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TOXICITY OF SEED EXTRACT FROM KADSURA ANANOSMA KERR AGAINST TRIBOLIUM CASTANEUM (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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ABSTRACT

The seed from *K. ananosma* was extracted by rotary evaporator and aqueous alcoholic extraction by fermentation and were tested for their insecticidal activities against red grain beetle *Tribolium Castaneum* (Herbst). The results revealed that the seed extracted from *K. ananosma* by rotary evaporation showed the mortality on the adult of *T. Castaneum* at 10.33% with treatment of 20% concentration by topical application method, but given percent mortality of the red grain beetle at 16.67% with treatment of 20% concentration by residual exposure method. Additional, the fumigant toxicity given the high mortality on *T. Castaneum* up to 40.01% with treatment of 50% concentration of seed extracts. Meantime, the seed extracts of *K. ananosma* by fermentation showed lower mortality on *T. Castaneum* only 13.33% at 50% concentration with testing by residual exposure method. Therefore, efficiency of seed extracts of *K.*

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Ananosma with rotary evaporator showed higher toxic on the tested insects than seed extracted by fermentation. Also fumigant toxicity had more effect on mortality of the *T. Castaneum* than contact poison.

Keywords: Kadsura ananosma Kerr; Tribolium Castaneum Herbst; Fumigant toxicity; Mortality.

1. INTRODUCTION

The plant species *Kadsura ananosma* Kerr currently found in Thailand. Its known as Noi Na Khay in Thai belongs to family Schisandraceae, which is a wild plant on the highland and nearly extinct in conservation of Plant Genetic Conservation Project under the Royal Initiative of HRH Princess Maha Chakri Sirindhorn in Thailand. It looks like a vine, fruit shape and smell like sugar apple *Annona squamosa* Linn. The ripen fruit is edible as sugar apple. The plants are rich in nutrients and contains high antioxidants as well. It is reported that the vines and roots are used as an herbal cure digestive disorders, and rheumatoid arthritis in China. Currently, found to be a valuable drug in the prevention of tumors, against HIV virus and anti-hepatitis. Therefore, the benefit derived from this plant as an alternative for insect control are considered. Since the application of various synthetic insecticides over the years has led to the development of insecticide resistance in various parts of the world. In recent years, various workers have been concentrating their efforts on the search for natural products.

There was never any previous research about this plant species before. Hence there has not yet been a report on its toxicity, however, there are many previous reports on the other plant known as sugar apple *Annona squamosa* Linn. which all parts similarly with the *Kadsura ananosma* Kerr. The extract from seeds of *Annona squamosa* L. has insecticidal activity to kill *Tribolium castaneum* and *Musca nebulo* [1]; human head-lice [2]; *Nepholettix virescens* [3] and *Boophilus microplus* [4]; [5].

Red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), distributed worldwide, is the most destructive pest of stored products. The application of various synthetic insecticides and fumigants to grain storage over the years has led to a number of problems, including the development of insecticide resistance in stored grain insect pests in various parts of the world. An alternative to synthetic pesticides is the use of essential oils; the

toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored-product insects [6]. Many essential oils are known to have fumigant activity against eggs, larvae and adults of *Tribolium castaneum*, and the toxicity progressively increased with increased exposure times and concentrations [7].

This study was carried out to investigate the toxic properties of seed extracts from this plant *Kadsura ananosma* Kerr against red flour beetle *Tribolium castaneum* Herbst.

Fig.1. Comparison fruit and seeds of the wild plant *Kadsura ananosma* Kerr. (a) and the fruit and seeds of *Annona squamosa* Linn. (b)

2. RESULTS AND DISCUSSION

2.1 Toxicity of seed extracts of *Kadsura ananosma* Kerr against *Tribolium castaneum* Herbst by bioassay methods

The efficacy test of seed extracts of *K. ananosma* Kerr by the topical application method as shown in Table 1. The result showed that contact toxicity to red flour beetle adult *T. castaneum* at 20% concentration of the seed extract by rotary evaporation was low efficient with mortality of 10.33 % after 72 h. The lower concentration from 5% to 10% showed that it had no effect on mortality of *T. castaneum*. Particularly, after 24 h, the result showed that mortality was not occurring on the treated adult *T. castaneum* at all of concentration from 5% to 20%. Meantime the fumigant toxicities to *T. castaneum* by residual exposure showed that 20% of the concentration produced a significant difference ($P < 0.05$) with mortality rate of 16.67% when compared to testing by the topical application method as shown in the Table 2. Therefore, the increased concentration of seed extracts by rotary evaporation to 30%, 40% to 50% showed that fumigant toxicities to the treated adult *T. castaneum* given mortality at 26.67%, 33.34% and 40.01% respectively. Although the result showed that % mortality had

not reached to 50 percent occurred on treated insects after 72 h, however the treatments produced a significant difference ($P < 0.05$) when compared to the control treatment (Table 2).

