Effectiveness of *Annona Squamosa* and *Annona Muricata* Seed Extracts as Ingredients in Bio-pesticides Spray

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Abstract. Pesticides released chlorofluorocarbon (CFC) that can harm to the environment and human health. Therefore, it is a need for development of an alternative methods in pest management due to negative effects of pesticides for human health and the environment. The objective of this study was to evaluate the effectiveness of *Annona Squamosa* and *Annona Muricata* seed extracts as ingredients in bio-pesticides spray on killing crickets (*Gryllidae*) and mealworm (*Tenebriomolitor*). The seeds of *Annona Squamosa* and *Annona Muricata* were dried, powdered and extracted in soxhlet’s apparatus using *n*-hexane as a solvent at 60°C. The extracted oil was placed in the rotary evaporator for purification process. As a result, mixture of extracts of *Annona muricata* and *Annona squamosa* induced high mortality rate to mealworm and cricket compared to extracts of *Annona squamosa* and extract of *Annona muricata* at all concentrations tested. Therefore, the extracts of these *Annona muricata* and *Annona squamosa* may be used as one of the alternatives as bio-pesticides that offer cheaper and environmentally friendly method.

1. Introduction

Pesticides are substance, that is being used as a plant growth regulator intended for preventing, destroying, or mitigating any pest. However, synthetic pesticides that has been widely used may cause harmful health effects to humans such as cancer, shorts and long-term injury to the lung damage, nervous system, reproductive dysfunction of the hormone and immune systems [1]. Despite, pesticides can affect environment by contaminating water, soil, turf, and other vegetation. Moreover, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants in addition to killing insects or weeds [2]. The common chemical ingredients that used in pesticides are Bendiocarb, Bifenthrin and Boric Acid. Due to its toxicity for human health and its impact on environment, it is become an urgent need to search for more environmental and toxicologically safe natural pesticides. Natural pesticides are one of the cheaper and safer alternatives in pest management. Bio-pesticides derived from natural materials such as plants, bacteria, animals, and certain minerals are naturally occurring substances that can control pests by non-toxic mechanisms [3]. It is usually less toxic than conventional pesticides which by contrast, produced by synthetic materials that directly kill or inactivate the pest.
Plant extracts were likely the earliest agricultural biocontrol, as history records that nicotine was used to control plum beetles as early as the 17th century. Experiments involving biological controls for insect pests in agriculture date back as far as 1835, when white-muscadine fungus (Beauveria bassiana) has been demonstrated as one of causes for an infectious disease in silkworm [4]. In most cases, the active ingredients of biopesticides are formulated in the same way as the synthetic pesticides and most convenient for farmers to use the same equipment for application [5]. Recently, several plants such as *Annona Muricata* and *Annona Squamosa* have been reported to have insecticidal properties and therefore can be used as bio-pesticide to control pests.

*Annona Muricata* is an evergreen blooming tree that is mostly distributed in tropical and subtropical regions of the world. It is a member of family *Annonaceae* known as soursop, graviola and guanabana. The fruit is locally referred as *durian belanda*. Research showed that more than hundreds of bioactive compounds such as alkaloids, acetogenins, and phenols were found in *Annona Muricata* plant. These bioactive compounds in vitro study have been identified as antioxidant, anti-inflammatory, antimicrobial, anti-cancer, insecticidal and larvicidal while in vivo studies found that the presence of these bioactive compounds as anti-stress, anxiolytic, immunomodulatory, anti-inflammatory, antidepressant, wound healing, antimalarial, hypoglycemic, hepatoprotective, gastro protective, anticancer and antitumoral activities [6]. Furthermore, phytochemical studies reveal that most of annonaceous acetogenins usually been found in leaves, barks, seeds, roots and fruits of *Annona Muricata* which this annonaceous acetogenins are the major constituents of *Annona Muricata* [6]. Active chemical compounds in the form of acetogenin found in leaves and seeds of soursop can be used to control the trips attack, suppress the intensity of the attack of green ladybugs and reduce the population [7]. Acetogenin also has a properties as an insecticidal (anti-insect-worm), antifeeding or lowering appetite properties [7]. Investigation on potential of soursop extract as larvicidal agent in larvae of *A. aegypti*. with modifications in biochemical constituents[8].

