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Research Article

Preliminary Valuation Activity of *Calotropis gigantea* L. Extracts against Several Insect Pests

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ABSTRACT

Calotropis gigantea are known to produces secondary metabolites with medicinal and insecticidal properties. Previous toxicity tests on several pest species had been conducted, yet the most susceptible pest species towards *C. gigantea* extract is still unknown. This study aimed to determine the susceptibility of several insect species and study their behavior after treated with *C. gigantea* extract. The test methods used in this research were leaf dipping and spraying upon four insect pest species from different orders (*Bactrocera carambolae* [Diptera: Tephritidae], *Nilapavata lugens* [Hemiptera: Delphacidae], *Sitophilus zeamais* [Coleoptera: Curculionidae], and *Plutella xylostella* [Lepidoptera: Plutellidae]). The concentration used to determine susceptible insects were 25 g.L^{-1} and control. Deterrence test of susceptible insects was done using a choice test and no-choice for 1.5 hours and observed every five minutes. Five replication were used for each concentration. Behavioral test of susceptible insects was done using a diet test. Five bok choy leaf discs were immersed into solutions for 1 minute and air-dried on a petri dish. Observations were carried out for 12 hours with observation interval period of every 1.5 hours. *P. xylostella* was more susceptible to *C. gigantea* leaf extract than *B. carambolae, N. lugens,* and *S. zeamais. C. gigantea* leaf extract showed toxic and feeding deterrent activity to *P. xylostella* larvae. The LC_{50} value of *C. gigantea* extract against *P. xylostella* by dipping was 16.9 µg.l^{-1} and 18.5 µg.l^{-1} by spray. The components of *C. gigantea* leaf extract consist of alkaloid, tannin, phenol, flavonoid, saponin, and terpenoid. The result of the research showed that *C. gigantea* leaf extract has a toxic and deterrent substance against *P. xylostella* and potential to control *P. xylostella*.

Keywords*: Calotropis gigantea*; mortality; *Plutella xylostella*; susceptible

INTRODUCTION

Calotropis gigantea (L.) (Asclepiadaceae) is one of the Indonesian native plants that have important ethnomedical values (Faradilla & Maysarah, 2019). It is known as a weed with milky white sap that is distributed over all parts of the plant and is able to produce secondary metabolites, which have medicinal plants and insecticidal properties (Ningsih *et al*., 2019; Yadav & Meena, 2021). *C. gigantea* belongs to the family Asclepiadaceae that includes more than 280 genera and around 2.000 species. *C. gigantea* and *C. procera* are two common species closely related to each other; they share similar botanical aspects and pharmacological effects. Bioactive compounds are spread in all parts of *C. gigantea*, especially in leaves with abundant sap, makes this plant resistant to insects and phytopathogen (Pandian *et al*., 2013).

Previous researches showed that *C. gigantea* root extract have repellent activity against adult of *Culex quinquefasciatus* (Dhivya & Manimegalai, 2013); mortality effect to adult of *Tribolium castaneum* (Parvin *et al*., 2014; Habib & Karim, 2016) and *Paracoccus marginatus* (Sumathi *et al*., 2017), larvicidal activity against *Helicoverpa armigera* (Prabhu *et al*., 2017) and *Aedes aegypti* (Koraag *et al*., 2017), and also oviposition deterrent and ovicidal activities against *Paraeucosmetus pallicornis* (Sjam *et al*., 2017).

Based on the previous research, it is still unknown which order of insect is most susceptible to *C. gigantea*

