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ORIGINAL ARTICLE

Oviposition deterrent and ovicidal properties of *Calotropis gigantea* (L.) leaf extract to *Paraeucosmetus pallicornis* (Dallas) in rice

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Abstract

Paraeucosmetus pallicornis (Dallas) is a new pest in Indonesia which decreases rice production and quality. This pest causes the grains to become flat, hollow, brownish, break easily when milled, and bitter. This research represents the first study on *Calotropis gigantea* extract as an oviposition deterrent and ovicide against *P. pallicornis*. The study was conducted under laboratory conditions using four extract concentrations i.e. 0.5, 1.0, 1.5 and 2.0%. The oviposition deterrent effect was determined by counting the number of eggs laid and hatched. Percent reproductive control (PRC) and ovicidal activity was calculated using Tennyson's formula. Results indicated that all concentrations of *C. gigantea* leaf extract reduced the number of eggs laid and hatched. The PRC also showed a gradual reduction of oviposition of the *P. pallicornis* and the ovicidal activity ranged between 86.5 and 100%. The extract concentration which showed the highest potential as an oviposition deterrent and ovicide against *P. pallicornis* was in the range of 1.0–2.0%. The overall results indicated that *C. gigantea* leaf extract has the potential to be used as an oviposition deterrent and ovicide against *P. pallicornis*.

Key words: *Calotropis gigantea*, leaf extract, ovicidal, oviposition, *Paraeucosmetus pallicornis*

Introduction

Paraeucosmetus pallicornis (Dallas) (Hemiptera: Lygaeidae) also known as black ladybug, is a new rice pest in Indonesia and notably in Sulawesi. This pest infests rice from the seedling stage until harvest. Rice grains from plants which have been attacked by this pest become flat, hollow, brownish, crumbly, and bitter. It also causes chlorosis symptoms or causes the plants' leaves to dry (Rosmana *et al.* 2014). Nowadays, this pest has also been found on newly harvested grain in storage.

Presently, the only way to control *P. pallicornis* is through the use of synthetic pesticides. The use of synthetic pesticides among farmers tends to be excessive and in the long run can have negative impacts such as environmental pollution, pest resistance, secondary pest population explosion, as well as on human health. Therefore, alternative and environmentally friendly methods are needed to control *P. pallicornis* such as using plant extracts.

Calotropis gigantea (L.) is a wasteland weed, better known as milkweed. This weed is widely distributed in sub-tropical and tropical countries (Khondkar *et al.* 2010) which include India, Indonesia, Malaysia, Philippines, Thailand, Sri Lanka and China (Kumar 2010). *Calotropis gigantea* has been reported for its anti-*Candida*, cytotoxic, antipyretic, and wound healing activities. Recently, another species of genus *Calotropis* (*C. procera*) has also been shown to be a potential biological control of some pests (Bakavathiappan *et al.* 2015). *Calotropis gigantea* and

C. procera are reported to have similar pharmacological characteristics (Krishnan 2010).

Plant extracts have been reported to be effective in controlling pests because they contain secondary metabolites that can have toxic effects, be feeding deterrents, and growth inhibitors. In addition, secondary metabolites from plant extracts have also been found to exhibit repellent, ovicidal, larvicidal, and sterility properties on pests (Islam 2010). *Calotropis gigantea* has also been reported to contain toxins such as cardenolides, cardiac glycosides, flavonoids, and other cytotoxins effective against pests (Prabhu *et al.* 2012). The effectiveness of *C. gigantea* extract in controlling *P. pallicornis* was studied by observing its ovicidal effects on the number of eggs laid and hatched. Therefore, the objective of this research was to analyse the effects of *C. gigantea* leaves on *P. pallicornis.*

Materials and Methods

Preparation of extracts and their concentrations

Leaves of *C. gigantea* were collected from areas in and around Takalar Regency of South Sulawesi, Indonesia and shade-dried for 2–3 days, macerated with methanol solvent for 7 days and filtered through filter paper. The solvent was removed by evaporation on a water bath for 6–8 h to obtain crude extract in the form of paste. It was then placed in a reagent bottle, and stored in the refrigerator as a stock solution prior to its use. Four different concentrations: 0.5, 1.0, 1.5 and 2.0% of the extract were prepared by diluting the stock solution with acetone.

