STORED PRODUCT PEST

Efficacy of *Eucalyptus citriodora* and *Syzygium aromaticum* essential oil as insecticidal, antiovipositant, and fumigant against *Callosobruchus maculatus* F (Coleoptera: Bruchidae)

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Abstract

Callosobruchus maculatus is the most pernicious pest of stored grain worldwide. Even though synthetic insecticides are commonly used to eliminate this insect pest, the negative effect of this pest management method on humans and the environment raises concern among people around the world. This study was done to identify the active ingredient of essential oils in *Eucalyptus citriodora* and *Syzygium aromaticum* and to evaluate the effectiveness of those essential oils in controlling *C. maculatus*. The results of gas chromatography/mass spectrometry analysis indicated that the essential oil extracted from the leaves of *S. aromaticum* are rich in Eugenol and β caryophyllene as much as 81% and 14.65% consecutively, while *E. citriodora* oil contains 86% of Citronella. According to the bioassay results, increasing the essential oil concentration from 1% to 3% resulted in a significant increase in insect mortality rate, oviposition deterrence, and fumigant toxicity. Additionally, *S. aromaticum* has significantly shown a higher insecticidal performance compared to *E. citriodora.* However, there are no synergistic effects observed on the use of essential oil of both plant species on *C. maculatus*. These results suggest that *S. aromaticum and E. citriodora essential* oils could be potential candidates as a natural insecticide in managing *C. maculatus* in stored products.

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Introduction

Cowpea seed beetle *Callosobruchus maculatus* F (Coleoptera: Bruchidae) is the major insect pest that causes both qualitative and quantitative losses of grain legumes during storage (Devi & Devi, 2014). Infestation of this insect pest will reduce the nutritional quality of grains and cause food contamination due to insect residue. The eggs and adults present on the grain, but larvae and pupae live within the seed. Its larvae make a hole in the grains and feed on the endosperm (Ahuchaogu & Ojiako, 2020). Feeding is done during the larval stage, the adults-only mate and oviposit (Nisar *et al.,* 2021). The age of adults is between 10 to 14 days, after which they will die (Beck & Blumer, 2011). *Callosobruchus maculatus* are able to complete their life cycle in several generations per year (Kebe *et al.,* 2020).

The estimated post-harvest losses caused by this insect to the pulses ranged from 30 to 40% within 6 months and when left unattended losses could be up to 100% (Mahendran & Mohan, 2002; Thein *et al.,* 2020). The pest management techniques in controlling this insect pest are generally done by using insecticide, fumigation (Phospine or Methyl bromide), or by radiation. However, these control methods are not environmentally friendly and are considered to have a negative impact on the environment and consumer health.

There is an increasing demand from grain buyers and consumers toward zero tolerance to contamination by insects in grains (Sarwar, 2012; Kaliramesh *et al.,* 2013). Owing to the negative impacts caused by previous control techniques, there is a need for environmentally friendly control measures for *C. maculatus* by using natural products. The efficacy of some natural products has been reported against *C. maculatus*, such as Neem (*Azadirachta indica),* Garlic (*Allium sativum)*, West African pepper (*Piper guineense),* Drum Stick (*Moringa oleifera)*, African Basil (*Ocimum gratissimum)*, Moss plant (*Barbula indica)* and *Clausena anisata* which is indicating to have potentials of controlling *C. maculatus* in the store (Muhammad *et al.,* 2017).

The use of garlic powder has significantly decreased egg laying, larval penetration and adult emergence as compared to turmeric powder (Sharma & Kaur, 2017). Otikai (*Alphitonia* sp.) leaf extract and pinang (*Areca catechu*) fruit extract also has the potential as natural insecticide material to control *Callosobruchus* sp. in the store (Gobaia *et al.,* 2015). While ginger oil, nutmeg oil, and clove oil have also affected the mortality of *C. maculatus* (Astuthi *et al.,* 2012). *Piper guineense* fruits, *Dennittia tripetala* fruits, *Allium sativum* bulbs, and *Zingiber officinale* rhizomes powders were also effective in controlling insect infestation by showing significantly higher mortality, reduction in the number of eggs laid, and suppression of the development and emergence of adult progenies from the treated mung bean seeds (Emeasor & Chukwu, 2019). Clove essential oil (*Syzygium aromaticum*) is toxic to *C. maculatus* (Bao *et al.,* 2015) followed by cinnamon (Cinnamomum sp), lengkuas (*Alpinia galanga*), citronella (*Cymbopogon nardus*), and kaffir lime (*Citrus hystrix*) essential oil (Thein *et al.,* 2020).