Table 1. Toxicities of seed extracts by rotary evaporation against *Tribolium castaneum* Herbst by topical application method

Treatment	Mortality (%)					
	12h	24h	36h	48h	72h	df
Control	0	0	0	0	0^{b}	ns
5%	0	0	0	0	0^{b}	ns
10%	0	0	0	0	0^{b}	ns
15%	0	0	3.33	3.33	6.66^{ab}	ns
20%	0	0	3.33	3.33	10.33^{ab}	ns

* = significant difference, means in the followed by the same letter are not significantly different at 5% level by DMRT

Table 2. Toxicities of seed extracts by rotary evaporation against *Tribolium castaneum* Herbst by residual exposure

Treatment	Mortality (%)					
	12h	24h	36h	48h	72h	df
Control	0	0	0	0	0^{c}	ns
20%	0	0	0	6.67	16.67^{b}	*
30%	6.67	10	13.33	13.33	26.67^{ab}	*
40%	0	6.67	13.34	20.01	33.34^{ab}	*
50%	6.67	23.34	30.01	30.01	40.01^{a}	*

* = significant difference, means in the followed by the same letter are not significantly different at 5% level by DMRT

2.2 Toxicity of seed extracts of *Kadsura ananosma* Kerr by extraction methods against *Tribolium castaneum* Herbst

Results of mortalities by residual exposure method due to the effect of seed extraction by fermentation as shown in Table 3. The result showed that toxicity to *T. castaneum* at 50% of

concentration was low efficient with a mortality rate of 13.33% after 72 hours. Particularly, after 36 hours, it had no effect on mortality occurred on the treated insects at all concentration of seed extracts by fermentation from 20% to 40% (Table 3). The toxicity of the *K. ananosma* seed by the rotary evaporator extraction show higher in insecticidal activity on the *T. castaneum* than fermentation extraction. The seed extract with rotary evaporator extraction given mortality at 40.01% when compared to the seed extracts with fermentation, it at only 13.33 % in the same concentration of 50% as shown in the Fig.2.

Table 3. Toxicities of seed extracts by fermentation against *Tribolium castaneum* Herbst By residual exposure

Treatment	Mortality (%)					
	12h	24h	36h	48h	72h	df
Control	0	0	0	0	0^{c}	ns
20%	0	0	0	3.33	3.33^{bc}	ns
30%	0	0	0	3.33	3.33^{bc}	ns
40%	0	0	0	0	10^{ab}	ns
50%	3.33	3.33	10	10	13.33^{ab}	ns

* = significant difference, means in the followed by the same letter are not significantly different at 5% level by DMRT

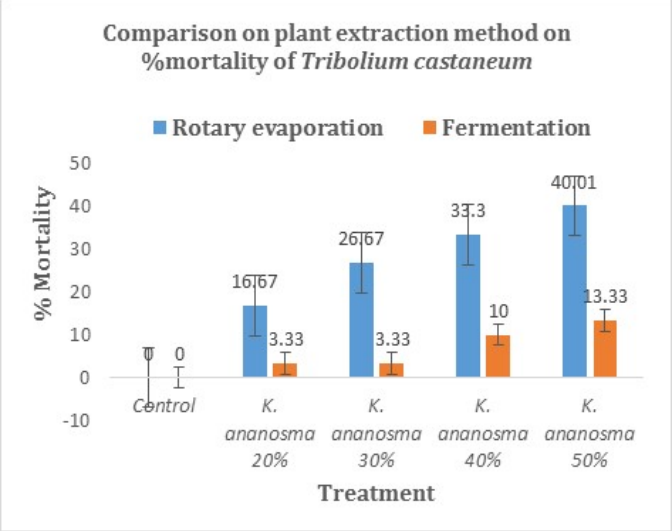

Fig.2. Comparison on the seed extraction method on the % mortality of *Tribolium castaneum* Herbst by residual exposure