Rearing the *Paraeucosmetus pallicornis*

Paraeucosmetus pallicornis were collected from rice plants in Bantaeng Regency of South Sulawesi for the insects to be raised in a laboratory. The insects were maintained in rice plants and reared in the insectarium at laboratory under controlled conditions of 26±1°C, 70±5% relative humidity (RH). Male and female adult insects were selected from a colony reared in the laboratory and placed in a cage with rice plants. The adults were allowed to grow until the F1 generation for testing.

Oviposition deterrent

The different crude extracts were sprayed on rice plants growing in cages. Pairs of new adults were introduced for four replication in each concentration in different cages containing rice plants. The impact of the oviposition deterrent under study is indicated by the number of eggs laid. Eggs were observed on separate Petri dishes until hatching. The percent reproductive control (PRC) was calculated following Rizvi *et al.* (1980):

$$\mathrm{PRC} = \frac{\text{The number of eggs laid by control} - \text{The number of eggs laid by treated}}{\text{The number of eggs laid by control}} \times 100.$$

Ovicidal effect

The egg mass was collected from the eggs laid by female in the laboratory rearing and observed for ovicidal activity. The ovicidal activity of plant extracts for each was tested on the *P. palliornis* eggs with the residual method. The extracts were transferred to Petri dishes (0.4 ml) and 10 eggs/replication (40 eggs) were then placed on other Petri dishes. The numbers of eggs hatched in control and treatments were recorded. The percentage of ovicidal activity (POA) was calculated using Tennyson's formula as follows:

$$\mathrm{POA} = \frac{\text{The number of eggs hatched by control} - \text{The number of eggs hatched by treated}}{\text{The number of eggs hatched by control}} \times 100\,.$$

The ovicidal effect of *C. gigantea* extract is determined by grouping the results of calculation into the following categories (Arivoli *et al.* 2013):

– no ovicidal activity;
+ ovicidal activity below 25% (low);
++ ovicidal activity between 25–50% (middle);
+++ ovicidal activity between 50–75% (high);
++++ ovicidal activity above 75% (very high).

The percentage of eggs hatched was calculated by formula:

$$\text{Eggs hatched} = \frac{\text{The number of eggs hatched}}{\text{The number of eggs laid}} \times 100.$$

Statistical analysis

Statistical analysis was carried out for the number of eggs laid and hatched using the statistical software SPSS for Windows version 10.0.1 to calculate the means, standard errors and standard deviations. One-way analysis of variance (ANNOVA) was applied to the data to determine differences. To check significant differences between the levels of the mean factor with Tukey's multiple comparison test were α = 0.05.

Results and Discussion

The results of the study indicated that all of the *C. gigantea* leaf extracts acted as oviposition deterrents against *P. pallicornis* by reducing the number of eggs laid and their hatching compared to the control and

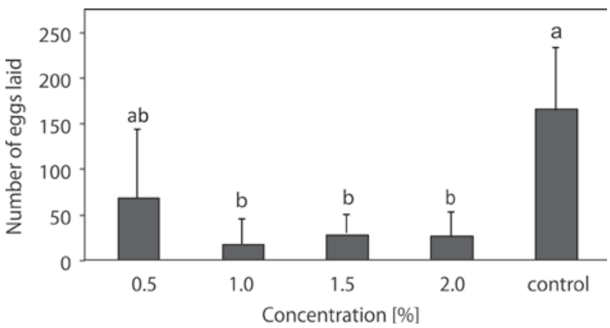

Fig. 1. The impact of different concentrations of *Calotropis gigantea* leaf extract on the number of *Paraeucosmetus pallicornis* eggs laid . Means with the same letter are not significointly different according to Tukey's multiple range test at p = 0.05