Eucalyptus (Family: Myrtaceae) is an Australian native, represented by around 700 species of tall, evergreen and magnificent trees cultivated around the world for their oil, pulp, timber, and medicine value. The essential oil found in its foliage possesses a broad spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal, and nematicidal (Daizy *et al.,* 2008).

Another species of Eucalyptus such as *Eucalyptus citriodora* containing essential oil (Citronellal - 64.7% and citronellol - 10.9%) has the potential to be used as bioherbicide and can constitute an alternative process of weed control (Benchaa et al., 2018). Eucalyptus essential oil has the potential as a repellent and antiovipositant against stored product insects of *Ephestia cautella* (Hasyim *et al.,* 2014). Nevertheless, there are only limited reports on the insecticidal activity of *S. aromaticum* and *E. citriodora* either in single or in combination against *C. maculatus*. Therefore, the objective of this study is to identify the main active ingredient of essential oils in *E. citriodora* and *S. aromaticum* and to evaluate the efficiency of these essential oils as insecticidal, antiovipositant, and fumigant against cowpea weevil *C. maculatus.*

Materials and Methods

Biological materials

The dried leaves of *E. citriodora* and *S. aromaticum* were obtained from the Manoko Experimental Station, Bogor Indonesia. Meanwhile, the colony of *C. maculatus was* reared on mung bean (*Vigna radiata*) seeds at Entomology Laboratory, ISMCRI. The insects were kept in the closed rearing box with ambient temperature at 28°C (±1ºC) and relative humidity of 68% (±5%) for further use.

Extraction and chemical analysis of the essential oil

Essential oils of *E. citriodora* and *S. aromaticum* were obtained from the distillation process of the leaves. The leaves of *E. citriodora* and *S. aromaticum* were harvested and dried indoors for 24 hours prior to distillation by steaming. The steam that came out flowed through the condenser and became liquid and was accommodated. The liquid consisted of both water and oil, with the oil floating on top and taken for further research. The active chemical content in the essential oil was analysed using Gas Chromatography (Agilent 6890 N) with an advanced electronic pneumatic control containing a Carbowax 20 M high polarity capillary column of 3 meters long and 0.25 mm in diameter. The temperature was programmed to rise to 60°C and then to 200°C at 3°C/ minute with injector and detector temperatures at 220°C and 250°C, respectively. The detector was a flame ionization detector and nitrogen was used as a carrier gas at a flow rate of 1ml/minute and the injection volume was 0.2 µl. The chemical constituents were identified by comparing their relative retention indices and mass spectra, with those published in the literature, and supplemented with NIST 1.7 and Wiley 7 gas chromatography/mass spectrometry libraries. The relative proportion of the essential oil constituent was computed in each case from the gas chromatography peak areas.

Insecticidal activities of *E. citriodora* and *S. aromaticum* essential oil on *C. maculatus*

A number of 100 mung bean seeds were sprayed with 0.2ml of 10 different essential oil treatments (Table 1) and then air-dried in the same place for approximately 10 minutes. After drying, 10 females were introduced to each treatment to observe their mortality rate on a daily basis for 4 days.

Oviposition deterrents and egg hatching

A plastic box (20 cm x 15 cm) was divided by paper into 12 similar segments. Each segment was filled with 20 mung beans that had been treated with 10 different treatments of essential oil (Table 1) while 2 segments in the middle were emptied for storing 20 pairs of *C. maculatus*. The number of eggs laid on the surface of the seeds was recorded after 4 days of application. Oviposition deterrent was calculated based on Meena and Gupta (2013).

Fumigant toxicity bioassay

The fumigant toxicity bioassay was done for 4 different treatments of essential oil (Table 2) using the fumigation testing tool consisting of a 170 cm^3 petri dish that was divided by screen cloth into lower and upper parts. 10 insects of *C. maculatus* were placed on the lower side and 0.1 ml of essential oil was dropped onto the cotton cloth on the upper side. The mortality rate of *C. maculatus* was recorded after 24 hours of incubation.

Statistical analysis

All experiments were arranged in a completely randomized design. The experiment for insecticide bioassay oviposition deterrents and egg hatching bioassay were done for 3 replications using 10 different treatments (Table 1). While 6 replications with 4 different treatments were designed for the experiment of fumigant toxicity bioassay (Table 2).

