

Toxicology study of *Eucalyptus citriodora* on the insect *Doleschallia bisaltidae*

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Abstract. Toxicological Study of *Eucalyptus citriodora* on *Doleschallia bisaltidae* as main pest of *Graptophyllum pictum*, was carried out at the Entomology laboratory, ISMECRI, Bogor from January to March 2021. The research consisted of toxicology study of *E. citriodora* and larval survival rate of *D. bisaltidae*. The toxicology study was carried by direct spray to 3rd instar larvae (contact) and spraying the leaves (residual). The study was designed in a completely randomized design with 3 replications and 6 treatments consisting of *Eucalyptus* oil concentration: (1) 20%; (2) 10%; (3) 5%; (4) 2.5%; (5) 1.25% and (6) control/water determined through a ranging test first. Contact application: thirty larvae were sprayed with each treatment, then placed in a plastic box where ten larva each then fed with fresh *Graptophyllum* leaves. Residual application: five treated *Graptophyllum* leaves were placed in a plastic box, then filled with ten larvae each. Observations were carried out every day. The results showed that *Eucalyptus* LC50 value by contact was 0.0584 ($Y = 8.39 + 2.75X$), more toxic than that applied residually with an LC50 value of 3.9487 ($Y = 4.51 + 0.81X$). The survival rate of larvae to imago was higher in the residual treatment compared to the contact.

Keywords: *Doleschallia*, essential oil, *eucalyptus*, pest entomology, toxicity

1. Introduction

Caricature plant (*Graptophyllum pictum*) has long been known as a medicinal plant that is often used by the public. This plant is usually propagated by stem cuttings, and is known to function as anti-bacterial, antioxidant, facilitate menstruation, can lower cholesterol levels, and has even been proven to be anti fungal in acrylic resin denture plates [1,2,3]. There are several pests that attack this plant, including *Doleschallia* spp., plant lice, and thrips [4]. In Indonesia, there are three types of *Doleschallia* spp. that attack this caricature plant, namely *Doleschallia bisaltidae* in West Java and South Sumatera, *D. nacar* in Maluku, and *D. hexophthalmos* in Papua [5,6]. The intensity of *Doleschallia* attacks on *G. pictum* in West Java, Maluku, and Papua was once recorded at 17.9%, 28%, and 1.9%, respectively [5]. This pretty high intensity of attack coupled with high reproductive ability makes *Doleschallia* a main pest of *G. pictum*. A study has shown that it has the highest larval

population in May and is positively correlated with rainfall [7]. Although several types of predators, parasitoids, and entomopathogenic fungi have been recorded [8,9], other methods are still needed to control this pest, especially by using bio pesticides.

The *Doleschallia* control with chemical insecticides cannot be carried out specifically on this medicinal plant, because it endangers the user. The negative effects of chemical pesticides, especially on human health and the environment (including non-target organisms), are some of the reasons why the use of this chemical pesticide is highly avoided [10]. As a type of plant compound, essential oil has the potential to be used as a bio pesticide because it is safe and biodegradable so that it can provide residue-free food ingredients [11,12]. When used as an insecticide, essential oils usually have insecticidal and insect repellent effects [13].

Eucalyptus is currently one of the essential oils that is often used as an insecticide [14,15]. One type of eucalyptus, *Eucalyptus citriodora*, has been tested several times and proven to be quite effective against several types of insects, namely *Anopheles gambiae* [16], *Aedes aegypti* [17], *Tribolium castaneum* [18], *Myzus persicae* [19], *Frankliniella schultzei* [19], *Plutella xylostella* [20], *Sitophilus oryzae* [21], *Lutzomyia longipalpis* [22], termites [23], and *Periplaneta americana* [24]. However, currently not much information has been found regarding the testing of *E. citriodora* against *D. bisaltidae*, so that in this study it will be studied further.

