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POTENTIAL OF CLOVE ESSENTIAL OIL AGAINST SUBTERRANEAN TERMITE *Macrotermes gilvus* HAGEN (BLATTODEA: TERMITIDAE)

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ABSTRACT

Macrotermes gilvus (subterranean termite) can play dual roles as decomposers of natural wood and pest in plantations and urban area. The damages caused by termites in the urban area reaches 35% and in the wood industry can reach 40%. Nowadays, termite control is still carried out by the application of synthetic termiticide. In termites' case, environmental pollution issue is more important than the resistance of termite to termiticide, so it's necessary to find alternative termiticide from natural materials. This study examined the potential of clove essential oils to control *M. gilvus* with two methods. The test methods were oil residue on filter paper and soil in a glass tube H. Clove essential oil on filter paper was applied and dried for one minute, after that put in the petri dish. For soil treatment, the soil was soaked with clove essential oil for one hour and dried for one day, after that the soil treatment put into the bridge tube. Each method was infested by 50 termites with ratio 90% workers and 10% soldiers. Termite mortality on treated filter paper was observed after 72 hours, and on treated soil were observed daily until 7 days. The result showed that clove essential oils caused mortality of *M. gilvus* more than 90% after consumed and contacted on treated filter paper and contacted on treated soil with concentration 0.80% and 1.20%, respectively. Based on the results, clove essential oil has the potential to be developed and can be used as an alternative against termites, that was environmentally friendly.

Keyword: *mortality, residue paper, soil injection, subterranean termite, Syzygium aromatcum* L.

ABSTRAK

[POTENSI MINYAK CENGKIH MENGENDALIKAN RAYAP TANAH *Macrotermes gilvus* HAGEN (BLATTODEA: TERMITIDAE)]. *Macrotermes gilvus* (rayap tanah) mempunyai peranan ganda yaitu sebagai pengurai kayu di habitat alami dan menjadi hama di perkebunan dan perkotaan. Kerusakan yang diakibatkan rayap di perkotaan mencapai 35% dan di industri kayu bisa 40%. Saat ini pengendalian rayap masih dilakukan dengan aplikasi termitisida sintetik. Dalam kasus rayap, masalah pencemaran lingkungan lebih penting untuk dimunculkan daripada ketahanan rayap terhadap termitisida, sehingga perlu dicari alternatif termitisida dari bahan alam. Penelitian ini mengkaji potensi minyak atsiri cengkeh untuk mengendalikan *M. gilvus* dengan dua metode. Metode pengujian yang digunakan yaitu residu minyak pada kertas saring dan tanah dalam tabung gelas H. Minyak atsiri cengkeh diaplikasikan pada kertas saring dan dikeringanginkan selama satu menit, selanjutnya dimasukkan ke dalam cawan petri. Untuk perlakuan tanah, tanah direndam selama satu jam dan dikeringanginkan selama seharian, selanjutnya tanah perlakuan dimasukkan ke dalam jembatan tabung H. Masing-masing metode diinfestasikan sebanyak 50 rayap dengan komposisi 90% kasta pekerja dan 10% kasta prajurit. Kematian rayap pada kertas saring yang diberi perlakuan diamati setelah 72 jam, dan pada tanah yang diberi perlakuan diamati setiap hari sampai 7 hari. Hasil pengujian menunjukkan bahwa minyak atsiri cengkeh menyebabkan kematian *M. gilvus* lebih dari 90% setelah dikonsumsi dan dikontakkan pada kertas saring yang diberi perlakuan dan dikontakkan pada tanah yang diberi perlakuan dengan konsentrasi masing-masing 0.80% dan 1.20%. Berdasarkan semua pengujian, minyak atsiri cengkeh sangat potensial untuk dikembangkan dan dapat digunakan sebagai alternatif terhadap rayap yang ramah lingkungan.

Kata kunci: *injeksi tanah, kematian, rayap tanah, residu kertas, Syzygium aromatcum* L.

