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Research Article

Insecticidal and Feeding Deterrent Effects of *Kaempferia galanga* L. and *Amomum subulatum* on *Pieris rapae* L. Larva

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Abstract

Background and Objective: *Pieris rapae* L., is one of the most widespread and destructive pests of cruciferous plants. At present, synthetic chemical insecticide is still the main approach to control this pest despite several disadvantages to human health and the wildlife environment as well as biological resistance. To search for plants having insecticidal activity, the biological effects of two medicinal plants *Kaempferia galanga* L. and *Amomum subulatum* on *Pieris rapae* L., were investigated. **Materials and Methods:** The methanol extracts of dry rhizomes and fruits of *Kaempferia galanga* L. and *Amomum subulatum* were used to determine the mortality, feeding and oviposition deterrence of larvae and adult of *Pieris rapae* L. **Results:** *Kaempferia galanga* L. and *Amomum subulatum* exhibited insecticidal activity against *Pieris rapae* L., with LC_{50} values of 2.11 and 11.80% (w/v), respectively. In the antifeedant test, *Kaempferia galanga* L., extract showed no significant difference with the control at the low concentration (0.5 and 1%). Whereas, with a concentration of 0.5%, *Amomum subulatum* extract demonstrated a high antifeedant effect on *Pieris rapae* L., larvae. In addition, plants treated with these two extracts reduced eggs laid by *Pieris rapae* L., in field conditions showing the oviposition deterrent properties. **Conclusion:** These results indicated that *Kaempferia galanga* L. and *Amomum subulatum* extracts have insecticidal substances against *Pieris rapae* L., which can be used for developing effective pesticides or/and oviposition deterrents for integrated pest management.

Key words: *Pieris rapae* L., *Kaempferia galanga* L., *Amomum subulatum*, insecticidal activity, feeding deterrent, raw extracts, limonoids

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pieris rapae L., (*P. rapae*-cabbage white butterfly) is one of the most serious pests of crucifers in the world[1]. As reviewed, this small cabbage butterfly has become the most abundant invasion butterfly on continents[2]. Common hosts of this pest are *Brassicaceae* vegetable crops such as cabbage, broccoli, cauliflower and radish. Normally, the larvae chew on the leaves, make holes and even break down the plants causing economic loss. Currently, due to the disadvantages of chemical insecticides on human health and the environment as well as resistant phenomena of insects, the demand for a safe eco-friendly pest management system has risen in recent years. In this situation, naturally derived plant products have achieved high attention and promising achievements to control several target insects[3]. In general, plant-based insecticides have different effects on the insects such as toxic, antifeedant, repellent and regulated insect growth through different mechanisms[4]. Plants were used as insecticidal substances with their essential oils, raw extracts, plant powder or isolated compounds[5]. On *P. rapae*, studies on the toxic activity, antifeedant or deterrent activities of various plant extracts have been conducted for the last thirty years[6]. As reported, some plant extracts showed feeding repellent or deterrent activities to *P. rapae* larvae including pumpkin leaf[7], rosemary[8], spearmint[8], *Artemisia annua* L.[9] and *Achillea millefolium* L.[9]. Notably, some plant extracts exhibited toxic activities such as *Artemisia annua* L.[9] and *Achillea millefolium* L.[9]. From various potential insecticidal plants, several bioactive compounds were isolated and evaluated for their effects on *P. rapae* such as deoxypodophyllotoxin[10], rhodojaponin-III[11], ginsenoside[12] and limonoids[13]. Despite the vast number of plant species, our knowledge about their insecticidal properties against insect pests is restricted. *Zingiberaceae* is one of 13 families that has the most publications on its insecticidal activity[5]. *Kaempferia galanga* L. and *Amomum subulatum* are species that belong to the *Zingiberaceae* family and have demonstrated numerous biological properties on several target species[14,15].

However, the effect of these plant extracts on *P. rapae* is still unknown. Therefore, this study was designed to investigate the toxicity, antifeedant and deterrent properties of extracts from *K. galanga* and *A. subulatum* on *P. rapae* under laboratory conditions.

MATERIALS AND METHODS

Study area: This study was conducted in the Plant Biotechnology Laboratory in the Department of Plant Biotechnology at the Faculty of Biotechnology, the Vietnam National University of Agriculture from August, 2020 to March, 2022.

