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Effectiveness of Kratom (*Mitragyna speciosa* Korth) leaf extracts against adult of sweet potato weevil (*Cylas formicarius* Fabricius) in laboratory conditions

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Abstract The result showed that the hexanolic leaf extract of Kratom presented rather high effect to this weevil with >70% mortality at 24 h after treatment. Besides, Kratom leaf extracted by using ethanol and acetone showed lower effect against this weevil. The hexanolic extract presented extreme toxicity to weevil with LC_{50} values of 3.50, 2.88 and 2.27% at 24, 48 and 72 h after treatment, respectively. This extract at 4% concentration exhibited repellency effect with > 77% significant difference compared with the control group. In addition, this extract showed high efficiency on oviposition inhibition for weevil (>89%) at 5% concentration, significantly different as compared with the control group.

Keywords: Insecticidal properties, Inhibited oviposition, Repellency effect, Crude extracts

Introduction

Sweet potato (*Ipomoea batatas* Lamk.) is an annual plant with a climbing vine and a fibrous root system. It produces small adventitious roots at its nodes, which can grow out of the joints. These roots are used for storing food, eventually expanding into tubers that remain underground. The flesh of sweet potatoes comes in a variety of colors depending on the species, and they are characterized by their sweet and starchy taste (Wang *et al.*, 2016). Native to the American continent, sweet potatoes are widely cultivated in many countries. In Thailand, various varieties of sweet potatoes are grown, with cultivation taking place in all regions and throughout the year. Sweet potatoes offer numerous benefits and medicinal properties, and their tubers can be used to prepare a wide range of dishes, both savory and sweet (Nedunchezhiyan *et al.*, 2012). This plant is often

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affected by infestations of insect pests, leading to reduced market value and quality of produce, the sweet potato weevil (*Cylas formicarius* Fabricius) has been particularly devastating, causing damage to all parts of the plant. The infested tubers lose market value due to bitterness, reduced weight, and unpleasant odor (Hue and Low, 2015). Currently, there has been an increased use of chemical insecticides to meet the rising demand of consumers. However, this has led to significant adverse impacts on the environment, including harm to beneficial insects and other vital components of ecosystems. Moreover, chemical residues on food crops can pose risks to both agricultural workers and consumers. This highlights the importance of sustainable and integrated pest management practices to minimize the environmental and health consequences associated with the escalating use of chemical insecticides while still meeting consumer demands. (Ali *et al.*, 2021).

Due to the adverse side effects of these chemical substances on the environment and human health, several studies have explored alternative approaches, such as essential oils and plant extracts. Compounds found in essential oils and plant extracts have been found to affect various insect species without causing harm to the environment or consumers. This shift towards natural and sustainable alternatives is driven by the need to reduce the negative impacts associated with the extensive use of chemical insecticides while still addressing consumer demands (Zenoozi *et al.*, 2022). In contemporary pest management, various plant-based extracts have emerged as effective alternatives for insect control. While chemical insecticides continue to be used, plant-based extracts have gained popularity due to their perceived safety and reduced environmental impact (Tembo *et al.*, 2018). It has been discovered that the insecticidal properties of the extract from star anise (*Illicium verum* Hook. F.), when dissolved in acetone, can lead to a 100% mortality rate in the larvae of the lesser mealworm (*Alphitobius diaperinus* Panzer) through a feeding method. The essential oils derived from peppermint (*Mentha piperita* L.) and black pepper (*Piper nigrum* L.) plants exhibited insecticidal properties effective against the rice weevil (*Sitophilus oryzae*) and rice moth (*Corcyra cephalonica*) (Khani *et al.*, 2012). Those plant extracts have demonstrated the insecticides properties, especially when applied through methods such as spraying, contact, fumigant or mixing with insect-consumed food.