extract. Different orders of insects have distinct bioecology and characteristics, causing their susceptibility to a chemical substance to be diverse. The susceptibility of several pests towards plant extract is affected by bioactive compound content, type of species, and the concentration of plant extract. Extracts of *Daphne mucronata* (Thymelaeaceae), *Tagetes minuta* (Asteraceae), *C. procera* (Apocynaceae), *Boenninghausenia albiflora* (Rutaceae), *Eucalyptus sideroxylon* (Myrtaceae), *Cinnamomum camphora* (Lauraceae), and *Isodon rugosus* (Lamiaceae) resulted in different activity against several insect pests species, including pea aphids (*Acyrthosiphon pisum*) (Hemiptera), fruit flies (*Drosophila melanogaster*) (Diptera), red flour beetles (*Tribolium castaneum*) (Coleoptera), and armyworms (*Spodoptera exigua*)(Lepidoptera) (Khan *et al*., 2017). Among extracts of *Piper retrofractum* (Piperaceae), *P. crocatum* (Piperaceae), *Chromolaena odorata* (Asteraceae), *Tagetes erecta* (Asteraceae), *Tithonia diversifolia* (Asteraceae), and *Ageratum conyzoides* (Asteraceae), *P. retrofractrum* extract exhibited the highest mortality compared to the other extracts against *Nilaparvata lugens* (Nuryanti *et al*., 2018). Other research results showed that extracts of *Citrullus colocynthis* (Cucurbitaceae), *Cannabis indica* (Cannabaceae), and *Artemisia a*rgyi (Asteraceae) produced different insecticidal activities and *C. colocynthis* showed higher insecticidal properties against cabbage aphid (*Brevicoryne brassicae*) (Ahmed *et al*., 2020)

Recent research used several species of insects from different orders including (*Bactrocera carambolae* [Diptera: Bactrocera], *Nilapavata lugens* [Hemiptera: Delphacidae], *Sitophilus zeamais* [Coleoptera: Curculionidae] dan *Plutella xylostella* [Lepidoptera: Plutellidae]). The four selected orders have different characteristics, especially their type of mouthparts. It determines the type of food they consume and feeding behavior (Antczak-Orlewska *et al*., 2021) as it is closely related to insect feeding behavior (Ma *et al*., 2013).

The characteristics of the mouth types, different metabolic levels, individual susceptibility, cuticle thickness and chemical compounds of plants greatly determine the amount of chemical compounds that enter into the insect's body. There is merit in understanding these factors to maximize the use of plant extracts against the correct insect target, especially considering the small amount of bioactive compounds contained in plants. This research aimed to study the effectiveness of *C. gigantea* extract to several insects belonging to different orders. The result is expected to help the development of *C. gigantea* in becoming an appropriate technology in pest insect management.

MATERIALS AND METHODS

Experimental Site

The research was conducted between February 2018 until November 2019 at the Pesticide Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta.

Rearing of Insect

Plutella xylostella. *Plutella xylostella* collected from Cangkringan Village, Sleman Regency, Special Region of Yogyakarta Province, was reared in the greenhouse. Rearing included the planting of plant seeds and rearing of *P. Xylostella* on bok choy plants. Bok choy (*Brassica rapa* var. *chinensis)* seeds were sown in plastic containers (sized 15×25×4 cm) containing a mixture of soil and manure (1:1). The seeds started sprouting after three days. After eight days, they were ready to be planted into polybags. Six days after planting, plants were ready to be used as oviposition sites. Bok choy plants were maintained by watering plants daily. Four plants were placed in rearing box (25×25×40 cm) together with twelve 3-day-old *P. xylostella* adults that were fed with 10% honey solutions on cotton placed on top of these rearing box. Once every three days, the plants were replaced from egg-laying box, and a new Bok choy plants placed until the adults died. The offspring larvae were maintained in a new rearing box until they transformed into adults. After that, they were moved to the egg-laying box, thus producing F2 offspring and third instar larvae were used as a test.

Nilaparvata lugens. *Nilaparvata lugens* individuals were obtained from the Laboratory of Pesticide Toxicology, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta. This population had been reared separately from other populations in the laboratory without additional field population and insecticide application (Trisyono *et al*., 2017). This population was reared with prescribed laboratory procedures upon rice nursery (Ciherang variety) at a temperature of ± 27ºC, relative humidity of 70–80%, and photoperiod of 12:12 hours (day:dark).

Twenty-five pairs of *N. lugens* were introduced into a plastic jar (9×9×19 cm) containing rice seedlings (age 5–7 days) for laying eggs. After the eggs hatched and the rice seedling turned yellow, nymphs were moved to a new jar containing new rice seeds. The rice plants were regularly changed once every four days.