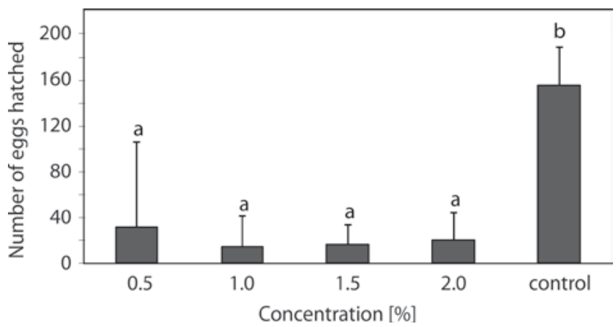

Fig. 2. The impact of different concentrations of C*alotropis gigantea* leaf extract on the number of *Paraeucosmetus pallicornis* eggs hatched. Means with the same letter are not significointly different according to Tukey's multiple range test at p = 0.05

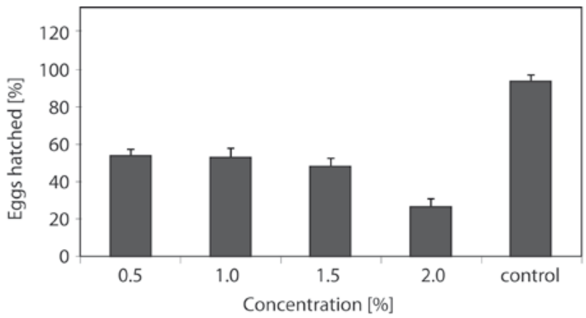

Fig. 3. Percent of eggs hatched of each concentration of *Calotropis gigantea* leaf extract

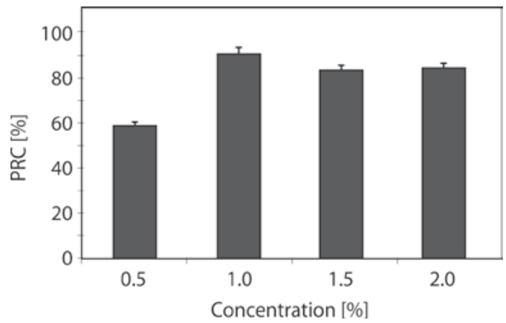

Fig. 4. Percent Reproductive Control (PRC) of *Calotropis gigantea* leaf extract at different concentrations

significantly different (Figs. 1–3). All exhibited high oviposition deterrent effects against *P. pallicornis*. The lowest number of eggs laid at 1.0, 1.5, and 2.0% respectively. The percent reproductive control (PRC) also showed a gradual reduction in oviposition of the *P. pallicornis*. The highest values of 1.0, 1.5 and 2.0% concentrations of C. *gigantea* were 84.15–90.2%, respectively. For 0.5% concentrations it was only 58.8% (Fig. 4). All these concentrations showed positive values, so that it can be concluded that *C. gigantea* is an effective oviposition deterrent against *P. pallicornis*. Values of PRC indicated that the greater the PRC value the less the number of eggs laid and eggs hatched.

Calotropis gigantea leaf extract with the highest ovicidal activity of 100% was seen at 1.0, 1.5 and 2.0% concentrations, while with 0.5% concentration it only reached 86.51% (very high ovicidal activity; Table 1). Data indicates that *C. gigantea* leaf extract has potential to be ovicidal against *P. pallicornis* with an effectivity greater above 75%. *Calotropis gigantea* leaf extract can be an oviposition deterrent and have an ovicidal effect because it contains some inhibitory compounds i.e. phenolic, flavonoids, alkaloids, tannin, saponin, glycosides, steroid and phytosterol (Kumar *et al.* 2012; Gouri *et al***.** 2014)**.** *Calotropis gigantea* has ovicidal effects against *Helicoverpa armigera* eggs and inhibits egg hatching up to 100% (Prabhu 2012). A chloroform extract of *C. gigantea* exhibited the best larvicidal activity and was the best anti-feedant against the *Spodoptera litura* (Bakavathiappan 2012). The activity was directly proportional to the concentration of the extract.