Preparation of plant material and extraction: Rhizomes of *K. galanga* and fruits of *A. subulatum* were collected in the North of Vietnam and allowed to dry at 35 degrees until no change in the weight of the samples. Dry materials were ground into fine powder by the electric blender. Crude extractions of two plants were prepared by following the procedure reported by Warthen *et al.*[16]. Briefly, 15 g of dried plant material was stirred with 150 mL of 85% methanol for 1 hr at room temperature. Then, the methanol solution was kept in the fridge at 4°C for 24 hrs. The procedure was followed by stirring for 1 hr, the solution was filtered through 2 layers of filter paper. The extraction solvent was completely removed by the rotary evaporator. The concentrated residue was suspended in 5 mL methanol as a stock extract. The crude extract was kept in the fridge at 4°C for further use.

Rearing of *Pieris rapae* L.: Larvae and adults of *P. rapae* were collected in a non-pesticide cabbage field of Vietnam National University of Agriculture, Hanoi, Vietnam. Larvae were grown in the box (65×25×20 cm), fed with cabbage leaves and cleaned every day. When larvae became pupae, the pupae were transferred into a net cage with pots of cabbage plants (1.5×1.2×1 m). The cages were kept in greenhouse conditions at 20-25°C, 65±5% RH. Hatching butterflies were fed with sweet solution (water, honey and sugar in a ratio of 8:1:1) and laid eggs on plants. After egg hatching, larvae were transferred to the lab and fed with cabbage leaves. The 3rd instar larvae were used for experiments.

Toxicity test: Five concentrations (0.5, 1, 2, 4 and 8%) of crude extracts were used for the toxicity test. Forty-five third instar larvae of *P. rapae* were used for each concentration in three replications. Larvae were starved for 4 hrs before exposure to treatment conditions. The leaf was dipped in diluted extract for 30 sec and let dry at room temperature. The larva was treated with a drop of 15 µL leaf extract with a tested concentration on the head. The LC_{50} values were calculated using GraphPad Prism 9.3.0 software with the mortality percentage at 24 and 48 hrs. The data were subjected to non-linear regression analysis.

Antifeedant test: Antifeeding effects were studied with *K. galanga* extract with two concentrations of 0.5 and 1% and with *A. subulatum* at 0.5% concentration. The control treatments were used with 0.5 and 1% methanol. The cabbage leaves were treated by dipping them into the testing solutions and then dried for 30 sec at room temperature. The third instar larvae were starved for 4 hrs and were fed treatment leaves. Leaf consumption of larvae was measured by using ImageJ software. Each replication of treatment was conducted on 10 of the 3rd instar larvae. The experiment was repeated three times. The index of antifeeding was calculated as:

$$\frac{C-T}{C}\times 100$$

where, C was the leaf consumption of the control treatment and T was the leaf consumption of the treatment. The leaf consumed area was collected at different time points up to 24 hrs of treatment.

Oviposition test: To evaluate the oviposition (repellent) activity, *K. galanga* and *A. subulatum* extract were applied to cabbage seedlings at the 6-8 leaf stage under field conditions. Each type of plant extract was assessed with different concentrations (1-4%). The control treatment was sprayed with water. Plants for each treatment were prepared in blocks randomly. Twelve plants were used for each treatment. The number of eggs on the plant was counted after 24 hrs of treatment.

RESULTS

Toxicity of plant extracts on 3rd instar *Pieris rapae*: The mortality data presented in Fig. 1 revealed the insecticidal activity of methanol extracts from *K. galanga* and *A. subulatum* against *P. rapae*. The percentage of mortality correlated to the concentration of the plant extracts and exposure period. After 24 hrs, 100% mortality of *P. rapae* larvae was recorded when exposed to *K. galanga* extract at a concentration of 8%. After 48 hrs of exposure to *K. galanga* extract, the mortality slightly increased. In addition, the mortality caused by *K. galanga* extract was higher than that of *A. subulatum*.

The toxicity of *K. galanga* and *A. subulatum* extracts on 3rd instar larvae of *P. rapae* at 24 and 48 hrs exposure to extracts was reported in Table 1. At 48 hrs of exposure, the LC_{50} value for *K. galanga* and *A. subulatum* against 3rd instar larvae were 2.11 and 11.80% (w/v), respectively. The data

Table 1: Dose-response parameter of 3rd instar larvae of *P. rapae* at 24 and 48 hrs

Parameters	*K. galanga*	*A. subulatum*
At 24 hrs		
log LC_{50}	0.46	-
HillSlope	2.13	-
LC_{50} (% w/v)	2.91	-
log LC_{50} (95% CI)	0.40-0.52	-
HillSlope (95% CI)	1.66-2.77	-
LC_{50} (95% CI)	2.53-3.33	-
At 48 hrs		
log LC_{50}	0.32	1.07
HillSlope	1.47	0.97
LC_{50} (% w/v)	2.11	11.80
log LC_{50} (95% CI)	0.24-0.40	0.97-1.24
HillSlope (95% CI)	1.14-1.90	0.74-1.25
LC_{50} (95% CI)	1.75-2.53	9.21-17.22