Results

Chemical constituents of *E. citriodora* and *S. aromaticum* essential oil

The yield of the essential oil from *E. citriodora* and *S. aromaticum* were 1.2% and 1.5% (v/w based on dry weight) respectively. A total of 43 components from the essential oil of *E. citriodora* were separated. Based on Gass Chromatography analysis, the main active ingredient of *E. citriodora* is Citronellal as much as 86.69% (Figure 1). Meanwhile, A total of 52 components from the essential oil of *S. aromaticum* were separated and the main active ingredient content was 81.04% Eugenol and 14.65% β caryophyllene (Figure 2).

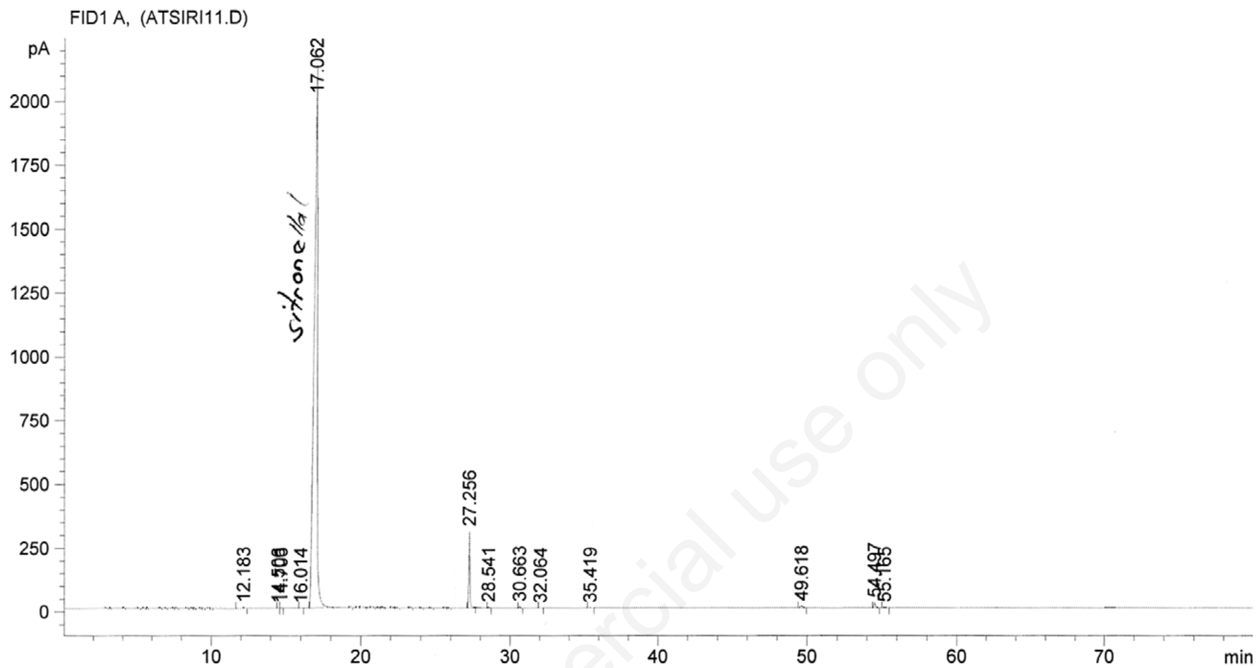

Figure 1. Retention time gas chromatogram (flame ionization detector) *E. citriodora*. FID, flame ionization detector.

Table 1. Treatment details for the experiment of insecticide bioassay and oviposition deterrents and egg hatching bioassay.

Treatment	Details
1	*E. citriodora* – 1% (10 ml EO/L water)
2	*E. citriodora* – 2% (20 ml EO/L water)
3	*E. citriodora* – 3% (30 ml EO/L water)
4	*S. aromaticum* – 1% (10 ml EO/L water)
5	*S. aromaticum* – 2% (20 ml/L water)
6	*S. aromaticum* – 3% (30 ml/L water)
7	*E. citriodora* + *S. aromaticum* (1 : 1) – 1% (10 ml/L water)
8	*E. citriodora* + *S. aromaticum* (1 : 1) – 2% (20 ml/L water)
9	*E. citriodora* + *S. aromaticum* (1 : 1) – 3% (30 ml/L water)
10	Control/water

Table 2. Treatment details for the experiment of fumigant toxicity bioassay.