Inhibition of egg hatching was influenced by a flavanoid compound. It inhibits the activity of some enzymes i.e. protease, lipase and chitinase (Botura *et al.* 2013). *Calotropis gigantea* contains α-amyrin acetate that can cause the ovicidal effect on insects (Kappusamy and Murugan 2012). In addition, *C. gigantea* also contains saponins steroidal compounds in large quantities (Seniya *et al.* 2011) which is probably caused by egg hatching's inhibition. In general, plant extract steroid compounds will inhibit protein by blocking sterol carrier protein, a protein needed for insects' development (Kumar *et al.* 2012). Protein is needed to produce an adult female insect juvenile hormone which is used for the development and maturation of ovarian eggs (Genc 2006). As a consequence of the lack of protein insect morphology becomes abnormal. Morphologically abnormal eggs are found in *P. pallicornis* insects treated with *C. gigantea* leaf extracts.

For the control, normal eggs of *P. pallicornis* are egg-shaped, elongated, oval and slippery (Fig. 5A). Abnormal *P. pallicornis* eggs resulting from treatment are torn, crushed, brown, flat, and hollow (Fig. 5B). Such morphological changes of eggs can be caused by saponin compounds of *C. gigantea*. They may lead to the destruction of the eggs and cause the egg shell

Table 1. Ovicidal effect of *Calotropis gigantea* leaf extract against *Paraeucosmetus pallicornis*

Plant extract	Concentration [%]	Ovicidal [%]	Ovicidal effect
C. gigantea	2.0	100	++++
	1.5	100	++++
	1.0	100	++++
	0.5	86.51	++++

++++ Ovicidal activity above 75%

chorion to be torn or destroyed. Saponin can also degrade a protein which is one constituent of egg chorion. It causes destabilization (unstable) and increases membrane permeability (Francis *et al*. 2002). Saponins also act as an astringent since they cause biological tissue to contract or shrink. Compounds such as soap due to saponins, can act as surface-active agents, which reduce the surface tension and damage the cell wall. As a result the cell membranes have no protector and there is a loss of cell membrane permeability (Astuthi *et al.* 2011).

Calotropis gigantea also contains steroid compounds (β-sitosterolacetate) that can cause growth inhibition (anti-juvenile hormone), by suppressing embryonic eggs' development (Islam *et al.* 2012). Steroids of the class terpenoida affect insects'growth and development (Manitto 1981). In general, steroid compounds of plant extracts will inhibit protein by blocking the sterol carrier protein (Kumar *et al*. 2012), leading to abnormal insect morphology. In 1970 it was found that steroids also cause abnormal development of the oocyte by blocking the oocyte reducing egg yolk contents (Veeravel and Manikantan 2007).

Morphologically abnormal eggs and ovicidal effects could also be caused by the penetration of chemical compounds into the chorion of eggs. Similar results were reported that the surfaces of eggs treated with mahlab *Prunus mahaleb* L. may allow the chemicals to penetrate the chorion of eggs through the micropyle. This would result in the mortality of the embryo or affect the respiratory activity by the formation of a granular protrusion on the chorionic surface that blocks aeropyles and causes respiratory impairments, probably affect metabolism and consequently other systems that would lead to egg mortality (Hala *et al*. 2016).

Other compounds contained by *C. gigantea* include asmudarin, anthocyanins, calactin, calotropin, 18, 20-epoxy-cardenolides, non-protein amino acid, protease