LC_{50}: Lethal concentration, CI: Confidence interval (95%), -: Not identified and Equation $Y = 100/(1+10^{((\log LC_{50}-X)\times \text{HillSlope})})$

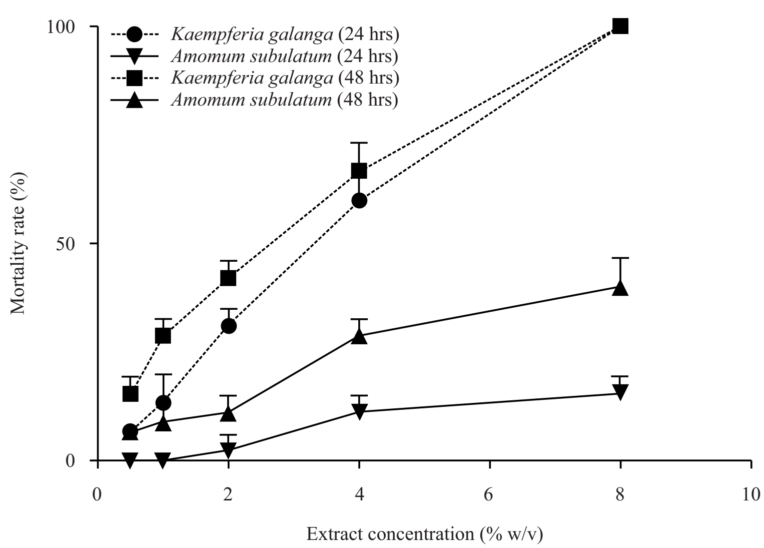

Fig. 1: Effect of *K. galanga* and *A. subulatum* extracts on the mortality rate of 3rd instar larvae of *P. rapae*

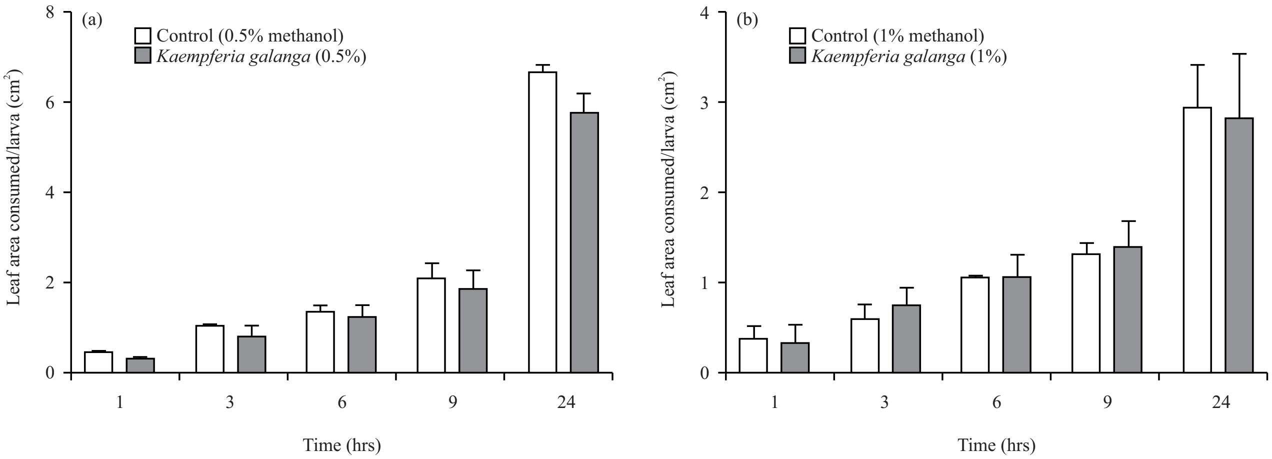

Fig. 2(a-b): Antifeedant effect of *K. galanga* extract on the third instar larvae of *P. rapae* at (a) 0.5% and (b) 1% concentration