Sitophilus zeamais. *Sitophilus zeamais* was reared according to methods of Ojo and Omoloye (2016). *S. zeamais* was collected from the culture of Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. Twenty pairs of seven-day-old *S. zeamais* were placed in a plastic jar (9×9×14 cm) with 250 g corn kernel covered with gauze and proceeded in three replication to achieve F1 generation. The *S. zeamaiz* were allowed to feed, copulate and oviposit for 10 days. In the second week, adults were separated into a plastic jar (9×9×14 cm) containing 250 g of corn kernel. *S. zeamais* individuals used for the test were from the first generation.

Bactrocera carambolae. *Bactrocera carambolae* was obtained from Laboratory of Entomology, Faculty of Agriculture, Universitas Gadjah Mada. *B. carambolae* was reared since 2006. This population was reared following the method of Chang *et al.* (2004) with necessary modifications. The rearing was done in the boxes (30×30×30 cm). *B. carambolae* adults were fed with sugar and water to maintain a healthy colony. Egg collecting was conducted by inserting an oviposition with holes on its surface. To attract oviposition, a black cloth immersed in pumpkin juice was placed into the oviposition cup. The eggs were harvested every two days and then placed on 25×15×5 cm tray containing artificial diet until they turned into pupas after approximately 14 days. The pupae were harvested and placed into rearing box until they turned into imagoes.

Extraction of *Calotropis gigantea* Leaves

The extraction used maceration method (Richardson & Harborne, 1985). As much as 250 g of *C. gigantea* leaves were added into a container, 1 L of ethanol was added and stirred to prevent sedimentation. The product was stored in room temperature and avoided exposure to direct sunlight. Immersion was done for 48 hours, filtered using filter paper and Buchner funnel. As the filtrate was obtained, separating solution from its solvent was done using a Vacum Rotary evaporator (Heidolph; Weirtheim and Waterbath Cole-Parmer type 7049-05) at temperatures of 40–180°C and 160–280 rpm for 3 hours.

***Calotropis gigantea* Leaf Extract Toxicity Test against Several Pest Insects**

Toxicity test was conducted upon larvae or nymph (*P. xylostella* or *N. lugens*) and adult insects (*S. zeamais* and *B. carambolae*). The methods used in this research were leaf dipping and spray assays. The concentration used were 25 μgL^{-1} and an untreated control. The extract was dissolved using distilled water until it was homogeneously mixed. Based on the test results, insect pests susceptible (highest mortality value) to *C. gigantea* extract were further tested for toxicity and deterrence behavior.

Bioassay

Plutella xylostella mortality was tested using leaf dipping method. *P. xylostella* larvae were first starved for one hour. Bok choy leaves were washed and then formed in a round shape using a 2.5 cm diameter of cork borer. Petri dish (9 cm diameter and 2 cm high) was used as the test arena. Five bok choy leaf discs were dipped in the extract solution for 10 seconds; for the untreated control was dipped in distilled water only and then dried and placed into the petri dish. As many as 20 larvae were inserted into the petri dish and five replications were applied. After 24 hours, larvae that were still alive were moved into new petri dishes and given untreated diets until up to 48 hours after bioassay. Percentage of mortality was observed every 24 and 48 hours

For *N. lugens* mortality test, each treatment required 20 third instar *N. lugens* nymphs. Five 14-day-old rice seedlings were soaked for 5 minutes in *C. gigantea* extract solution, dried and then put into a container (5 cm high, 4 cm diameter) containing 5 rice seedlings. Twenty nymphs were introduced to the test container and five replications were conducted.

As for *S. zeamais* mortality test, it was conducted on adult *S. zeamais*. Five gram of corn seeds were soaked in *C. gigantea* extract for 5 minutes and then air dried. Afterward, they were inserted to a plastic container (4 cm high, 4 cm diameter). Twenty test insects were put in and for 5 times replication.

B. carambolae mortality was tested using 200 adults of *B. carambolae* that were placed in a container (12 cm high, 8 cm diameter). Insect were made unconcious by storing insects in freezers for 1 minute. Later on, 20 insects were put in a treatment container and five replications were carried out. Treatment containers were jar modified to possess a hollowed lid covered with mesh cloth. The concentration used was slowly wetting mesh cloth by applying 2 mL until throughly wet by treatment solution or distilled water for the control. Percentage of mortality was observed at 24 and 48 hours.

