau de liberdade (g.l.).*

Essential oil	*n*	LC_{50} (%)(95% C.I.)	Slope ± SE	x^2	d.f.
E. camaldulensis	400	3.16(0.92 – 8.94)	3.40 ± 0.69	5.57	2
E. citriodora	500	0.63(0.55 – 0.70)	2.98 ± 0.24	2.18	3
E. tereticornis	500	2.95(1.20 – 4.84)	4.92 ± 1.36	7.04	3
E. pseudoglobulus	400	3.66(2.30 – 5.83)	4.56 ± 0.69	3.96	2
E. maidenii	400	3.06(1.92 – 4.83)	5.17 ± 0.83	5.31	2

lethal time (LT_{50}) of 42.5 hours at the concentration of 1.25%, and less than one hour at the concentration of 10%. At the highest concentration of the product (10%), 100% mortality was observed in the first 60 minutes (Table 4).

The LT_{50} for *E. citriodora* oil at the concentration of 1.25% was significantly lower than that of *E. camaldulensis*, *E. pseudoglobulus*, and *E. maidenii* oils. The *E. tereticornis* oil did not differ statistically from that of *E. citriodora*, with LT_{50} of 71.6 hours at the concentration of 1.25%, and less than one hour at the concentration of 10% (Table 4). The *E. pseudoglobulus* oil presented the longest LT_{50} (473.1 h or 19.7 d), at the concentration of 1.25%, with a value 11.1 times higher than that estimated for *E. citriodora*.

4. DISCUSSION

With the exception of *E. pseudoglobulus*, for which no citations were found regarding the yield of essential oils, the other eucalyptus species tested (*E. citriodora*, *E. maidenii*, *E. tereticornis*, *E. camaldulensis*) presented yields similar to or slightly higher than those reported in literature. Yields of essential oils lower than those observed in the present study were reported by some authors for *E. maidenii* (1%), *E. tereticornis* (0.4 to 0.5%), and *E. camaldulensis* (0.32%) (GUENTHER, 1950; SILVA et al., 2006). Variable yields of essential oils were reported for *E. citriodora* (0.5 to 2.0%), *E. camaldulensis* (0.2 to 2.8%), and *E. tereticornis* (0.9 to 1.0%) (LASSAK, 1988; DORAN, 1991; VITTI; BRITO, 2003; VIEIRA, 2004; SILVA et al., 2006).

The effectiveness of *E. citriodora* essential oil in termite (*Reticulitermes speratus*) control was also reported by Park and Shin (2005), who observed a highly toxic fumigant effect of the product (at 3.5 μL/L of air), one day after application.

Siramon et. al. (2009) also reported the toxic effect of essential oils from leaves of *E. camaldulensis* to *Coptotermes formosanus* Shiraki. The tests showed that *E. camaldulensis* essential oils had toxicity by fumigant effect and residual contact, with LC_{50} values ranging from 12.68 to 17.50 mg/g filter paper (8.7 cm in diameter) by residual contact and between 12.65 and 17.50 mg/Petri dish (100 cm^3) by fumigation. The compounds p-cymene and gamma-terpinene were the main responsible components for residual contact toxicity, and 1.8-cineol (eucalyptol) was responsible for the fumigant effect. In studies of mechanisms of action of the essential oils of *E. camaldulensis*, acetylcholinesterase inhibitory activity was observed, demonstrating common symptoms of neurotoxic compounds for *C. formosanus* (SIRAMON et al., 2009).

The LC_{50} reported by Siramon et. al. (2009) for *E. camaldulensis* essential oil by residual contact (12.7 to 17.5 mg/g filter paper) for *C. formosanus* was similar to that observed (15.8 mg/g filter paper) for *C. gestroi*, in the present experiment.

Long lethal times required to kill termites, as observed for *E. maidenii* and *E. pseudoglobulus* (up to 19.7 d) in *C. gestroi*, were also reported by Chang and Cheng (2002), when evaluating the effect of essential oils of *Cinnamomum osmophloeum* Kaneh on *C. formosanus*. For several components (eugenol, α-terpineol, geraniol, benzaldehyde, and neral) of the essential oils of this plant, an increase in termite mortality was observed when the evaluation time was extended up to 14 days.

These results indicate the potential of using eucalyptus oils, especially *E. citriodora* oils, for the control of *C. gestroi*. Studies under field conditions, through tree applications and/or wood or soil treatment, are needed to prove the efficacy of eucalyptus essential oils in the control of the subterranean termite.

Table 4 – Lethal time (hours) of eucalyptus essential oils, at the concentrations of 1.25% and 10%, for *Coptotermes gestroi*: botanical species, medial lethal time (LT_{50}), slope and standard error, chi-square (X^2), and degree of freedom (d.f.).

Tabela 4 *– Tempos letais (horas) para óleos essenciais de eucalipto, nas concentrações de 1,25% e 10%, para* ***Coptotermes gestroi****: espécie botânica, tempo letal médio (TL_{50}), coeficiente angular e erro padrão da média, qui-quadrado (X^2), grau de liberdade (g.l.).*

Essential oil	Concentration(%)	LT_{50} (h) (95% C.I.)	Slope ± SE	x^2	d.f.
E. camaldulensis	1.25	104.74 (80.48 – 158.16)	1.83 ± 0.25	6.02	5
	10.0	0.43 (0.03 – 0.97)	1.18 ± 0.26	2.24	5
E. citriodora	1.25	42.45 (28.08 – 73.15)	1.94 ± 0.31	8.70	5
	10.0	< 1*	-	-	-
E. tereticornis	1.25	71.62 (58.45 – 93.45)	1.84 ± 0.20	9.56	5
	10.0	< 1*	-	-	-
E. pseudoglobulus	1.25	473.13 (223.07 – 2265.63)	1.21 ± 0.23	4.83	5
	10.0	11.09 (7.43 – 16.93)	2.67 ± 0.33	8.87	5
E. maidenii	1.25	136.85 (97.80 – 234.14)	1.59 ± 0,22	8.41	5
	10.0	7.03 (6.19 – 8.15)	3.44 ± 0.25	6.39	5

*Mortality of 100% in less than 1 hour.

5. CONCLUSIONS

Based on our results, the following conclusions can be drawn: a) *C. gestroi* termites were susceptible to the treatments with essential oils of *E. camaldulensis*, *E. citriodora*, *E. tereticornis*, *E. pseudoglobulus,* and *E. maidenii*, with mortality rates up to 100%; b) *E. citriodora* oil was the most toxic to *C. gestroi* (LC_{50}: 0.63%), whereas the least toxic oil was from *E. pseudoglobulus* (LC_{50}: 3.66%); c) The use of *E. citriodora* and *E. tereticornis* oils resulted in the shortest lethal times for *C. gestroi* ($LT_{50} \leq 72$ h at 1.25%); d) *E. pseudoglobulus* oil presented the slowest action for the control of the subterranean termite *C. gestroi* (LT_{50}: 473 h at 1.25%).

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