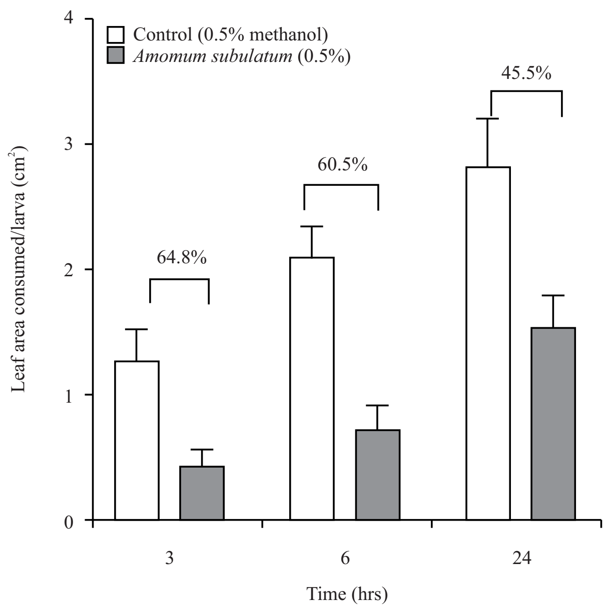

Fig. 3: Antifeedant effect of *S. subulatum* extract on the third instar larvae of *P. rapae*
Number indicated the feeding deterrent index

indicated that *K. galanga* was more toxic to the 3rd instar larvae of *P. rapae* than to *A. subulatum*.

Antifeeding effect: The antifeedant activity of *K. galanga* and *A. subulatum* extracts was assessed by measurement of leaf area consumption 24 hrs after the third instar larvae of *P. rapae* were exposed to extracts. Extract of *K. galanga* at 0.5 and 1% concentration showed no significant difference with control treatment in leaf consumption ($p>0.05$) (Fig. 2a-b). The results indicated that the methanol extract of *K. galanga* has no antifeeding activity. In contrast, the methanol extract of *A. subulatum* at 0.5% concentration showed an antifeedant index of 64.8, 60.5 and 45.5%, after 3, 6 and 24 hrs, respectively (Fig. 3). The result suggested that the methanol extract of *A. subulatum* has high antifeedant efficiency (>50%) to *P. rapae* larvae.

Oviposition-deterrent activity: In the oviposition deterrence assay, different concentrations of two extracts (1-4%) were applied to cabbage at the 6-8 leaves stage under field conditions. Water was used as a control treatment for this experiment. The *P. rapae* adult preferred to lay eggs in the distilled water-sprayed plants to treated plants (Fig. 4). However, the level of preference was dependent on the type

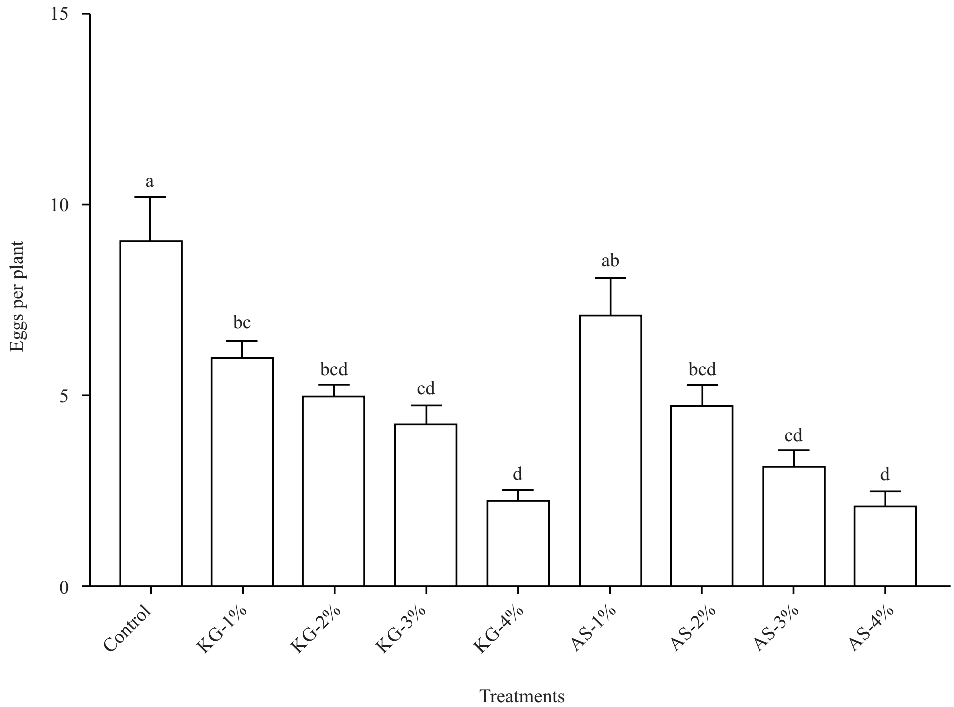

Fig. 4: Number of eggs laid on plants treated with indicated extracts in field conditions after 24 hrs of treatment
Control: Sprayed with water, KG: *K. galanga* and AS: *A. subulatum*. Bars marked with different letters were significantly different from one another with $p<0.05$

and concentration of extract. The number of eggs laid on the plants treated with *K. galanga* extract was significantly lower than that of the control in all four tested concentrations. *K. galanga* extracted at 4% concentration showed the lowest number of eggs. In addition, the number of eggs laid in leaves treated with *A. subulatum* extract at different concentrations (1-4%) was also reduced compared with the control treatment. A low concentration of *A. subulatum* (1%) exhibited no statistical difference in the number of laid eggs per plant with the control treatment. There was no statistical difference between *K. galanga* and *A. subulatum* extract in oviposition deterrent activity at higher applied concentrations (2-4%). The data indicated that both *K. galanga* and *A. subulatum* extracts have oviposition deterrence activity.

