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Short Communication: Larvicidal and antifeedant activities of *Kalanchoe daigremontiana* against *Plutella xylostella* larvae

DEWI HIDAYATI♥, YUDI DARMANTO, TUTIK NURHIDAYATI, NURLITA ABDULGANI
Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Teknologi Sepuluh Nopember. Keputih, Sukolilo, Surabaya 60111, East Java, Indonesia. Tel. +62-31-5963857, Fax.. +62-31-5963857, ♥email: dewi_hidayati@ymail.com

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Abstract. *Hidayati D, Darmanto Y, Nurhidayati T, Abdulgani N. 2016. Short Communication: Larvicidal and antifeedant activities of* Kalanchoe daigremontiana *against* Plutella xylostella *larvae. Nusantara Bioscience 8: 312-315.* Larvicidal and antifeedant activities of *Kalanchoe daigremontiana* polar extract were tested on fourth instar of *Plutella xylostella* larva using choice test, leaf dipping method and antifeedant capacity test (no choice test). *K. daigremontiana* polar extract in level of 0.25, 0.5, 0.75, 1, 1.25, and 1.5% were used on choice test to determined the larvae behavior tendencies of an attractant or to be an antifeedant, while on toxicity test were used extract in level of 0.25% and 0.5%, whereas on antifeedant capacity test were in level of 0.75, 1, 1.25, and 1.5%. The Probit analysis resulted that 50% of lethal concentration (LC_{50}) was found at 72 hours exposure of 0,5% polar extract. Effective concentration for antifeedant at 1.25%, while antifeedant capacity percentage was in level of 84.85%.

Keywords: Antifeedant, *Kalanchoe daigremontiana*, larvicidal, *Plutella xylostella*

INTRODUCTION

Larvae of *Plutella xylostella* L. (Lepidoptera: Plutellidae) is a major pest of Brassicaceae plants in Indonesia, especially cabbage, mustard and choi sum that mostly is still controlled by the insecticides. *P. xylostella* is a the most destructive cosmopolitan pests of cruciferous crops, spread in the highlands and low (Talekar and Shelton 1993; Herlinda 2003) Pest control efforts against *P. xylostella* still mostly relies on the use of synthetic insecticides and shown significant resistance to almost every synthetic insecticide applied in the field (Herlinda 2005; Sarfraz et al. 2005). *P. xylostella* reportedly been resistant to some insecticides, such as organic phosphate compounds, synthetic pyrethroid (Maienfisch et al. 2012), carbamates, and the latest generation insecticide compound insect growth regulator (Kobayashi et al. 1992). The population of *P. xylostella* at 1989 also reported to have been resistant to 51 types of insecticides compounds (Vasquez 1995).

Moreover, the usage of chemical insecticides have several risks included the pest resistance, mortality of the non-target organism and presence of residues in food as well as the decreasing the environmental quality (Brouwer et al. 1999; Munandar and Madyawati 2002). Therefore, the environmentally friendly insecticides are needed, such as insecticide made from plants or biopesticide (Kasmara 2004).

Among several compounds of plant secondary metabolites generally has a biological activeness hence can use as a natural products of insecticide. Plants containing active insecticidal phytochemicals which potentially to answer the economic and ecological challenge at sustainable agriculture. Hence, there is need the exploration of new active molecules with different mechanisms of action (Rattan 2010). Secondary metabolites are formed and stored in parts of the plant itself, and beneficially for protection against herbivores and as the body's defense of pest (Jacobson 1989; Hermawan et al. 2005).

Kalanchoe daigremontiana Raym.-Hamet & H. Perrier. (Javanese: *Cocor bebek*) has been used as an ornamental plants and traditional medicines for drug infections, rheumatism, cough and fever (Melanie et al. 2004), is known to contain a compound bioactive potential to be developed as a material botanical pesticides. Bioactive compounds in plants *K. daigremontiana* contained in part of leaves and stems. It was reported that leaves containing higher cardiac glycoside than rod hence it more toxic (William and Smith 1984; Melanie et al. 2004). Extraction is the one of method for obtaining the organic compounds in the plant tissue Extraction using methanol as well as ethanol (alcohol) are aimed to get a more polar compound (Harborne 1973), which namely the polar fraction extract (Hamidah et al. 1999). According its ingredient, *K. daigremontiana* has potential for alternative plant pest control or a botanical pesticides.