DISCUSSION

Although there has not yet been a report on its toxicity from this plant on the insect pest. There are many previous reports on the insecticidal activity of the sugar apple *Annona squamosa* Linn. against many insects. The extract from seeds of *Annona squamosa* L. has insecticidal activity to kill *Tribolium castaneum* and *Musca nebulo* [1]. The seed oil of *A. squamosa* has high insecticidal activity against beetles, houseflies [8]; leafhoppers [3]; tropical cattle tick [4]; [5]. Particularly, the ethanol extracts of seed from *Annona squamosa* L. contained more larvicidal activity than that from the leaves [9]. The toxicity of seed oil of *A. squamosa* and its petroleum ether extracts on tested insects is also poor, but aqueous fractions of water distillation of the seeds has moderate toxicity [10]. Most previous works have been carried out by using the rotary evaporation extracts of the plants. In this work, the aqueous alcoholic extraction by fermentation and rotary evaporation of the seed *Kadsura ananosma* were tested. It has been shown that the rotary evaporator extraction of *K. ananosma* seeds is more intense in insecticidal activity on the tested insects. Additional, the seed extract with rotary evaporator showed high toxicity on *T. castaneum* within 24h after treatment by residual exposure and showed significant different in corrected mortality between 24h, 36h, 48h and 72 h as shown in Table 2. In general, essential oils may be a potential grain protectant by killing various life stages of *T. castaneum* through contact and fumigant actions [11]. In addition, essential oils have been shown to have higher fumigation toxicity than contact toxicity against the red flour beetle *T. castaneum* [12]. Furthermore, plant extracts contain compounds that show ovicidal, repellent, antifeedant, sterilization and toxic effects in insects [13]. The toxicity may be by contact, ingestion or through fumigant action. The use of plant products in general, against insect pests of stored products [14];[15].

3. EXPERIMENTAL

3.1 Materials and Methods

3.1.1 Insect preparation

Red flour beetle *Tribolium castaneum* Herbst was collected from rice storage silos in Phitsanulok province, Thailand, and was laboratory-reared in plastic containers (20 cm length×14 cm width×8 cm height) containing grain rice, with eggs laid on grain rice and

hatched at room temperature. Two-day old adults of red flour beetle were selected and prepared for the bioassay tests.

3.1.2 Plants extract preparation

Two methods were used in the extraction:

Rotary evaporation; seeds of *Kadsura ananosma* Kerr were collected, the seed was cleaned and grinded with grinder, and was mixed with 95% ethyl alcohol at a ratio of 1:10 (powder/solvent) for 72 h and shaken during the extraction time to ensure complete extraction. The mixtures were first filtered with cheesecloth, then with WhatMan No 1 filter paper (24cm). The filtrates were then separately concentrated in vacuum using rotary evaporator. Ethyl alcohol was evaporated from the supernatants on a rotary evaporator at 95 mm Hg pressure and 65 °C. The evaporated seed extracts were kept in the refrigerator at 4°C and was diluted for testing on the red grain beetle. Tween 80 was used for emulsion to stabilize the sample before testing (Fig 3).