Treatment	Details
1a	*E. citriodora oil* (0.1 ml/170 cm^3)
2a	*S. aromaticum oil* (0.1 ml/170 cm^3)
3a	*E. citriodora* + *S. aromaticum oil* (0.1 ml/170 cm^3, 1:1)
4a	Control/water (0.1 ml/170 cm^3)

Insecticide bioassay

The result showed that on the first day after application, there was no significant effect of *E. citriodora* and *S. aromaticum* essential oil on the mortality of the tested insect. On the second day after application, *S. aromaticum* essential oil at concentrations of 2% and 3% (Treatment 5 and 6) began to show its effect on insects as much as 13% to 16% of mortality (Table 3) and the results are significant compared to control. Other treatments have not yet shown their effect on the insect's mortality on day 2 after treatment.

All treatments showed mortality of *C. maculatus* after 3 days of application. *E. citriodora* essential oil at concentrations of 1%, 2%, and 3% (Treatment 1, 2, and 3) caused mortality to the tested insect as many as 16.6%, 23.3%, and 26.6% respectively. *S. aromaticum* essential oil at 2% and 3% (Treatment 5 and 6) showed higher and significant mortality rates compared to other treatments. On the fourth day after treatment, the results were significant for *S. aromaticum* essential oil at concentrations of 1%, 2%, and 3% (Treatment 4, 5, and 6) causing 43.3%, 50%, and 56.6% insect mortality respectively. Meanwhile, a combination of *E. citriodora* and *S. aromaticum* essential oil (Treatment 7, 8, and 9) showed lower results compared to the use of a single application of essential oil. Mortality rates of *C. maculatus* for Treatment 7, 8, and 9 showed not much difference and were not significant to the treatment with single usage of *E. citiriodora* essential oil (Treatment 1, 2, and 3).

Oviposition deterrent and egg-hatching

The different types and concentrations of essential oil oviposition deterrence activity in the females of *C. maculatus* are shown in Table 4. The oil's oviposition deterrence increased with increased concentrations of the oil. The results showed that the number of eggs laid by the females decreased as concentrations of

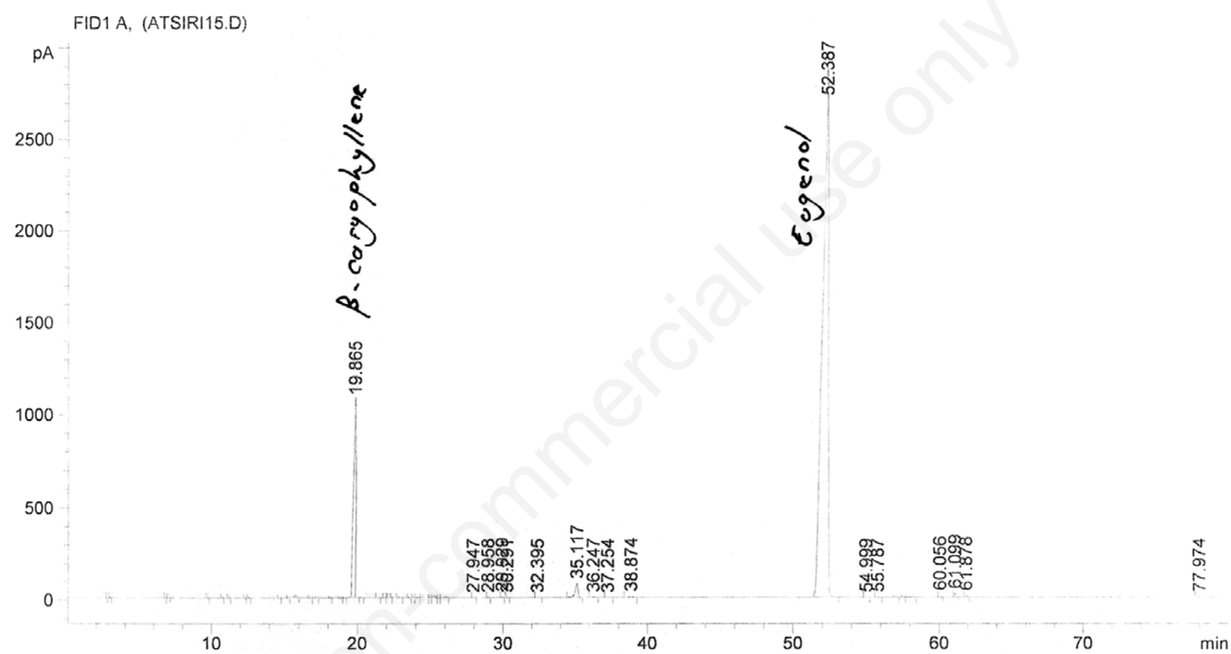

Figure 2. Retention time gas chromatogram (flame ionization detector) *S. aromaticum*. FID, flame ionization detector.

