Bioactivities of *Annona muricata* L. leaf extracts against *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

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Abstract

Three different extracts of *Annona muricata* L. leaves at five concentrations (1, 2, 3, 4 and 5%) were evaluated for their effects on mortality, repellency, inhibition of F_1 adult emergence and grain weight loss caused by rice weevil, *Sitophilus oryzae*. The adult weevils were exposed to the plant extracts by grain treatment test. The repellent activity of the previous extracts was also studied using the area preference bioassay. The insect mortality increased proportionally with the increase of the extract concentration and exposure time. All the three extracts at different concentrations were highly toxic to the insect. Among them, the ethanol extract at the highest concentration showed the strongest insecticidal activity against *S. oryzae* with 100.00% mortality at 4 d after treatment followed by ethyl acetate (97.50%) and petroleum ether extracts (87.50%), respectively. In addition, the ethanol, ethyl acetate and petroleum ether extracts at all concentrations gave over 90% inhibition rate of of F_1 adult emergence. The ethanol extract at 4% and 5% produced 100.00% inhibition rate followed by ethyl acetate (99.04%) and petroleum ether extracts (98.91%), respectively. Moreover, all the three extracts significantly reduced percentage of grain weight loss compared to control. The ethanol extract at 5% produced the lowest grain weight loss with 2.33% followed by ethyl acetate (2.47%) and petroleum ether extracts (2.48%), respectively. All the extracts also exhibited strong repellent activity against *S. oryzae*. The petroleum ether extract had the strongest repellent activity with 64.95-87.59% mean repellency (class IV to class V). The mean repellency for ethanol and ethyl acetate extracts were 66.17-82.28% (class IV to class V) and 58.08-83.99% (class III to class V), respectively. The results suggested that *A. muricata* has a great potential for development as botanical insecticide for *S. oryzae* control.

Keywords: *Sitophilus oryzae*, *Annona muricata*, mortality, repellency, adult emergence

1. Introduction

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is one of the most serious insect pest of stored cereals throughout the world (Yankanchi and Gadache, 2010). The larva feeds within the kernel and consumes the endosperm. The adult leaves a large, ragged exit hole in the kernel and feeds on damaged kernels. Feeding by *S. oryzae* larva and adult cause weight losses as much as 75% (Dal Bello et al., 2001) and decreases nutritional value of the grains. Moreover, *S. oryzae* reduces germination resulting in the lower prices for seed grains (Moino et al., 1998).

Use of natural compounds from plant extracts has been suggested as a viable source of alternative treatments for insect control because many of such compounds have novel modes of action, no or low toxicity to non-target organisms, and are less harmful to the environment (Schmutterer, 1997). In addition, plant based insecticides are cheaper than some synthetic insecticides. Numerous plant extracts have been reported to have a variety of biological activities against insects including insecticidal, repellent, antifeedant, fumigant, growth regulatory, anti-oviposition activities (Isman, 2006).

Annona muricata L. is a member of Annonaceae family and cultivated throughout the tropical countries including Thailand. To date, research on pharmacological activity of active compounds from the leaf of this plant is growing rapidly. The leaf extracts of *A. muricata* have been reported to have molluscicidal (Santos and Sant'Ana, 2001), antiarthritic, anti-inflammatory (Foong and Hamid, 2012), antitumor, antidiabetic and antioxidant activities (Florence et al., 2014). They also have insecticidal activity against a range of insect pests (González-Esquinca et al., 2012). However, in Thailand *A. muricata* is categorized as an underutilized tree species. Only a limited amount of researches has been conducted on bioactivity *of A. muricata* leaf against insect pests. Thus, the objectives of this study were to assess both insecticidal and repellent activities of *A. muricata* leaf extracts against *S. oryzae* as well as their effects on F_1 adult emergence and grain weight loss.

2. Materials and Methods

2.1. Insects

S. oryzae adults were collected from naturally infested rice grains. They were reared on sterilized rice grains in the laboratory at 26±1°C and 75 % RH under 14: 10 (L: D). Adults were allowed to mate and oviposit for two weeks. The parental insects were then removed and the medium containing the eggs were kept in the same condition until adult emergence. Seven to 10-day old adults were used in all the experiments.