2. Materials and Methods

Toxicological Study of *E. citriodora* on the insect *D. bisaltidae* which is the main pest of the medicinal crop *G. pictum* was carried out at the Entomology laboratory, Indonesia Spices and Medicinal Crops Research Institute, Bogor from January to March 2021 which is consisted of (1) toxicology study of *E. citriodora* and (2) larval survival rate of *D. bisaltidae* (to pupa and imago). Eucalyptus application is carried out in two ways, namely direct spray to the 3rd instar larvae (contact) and spraying the leaves (foliar spray residue). The study was designed in a completely randomized design with 3 replications and 6 treatments consisting of Eucalyptus oil concentration: (1) 20%; (2) 10%; (3) 5%; (4) 2.5%; (5) 1.25% and (6) control/water determined through a ranging test first. This study used plastic boxes measuring 10 cm x 7 cm x 5 cm. Contact application: thirty larvae were sprayed with each treatment, then placed in a plastic box where ten larva each then fed with fresh *Graptophyllum* leaves. Residual application: five leaves of *Graptophyllum* that had been treated were placed in a plastic box, then filled with ten larvae each. If the treated leaves were eaten by the larvae, they were replaced with fresh leaves. Observations of mortality and larval development were carried out every day.

3. Results and discussion

A study was conducted to determine the toxicity of *E. citriodora* to *D. bisaltidae* by calculating the mortality caused by direct spraying and residual treatment where the surviving larvae were then seen for their survival rate. The results of the study are shown as follows:

3.1. Effect of Eucalyptus on the mortality of *D. bisaltidae* applied by contact (direct spray)

From the first day, it was seen that 20% eucalyptus treatment gave a significant mortality value. On the second day of observation, all concentrations began to have a significant effect on the mortality test, when compared to the control. On the third day, eucalyptus treatment at 20% concentration resulted in 100% mortality, followed by concentrations of 10% and 5% which resulted in 80% and 46.6% mortality, respectively (Table 1).

Table 1. Effect of Eucalyptus on the mortality of *D. bisaltidae* in contact treatment

Treatments (<i>E. citriodora</i>)	Mortality (%) of larvae at the day of		
	1 st	2 nd	3 rd
20 %	33.3 a	93.3 a	100 a
10%	6.6 b	80.0 b	80.0 b
5%	6.6 b	33.3 c	46.6 c
2,5%	6.6 b	13.3 d	13.3 d
1.25%	0 b	6.6 de	6.6 de
0% (control)	0 b	0 e	0 e

Notes: Numbers followed by the same letter at the same column are not significantly different at 5% DMRT

3.2. Effect of Eucalyptus on the survival rate of insect applied by contact (Direct spray)

At 20% Eucalyptus concentration, no larvae can survive become pupa or imago, since in this treatment the larvae were 100% dead. Furthermore, at concentration of 10% to control, the number of larvae that successfully became pupae ranged from 20% to 80% and those that succeeded in becoming imago ranged from 13% to 73.3%. This is in line with the number of larvae that died previously as a result of the treatment. From this data, it can be seen that the survival larvae may develop (80%) into pupae and then into imago (66.6%) just like in control treatment (Table 2).

Table 2. Survival rate of larvae after treated by Eucalyptus by contact treatment

Treatments (<i>E. citriodora</i>)	Survival rate of larvae		
	Larvae	Pupa	Adult
20 %	30	0 (0%)	0 (0%)
10%	30	6 (20%)	4 (13.3%)
5%	30	10 (33.3%)	8 (26.6%)
2,5%	30	20 (66.6%)	20 (66.6%)
1.25%	30	20 (66.6%)	20 (66.6%)
0% (control)	30	24 (80%)	22 (73.3%)

3.3. Effect of Eucalyptus on the mortality of insect applied by foliar spray residue

Application through foliar spray residue showed lower mortality compared to direct application. On the first day after application, it can be seen that the larval mortality rate at a concentration of 20% is only 13.3%, while at the lower concentrations showed no significant difference with the control. On the second day observation, there was a slight increase in mortality, especially at concentrations of 20% and 10%, while at concentrations below it did not show the significantly difference with the control and on the third day after application it was seen that only the treatment with concentrations of 5% and above gave a higher mortality rate significantly different from the control. The highest mortality rate achieved at 20% concentration was only 66.6%, followed by 10% and 5% concentrations which resulted in 33.3% and 20% mortality, respectively (Table 3).