Table 3. Effect of *E. citriodora* and *S. aromaticum* essential oil on the mortality of *C. Maculatus*.

Treatment	Mortality rate (%)			
	Day 1	Day 2	Day 3	Day 4
1	0.0 a	3.3 a	16.6 b	32.6 b
2	0.0 a	6.3 a	23.3 c	30.0 b
3	0.0 a	6.6 a	26.6 c	33.3 b
4	0.0 a	10.0 ab	23.3 c	43.3 c
5	0.0 a	16.6 b	30.0 cd	50.0 cd
6	3.3 a	13.3 b	43.3 d	56.6 d
7	0.0 a	0.0 a	26.6 c	33.3 b
8	0.0 a	6.6 a	23.3 c	30.0 b
9	3.3 a	3.0 a	22.0 c	30.0 b
10	0.0 a	0.0 a	0.0 a	0.0 a

Numbers with the same letters in the same column are not significantly different at 5% Duncan's multiple range test.

the oil were increased. *S. aromaticum* essential oil had better oviposition deterrent ability compared to the use of single or in combination with *E. citriodora.* The oviposition deterrent ability of *S. aromaticum* was 46-54.9%, *E. citriodora* was 27-41% and the mixture of both essential oils was 35% to 42%.

Fumigant toxicity test

The data for fumigant toxicity was collected after 24 hours of application and presented in Table 5. The result showed that all treatments gave a significantly different fumigant toxicity ability. *E. citriodora* essential oil (Treatment 1a) gave the highest insect mortality (93.3%), followed by the combination of the two essential oils (Treatment 3a) with 76.6% insect mortality while *S. aromaticum* (Treatment 2a) showed the lowest mortality rates of 53.3%.

Discussion

Legumes are rich in proteins, vitamins, and minerals (Shevkani *et al.,* 2019). These characteristics attract insect attacks that are usually interested in seeds that contain carbohydrates and protein. The process of damage and loss of green beans stored in the warehouse by warehouse pests *C. maculatus* begins when insects lay eggs on the surface of the seeds. The shape and size of the seeds and the presence of certain substances on the surface of the seeds will influence insect pests to choose places for laying eggs, including avoiding them (Kaliramesh *et al.,* 2013). The presence of *S. aromaticum* and *E. citriodora* essential oil on the surface of mung bean seeds has significantly decreased the oviposition of the insect on the surface of mungbean. This is in line with Bao *et al.* (2015) statement that clove oil (*Syzygium aromaticum*) was toxic to *C. maculatus*. While Eucalyptus essential oil has the potential as an insecticidal repellent and antiovipositant against stored product insect (Daizy *et al*., 2008; Hasyim *et al.,* 2014).

The results from the insecticide bioassay showed that *S. aromaticum* has significantly killed an average of 56% and 59% more insects compared to the use of *E. citriodora* alone or in combination with *S. aromaticum* respectively after 4 days of treatment. It was the same as in oviposition deterrent ability assay where *S. aromaticum* showed an average of 43% and 26% respectively higher results compared to *E. citriodora* alone or in combination with *S. aromaticum*. The number of eggs hatching also decreased as the concentration of every essential oil increased either alone or in a combination. However, the result from fumigant toxicity showed that *E. citriodora* gave significantly 22% and 75% higher mortality rates respectively compared to the use of *S. aromaticum* either alone or in combination with *E. citriodora*. Thus, the results from the three bioassays showed that there are no synergism effects between *S. aromaticum* and *E. citriodora* essential oils against *C. maculatus* attacks on mung beans.

A combination of essential oils usually will produce synergistic effects. However, it depends on a vigorous set of criteria including the dose of the oils and the complexity of chemical molecules each of which can act on different targets (Kachkoul *et al.,* 2021). The mixture of essential oils compounds does not necessarily always synergism because antagonism can occur as well as potentiation both situations can exist depending on the dose, application and properties of the oils (Harris, 2002). The major and minor components of essential oil could also be the factor that affects their effectiveness such as in the case of the antimicrobial property of Eucalyptus species where the major components are often relatively inactive but the minor components that actually the main synergists (Zakaraya *et al.,* 1993). These factors may be contributing to the results obtained in this study where there is no synergism effect recorded for the combination of *E. citriodora* and *S. aromaticum* on insecticidal, oviposition, and fumigant effects on *C. maculatus*.