Bioassay for Contact Toxicity

Contact toxicity of *C. gigantea* was assessed by directly spraying upon the test insects using Potter Precision Laboratory Spray Tower (Burkard Manufacturing Co. Ldt. Rickmansworth Herts. UK) with aperture nozzle of 0.6985 mm at 1.12 $kg.cm^{-2}$ operational pressure.

P. xylostella larvae were placed on petri dish layered with a paper tissue and then sprayed directly with 2 mL *C. gigantea* extract or distilled water for the control. After spraying, larvae were fed with untreated bok choy leaves. Each petri dish contained 20 larvae and replications were done five times. After 24 hours, larvae that were still alive were moved to a new container and fed repeatedly until up to 48 hours after application.

N. lugens mortality was tested by inserting third instar nymphs into a plastic cup (4 cm high, 4 cm diameter) and covered with mesh cloth, sprayed directly with 2 mL *C. gigantea* leaf extract. After spraying, the nymphs were inserted into a plastic cup containing 14-days-old rice plant. After 24 hours, the nymphs were moved to a new plastic container containing another rice plant up to 48 hours after application.

As for *S. zeamais*, *S. zeamais* were inserted into a plastic container (4 cm high, 4 cm diameter) modified and covered with mesh cloth, sprayed directly using 2 mL *C. gigantea* leaf extract. Afterward, the insect was moved to a container filled with 5 g of corn.

B. carambolae mortality was tested by making 200 *B. carambolae* unconscious in the freezer for 1 minute and then placed in a plastic container (15 cm high, 9 cm diameter) with hollowed lid, covered with mesh cloth, and layered with paper tissue. After they started to move and began their activities, the direct spray was done using 2 mL *C. gigantea* extract. After spraying, *B. carambolae* were fed with sugar and water. Each treatment was replicated five times. The observation was conducted at 24 and 48 hours after treatment by calculating the mortality percentage of each test insect.

***C. gigantea* Extract Deterrence Test against *P. xylostella* Larva**

This deterrence test aimed to determine the antifeedant ability of *C. gigantea* leaf extract against *P. xylostella* larvae. The method used in this research is the leaf dipping method. This test used the method of Cameron *et al.* (2014) and Yang *et al*., (2017), namely the Non-Choice Antifeedant Test. The concentrations used in this study were 12.5 μgl^{-1} and control. The prepared bok choy leaves were washed and molded in a round shape using a cork borer with a diameter of 2.5 cm. Each leaf was placed in a circular Petri dish so that in the middle of the Petri dish there is space for the larvae to be placed. A total of 20 larvae were placed in the middle of petri dish and allowed to choose diets. These settings were replicated five times. The parameters observed included the percentage of larvae that chose or stayed on diets, percentage that did not choose feed or avoided diet, the mortality of larvae and surviving larvae (on the feed and not on the feed).

C. gigantea* Extract Toxicity Test against *P. xylostella

Toxicity test was carried out to determine the LC_{50} value using Probit analysis. The concentration used was 1 g of *C. gigantea* leaf extract paste diluted with 40 ml of distilled water. *P. xylostella* larvae were tested using feeding assay. Bok choy leaves were washed and made into circular leaf discs using a 2.5 cm diameter cork borer. Petri dishes were used as bioassay arena. Concentration used were 25, 12.50, 6.25. 3.12, 1.56, 0.78, 0.39, 0.19, 0.09, 0.04 μgL^{-1}. Five bok choy leaf discs were immersed into solutions for 10 seconds and air-dried on a petri dish. Twenty larvae were placed onto the petri dish according to the designated treatment. Mortality percentage was observed at 24, 48, and 72 hours. Contact assay was done using a spraying assay. Twenty larvae were placed on a petri dish, and each concentration was replicated 3 times. As much as 2 ml of *C. gigantea* leaf extract were sprayed using Potter Precision

Laboratory Spray Tower with aperture nozzle of 0.6985 m, pressure at 1.12 kg.cm^{-2}. Percentage of mortality was observed at 24, 48, and 72 hours.