2.2. Plant material and extraction

The air-dried leaves of *A. muricata* were ground into powder using an electric grinder and screened through an 80-mesh screen. For extraction, 100 g of leaf powder was soaked with 500 ml of petroleum ether, ethanol or ethyl acetate at room temperature (26±1°C) for 3 d. After filtering, the extracts were concentrated to dryness by a rotary evaporator under low pressure to obtain the crude extracts. Five concentrations (1, 2, 3, 4 and 5%) of each extract were prepared using acetone as a solvent. The diluted concentration was used for subsequent experiments.

2.3. Effect on adult mortality, F_1 adult emergence and grain weight loss

The insecticidal activity of three extracts of *A. muricata* leaf against adults of *S. oryzae* was evaluated by grain treatment test. An aliquot of 0.5 ml of each concentration of each extract was mixed thoroughly with 20g of sterilized rice grains. After evaporation of solvent, the treated rice grains were placed in a 250 ml plastic jar. Ten pairs of *S. oryzae* adults were introduced into each container. Adult mortality was monitored daily over 4 days. On day 4, both dead and alive insects were removed from each container. The rice grains were returned to their respective containers and kept at the same conditions. On day 49, the number of emerged F_1 adults were recorded. Inhibition rate (%) or reduction in the number of emerged adults was calculated by the method of Tapondjou et al. (2002) as follows:

$$\text{Inhibition rate (\%)} = \frac{(\text{Cn}-\text{Tn})}{\text{Cn}} \times 100 \qquad (1)$$

Where Cn is the number of emerged adults in the untreated grains and Tn is the number of emerged adults in the treated grains. The rice grains were also reweighed and weight loss (%) was determined by the method of Odeyemi and Daramola (2000) as follows:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight}-\text{final weight}}{\text{Initial weight}} \times 100 \qquad (2)$$

2.4. Repellent effect

An area preference bioassay described by Obeng-Ofori et al. (1998) was adapted to evaluate repellent activity of *A. muricata* leaf extracts against *S. oryzae* adults. Whatman No.1 fillter paper of 9 cm diameter was divided into two equal parts. The first half was treated with 0.5 ml of each concentration while the other half was treated with 0.5 ml of acetone alone as control. After evaporation of solvent, a full disc was remake by attaching the treated half and the control half with clear adhesive tape. Each filter paper disc was placed in a Petri dish. Twenty unsexed adults were released at the center of filter paper disc and the Petri dish was covered. Each treatment was replicated four times. The number of insect presented on control and treated sides were recorded at 1, 2, 3, 4 and 24 h after treatment. Repellency rate (%) was calculated by using the following formula from Abbott (1925):

$$\text{Repellency rate (\%)} = \frac{(A-B)}{A} \times 100 \qquad (3)$$

Where A was average number of insects present on untreated portion and B was average number of insects present on treated portion.

2.5. Statistical analysis

All data were analyzed with one-way ANOVA followed by Duncan's multiple range test at $P<0.05$ using software SPSS V 17. The repellent rate was categorized to repellency class from O to V: class O = > 0.01%- <0.10%; class I = 0.10%-20.00%; class II = 20.10%-40.00%; class III = 40.10%-60.00%; class IV = 60.10%-80.00%; class V = 80.10-100.00% (Amin et al., 2000; Roy et al., 2005).

3. Results and Discussion

3.1. Effect on adult mortality

All three extracts of *A. muricata* leaf at different concentrations exhibited insecticidal activity against *S. oryzae* as dose-and time-dependent variables (Table 1). There were significant differences between tested extracts at different hours. At 1 d, all three extracts showed little or no insecticidal activity against the insect with mortality of 0.00 to 25.00%. At 2 d, the rice grains treated with ethanol extract at 5% cause the highest mortality of 47.50%. By 3 d, mortality in all extracts at all concentrations increased considerably compared to those at 2 d, and ethanol extract at 5% remained the strongest one with insect mortality of 82.50%. At 4 d, the highest mortality of 100% was produced from the rice grains treated with ethanol extract at 5% and the other concentrations of ethanol extract also showed strong insecticidal activity of 95.00 to 97.50%. In addition to ethanol extract, the rice grains treated with ethyl acetate and petroleum ether extracts also gave high mortality to the insect, ranging from 72.50 to 97.50% and 70.00 to 87.50%, respectively.