Kratom (*Mitragyna speciosa* Korth.) is indigenous to Southeast Asia. The leaves of Kratom is often used as traditional medicine to relieve tiredness and muscle fatigue, and to treat some common illnesses (Srichana *et al.*, 2015). It has bioactive constituents, such as a major alkaloid mitragynine and a minor component 7-hydroxymitragynine (Prozialeck *et al.*, 2012). Previously researches reported that Kratom extracts have insecticidal properties such as, Rashid *et al.* (2012) have applied Kratom leaves extract mixed into beef liver could be inhibit the growth rate of blowfly (*Chrysomya megacephala* (Fabricius)). In addition, mitragynine has also been detected in samples of blowfly maggots that ate this beef livers impregnated with substances from Kratom leaves as food. Hematpoor *et al.* (2022) reported that the methanol extract of Kratom was the most potent extracts against *Sitophilus oryzae* and *Rhyzopertha dominica* with LC_{50} values of 0.30 mg/mL and 0.49 mg/mL, respectively by rice grain treated with each extract method. Furthermore, Kratom leaves extract have shown effectiveness in preventing and controlling the larvae of the *Aedes aegypti* (L.) mosquito, commonly known as the yellow fever mosquito (Phaophanplaek *et al.*, 2022). The application of Kratom extracts for testing against the sweet potato weevil to evaluate its potential as an insecticidal agent were interested.

This study aimed to evaluate the effects of Kratom (*Mitragyna speciosa* Korth.) leaf extracts by using hexane, acetone, and ethanol as solvents against adult of sweet potato weevil (*Cylas formicarius* Fabricius) in laboratory conditions. The insecticidal properties, killing, repellency, and oviposition inhibition activities of this insect were evaluated by filter residue contact method.

Materials and methods

Plant collection and preparation of phytoextracts

Old leaves of Kratom (*Mitragyna speciosa* Korth.) were collected from Kratom plantation located in Bangkok, Thailand and then they were cleaned, followed by drying in an oven at 45°C for 2 days. Then, the dried leaves were finely ground and soaked in solvent solutions at a ratio of 1:4 (w/v) for 3 days, according to method adapted from Pumnuan *et al.* (2022). The first extraction was performed by using hexane as solvent, and the remain from hexane extraction was then immersed with acetone and ethanol, respectively. All extractions were filtered through a Buchner funnel and filter paper (Whatman^{TM} No.1), concentrated under low pressure using rotary evaporator at 40°C to obtain the hexane, acetone and ethanol crude extracts, respectively. These crude extracts were stored at 4°C in a refrigerator for future experiments. Tween-20 used as dissolving agent for crude extracts and water of ratio 2:1 and diluted in water in different concentrations.

Culture of sweet potato weevil (Cylas formicarius Fabricius)

Sweet potato (*Ipomoea batatas* (L.) Lam) tubers damaged by sweet potato weevil (*Cylas formicarius* Fabricius) were collected and used to establish a culture in laboratory. The tubers were placed in a square plastic rearing box (30 cm width × 30 cm length × 20 cm height), with air holes for proper ventilation to prevent moisture buildup and fungal growth. The weevil culture was maintained in room temperature (25±2°C) until developing to adult, 5-7 days old in 2^{nd} to 3^{rd} generations of weevils and they were utilized for subsequent experimental testing.

Contact toxicity test

The insecticidal property by filter paper residue contact method was evaluated and prepared by modified experimental method according to Bhavya *et al.* (2018). The test was conducted by dropping 1 ml of each extract solution at 1.0-5.0% concentrations onto the evenly paper discs (Whatman^{TM} No.1, 90 mm diameter) placed in 90 mm glass petri dish. The filter paper was then air dried for approximately 3 minutes. Three replicates were maintained for each test concentration containing 10 unsexed adult weevils at the middle of the filter paper disc and then the dish was sealed. These dishes were placed at room temperature (25±2°C), the mortality rate of weevils was recorded at 24, 48, and 72 hours after exposure and compared with the control (10%v/v Tween-20 in water). The lethal concentrations of Kratom leaf extracts needed to kill 50% and 90% of the insects (LC_{50} and LC_{90}, respectively) were calculated and presented for insecticidal property value.