Identification of *C. gigantea* Leaf Extract Compound Group

Two grams of *C. gigantea* were extracted and then tested to determine the class of compounds, which included total alkaloids, tannins, phenols, flavonoids, saponins and quantitative tests of terpenoids. The total alkaloids, tannins, phenols, flavonoids, and saponins were performed using spectrophotometry, while terpenoids were performed using thin-layer chromatography (Chanwitheesuk *et al*., 2005). The extract's chemical composition identification was carried out by *Laboratorium Penelitian dan Pengujian Terpadu* (LPPT; The Integrated Research and Testing Laboratory), Universitas Gadjah Mada.

Data Analysis

Data were analyzed using One-Way ANOVA and Independent t-test with 95% confidence level. When significant difference appeared, analysis was then proceeded to DMRT test 95%. Probit analysis was done using SAS JMP Pro.v13.2.1 to obtain LC_{50} and LC_{95} on test insects.

RESULTS AND DISCUSSION

Calotropis gigantea Leaf Extract Toxicity against Several Pest Insects

The results of extract toxicity by leaf-dipping method against *P. xylostella* and *B. carambolae* were significantly different to *N. lugens,* and *S. zeamais* (Table 1). The highest mortality occurred on species of *B. carambolae* and *P. xylostella* with 87.13%, and the lowest occurred in *N. lugens* and *S. zeamais* with 5.98 and 9.09%, respectively. Meanwhile, the highest mortality of the four species of insect by spray method occurred on *P. xylostella* with 50.32%, followed by *S. zeamais* with 14.02%, *N. lugens* with 14.70%, and *B. carambolae* with 7.99%. The analysis of variance showed that *P. xylostella* mortality percentage was significantly different to *B. carambolae, N. lugens,* and *S. zeamais*.

The mortality rates from diet bioassay was determined by the contents of the bioactive compound contained in *C. gigantea* leaf extract and the characteristic of the pest insects. The high mortality of *B. carambolae* and *P. xylostella* was mainly due to the type of mouthpart; this indicates that the extract of *C. gigantea* is a stomach poison. The low mortality of *S. zeamais* was highly influenced by the thickness of the corn kernel pericarp since it determines how much extracts were being absorbed to the outer skin of the corn as it is for *N. lugens*.

The four insects mortality test by spray showed that *P. xylostella* showed the highest mortality compared to the other test insects of *S. zeamais, N. lugens,* and *B. carambolae*. The difference between those four test insects' mortality happened mainly due to the characteristic of each insect's integument and *C. gigantea* leaf extract. *P. xylostella* possessed a softer integument allowing the *C. gigantea* leaf extract to penetrate the integument layer. According to Yu (2011), the factors affecting cuticular penetration rates were the effect of solvent, the polarity of insecticides, and cuticular composition. Insecticides would penetrate the thinner, less complex region of the cuticle more rapidly than in the thicker part. Evidence shows that the thin-cuticle area around seta and sensilla and at intersegmental membranes offer less resistance to the diffusion of insecticide than other cuticle structures. In addition, it appears that insecticides can enter through pore canals and dermal gland ducts. Botanical insecticides affect various types of insects in a number of way possible depending on the physiological characteristic of the insect species and the types of plants (Hikal *et al*., 2017).

Table 1. Toxicity of *Calotropis gigantea* leaf extract against *Bactrocera carambolae, Nilaparvata lugens, Sitophilus zeamais,* and *Plutella xylostella* using leaf dipping and spray methods at 48 hours after treatment

Methods	Mortality (%)			
	B. carambolae (adult)	*N. lugens* (3rd instar nymphs)	*S. zeamais* (adult)	*P. xylostella* (3rd instar larvae)
Feed (Leaf-dipping)	87.13 ± 0.00^{a}	5.98 ± 6.96^{b}	9.09 ± 8.53^{b}	87.13 ± 0.00^{a}
Contact (Spray)	7.99 ± 7.28^{b}	14.02 ± 2.47^{b}	14.70 ± 11.18^{b}	50.32 ± 6.79^{c}

Note: Values (Mean ± SD) followed by the same letter in the same lines are not significantly different according to Duncan (0.05).

