Insecticidal activity of soursop leaves (*Annona muricata L.*) against *Sitophilus zeamais*

W.I.U. de Silva[a]*, M.H.Haroon[b]

[a, b]Department of Chemical Sciences, Faculty of Applied Sciences, South Eastern University of Sri Lanka

([a]ishadesilva94@gmail.com, [b]haroonnmh@fas.seu.ac.lk)

Keywords: *Annona muricata*, *Sitophilus zeamais*, insecticidal activity.

Introduction

The varied behavioral and physiological responses which insects express against various synthetic and natural toxins convey how it influences pest behavior and reflects the toxin's mode of action. Insects show resistance through behavioral mechanisms that reduce their subjection to insecticides and through the evolution of physiological mechanisms allowing them to deal with high insecticide levels in or on the body [1].

The conditions of relatively high humidity and temperature in tropical and subtropical countries are ideal for cultivating several grain types as well as for the large-scale propagation of all kinds of insects that can cause unsatisfied storage infrastructure for the grain stocks [2].

The harmful effects on the environment and health, greater selection pressure and the increment in the genetically resistant strains to the insecticidal active ingredients, and undesirable failures in supply and control management systems have been identified as the major limitations of the endless usage of synthetic insecticides. To get rid of these issues, discovering efficient methods for pest control is necessary. Therefore, it is essential to find out new substances that will fulfill the requirements of toxicological safety, low environmental impact, and agronomic efficiency for the beneficial control of insect pests of stored grains [3].

Recent investigations have proven that the efficiency of plant extracts as promising stored-grain protectants and several bioactive compounds which can be used as alternative insect control agents have been recovered. Phytochemical pesticides are advantageous for providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for designing target-specific molecules [4-6].

According to the literature of *Annona muricata L.*, although the seed is reported to have strong insecticidal effects, the leaves have not yet been recommended as the chemical and nutritional contents in recent times.

Therefore, the current study has been conducted to separate the bioactive compounds according to the polarity and evaluate the bioactivity of methanol extract and fractions obtained from *A. muricata* leaves against *Sitophilus zeamais* Motschulsky which is the main pest species of stored cereals in tropical conditions with the aim of discovering new eco-friendly natural insecticides.

Methodology

The leaves of *Annona muricata* were collected from areas where it grows naturally in the Ampara district, cleaned, shade dried at room temperature, and then pulverized.

Powdered plant leaves (500 g) were macerated in methanol (1000 ml) (1:2). After the filtration, the clear filtrate was concentrated by using a rotary evaporator under reduced pressure at (30-40) ^{o}C. A thick greenish-black oily residue (60 g) was obtained.

The dried crude extract was then fractionated using a column (length =110 cm, diameter =8.0 cm; pressure 1 bar) of silica gel (1000 g, Merck Kieselgel 60, 230-400 mesh ASTM) by using organic solvents as a combination of hexane: ethyl acetate and ethyl acetate: methanol in different ratios as in gradually increasing polarities. Eight fractions were separated using the TLC technique and dried. Methanol extracts (1000 ppm) of each of these fractions were applied to one week old S. zeamais to conduct the contact bioassay for 10 days. Mortality was recorded every 24 hours and the full bioassay was carried out under 27±2 ºC and 65±5 % relative humidity at constant laboratory conditions. Dead insects were removed at each assessment, counted, and recorded.

Mortality % = (Number of dead insects)/ (Number of total insects) × 100%

SAS university edition was used for the data analysis.

Results and Discussion

Table 1. Shows various fractions obtained from the column chromatography.

Fraction no	Combination of used eluent
F-1	0% to 25% Ethyl acetate with hexane
F-2	25% to 60% Ethyl acetate with hexane
F-3	60% to 95% Ethyl acetate with hexane
F-4	95% to 20% Ethyl acetate with hexane, Methanol with ethyl acetate
F-5	20% to 35% Methanol with ethyl acetate
F-6	35% to 50% Methanol with ethyl acetate
F-7	50% to 70% Methanol with ethyl acetate
F-8	70% to 100% Methanol with ethyl acetate

Table 2. Shows contact mortality of each fraction with time.

	Mean% Mortality ± SE									
	Day 01	**Day 02**	**Day 03**	**Day 04**	**Day 05**	**Day 06**	**Day 07**	**Day 08**	**Day 09**	**Day 10**
F1	56± 22.93	58 ± 2	66 ± 5.83	74 ± 5.83	74 ± 0	78 ± 2.44	82 ± 4	82 ± 0	82 ± 0	82 ± 0
F2	60 ± 7.07	82 ± 8.6	86 ± 2.44	90 ± 2.45	94 ± 4	96 ± 2	100 ± 2.45	100 ± 0	100 ± 0	100 ± 0
F3	8 ± 2	14 ± 4	32 ± 3.74	42 ± 3.16	46 ± 2.45	46 ± 0	54 ± 3.74	58 ± 2.45	58 ± 0	58 ± 0
F4	14 ± 8.72	16 ± 2	22 ± 4	44 ± 2	58 ± 6	68 ± 3.16	76 ± 3.74	76 ± 0	76 ± 0	76 ± 0
F5	70 ± 20	74 ± 4	74 ± 0	74 ± 0	74 ± 0	74 ± 0	76 ± 2	76 ± 0	76 ± 0	76 ± 0
F6	66 ± 19.9	70 ± 2.45	70 ± 0	74 ± 2.44	76 ± 2	80 ± 2.45	100 ± 15.49	100 ± 0	100 ± 0	100 ± 0
F7	32 ± 17.72	42 ± 5.48	46 ± 2.45	60 ± 7.48	80 ± 9.49	86 ± 4	92 ± 4	92 ± 0	92 ± 0	92 ± 0
F8	48 ± 18.28	50 ± 2	50 ± 0	54 ± 4	62 ± 5.83	64 ± 2	66 ± 2	66 ± 0	66 ± 0	66 ± 0
P.C.E.	50 ± 7.75	76 ± 4	86 ± 0	92 ± 4	100 ± 3.74	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
CONTROL	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Mean percentage contact mortality ± SE for five replicates.