INTRODUCTION

Termite is a social insect that lives in the colony. Termite are classified in order Blattodea (Inward *et al*., 2007; South *et al*., 2020), they spread widely around America, Europe, Africa, Asia, and Australia continents (Pearce, 1997). Reproduction of termite can be optimum in temperature around 25-32 °C (Cao & Su, 2015) and humidity around 75-90% (Zukowski & Su, 2017). More than 200 species of termites are found in Indonesian, and *Macrotermes gilvus* (Blattodea: Termitidae) is the one of many spesies that become an important pest. Damage caused by *M. gilvus* in Indo-Asia can reach 13% (Kuswanto *et al*., 2015). *M. gilvus* become a pest in urban area (Savitri *et al*., 2016), plantation (Sayuti 2012), and become a potential pest in palm oil plantation (Nandika, 2014). According to Yusuf & Utomo (2006), damage caused by subterranean termites in urban area reach 35% and in wood industry can 40%. The symptoms of subterranean termite attack there is a hole in the wood or branches that connecting like a tunnel, and we can find "tabung kembara" in the surface of wood or branches that attack by termite. In the heavy attack by termites the wood can broke easily (Kurniawan *et al*., 2015). Research by Nandika (2014), shown that the economic loss caused by termites can reach 90 billion rupiah in Indonesian, and it can increase with increasing the urban area and the selling value of wood.

Nowadays, termite control is still carried out with the application of synthetic termiticide. In termite cases, the environmental pollution issue is more important to pose than the resistance of termites to termiticide (Djojosumarto 2008). To reduce that problem, it's necessary to find alternative termiticide from natural materials (Hutabarat *et al*., 2015). The research of Meisyara *et al*. (2021) showed that clove essential oil caused the highest mortality than lemongrass and cubed pepper on drywood termites, *Cryptotermes cynocephalus* (Blattodea: Kalotermitidae). The same result showed in Xie *et al*. (2015) and Indrayani *et al*. (2016), clove oil caused 100% mortality to *Reticulitermes chinensis* (Kalotermidae) Coptotermes formosanus (Rhinotermitidae) of 0.50 µg/g and 0.1%, respectively. Other research using clove oil to control pest storage (*Cryptolestes ferrugineus*) (Ikawati *et al*., 2021). Clove is one of many plants that have potential to be developed as bio-insecticide, because it produced terpenoid for the secondary compound that can kill termites and other insects (Braga *et al*., 2007). So, this study examined the potential of clove essential oils to control *M. gilvus*

MATERIALS AND METHODS

This research was conducted at the Laboratory of Physiology and Toxicology Insect, Department of Plant Protection, Faculty of Agriculture, IPB University from December 2019 until April 2020. The termites were found in Yanlappa Nature Reserve, Jasinga, and the termites were identified using the identification key book Tho (1992) and Mubin *et al*. (2019). The clove essential oil was obtained from the Laboratory of Entomology, Southeast Asian Regional Center for Tropical Biology (SEAMEO BIOTROP), Bogor.

Clove essential oil is used for this treatment, fipronil is used for control positive and acetone used for control negative treatment. Clove essential oils were dissolved using acetone. The test methods were oil residue on filter paper with concentration 0.80, 0.40, 0.20, 0.10, 0.05% and soil in a glass tube with concentration 1.20, 0.60, 0.30, 0.15, 0.07%. Clove essential oil on filter paper was applied using pipet with spiral motion and dried for one minute. After that, put the filter paper in a petri dish and infest 50 termites with ratio 90% workers and 10% soldiers. For soil treatment, the soil is soaked with clove essential oil for one hour and dried for one day (Salam *et al*., 2014). After that, put 5.5 g of soil into the bridge tube. In glass tube infest 50 termites with ratio 90% workers and 10% soldiers, in another glass tube put rubberwood with the size of 2.5 x 2.5 x 1 cm. Closed the top of glass tubes using aluminum foil to keep the humidity. Termite mortality on treated filter paper was observed after 72 hours, and on treated soil were observed daily until 7 days. The analyzed using POLO PC, R Studio v 1.2.5001, and SPSS v 20.0 programs, followed by Tukey test with alpha 5%.

Percentage of termites' mortality:

$$\text{Mortality} = \left(\frac{N}{No}\right) \times 100\%$$

N = The number of termites that died during treatment
No = The number of total termites used for treatment

RESULTS AND DISCUSSION

Residue on Filter Paper Treatment

Filter paper treatment test using clove essential oil showed an increased mortality percentage of *M. gilvus* at each concentration (Table 1). Clove essential oil with a concentration of 0.08% (the highest concentration in this test) can cause 100% mortality of *M. gilvus* and in the lowest concentration (0.05%) can cause 19.73% mortality of *M. gilvus*. The mortality of *M.*

gilvus in the control treatment showed different results for all treatment concentrations with a mortality of 1.72%. The mortality of *M. gilvus* at a concentration of 0.80% had the same result as the fipronil treatment, which was 100%. This shows that the application of 0.80% clove essential oil in filter paper has the same ability as the active ingredient fipronil in increasing termite mortality.