Deterrence Effect *C. gigantea* Extract against *P. xylostella* Larvae

Deterrence test result of the extracts (Table 2) showed that *C. gigantea* leaf extract clearly influenced insect behavior on choosing diets, not choosing diets, or avoiding diets. Larvae mostly did not chose or avoided diets (18.03 larvae) compared to ones choosing or staying on them (1.93 larvae). Meanwhile, in control, larvae mostly chose or stayed in the feed (18.83 larvae) rather than not choosing feed or staying away from feed (1.24 larvae). Greater number of larvae did not choose or avoided treated diets (18.03 larvae) compared to control (1.24 larvae) while ones that staid on untreated control diets were higher (18.83 larvae) compared to treatment (1.93 larvae) indicated that *C. gigantea* leaf extract contained deterring substance. Compared to control, larvae not choosing or staying away from the feed indicated that larvae were not constantly hungry, but there were occasions when they stop feeding. On top of that, the odor and extract substances heavily affected larva behavior of not choosing or staying away from diets.

The percentage of larvae chose or decided to stay on diets fluctuated together with the ones that refused or avoided diets (Figure 1). After 1.5 hour, the percentage of larvae exposed to treated diets that did not choose or avoided diets ranged from 82 to 98.5%. Meanwhile for the control, the percentage of larvae not choosing and staying away from feed ranged between 1.5 to 8.5 percent. The percentage of larvae in treatment choosing or staying on diets ranged from 1.5 to 17%, while on untreated control, it ranged between 91.5 to 98.5%. On the chosen diets, the presence of larvae was influenced by the selection of host because *C. gigantea* leaf extract compounds were found in diets. As for the treated feeds, larvae tended to reject and avoid them due to the strong odor in the feed. Plants containing repellent compounds cause insects to move away from odor sources without physical contact (Deletre *et al.*, 2016). Results from Wallingford *et al.* (2017) showed that odor of treated diets inhibited the eating behavior of *Drosophila suzukii*. According to Fleischer *et al.* (2018), the insect olfactory system is highly sensitive and discriminates appropriately against relevant cues from the odor world. Insects have a highly sensitive and discriminatory olfactory system to detect relevant volatile compounds and odors

Table 2. The influence of *Calotropis gigantea* leaf extract on *Plutella xylostella* third instar larvae food selection behavior

Treatment	Number of larvae	
	Choosing or staying in the feed	Not choosing feed or staying away from feed
Control	18.83 ± 0.66[a]	1.24 ± 0.59[b]
C. gigantea leaf extract	1.93 ± 1.02[b]	18.03 ± 0.95[a]

Note: Values (mean ± SD) followed by the same letters in the same column are not significantly different according to the independent sample t-test (0.05).

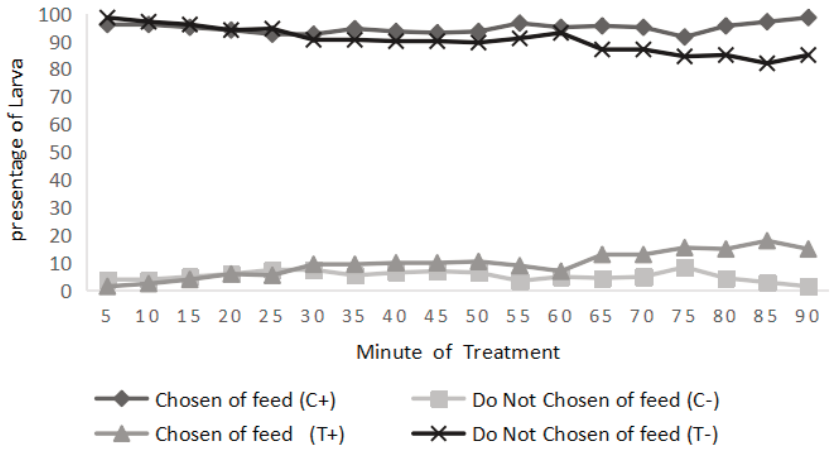

Figure 1. Feeding choice behavior among two food sources; T = Treatment, (did not choose diet on treatment [T-] and chose diet on treatment [T+]; C = control, (did no chose diet on control [C-] and chose diet on control [C+]