Repellent activity test

The repellent activity by the wat of filter paper residue contact method was used by modified experimental method according to Kerdchoechuen *et al.* (2010). The filter paper discs (Whatman^{TM} No.1, 90 mm diameter) were cut into two halves, the first half treated with the treatment group and the other half with as the control group (10% Tween-20 in water) placed in 90 mm glass petri dish. The treatment group as each Kratom leaf extract solutions at 0.5-5.0% were evaluated. This test was dropped 0.5 ml of each extract solution was applied onto each half filter paper, and air dried for approximately 3 minutes. For each test containing 10 unsexed adult weevils at the middle of the dish and the dish was sealed. These dishes were placed at room temperature (25±2°C), and the number of weevils on each half filter paper side was evaluated at 24 hours. Those response effects (repellent and attractant percentage rates) were classified using the method of Doungnapa *et al.* (2021).

Oviposition inhibition test

The oviposition inhibition test by tubers dipping method was evaluated with each extract solvents at 1, 3 and 5% concentrations, compared with the control group (5% Tween-20 in water) and blank (non-dipped with any solvent). The same size of sweet potato tubers was dropped in each extract solution of 1 min and after air-drying for approximately 3 min. The treated tubers were placed in a square plastic box (size 45 L), with air holes for proper ventilation to prevent moisture buildup and fungal growth. All treatments with three replications were placed in this container box which supported stainless steel rack. Next, five hundred unsexed adult weevils were introduced into the container, 48 hours for allow oviposition. Subsequently, the adult weevils were removed, and the number of eggs in the sweet potato tubers were counted after treated 10 days and calculated oviposition inhibition rate.

Statistical analysis

The experiment was performed in a completely randomized design (CRD) with three replicates per treatment. The insecticidal property test results, represented by LC_{50} and LC_{90} values (lethal concentration of extract solvents required to kill 50% and 90% of weevils, respectively) were determined using probit analysis. The data from the repellency test were analyzed by the $\chi 2$ test. In addition, oviposition inhibition test was analyzed by ANOVA (analysis of variance), and the difference between treatments was compared by DMRT (Duncan's multiple range test).

Results

Contact toxicity test

The killing activity of hexane, acetone, and ethanol extracts from Kratom leaves at 1% concentration against adult of sweet potato weevil showed that the hexane Kratom leaves extract presented highest mortality rates of 73.3, 86.7 and 93.3% at 24, 48 and 72 hours after treatment, respectively. Whereas, the acetone and ethanol Kratom leave extracts at 1% concentration presented low insect mortality rates (<10%) (Figure 1). The hexanolic Kratom extract demonstrated the highest insecticidal activity and showed the LC_{50} and LC_{90} values of 3.499

and 5.703%, respectively at 24 hours after treatment, 2.879 and 5.135%, respectively at 48 hours after treatment, and 2.271 and 4.528%, respectively at 72 hours after treatment (Table 1).

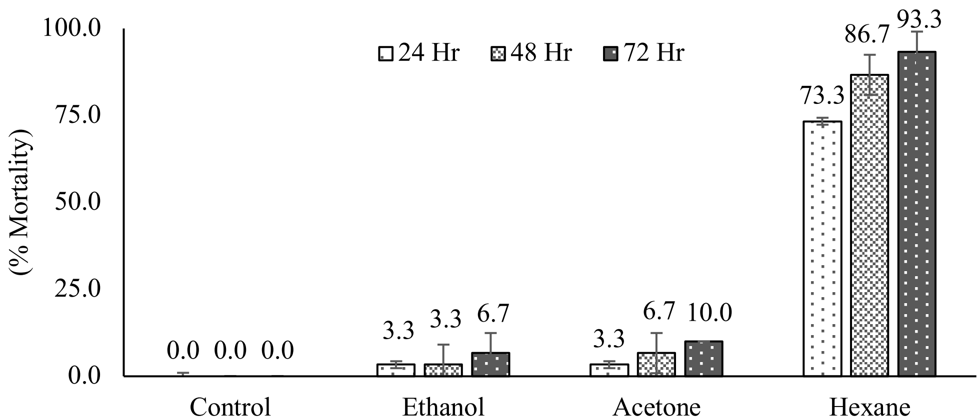


Figure 1. Effectiveness of hexane, acetone and ethanol extracts from Kratom (*Mitragyna speciosa* Korth) leaves at 1% concentration against adult of sweet potato weevil (*Cylas formicarius* Fabricius) via filter paper residue contact method