Table 1. Percentages of *M. gilvus* mortality caused by clove essential oil in filter paper treatment

Concentration (%)	Mortality (%)[a]
Control	1.72 ± 1.36 d
0.05	19.73 ± 4.13 cd
0.10	44.22 ± 4.13 bc
0.20	51.70 ± 8.84 b
0.40	55.78 ± 8.92 b
0.80	100.00 ± 0.00 a
Fipronil	100.00 ± 0.00 a

[a]Based on Tukey test with alpha 5%, the numbers in the same column followed by the same letter aren't significantly different.

Residue on Soil Treatment

Soil treatment test using clove essential oil showed an increased mortality percentage of *M. gilvus* at each concentration (Table 2).

Table 2 Percentages of *M. gilvus* mortality caused by clove essential oil in soil treatment

Concentration (%)	Mortality (%)[a]
Control	0.00 ± 0.54 e
0.07	18.67 ± 4.24 d
0.15	30.33 ± 4.24 d
0.30	50.00 ± 9.07 c
0.60	68.67 ± 9.15 b
1.20	93.33 ± 0.00 a
Fipronil	100.00 ± 0.00 a

[a]Based on Tukey test with alpha 5%, the numbers in the same colum-followed by the same letter aren't significantly different.

The mortality of *M. gilvus* was increased with increasing the concentration of essential oil used. Clove essential oil with a concentration of 1.20% (the highest concentration in this test) can cause 93.33% mortality of *M. gilvus* and in the lowest concentration (0.07%) can cause 18.67% mortality of *M. gilvus*. Clove essential oil in concentrations of 0.15% and 0.07%, didn't have significant differences that can cause mortality of termites respectively 30.33% and 18.67%. The mortality of *M. gilvus* in the control treatment showed different results for all treatment concentrations with a mortality of 0%. The mortality of *M. gilvus* at a concentration of 1.20% was lower (93.33%) than the fipronil treatment (100%) (Table 2). This showed that the application of fipronil was better than 1.20% clove essential oil in soil treatment for increasing termite mortality.

Mechanism of Clove Essential Oil Against M. gilvus

Mortality of *M. gilvus* (Tables 1 and 2) caused by several compounds were contained in clove essential oil, such as eugenol (65.02%), trans-caryophyllene (15.64%), ethanol (5.55%), and phenol (2.69%) (Hadi, 2012). Eugenol compounds have potential action to be used as insecticides, fungicides, bactericides, and nematicides (Kardinan, 2005). Cloves essential oil with a concentration of 0.02 mL/L fumigant can cause mortality of *Cryptolestes* sp. 94% within 72 hours of application (Bertus, 2015). Cloves extract with a concentration of 1.25% can kill and reduce the population of *Planococcus minor* mealybugs by 52.5% (Balfas, 2008), and can suppress the growth of the pathogenic fungal colony *Sclerotium rolfsii* by 22.75% (Tawa *et al*., 2017). Eugenol and aceteugenol work simultaneously in killing insects, these compounds can disturb the nervous system and ion transport in cells that can cause death (Kumala & Indriyani, 2008). The mechanism of eugenol and aceteugenol can disrupt the nervous system by inhibiting the action of the acetylcholine enzyme which acts as a chemical transmitter substance remover in synaptic nerves. Inhibition of this enzyme can cause insects to experience seizures and death (Asyiah, 2011). According to Adkhi (2007), giving 2% clove extract with n-hexane solvent can causes mortality of *Coptotermes* sp. 98.79% within 4 days after application. In addition, eugenol can also cause a knockdown effect on several insects, one of that is *Culex* sp. Clove flower extract with concentration of 1.25% can kill *Culex* sp. mosquitoes by 72% within one hour after application (Nopitasari *et al*., 2014).

Morphology of *M. gilvus* after treated with clove essential oil showed discoloration of the abdomen to blackish brown, dry out, and wrinkles. In the normal condition, the abdomen of *M. gilvus* soldier caste is pale brown and the warrior caste is reddish-brown (Subekti, 2010). The discoloration of the abdomen is caused by disruption of the ion balance in the body so that insects become dehydrated and shrink (Saenong, 2016).