control their behavior to direct them to or away from the source of the odor (Renou & Anton, 2020). Besides being influenced by the odor effect of *C. gigantea* extract, larval feeding behavior was also influenced by the taste and content of compounds in the extract. *Fistula cassia* leaf extract test, *Jatropha, Piper longum, Tephrosia purpurea,* and *Vernonia cinerea* against *Spodoptera litura* showed that the treated feed had an unpleasant taste that inhibited (Arivoli & Tennyson, 2013). The extract toxicity in 24 hours (Figure 2) showed that *C. gigantea* leaf extract possesses a toxicity level causing *P. xylostella* mortality of 35%, leaving the rest to both be in and off the diet. Observation of larvae mortality observed every 1.5 hours for 24 hours indicated an increasing rate of larva mortality starting from 0 to 35%. Some of the larvae in or nearby the diet indicated a decline of approximately 94 to 49%. In comparison, larvae far away from the feed experienced an increase from 6 percent to 51 percent. The declining larva behavior towards the feed showed that larvae moved aimlessly due to the pungent odor of *C. gigantea* leaf extract present in the feed. As time went by, some larvae approached the diet and consumed it with small bite marks since they only ate the upper epidermal layer. The increasing rate of larvae off of the feed is heavily influenced by the components present in *C. gigantea* leaf extract in the feed.

P. xylostella larvae on and off the diet was the process of finding a suitable host. After extract treatment, larvae tended to avoid the diet and wandered aimlessly. There were also some larvae that scattered and were confused, which eventually moved towards the diet even though they did not eat it. As soon as they approached the diet, they quickly moved back to another place to avoid the diet. This indicated that secondary metabolite compounds affected insects' behavioral and physiological responses. According to Lina *et al.* (2015), secondary metabolites are classified into two major groups, namely compounds that influence behavioral responses and physiological responses. Insect feeding cessation activity and mortality within 24 hours after the application occurred with 51 percent in stopping position and 35 percent in mortality. Larvae started approaching and eating the diet due to the fading odor of the extract. Behaviors conducted by the larvae showed that *C. gigantea* leaf extract is repellent, deterrent, and toxic.

C. gigantea Extract Toxicity Test against *P. xylostella*

Toxicity against *P. xylostella* from the leaf dipping bioassay demonstrated probit analysis with LC_{50} estimation of 16.99 μgL^{-1} with 95% CI between 13.89 μgL^{-1} to 21.56 μgL^{-1}. Toxicity of *P. xylostella* larvae using spraying application (Table 3) had a probit estimation of 18.55 μgL^{-1} with 95% CI between 11.23 μgL^{-1} to 35.77 μgL^{-1}. Mortality level is affected by concentration, the content of bioactive compounds and physiological characteristics of *P. xylostella.* Toxicity of *C. gigantea* leaf extract, when applied using the dipping and spraying method, caused higher mortality as concentration increased. The *C. gigantea* leaf extract has a potentially toxic

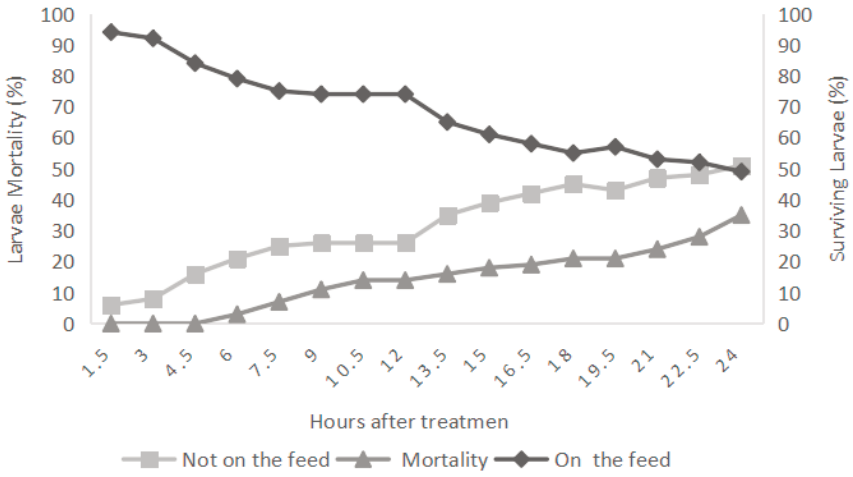

Figure 2. Effects of *Calotropis gigantea* leaves extract on the third instar *Plutella xylostella*