Kalanchoe daigremontiana has potential to be developed as a bio pesticide due to its bioactive compound, i.e. cardiac glycosides (Melanie et al. 2004). Cardiac glycosides is the highly toxic plant secondary compounds, which block Na K-ATPase, the essential enzyme for the nerve cells function (Emery et al. 1998; Dobler et al. 2015). The study of insecticidal activity of *K. daigremontiana* using silkworm (*Bombyx mori* L.) indicated that

bufadienolides, orthoacetate and α-pyrone moiety showed strong activity and the present of oxygenated substituents at C-11 and C-12 on C-ring enhanced the insecticidal activity (Supratman et al 2001; Maharani et al. 2008). This study was performed to develops the insecticidal *K. daigremontiana* application to the other crops including biocontrol of *P. xylostella* at Brassicaceae plants. This research was focused in how the influence of polar extracts of *K. daigremontiana* leaf on the mortality and antifeedant capacity of *P. xylostella*. The research was aimed to obtain the lethal concentration (LC_{50}) of extracts *K. daigremontiana* to larvae *P. xylostella*, as well as to get the effective concentration of polar leaf extracts *K. daigremontiana* as an antifeedant.

MATERIALS AND METHODS

The extract polar of *K. daigremontiana* preparation: Firstly, the leaves were cleaned with distilled water, then it was dried-aired in the open air (not direct sunlight) at room temperature prior to be crushed using a mortar to form a *K. daigremontiana* leaves powder. The powder was then macerated in the ratio 1:3 (1 g powder with 3 mL of ethanol 70%) (Hermawan et al. 2005) in a glass beaker 250 mL and placed on a shaker for 24 hours until obtained a clear filtrate.

Moreover, the filtrate was removed into the 50 mL microfuge tube and centrifuged for 30 minutes at a speed of 4000 rpm. The obtained supernatant (*K. daigremontiana* polar extract) was then collected into a 250 mL Erlenmeyer flask. *K. daigremontiana* polar extract was diluted to obtain varying concentrations ie, 0% (control), for choice test were in level of 0.25, 0.5 0.75, 1, 1.25, and 1.5%. While the range of *K. daigremontiana* polar extract concentration for test of toxicity and antifeedant were determined refer to the result of choice test.

The "Choice Test" and "No Choice Test" (the antifeedant test): At first, the leaf discs of mustard with a diameter of ± 10 cm were dipped for ± 10 seconds in a solution of. *K. daigremontiana* polar extract which coded as the treatment mustard leaves or it called as T. While, the mustard leaves discs that dipped in distilled water was coded as a control mustard leaf or it called as C. The T and C mustard leaves were-dried on air for ± 30 minutes at room temperature. Secondly, the fourth-instar larvae of *P. xylostella* (L IV) that have fastened for 3 hours were placed in pored transparent chamber that contain both of C and T mustard leaves. The larvae were left to eat the leaves for 24 hours. The choice test of larvae against both types of leaves offered two possible tendencies, i.e. to be an attractant (when the larvae pick the T leaf or choose both types of leaves, but the T leaf was eaten more) or to be an antifeedant (when the larvae only eat C leaf or both types of leaves, but the C leaf was eaten more).

By using the similar preparation, the *P. xylostella* larvae for the antifeedant test was exposed with the single mustard leaf (no choice test= exposed with C or T leaf only). The antifeedant capacity was measured using followed formula:

$$\text{The antifeedant capacity} = \frac{C-T}{C+T} \times 100\%$$

Where,
C= The control mustard leaf that eaten by *P. xylostella*
T= The treatment mustard leaf that eaten by *P. xylostella*

The toxicity test (the leaf dipping method): The T and C mustard leaves were prepared in the same direction with the previous methods. Every treatment consist of two mustard leaves and 10 larvae that placed in a transparent plastic box and covered gauze. The percentage mortality of larvae was observed every 24 hours, 48 hours, and 72 hours (Nurtiati et al. 2001). The 50% of lethal concentration (LC_{50}) was analyzed with Probit analysis. Statistic analysis was performed by One way ANOVA that followed with Duncan analysis.

RESULTS AND DISCUSSION

Results

Refer to the result of the choice test that showed in Table 1, the higher concentration of *K. daigremontiana* polar extract (0.75 to 1.5%) have tendency to be an antifeedant. Meanwhile, in the lower concentration (0.25 and 0.5%) have tendency to be attractant.

According to the choice test, concentration of *K. daigremontiana* polar extract for an antifeedant test or "no choice test" can be determined i.e. 0.75%, 1%, 1.25%, and 1.5%. Based on the result of antifeedant test at Fig 1 indicated that the increasing concentration of *K. daigremontiana* polar extract is followed by the decreasing weight of mustard leaf that eaten by *P. xylostella* larvae. Furthermore, Figure 2 exhibited that the highest level of capacity antifeedant occurred at concentration of 1,5%. However, as showed at Figure 2, there were no statistically significant differences with the capacity antifeedant at concentration of 1.25% ($p>0.05$). Accordingly, the effective concentration *K. daigremontiana* polar extract for antifeedant is at 1.25%.

The concentrations of *K. daigremontiana* polar extract that have tendency to be an attractant i.e. 0.25%. and 0.5% as previously explained, were used for toxicity test. The result of toxicity test was presented in Table 2. Moreover, the Probit analysis showed that LC_{50} was found at 72 hours exposure of *K. daigremontiana* polar extract of 0.5%

Discussion

The concentration of *K. daigremontiana* polar extract of 1.25% has antifeedant capacity at level of 84.85% hence in it is qualified to be categorized as an effective antifeedant for *P. xylostella* larvae. Refer to Koul (1993) and Munandar and Madyawati (2002), bioactive substance is stipulated as an effective antifeedant when it has capacity antifeedant 80%.