DISCUSSION

One of the functions of secondary plant metabolites is to protect plants from herbivores[17]. Several compounds proved to have defense responses such as phenolics, terpenes, nitrogen-containing compounds and sulfur-containing compounds[18]. The present work revealed the insecticidal activity of both *K. galanga* and *A. subulatum* extracts against *P. rapae* larvae and adults. According to the current finding, the mortality of *P. rapae* with tested extracts increased when increasing concentrations and exposure time. The crude methanol extract of *K. galanga* was more toxic than that of *A. subulatum*. At a high concentration (8%) of *K. galanga* extract, all larvae dead after 24 hrs of exposure. Extract of *A. subulatum* exhibited low toxicity activity. Notably, *K. galanga* and *A. subulatum* were identified as insecticidal activities against *P. rapae* for the first time although their insecticidal properties have been proven. Previously, it was reported that *K. galanga* had insecticidal properties on different target insects[15]. As reported, the ethyl esters of cinnamate and p-methoxycinnamate in *K. galanga* exhibited insecticidal properties to *Spodoptera littoralis*[19]. In another study, three constituents of *K. galanga* ethyl cinnamate, *trans*-cinnamaldehyde and ethyl p-methoxycinnamate showed potent toxicity against *Liposcelis bostrychophila*[20]. In addition, all extracts of *K. galanga* with different solvents showed insecticidal effects on *Sitophilus oryzae*[21]. To date, the mechanism of *K. galanga* rhizome derivatives that work as insecticidal compounds remains unclear. Similarly, *A. subulatum* has demonstrated insecticidal activities on insect species, for example, *Callosobruchus chinensis*[22] and *Callosobruchus maculatus*[23]. Additionally, *A. subulatum* essential oil has been shown moderately toxic to *Drosophila melanogaster*[24]. Current results on the mortality of *P. rapae* at different concentrations of *A. subulatum* extract were consistent with these findings.

Current results have shown that *K. galanga* had no antifeedant effect against the third instar of *P. rapae* larvae. In contrast, the crude methanol extract of *K. galanga* caused a significant oviposition deterrent to *P. rapae.* Supporting this data, previous studies have shown *K. galanga* had repellent activity on several species such as *Aedes aegypti*[25], *Sitophilus zeamais*[26], *Liposcelis bostrychophila*[20]. Liu *et al.*[20] demonstrated that *trans*-cinnamaldehyde could be a potential compound for repellent. This was indeed the first report of oviposition deterrent on *P. rapae* adults of *K. galanga*.

According to current findings, the extract of *A. subulatum* exhibited antifeedant and oviposition deterrent effects on *P. rapae*. At a low concentration of *A. subulatum* extract (0.5%), the antifeedant index was 45.5%. Additionally, *A. subulatum* extract with concentrations of 2-4% showed a significant difference in egg numbers compared to the control. A literature review survey regarding the antifeedant activity or oviposition deterrent of *A. subulatum* against *P. rapae* or other pests showed that no studies reported on this information. This data is the very first report about antifeedant and oviposition deterrent activities against *P. rapae* of *A. subulatum*.

CONCLUSION

In conclusion, the results of this investigation indicated that *K. galanga* and *A. subulatum* extracts exhibited insecticidal activities and oviposition deterrent effects on *P. rapae*. In addition, *A. subulatum* extract proved the antifeedant effect at low concentrations. Therefore, these plant materials could be good resources for developing alternative approaches to control *P. rapae* in the future. Future studies on the toxic, active compounds/substances needed to be investigated.

SIGNIFICANCE STATEMENT

This study revealed the positive effects of *K. galanga* and *A. subulatum* extracts to control a specific pest *P. rapae*. *K. galanga* extract showed a high mortality rate and significant oviposition deterrent property. Moreover, *A. subulatum* extract gave strong antifeedant and oviposition deterrent properties besides the moderate insecticidal effect. This study will help researchers to provide better alternative plant-based pesticides in the integrated management of *P. rapae*.

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