Many essential oils extracted from different plant spices have

Table 4. Effect of *E. citriodora* and *S. aromaticum* essential oil as oviposition deterrent.

Treatment	Number of eggs laid	Oviposition deterrent (%)
1	44.0 c	27.4 d
2	37.3 bc	38.4 bc
3	35.6 b	41.2 b
4	32.6 ab	46.2 ab
5	31.0 ab	48.8 ab
6	27.3 a	54.9 a
7	39.3 bc	35.1 cd
8	35.0 b	42.2 b
9	35.0 b	42.2 b
10	60.6 d	0 e

Numbers with the same letters in the same column are not significantly different at 5% Duncan's multiple range test.

Table 5. Effect of *E. citriodora* and *S. aromaticum* essential oil as fumigant against *C. maculatus* after 24 hours of treatment.

Treatment	Mortality of insect (%)
1a	93.3 a
2a	53.3 c
3a	76.6 b
4a	3.3 d

Numbers with the same letters in the same column are not significantly different at 5% Duncan's multiple range test.

already been screened for toxicity as potential insecticides against *C. maculatus*. Essential oils are generally broad spectrum due to the presence of several active ingredients that may operate through various modes of action. Eugenol and β-caryophyllene are two major chemical constituents found in the essential oil of clove oil with varying percentages (Selles *et al.,* 2020). The percentage of eugenol and β-caryophyllene in this study is in line with previous studies done by other researchers. Eugenol is the phenolic compound and accounts for about 70-90% of the oil (Barceloux, 2008). It is responsible for the characteristics of clove aroma and taste. Eugenol has been found to have antioxidant, antimicrobial, anti-inflammatory, anticancer, and insecticide activity (Khalil *et al*. 2017; Gonzalez *et al.,* 2021). Meanwhile, β-caryophyllene is a sesquiterpene class of terpenoids and is present in clove oil at a concentration of about 12-17% (Nurdjannah & Bermawie, 2012). It has a spicy aroma and is commonly found as a major constituent of cloves, rosemary, cannabis, and more (Hartsel *et al.,* 2016).

Eugenol and β-caryophyllene have been found to have insecticide and repellent properties. Several studies have investigated the effectiveness of eugenol and β-caryophyllene as natural insect repellents against a variety of pests such as *Spodoptera littoralis* and cotton aphids (Tuni & Sahinkaya, 1998; Najla *et al.,* 2013). The yield for *S. aromaticum* essential oil in this study is lowered compared to other studies because generally essential oil extraction by water distillation yields 2% to 3% volatile oils (Khan & Abourashed 2010). This might be due to the chemical compositions of clove oils that vary due to the differences in plant growing conditions, genetic traits, plant parts used, and extraction methods (Alma *et al.,* 2007). While eugenol and β-caryophyllene have shown potential as natural insect repellents, it is important to note that their effectiveness may vary depending on the type of insect, variability, and the concentration of the active substances in that oil (Baker *et al.,* 2018).

Meanwhile, *E. citriodora* essential oil also known as lemon eucalyptus oil, has a variety of applications due to its pleasant scent and potential health benefits. Other than aromatherapy, it also has insecticidal properties because of its strong antifungal, antimicrobial, antiacetylcholinesterase, an antioxidant property that can be used as a successful spray deterrent against pests (Khan *et al.,* 2017). The main compound of essential oil is the monoterpenoid citronellal with 60-80% concentration (Hasegawa *et al.,* 2008). Thus, this study has identified a high citronellal compound (86.69%) in *E. citriodora* compared to other research.

Conclusions

The essential oils from *S. aromaticum* and *E. citriodora* in this study are toxic to *C. maculatus* causing significantly high mortality rates, high oviposition deterrent as well as a reduction in the number of eggs hatching and high fumigation rates at the concentration of 1% to 3%. Higher concentrations of essential oils caused higher toxic effects on *C. maculatus* and no synergism between *S. aromaticum* essential oil and *E. citriodora* essential oil. The essential oil investigated in this study is a natural compound that is commonly known and used in the flavoring and pharmaceutical industry and is therefore considered less harmful to humans and non-target organisms than most conventional pesticides. However, further study is needed to evaluate the other compounds and potential of the essential oils in *E. citriodora* and *S. aromaticum* to optimize their insecticidal effects and their usage as an effective safe insecticide in storage systems.

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