Table 1. The LC_{50} and LC_{90} values of hexane extract from Kratom (*Mitragyna speciosa* Korth) leaves against adult sweet potato weevil (*Cylas formicarius* Fabricius) via filter paper residue contact method

After treated (h)	Toxicity[1/]			SE	χ^2	P[2/]
	Regression[3/]	LC_{50} (%) (range)	LC_{90} (%) (range)			
24 h	Y = 0.581x – 2.034	3.499 (2.977-4.147)	5.703 (4.869-7.349)	0.044	12.385	0.015*
48 h	Y = 0.568x – 1.636	2.879 (2.439-3.347)	5.135 (4.466-6.263)	0.041	8.69	0.690ns
72 h	Y = 0.568x – 1.289	2.271 (1.467-2.996)	4.528 (3.647-6.550)	0.041	21.256	<0.001**

[1/] Data were determined based on n = 10 adults of sweet potato weevil / three replications, lethal concentrations of Kratom leaf extracts needed to kill 50% and 90% of the insects (LC_{50} and LC_{90}, respectively) at 24, 48 and 72 h after treatment.
[2/] *, ** : Significant difference at P < 0.01 and P < 0.01, respectively, ns: nonsignificant difference.

Repellent activity test

The repellent activity of hexane extracts from Kratom leaves at various concentrations (0.1-5.0% concentrations) against adult of sweet potato weevil showed that this extract at 5.0% concentration presented highest repellency efficacy as 80.0%, significant difference at P < 0.01 compared with the control group. In addition, the concentration of 4.0% presented repellency efficacy as 77.5%, significant difference at P < 0.05 compared with the control group. While the concentrations less than 0.4% showed non- repellency efficacy with <77.5% (Table 2).

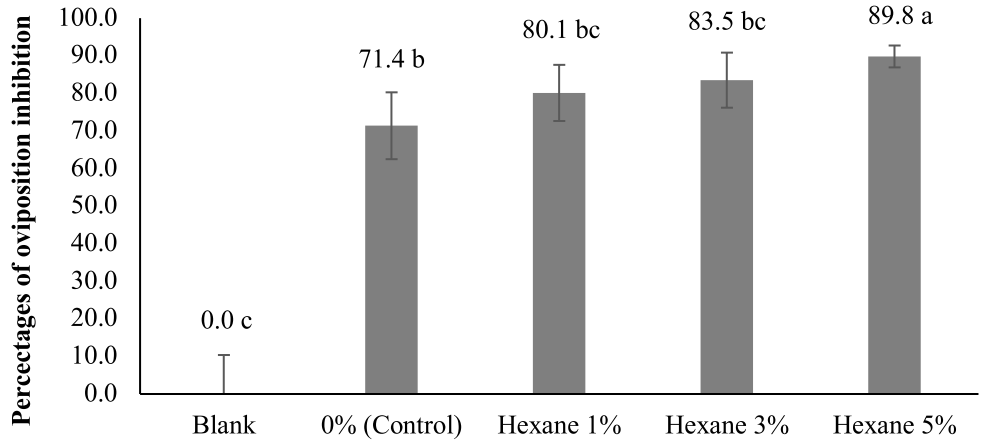
Table 2. Response percentage (repellency and attraction) of sweet potato weevil adult (*Cylas formicarius* Fabricius) to different concentrations of hexane extract from Kratom (*Mitragyna speciosa* Korth) leaves via filter paper residue contact method at 24 after treatment

Concentration percentages of extracts[2/]	%Response[1/]		χ^2	P
	%R	%A		
0.1	55.0	45.0	0.2005	0.654317^{ns}
0.5	65.0	35.0	1.8414	0.174783^{ns}
1.0	65.0	35.0	1.8414	0.174783^{ns}
2.0	67.5	32.5	2.5274	0.111884^{ns}
3.0	72.5	27.5	4.2660	0.388830^{ns}
4.0	77.5	22.5	6.5450	0.010518*
5.0	80.0	20.0	7.9121	0.004911**
Control	50	50	-	-

[1/] Data were determined based on n = 10 adults of sweet potato weevil / three replications. %R: indicates the percentage response to the treatment (repellency), %A: indicates the percentage response to the control (attraction) at 24 h after treatment, *, **: Significant difference at P < 0.05 and P < 0.01, respectively, ns: nonsignificant difference. SE: standard error, χ2: chi-square value.