Our extracts seem to be more active than some of the other extracts reported previously against *S. oryzae.* For example, Saljoqi et al. (2006) obtained 82.00% mortality within 6 d using ethanol extract of *Melia azedarach* leaf at 10% against *S. oryzae* in wheat. Yankanchi and Gadache (2010) evaluated the insecticidal activity of *Clerodendrum inerme*, *Withania somnifera*, *Gliricidia sepia*, *Cassia tora* and *Eupatorium odoratum* leaf extracts at 5% against *S. oryzae* by grain treatment test and found the highest mortality of 86.66% from *C. inerme* leaf extract at 21 d after treatment. Buatone and Indrapichate (2011) investigated the toxicity of leaf extracts of *Metha cordifolia* with two solvents at 6.4% against *S. oryzae* in milled rice and reported the mortality of 100.00 and 76.67% for ethanol and water extracts within 20 d, respectively.

Table 1 Mean mortality of adults of *Sitophilus oryzae* in rice grains treated with *three Annona muricata* leaf extracts.

Solvent	Conc. (%)	Mortality (mean±SE, %)[a] 1 d	2 d	3 d	4 d
Petroleum ether	5	25.00±2.89a	27.50±4.79b	67.50±2.50a-e	87.50±2.50a-c
	4	15.00±2.89b	25.00±2.89bc	62.50±4.79c-f	80.00±0.00b-d
	3	15.00±2.89b	20.00±0.00bc	52.50±4.79e-g	72.50±2.50cd
	2	15.00±2.89b	20.00±4.08bc	50.00±5.78fg	70.00±5.78d
	1	12.50±2.50bc	17.50±2.50b-d	45.00±2.89g	70.00±7.07d
Ethyl acetate	5	5.00±5.00cd	15.00±2.89b-d	80.00±0.00ab	97.50±2.50a
	4	0.00±0.00d	15.00±2.89b-d	75.00±2.89a-c	92.50±4.79ab
	3	0.00±0.00d	12.50±4.79c-e	70.00±7.07a-d	90.00±4.08ab
	2	0.00±0.00d	5.00±2.89de	65.00±5.00b-f	80.00±5.78b-d
	1	0.00±0.00d	5.00±2.89de	42.50±2.50g	72.50±2.50cd
Ethanol	5	0.00±0.00d	47.50±2.50a	82.50±2.50a	100.00±0.00a
	4	0.00±0.00d	27.50±2.50b	77.50±4.79a-c	97.50±2.50a
	3	0.00±0.00d	25.00±2.89bc	75.00±2.89a-c	97.50±2.50a
	2	0.00±0.00d	15.00±2.89b-d	67.50±4.79a-e	95.00±5.00ab
	1	0.00±0.00d	12.50±2.50c-e	55.00±2.89d-g	95.00±5.00ab
control	-	0.00±0.00d	0.00±0.00e	0.00±0.00h	0.00±0.00e

[a]Mortality within a column followed by the same letter are not significantly different at P <0.01 by DMRT

Our current laboratory tests on *A. muricata* of three different solvents against adults of *S. oryzae* indicated that all three extracts had strong insecticidal activity. The effect was slow-acting. The three organic solvent extracts required 3 d to achieve greater than 50% mortality. These results also revealed that the efficacy of *A. muricata* leaf extracts for *S. oryzae* control depended on the organic solvent used for extraction, concentration and exposure time. Ethanol extract was the most toxic against *S. oryzae* followed by ethyl acetate and petroleum ether extracts, respectively.

The main active components found in *A. muricata* leaf extracts are acetogenins (Chang et al., 2003) alkaloids (Leboeuf et al., 1981) and essential oils (Owolabi et al., 2013). Among them, acetogenins have been reported to have insecticidal activity on insect pests including *Spodoptera littoralis*, *Leptinotarsa decemlineata*, *Myzus pesicae* (Guadaño et al., 2000). Álvarez Colom et al. (2008) found that acetogenins such as annonacin, annonacin-A, and *cis*-annonacin-10-one isolated from *A. montana* leaf were toxic to adults of *Oncopeltus fasciatus*. These acetogenins have also been found in *A. muricata* leaf (Wu et al., 1995). Besides acetogenins, benzylisoquinoline alkaloids derived from *Annona* species also have insecticidal activity (Saxena et al., 1993). Therefore, the insecticidal properties of *A. muricata* leaf extracts are attributed to the acetogenins and benzylisoquinoline alkaloids. However, the studies on insecticidal activity of other components from *A. muricata* leaf extracts should be clarified.