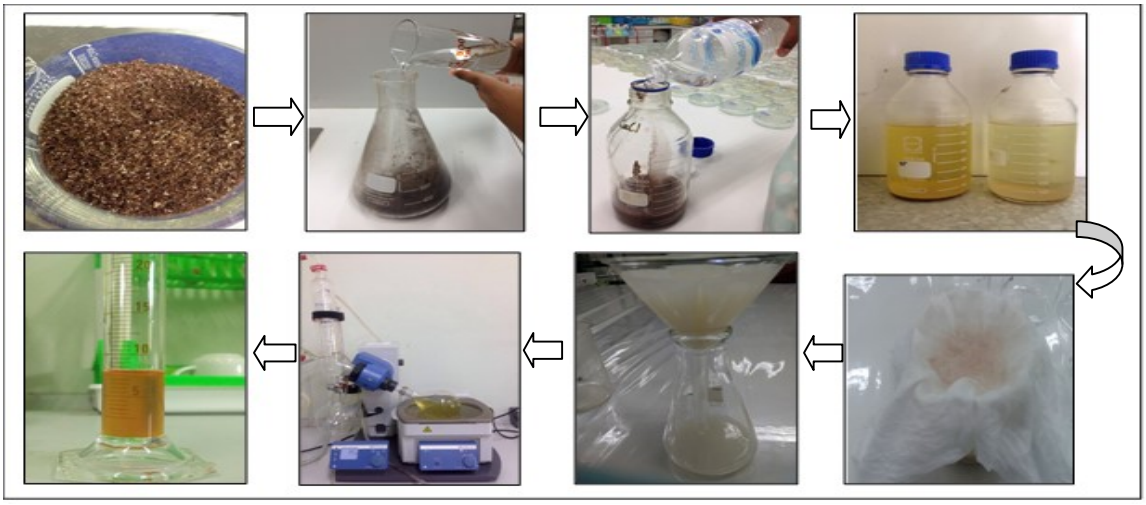

Fig.3. Process of seed extraction of *Kadsura ananosma* with Rotary evaporator

Aqueous alcoholic extraction by fermentation; the seed powder was mixed and immersed with 95% ethyl alcohol at a ratio of 1:5 (powder/solvent) for 3 days and shaken during the fermentation time to ensure complete extraction. The mixtures were first filtered with cheesecloth, then with What Man No 1 filter paper. The seed extract was kept in containers covered with aluminum foil at 4°C (Fig.4).

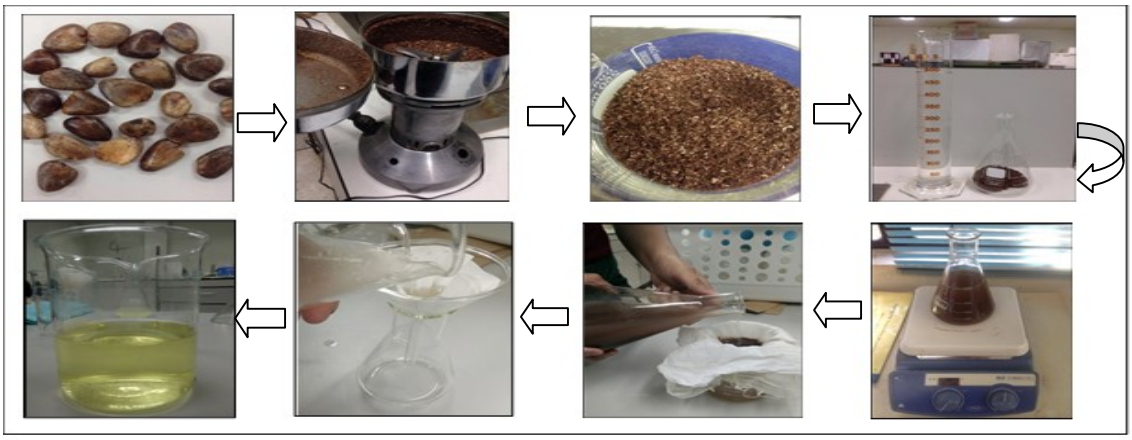

Fig.4. Process of seed extraction of *Kadsura ananosma* with aqueous alcoholic extraction by fermentation

3.1.3 Insects bioassay test

Two methods were used in the experiment:

Topical Application assesses contact toxicity; a 0.5 µl droplet of the diluted essential oil at a ratio of 1:9 for each treatment was placed on the head area of the red flour beetle with a micro-applicator. The treated insects were moved to a cup with grain rice (10 insects /cup) for observation of mortality.

Residual Exposure assesses fumigant potential; the diluted essential oil at a ratio of 1:9 was sprayed on filter paper and placed in a petri dish. Red flour beetles were added after the treatment evaporated (10 insects / petri dish). Each treatment was carried out in 4 replicates. Mortality was observed 12, 24, 36, 48 and 72 hours after adding the red grain beetles. When control mortality occurred on the experimental test, the mortality was corrected by Abbott's formula [16]:

$$\% \text{ Mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

3.1.4 Statistical Analysis

The significance of treatments was calculated by one-way Analysis of Variance (ANOVA) and effective treatment was separated by the Duncan's New Multiple Ranges Test (DMRT). Differences between means were considered significant at $P< 0.05$.

4. CONCLUSION

In conclusion, seed extracts from the plant species *Kadsura ananosma* Kerr given potential to control *Tribolium castaneum* Herbst by fumigant toxicity, the topical application method revealed lower toxic for bioassay test. The extraction method from the seed by rotary evaporation given higher efficiency than fermentation extracted. The seed of *K. ananosma*, or its purified bioactive compounds, may prove to be very useful as a grain protectant, Further research into the bioactivity of *K. ananosma* and its constituents against other insects can be considered.

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