The antifeedant potential in *K. daigremontiana* polar extract might be related to its bioactive substances included triterpenoid and alkaloid. Those known as a bitter substance and enzyme inhibitor (Singh and Bharate 2005).

Table 1. Result of the choice test

Concentration of *K. daigremontiana* polar extract (%)	Mean weight of mustard leaf that eaten by *P. xylostella* larvae (g)		Bioactive tendency of *K. daigremontiana* polar extract
	Control mustard leaf (C)	Treatment mustard leaf (T)	
0.25	0.02	0.06	C<T , attractant
0.5	0.02	0.07	C<T , attractant
0.75	0.04	0.01	C>T , antifeedant
1	0.1	0.005	C>T , antifeedant
1.25	0.11	0.002	C>T , antifeedant
1.5	0.14	0.00	C>T , antifeedant

Table 2. Result of toxicity test of *K. daigremontiana* polar extract to *P. xylostella* larvae

Concentration of *K. daigremontiana* polar extract	*P. xylostella* larvae mortality (%)		
	24 hours	48 hours	72 hours
0% (control)	0 [a]	0 [a]	0 [a]
0.25%	6.67 [b]	17.78 [b]	32.22 [b]
0.5%	12.22 [c]	27.78 [c]	50 [c]

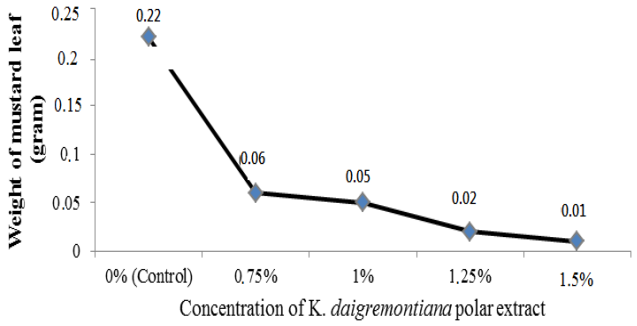

Figure 1. Result of antifeedant capacity of *K. daigremontiana* polar extract against *P. xylostella* larvae.

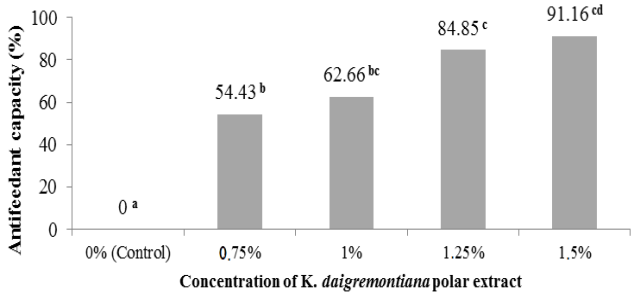

Figure 2. Result of analysis antifeedant capacity according to the antifeedant test

In addition, alkaloid has a repellant smell for insect (Salisbury and Ross 1997). It also contains tannin that can inhibit the digestive enzyme and decreasing feeding activity (Munandar and Madyawati 2002).

Based on Chapman (1995), *K. daigremontiana* chemical compositions that previously explained as well as its effect to the feeding behavior. Reveal the theory of *K. daigremontiana* antifeedant mechanism as follows: The anti-feeding behavior that exhibited by *P. xylostella* larvae during antifeedant test mostly affected by the taste and smell of *K. daigremontiana* polar extract. The central nervous system of *P. xylostella* receive that both stimulus as an information of quality and quantity of food chemicals. By that, the anti-feeding behavior can occurs.

Other toxic chemicals in *K. daigremontiana* such as flavonoid, saponin, cardiac glycoside, polyphenol and sterol might be responsible in *P. xylostella* mortality. The hydrogen bound in flavonoid and saponin can make a complex with protein cell membrane which causing the change of cell membrane permeability and followed by mortality (Harborne 1994; Hendrick 2006). While the toxicity of cardiac glycoside, begins from the its formation complex with alpha sub unit of Na/K-ATP-ase which generates the inhibition of sodium potassium pump and then followed by necrotic cells (Alberts et al. 1994; Supartinah and Kusmoro 2003). Accordingly, the higher concentration of *K. daigremontiana* polar extract will give the higher complex protein-toxic substance and by then causing the significant higher ($p<0.05$) mortalities of *P. xylostella* (Table 2).

In conclusion, concentration of *K. daigremontiana* polar extract of 1.25% have antifeedant capacity in level of 84.85% and stipulated as an effective antifeedant for *P. xylostella* larvae. The LC_{50} of *K. daigremontiana* polar extract to *P. xylostella* larvae occurred at concentration of 0.5% with exposure time 72 hours.

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