Table 3. Toxicity of *Calotropis gigantea* leaf extract using dipping and spraying method on 3rd instar *Plutella xylostella*

Method	Volume (mL)	N	Slope (± SE)	LC_{50} (95% CL, μgl^{-1})	df
Dip	40	600	0.488 (± 0.031)	16.99 (13.89-21.56)	8
Spray	40	600	0.414 (± 0.059)	18.55 (11.23-35.77)	8

Note: N= Number of individuals used; CL= Confidence Limit.

effect on *P. xylostella*. The composition of compounds contained in the extract of *C. gigantea* affects its toxicity on *P. xylostella*. Based on the identification of compound composition (Table 4), *C. gigantea* extract contains secondary metabolic compounds (alkaloids, tannins, phenols, flavonoids, saponins, and terpenoids). Alkaloid, saponin, glycoside, tannin, flavonoid, and terpenoid are used for plant defense against herbivores and have been reported to possess insecticidal properties (Hikal *et al*., 2017).

Identification of *Calotropis gigantea* Leaf Extract Compound

Spectrophotometry analysis showed that *C. gigantea* contained six secondary metabolite compounds, including alkaloid, tannin, phenol, flavonoid, saponin, and terpenoid (thin layer chromatography) (Table 4). According to Pandian *et al.* (2013), the composition of compounds in *C. gigantea* leaf extracts were alkaloid, carbohydrates, glycoside, phenolic/tannin, protein or amino acid, flavonoid, saponin, sterol, and resin. The toxicity of *C. gigantea* extract against several insect pests was toxic and the deterrence test against susceptible insects showed deterrent activity and affected the behavior of *P. xylostella*. The results of research by Tripathi *et al.* (2013), the ethanolic extract of *C. gigantea* leaves contains glycosides, alkaloids, flavonoids, tannins, calotroxins, uscharin, uschridin, and proceroside cardenolide, flavonoids, terpenes, and non-protein amino acids (gigantine and calotropin). Alkaloids, tannins, phenols, flavonoids, saponins, free fatty acids and carbohydratesin the extract of *C. gigantea* caused mortality of *Paracoccus marginatus* (Sumathi *et al*., 2017). According to the results of Arivoli and Tennyson (2013), ethanol extract of *C. gigantea* leaves has antifeedant activity against *Spodoptera litura*, *Helicoperva armigera* (Prabhu *et al.,* 2017), toxic and antifeedant activity against *Plutella xylostella* (Khasanah *et al*., 2021). Activity of saponin compounds in extract *C. gigantea* is larvicidal against *Aedes aegypti* (Aini & Mardiyaningsih, 2016). Secondary metabolite compounds act as poison, deterrent, repellent and reduce the nutritional value of food (Belete, 2018). According to Laxmishree and Nandita, (2017), generally plant secondary metabolites are repellent, deterrent, toxic, and interfere with the physiological activities of insects.

Table 4. Chemical composition of *Calotropis gigantea* leaf extract

Group of compound	Total compound in material (%w/w)
Alkaloid	0.90
Tannin	5.94
Phenol	9.88
Flavonoid	2.20
Saponin	4.95
Terpenoid	Positive

Note: Positive (extract contains terpenoids)

CONCLUSION

C. gigantea leaf extract had toxic and deterrent activity against *P. xylostella*. *P. xylostella* was the insect pest that was more susceptible to *C. gigantea* leaf extract than *B. carambolae, N. lugens,* and *S. zeamais* based on by diet and contact bioassays. The increase in mortality of *P. xylostella* larvae and the decrease in larval survival (on or near food or not on food) for 24 hours were influenced by the time and toxicity of *C. gigantea* leaf extract. The LC_{50} value of *C. gigantea* extract against *P. xylostella* by dipping was 16.99 μgl^{-1} and 18.55 μgl^{-1} by spray.

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