Table 3. Effect of Eucalyptus on the mortality of *D. bisaltidae* in residual treatment

Treatments (<i>E. citriodora</i>)	Mortality (%) of larvae at the day of		
	1 st	2 nd	3 rd
20 %	13.3 a	33.3 a	66.6 a
10%	6.6 b	13.3 ab	33.3 b
5%	0 b	6.6 bc	20.0 c
2,5%	0 b	0 c	6.6 d
1.25%	0 b	0 c	0 d
0% (control)	0 b	0 c	0 d

Notes: Numbers followed by the same letter at the same column are not significantly different at 5% DMRT

3.4. Effect of Eucalyptus on the survival rate of insect applied by foliar spray residue

In line with the mortality rate, the success of larvae into pupae achieved by a concentration of 20% was 30%, followed by a concentration of 10%; 5%; 2.5%; and 1.25% by 63.3%; 73.3%; 86.6% and 93.3% respectively (Table 4), while those that successfully become imago from a concentration of 20% to 1.25% were 23.3% to 83.3%.

Table 4. Survival rate of larvae after treated by Eucalyptus by residual treatment

Treatments (<i>E. citriodora</i>)	Survival rate of larvae		
	Larvae	Pupa	Adult
20 %	30	9 (30%)	7 (23.3%)
10%	30	19 (63.3%)	15 (50%)
5%	30	22 (73.3%)	19 (63.3%)
2,5%	30	26 (86.6%)	22 (73.3%)
1.25%	30	28 (93.3%)	25 (83.3%)
0% (control)	30	27 (90%)	25 (83.3%)

From the test results above, especially the contact test, it can be seen that *E. citriodora* is toxic to *D. bisaltidae*. *E. citriodora* is known as one of the richest and most economical sources of citronellal. And as main constituent, citronellal is usually present in varying concentrations depending on the location of the plant [25]. Several previous studies mentioned some variations in citronellal concentrations ranging from 29.3% [19], 52.8% [16], 63.9% [26], 64.7% [27], 78.6% [17] to 83.95% [28]. In addition to citronellal, citronellol is also a fairly major compound found in *E. citriodora* where the concentration also varies from 5.24% [26], 10.9% [27], 12.1% [17], to 20% [16]. Previous studies that have been carried out on *Ascia monuste* and *P. xylostella* with the separate compounds in *E. citriodora* showed that citronellal and citronellol compounds were the most toxic compounds, where the other compounds presented lower toxicity with similar doses [20]. There are even studies that tested the compounds contained in *Geranium maculatum* against *Pediculus humanus capitis*, where even though it is often noted that complex mixtures of monoterpenoids are significantly more efficacious than the pure compound, but results show that there is the greatest reduction in toxicity when citronellol is removed [29]. This shows that citronellal and citronellol contained in *E. citriodora* are one of the main factors that support its toxicity. This is in accordance with many previous studies that have been carried out. Citronellal and citronellol which are also contained in several other plants such as *Cymbopogon nardus* and *C. winterianus* have also been shown to be toxic to other insect pests such as *Bemisia tabacci* and *S. oryzae* [30,31,32]. Both of these compounds are classified as monoterpenoid compounds which are lipophilic and have high volatility. This allows the compounds to penetrate the insect's skin more quickly so that in the end it can affect the physiological function (disrupts the metabolism of the insect and deters feeding) of insects [33,34].

However, the eucalyptus effect on mortality of *D. bisaltide* in the residual treatment was much lower than in the contact treatment. This shows that although *E. citriodora* has toxic properties, the effect will be lower when applied through feed. There are several possibilities regarding the factors that support this occurrence, including its volatile nature and the presence of a repellent effect on *E. citriodora*. As one of the major components of *E. citriodora*, citronella is known to have fast evaporation and repellent properties [34]. Several studies have shown *E. citriodora* to have a repellent effect against some insects such as *Zabrotes subfasciatus* [35], *S. zeamais* [36], and *Prostephanus truncatus* [37]. When *E. citriodora* essential oil was sprayed onto the leaves of *G. pictum*, a small part of the essential oil would evaporated while the rest would still have the repellent properties to the *D. bisaltidae*. That made the leaves which eaten by *D. bisaltidae* might be eaten longer than they should be. Thus resulted in the amount of *E. citriodora* toxin that entering the body of *D. bisaltidae* at the same time would not as high as in the contact treatment. This of course resulted in a significant reduction in the mortality percentage. These results prove that one of the main weakness essential oil as a natural insect repellent is its volatile nature, which reduces effectiveness in controlling the pest. But in the other hand, it also proves that it is safer for humans and the environment.