Oviposition inhibition test

The oviposition inhibition activity of hexane extract from Kratom leaves at 0.0, 1.0, 3.0 and 5.0% concentrations against adult of sweet potato weevil were evaluated by comparing with the blank group (non-dipped with any solvent). This result showed that the Kratom hexanolic extract at 5% concentration presented highest oviposition inhibition activity against sweet potato weevils as 89.8% oviposition inhibition, significant difference at P < 0.05 compared with the other groups. It was interesting that the control group (5% Tween-20 in water) presented high oviposition inhibition activity (71.4%), with less oviposition inhibition value than the treatment groups significant difference (Figure 2).



Means value on the bar graph followed by the same common letter are not significantly different as determined by DMRT at $P<0.05$.

Figure 2. Oviposition inhibition percentage of different concentrations of hexane extract from Kratom (*Mitragyna speciosa* Korth) leaves against sweet potato weevil (*Cylas formicarius* Fabricius) via tubers dipping method

Discussion

The results found that the hexane extract from Kratom leaves presented highest killing adult of sweet potato weevil than acetone and ethanol extracts. There were many researches proved that the extract from medical plants extracted by hexane showed higher insecticidal properties than extracted by acetone or ethanol. Pumnuan *et al.* (2022) reported that hexane extracts from long pepper (*Piper retrofractum* Vahl) presented the higher toxicity to kill seed beetles (*Callosobruchus chinensis* L., *Callosobruchus maculatus* Fab., and *Sitophilus zeamais* Motschulsky) than acetone and ethanol extracts. The hexane extract of three indigenous herbs of northeastern Thailand (*Anethum graveolens* Linn., *Oroxylum indicum* Linn. and *Polygonum odoratum* Lour.) were highly effective in controlling tobacco cutworm (*Spodoptera litura* F.) more than acetone and ethanol extracts (Charoensak *et al.*, 2009). Efficacy of hexane crude extract of Kratom leaf against *A. aegypti* larvae was more than ethanol extract (Phaophanplaek *et al.*, 2022). Ogunsina *et al.* (2011) was also proved that medical plant extracts from *Lantana camara* L. and *Monodora myristica* G. extracted by hexane exhibited insecticidal properties against the *C. maculatus* and *S. zeamais*. Specifically, *M. myristica* extract concentration of 0.1 g/ml presented greater mortality action against *C. maculatus* (100%) and *S. zeamais* (96%) after 24 h of treatment.

Aryani and Auamcharoen (2016) investigated the repellency activity of crude extracts from Thai plants extracted by various solvents against *S. zeamais* in laboratory. It was found that *Z. cassumunar* hexane extract scored the highest repellency up to 99% at concentration 1,415 µg/cm^2, 8 h after application. In the other hand, this extract caused lower mortality than crude extract with methanol solvent. Adedire *et al.* (2011) reported that cashew kernel extracted by hexane was most effective against *C. maculatus* evoking 98.75% mortality, after 72 h of exposure. Ethanol extract was least toxic causing 85.50% insect mortality after 96 h of exposure. All crude extracts from cashew nut seed extracted by hexane, acetone and ethanol effectively reduced oviposition by *C. maculatus*. And also, no adult emerged in cowpea seeds treated with cashew kernel oil extract.

In this research, crude extract from Kratom leaves extracted by hexane showed high effectiveness against adult of sweet potato weevil (*C. formicarius*) in the form of insecticidal properties, killing, repellency, and inhibited oviposition activities to insect. Thise result could verify the potentiality of Kratom extract in controlling insect pest. Previous reports substantiated that could inhibit the growth rate of blowfly (Rashid *et al.*, 2012), and controlling the stored product insect pests (Hematpoor *et al.*, 2022) and larvae of the mosquito (Phaophanplaek *et al.*, 2022). The results obtained from this study revealed that extract of Kratom was effective in controlling sweet potato weevil and could serve as an alternative to synthetic insecticides in the future.

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