3.2. Effect on F_1 adult emergence and grain weight loss

The number of adults that emerged from all three extracts of *A. muricata* leaf at different concentrations treated rice grains was significantly lower than the adults in control (Table 2). No insects emerged from grains treated with ethanol extract at 4 and 5%. The inhibition rate of F_1 adult emergence was also significantly lower in all treated grains compared to control. The inhibition rate increased with increase in concentration of plant extract. The highest inhition rate (100.00%) was observed in grains treated with ethanol extract at 4 and 5% followed by ethyl acetate (99.04%) and petroleum ether extracts (98.91%) at the same concentrations. The weight loss in grains treated with all extracts at all concentrations was significantly lower than that of control. The grains treated with ethanol extract at 5% produced the lowest weight loss of 2.33% followed by ethyl acetate (2.47%) and petroleum ether extracts (2.48%), respectively (Table 2).

Table 2 Effect of three *Annona muricata* leaf extracts on F_1 adult emergence of *Sitophilus oryzae* and rice grain weight loss.

Solvent	Conc. (%)	No. of F_1 adult emerge (mean±SE)[a]	Inhibition rate (mean±SE, %)[a]	Weight loss (mean±SE, %)[a]
Petroleum ether	5	0.25±0.25cd	98.91±1.09ab	2.48±0.18f
	4	0.25±0.25cd	98.91±1.09ab	4.99±0.10e
	3	1.00±0.00b-d	96.64±1.13a-c	7.42±0.18d
	2	1.00±0.00b-d	96.64±1.13a-c	11.81±0.22b
	1	2.00±0.00b	91.35±0.37d	12.64±0.18b
Ethyl acetate	5	0.25±0.25cd	99.04±0.96ab	2.47±0.14f
	4	0.25±0.25cd	99.04±0.96ab	4.68±0.31e
	3	0.50±0.29cd	97.95±1.19ab	6.71±0.21d
	2	1.00±0.00b-d	95.67±0.19bc	9.03±0.20c
	1	1.50±0.29bc	93.62±1.07cd	10.01±0.05c
Ethanol	5	0.00±0.00d	100.00±0.00a	2.33±0.09f
	4	0.00±0.00d	100.00±0.00a	3.79±0.08e
	3	0.50±0.29cd	97.96±1.19ab	6.26±0.31d
	2	0.50±0.29cd	97.72±1.32ab	7.43±0.34d
	1	0.50±0.29cd	97.72±1.32ab	9.19±0.23c
control	-	23.25±1.03a	0.00±0.00e	31.89±0.92a

[a]Mean within a column followed by the same letter are not significantly different at P <0.01 by DMRT

Our results showed that the number of adults that emerged from the treated grains after 49 d of storage was dose-dependent. The reduction in F_1 adult emergence is probably due to egg mortality or larval mortality or even reduction in hatching of eggs and also might be due to the toxic substance (Mathur et al., 1985). Grain weight loss indicated the quantitative loss in stored grains due to the insects showing a direct relationship between insect population and weight loss.

3.3. Repellent effect

The repellent activity of three extracts of *A. muricata* leaf at different concentrations was tested against adults of *S. oryzae* using the area preference bioassay (Table 3). There was significant difference among the tested extracts at different hours. Overall, all extracts showed potent repellent activity against the pest. Among them, petroleum ether extract exhibited the strongest repellent activity with class IV to class V (64.95 to 87.59%). In addition to petroleum ether extract, strong repellency of 66.17 to 82.28% (class IV to class V) was also observed from ethanol extract. Repellency values of ethyl acetate extract ranged between class III and class V (58.08 to 83.99%). Moreover, the highest concentration (5%) of all extracts showed the strongest repellent activity to the insect with mean repellency values of 87.59, 83.99 and 82.28% for petroleum ether, ethyl acetate and ethanol extracts, respectively. These results revealed that repellent activity was dose dependent.

In previous studies, many plant extracts have been reported to have repellent activity against *S. oryzae*. Fernando and Karunaratne (2013) tested leaf extracts of *Olax zeylanica* with three solvents and reported the repellency of 96.0, 94.0 and 83.0% for methanol, ethanol and hexane extracts, respectively. Waliullah et al. (2014) evaluated the repellent activity of different extracts of *Clerodendrum viscosum* and found the highest repellency of 100% from leaf, root and stem extracts. However, *A. muricata* leaf extracts had higher repellent activity than some of the other plant extracts in the previous studies. Rahman et al. (2007) exposed *S. oryzae* to *Macaranga postulata* fruit extract and reported repellency of 9.84, 12.76 and 22.43% for concentrations of 1, 2 and 3%, respectively. Akhtar et al. (2013) tested the repellent effects of *Psidium guajava* leaf extract at 5, 10, 15 and 20% against *S. oryzae* and found the highest repellency of 70.33% at concentration of 20%.