The results of the survival rate of larvae after being given contact and residual treatment with *E. citriodora* showed that in this study there was no significant difference between the control and treatment. Larvae that did not die from treatment can mostly develop and became imago. There are some studies on *Z. subfasciatus* and *Spodoptera frugiperda* with results showing that *E. citriodora* has an IGR (Insect Growth Regulator) effect and can reduce the number of emerged insects [35,38]. However these things do not apply in this test. As we know that there are three basic principles and relationships of toxicology, namely dose-response, hazard - exposure, and individual sensitivity. Each species has its own level of sensitivity to toxins and usually, the larger the insect's body size and weight, the lower its sensitivity to toxins [39,40,41,42]. In this regard, *D. bisaltidae* has a larger body size and weight than *S. frugiperda* and *Z. subfasciatus*, so this may be one reason the development of *D. bisaltidae* is less affected. These results also prove that for this test with *D. bisaltide*, *E. citriodora* has a reversible effect. This reversible effect can occur when the toxin exposure is stopped and the remaining toxins in the body can be immediately metabolized/neutralized so that it can recover and return to normal function and structure [42,43].

3.5. Toxicity of Eucalyptus against *D. bisaltidae* Insect

LC50 value by contact application (0.0584) is more toxic compared to by foliar spray residue (3.9487) (Table 5 and Figure 1). It is indicated that eucalyptus essential oil with its active ingredient of citronella is as a contact poison. These results are quite in line with studies which showed LC50 values for *Tribolium castaneum* were 6.9% (24 hours), 5.1% (48 hours), and 4.5% (72 hours) for contact treatment [18].

Table 5. LC50 value of *E. citriodora* on *D. bisaltidae*

Application method	LC50 value	a (intercept)	b (slope)
Contact	0.0584	8.3957	2.7531
Residual	3.9487	4.514	0.8149

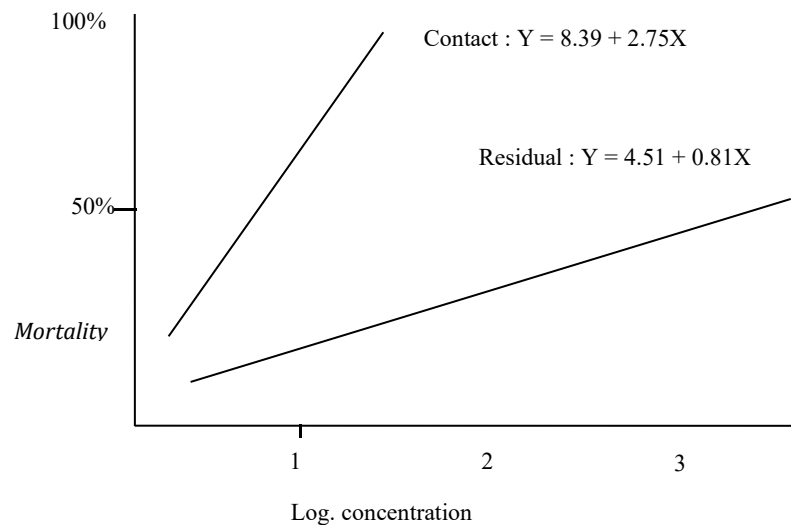


Figure 1. Toxicity of Eucalyptus against *D. bisaltidae* insect by residual and contact treatment

4. Conclusion

From the test results above, it can be seen that *E. citridora* is toxic to *D. bisaltidae*, especially by contact treatment. Eucalyptus LC₅₀ value at the third day after application by contact was 0.0584 ($Y = 8.39 + 2.75X$) whereas in residual treatment the LC₅₀ value was 3.9487 ($Y = 4.51 + 0.81X$). The survival rate of larvae to imago was greater in the foliar spray residue application (23% – 83%) compared to the contact application (0% – 73%).

5. Acknowledgments

We would like to express our special thanks to our technical staff, Nurbetti Tarigan and Galih Perkasa, for the support during this research.

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