According to the results shown in Table 2, the plant crude extract (P.C.E.) of Annona muricata has been recorded 100% of maximum contact mortality after five days. The CONTROL samples that were treated only with methanol showed 0% mortality throughout the experiment and corroborated that the insecticidal effects only depend on the added plant material. The F-2 shows the greatest bioactivity against the insects as it contains the highest mean percentage of mortality. This revealed that F-2 contains the highest concentration of bioactive chemical compounds even than that of the plant crude extract and might be containing a specific promising bioactive compound that can be used as a potent insecticide. Finally, fractions 2 and 6 were resolved to be active against the maize weevil, and fraction 2 was the most active hence it has the greatest mean value in mortality according to the HSD Test (Figure 2).

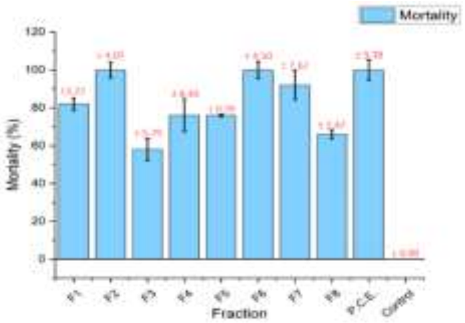

Figure 1. Distribution of Mortality after 10 days.

The mean comparisons after 10 days in HSD Test for Mortality are presented in Figure 2.

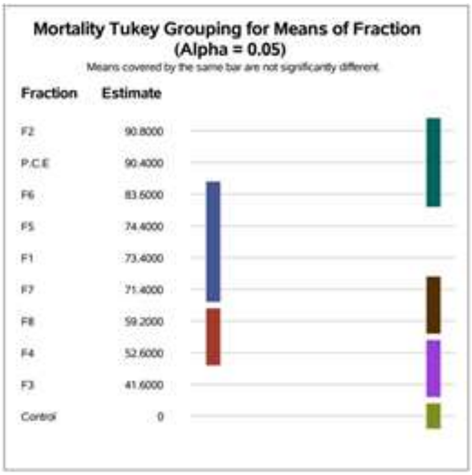

Figure 2. Tukey's grouping for Means of Mortality.

Table 3. Shows the Tukey's Studentized Range (HSD) Test results for Mortality after 10 days.

Alpha	0.05
Error Degrees of Freedom	81
Error Mean Square	89.59457
Critical Value of Studentized Range	4.60115
Minimum Significant Difference	13.772

Conclusion

The toxicity of the fractions of *Annona muricata L* against grown-up *S. zeamais* derives that they could contain promising bioactive (insecticidal) compounds and could be used to diminish the weevil invasions in stored corn. However, it is recommended that further research be conducted into their toxicity to mammals and other pests.

Acknowledgment

The authors would like to thank the Department of Chemical Sciences, Faculty of Applied Sciences, the South Eastern University of Sri Lanka for their guidance and support.

References

[1] Bermejo, A., Figadere, B., Zafra-Polo, M. C., Barrachina, I., Estornell, E., Cortes, D., Acetogenins from Annonaceae: recent progress in isolation, synthesis and mechanisms of action. Nat. Prod. Rep., 2005. 22(2), pp. 269-303.

[2] Wyckhuys, K., Neil, R., Local agro-ecological knowledge and its relationship to farmers' pest management decision making in rural Honduras. Agriculture and Human Values 2007. 24: pp. 307-321.

[3] Maciel, M., Morais, S., Bevilaqua, C., Silva, R., Barros, R., Sousa, R., Sousa, L., Brito, E., Souza-Neto, M. J. V., Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. 2010. 167(1): pp. 1-7.

[4] Liu, Z. L., Chu, S. S.; Liu, Q. R., Chemical composition and insecticidal activity against Sitophilus zeamais of the essential oils of Artemisia capillaris and Artemisia mongolica. Molecules 2010. 15(4), pp. 2600-8.

[6] Isman, M. B., Akhtar, Y., Plant Natural Products as a Source for Developing Environmentally Acceptable Insecticides. In Insecticides Design Using Advanced

Technologies, Ishaaya, I.; Horowitz, A. R.; Nauen, R., Eds. Springer Berlin Heidelberg: Berlin, Heidelberg, 2007. pp. 235-248.
[7] Tawfeek, M. E., Abu-shall, A., Gad, A., Mohey, M., Evaluation of Six Plant Essential FOils against Three Stored Product Insects and Their Effects on the Haemogram under Laboratory Conditions. AJAS, 2017. pp. 291-301.