Our results are the first to demonstrate the strong repellent activity of *A. muricata* leaf against *S. oryzae*. The results indicated that petroleum ether extract had the strongest repellent activity followed by ethanol and ethyl acetate extracts, respectively. The reason for this could be explained by the fact that repellent constituents of petroleum ether extract of *A. muricata* leaf are non-polar compounds with high volatility. Thus, petroleum ether extract showed strong repellent activity at the first hour and persisted upto 4 h. The repellent potential of the extract might be a result of the combined activities of constituents found in *A. muricata* leaf. However, isolation and identification of the active compounds responsible for the observed repellent activity of *A. muricata* leaf are necessary to study.

4. Conclusions

Three extracts of *A. muricata* leaf demonstrated have a broad spectrum activity against adults of *S. oryzae*. Among them, ethanol extract had the strongest insecticidal activity and produced the lowest F_1 adult emergence and grain weight loss. Petroleum ether extract showed the strongest repellent activity. Therefore, we conclude that *A. muricata* leaf extracts could be used as protectant against *S. oryzae*. Further work is in progress to isolate and identify the insecticidal and repellent constituents of this plant.

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Table 3 Repellent activity of *Annona muricata* leaf extracts against adults of *Sitophilus oryzae* using area preference bioassay.

Solvent	Conc. (%)	Repellency rate (mean ± SE)[a] 1 h	2 h	3 h	4 h	24 h	Mean Repellency (%)	Repellency class
Petroleum ether	5	88.71±2.53ab	87.08±2.98ab	91.67±2.78a	91.81±1.69a	78.68±2.12ab	87.59±1.47a	V
	4	85.24±4.25a-c	81.52±5.75a-f	90.03±3.66ab	90.18±2.95a	74.76±3.20a-c	84.35±2.10ab	V
	3	84.72±6.05a-c	85.62±1.89a-c	88.72±2.53ab	88.40±4.16a	70.84±2.41a-c	81.88±2.30a-c	V
	2	75.84±6.85a-c	68.75±2.08ef	70.84±2.41c-e	80.31±3.34ab	68.45±4.25a-d	74.61±2.18b-e	IV
	1	65.08±7.94c	68.45±4.25f	68.75±2.08d-f	66.37±3.65c-e	56.11±6.81d-f	64.95±2.39e-g	IV
Ethyl acetate	5	89.65±5.39ab	94.73±0.00a	74.76±3.21cd	78.68±2.12a-c	82.15±2.84a	83.99±2.10ab	V
	4	74.75±3.21a-c	87.26±1.64ab	66.37±3.65d-f	66.37±3.65c-e	76.84±1.84a-c	74.32±2.13b-e	IV
	3	79.86±5.48a-c	83.94±1.64a-d	70.84±2.41c-e	70.84±2.41b-d	72.67±3.78a-c	75.64±1.83b-e	IV
	2	36.54±3.21d	82.15±2.84a-e	59.52±2.38ef	63.99±4.30de	74.76±3.20a-c	63.39±3.81fg	IV
	1	29.54±3.79d	81.94±4.01a-f	56.41±5.92f	54.03±4.95e	68.45±4.25a-d	58.08±4.40g	III
Ethanol	5	94.54±2.27a	80.51±1.84b-f	83.99±1.64a-c	85.62±1.89a	66.67±0.00b-d	82.28±2.19a-c	V
	4	87.08±2.98ab	72.92±2.08c-f	78.68±2.12b-d	82.15±2.84ab	61.91±2.75c-e	76.55±2.24b-d	IV
	3	80.31±3.34a-c	72.23±5.56c-f	74.76±3.20cd	82.15±2.84ab	48.90±2.75ef	71.67±3.11c-f	IV
	2	74.30±5.24a-c	70.84±2.41d-f	72.92±2.08cd	78.68±2.12a-c	46.15±0.00f	68.58±2.88d-g	IV
	1	72.92±2.09bc	68.75±2.08ef	72.67±3.78cd	70.84±2.41b-d	45.69±4.87f	66.17±2.70d-g	IV

2 [a]Repellency within a column followed by the same letter are not significantly different at $P < 0.01$ by DMRT

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