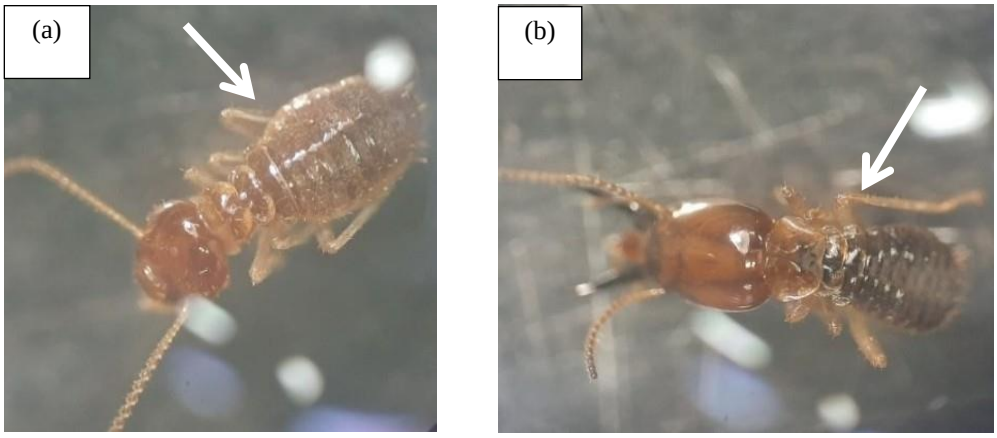

Figure 1 Discoloration of the abdomen *M. gilvus* after being treated with essential oil to blackish brown on worker (a) and soldier (b)

Effectivity of Termite Control in Two Methods Using Clove Essential Oil

Lethal concentration (LC_{50} and LC_{95}) showed the concentration that can cause 50 and 95% mortality of *M. gilvus* from the total sample population. The calculation of lethal concentration in two methods showed the variance results (Table 3).

Table 3. Number of LC and regression in two methods

Methods	LC_{50}(%)	LC_{95}(%)	Y = a + bx	
			a	b
Residue on filter paper	0.192	1.259	1.327	1.728
Residue on soil	0.267	2.191	1.697	2.982

Descriptions: Y: linear regression, a: intercept, b: slope, x: independent variable (lethal concentrations)

The testing result of clove essential oil using filter paper treatment showed an LC_{50} value of 0.192%, meaning that the concentration of clove essential oil that can cause mortality 50% of termites was 0.192%. This value is lower than the LC_{50} in the soil treatment, which is 0.267%. The test using the filter paper treatment showed an LC_{95} value of 1.259%, meaning that the concentration of clove essential oil that can cause mortality 95% of termites was 1.259%.

Meanwhile, the LC_{95} value in the test using the soil treatment was higher, which was 2.191%. Overall, the LC_{50} and LC_{95} values for the application of clove essential oil using the filter paper treatment had lower values compared to the LC_{50} and LC_{95} in the soil treatment. The regression value obtained from both tests showed a positive value, meaning that the relationship between the concentration of clove essential oil and the percentage of mortality of *M. gilvus* was directly proportional. This showed that there was an increase in the mortality percentage of *M. gilvus* along with the increase in the concentration of the clove essential oil used.

The effectiveness of essential oils in tests with filter paper treatment and soil treatment can cause by differences in the way that the active compounds enter the insect body. In the test using the filter paper treatment, the active compound works double as a contact and stomach poison, whereas in the soil treatment only works as a contact poison. It's related to the concentration of essential oils using filter paper treatment was lower than the soil treatment but can cause the mortality of *M. gilvus* higher than the soil treatment (Tables 1 and 2). This is similar to Prasta (2018) research, which explains the testing result of *Tephrosia vogelii* and *Annona muricata* extracts using the towel tissue residue method has a higher mortality rate of *C. curvignathus* compared with the soil treatment method.

Nowadays essential oils are one of the potential pest controls because it has several advantages, that doesn't cause resistance to pests and safe for the environment (it's easy to decompose and isn't harmful to other living things) (Kardinan 2005). However, controlling using essential oils also has a weakness, the compounds contained in essential oils are volatile and unstable (Hartati 2012). These properties affect the resistance of the compounds contained in essential oils so that the use of essential oils in the

field needs to be mixed with additional chemical compounds/adjuvants which can reduce the evaporation of essential oils and are more stable.

CONCLUSION

Clove essential oils caused mortality of *M. gilvus* more than 90% after consumed and contacted on treated filter paper and contacted on treated soil with concentration 0.80% and 1.20%, respectively. So, the conclusion was clove essential oil has very potential to be developed and can be used as an alternative against termites, that was environmentally friendly.

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