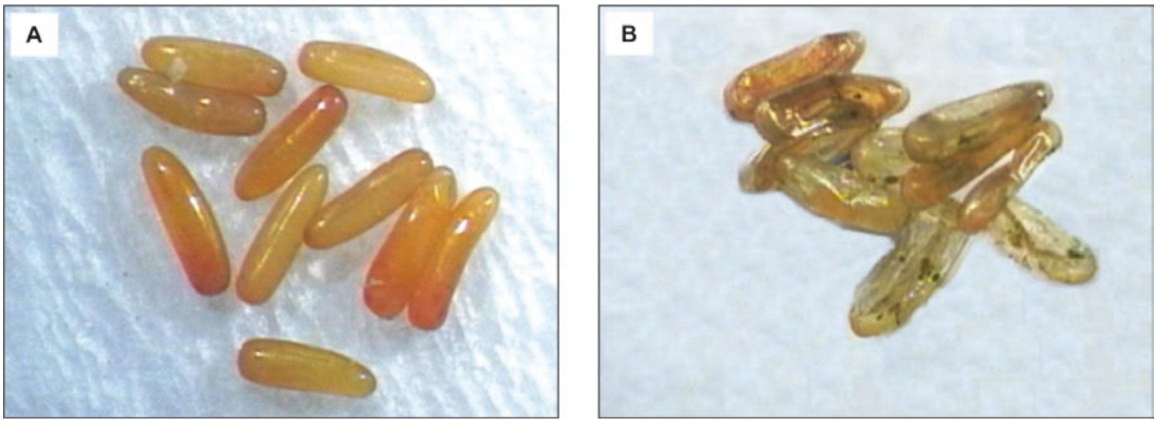

Fig. 5. Eggs of *Paraeucosmetus pallicornis:* untreated (A) and treated with *Calotropis gigantea* leaf extract (B)

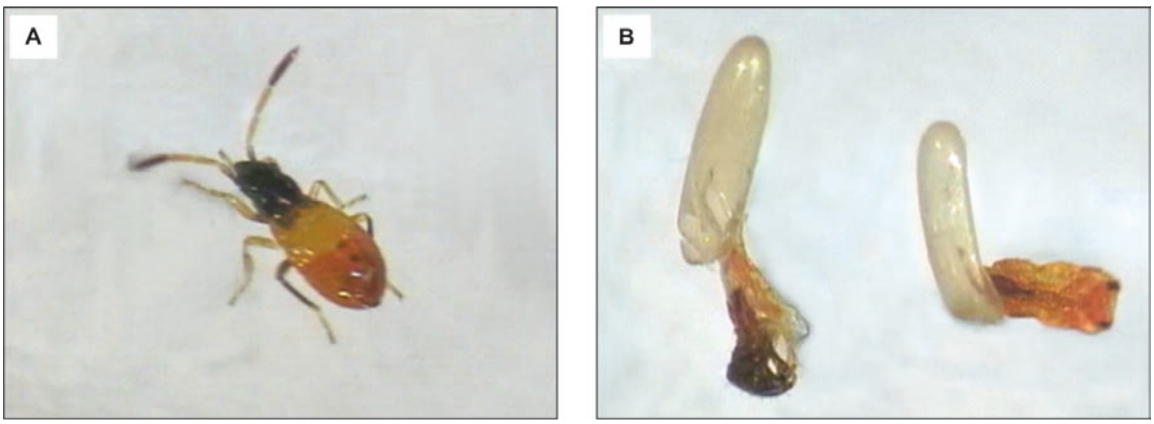

Fig. 6. Nymphs of *Paraeucosmetus pallicornis:* untreated (A) and treated with *Calotropis gigantea* leaf extract (B)

inhibitor, α-amylase inhibitor constitutive, and lectins (David *et al.* 2011). Protease inhibitors found in *C. gigantea* can easily affect the development of nymphs. Nymphs hatched after being treated by the extract will become abnormal (Fig. 6). Steroid compounds which can affect the development of insects by inhibiting the growth hormone also cause abnormality in nymphs.

Therefore, research showed that *C. gigantea* as an ovipositional detterent and ovicide that could be used for the management of pests especially *P. pallicornis*.

Conclusions

The leaves of *C. gigantea* can potentially act as an oviposition deterrent and ovicide against *P. pallicornis* (Dallas) (Hemiptera: Lygaeidae) on rice. Further field studies and research on the preparation of formulas which would enhance potential and stability, toxicity and effects on non-target organisms and the environment are needed to recommend *C. gigantea* as an eco-friendly botanical that could be used for the management of pests in rice, especially towards *P. pallicornis,* and can replace the harmful use of conventional insecticides.

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