*Annona Squamosa*, commonly known as Custard Apple, is a native of West Indies and is cultivated throughout India, mainly for its edible fruit. The plant contains aporphine alkaloids, carvone, linalool, limonene, squamosin and quercetin [9]. Acetogenins, another a characteristic group of compounds isolated from *Annona Squamosa* seeds have been suggested to act as potential anti-neoplastic agents and are also the principal insecticidal constituents of *Annona* seed extract [10]. Custard apple extracts have shown promise for pest control against a range of insect pests. Laboratory and field tests showed that extracts from custard apple kernels were effective against crop pests like caterpillar. The result showed that soursop leaf extract is effective as a biopesticide material to control leaf caterpillar pest for delima rose apples. [11]. Insecticidal activity of the seed extracts of custard apple, *Annona Squamosa* in petroleum spirit, ethyl acetate, acetone and methanol against on larvae and adult beetles was also studied. It is reported that the seeds of *Annona Squamosa* were reported to have insecticidal and abortifacient properties. The crude oils from seeds of *Annona Squamosa* at 2.5 percent concentrations significantly reduced the leaf damage caused by larvae and adult beetles [10].

Recent research has been focusing on extraction of mixture of *Annona Squamosa* and *Annona Muricata* to increase its performance as bio-pesticides. Research by [13] found that the seed extracts of mixture of *Annona Squamosa* and *Annona Muricata* can be used as one of the alternatives in controlling mosquito that offer a new a cheaper and environmentally friendly method. Meanwhile, research by [12] proved the potential utilization of secondary metabolites in soursop (*Annona Muricata*) and sugar apple (*Annona Squamosa*) as plant-based pesticides to control pests and diseases of plants. Due to its (extraction of mixture of *Annona Squamosa* and *Annona Muricata*) potential as natural bio-pesticides, therefore, the objective of this research was to determine the effectiveness of mixture of *Annona Squamosa* and *Annona Muricata* extract in killing crickets (*Gryllidae*) and mealworm (*Tenebriomolitor*). The extraction using soxhlet extractor method is used with *n*-hexane as solvent.
2. Research Method

2.1. Materials/Instruments
The materials and instruments that are used to prepare the bio-pesticide are *Annona Squamosa* and *Annona Muricata* seed, 250ml flask of Soxhlet apparatus for extraction, rotary evaporator, the n-hexane as a solvent for the extraction of seed.

2.2. Method
After collection of seeds, both *Annona Squamosa* and *Annona Muricata* seeds were removed manually from these fruits, washed in water, and dried under the fan and was crushed into fine powder by using blender. The seeds were then further dried for 24 hours inside the laboratory oven to remove moisture content. A total of 500 grams of dried *Annona Squamosa* and *Annona Muricata* seeds were obtained, respectively.

2.3. Extraction Bio-Pesticide
After the drying process, 30 grams of each sample were weighed and placed inside the Soxhlet extraction thimble. 250ml of n-hexane was taken in the round bottom flask. The temperature was maintained at 60°C for boiling 4 hours. After 4 hours hexane with the extracted oil was taken out from the flask; and the seed sample was allowed to be cooled before further process. The sample then is placed into the rotary evaporator for purification process; to remove the solvent and the extract was separated out.

2.4. Preparation and Application of Bio-Pesticide
Bio-pesticide spray was categorized into three types as tabulated in table 1; *Annona Squamosa* with water based, *Annona Muricata* with water based and the mixture of both *Annona Squamosa* and *Annona Muricata*. These three types of sprays were prepared in different ratio of oil and base.

| Percent of *Annona Squamosa* (%) | Percent of *Annona Muricata* (%) | Percent of mixture *Annona squamosa* and *Annona muricata* (%) |
|----------------------------------|----------------------------------|---------------------------------------------------------------|
| 90                               | 90                               | 90                                                           |
| 45                               | 45                               | 45                                                           |
| 15                               | 15                               | 15                                                           |

2.5. Testing Against Insects
The different ratio of sprays was tested to two types of insects which are mealworms and crickets as shown in figure 1. The prepared sprays were tested against two types of insects: mealworms and cricket. The insects were placed in the dish containing food and drink and the insects were sprayed in various proportion and composition of the extract. Observation on the mortalities of the insects were done day by day. Mortality is a major change observed in the toxicity test. Animal mortality data were required to determine the efficacy of the extract. Observation on mealworms mortality was done within 1-3 days. Test of mealworms mortality was performed on 3 treatment groups with 3 replications. Each treatment was given 10 mealworms. The dead mealworms were calculated to find out the average of mealworms mortality. The data were obtained by determining the average number of mortality of insects within 3 days to know if there is a mortality difference of each treatment and find out the best concentration for custard apple, soursop, and mixture of custard apple and sour soup.
Figure 1. (A) Mealworm (Tenebriomolitor); (B) Cricket (Gryllidae); (C) Spray of extracts of Annona Squamosa, Annona Muricata and mixture of Annona Squamosa and Annona Muricata

3. Results and Discussion

3.1. Effectiveness of Annona Squamosa, Annona Muricata and mixture of Annona Squamosa, Annona Muricata extracts in controlling mealworms. From the figure 2, the graph showed how many mealworms killed by three different types of sprays at (A) 15%, (B) 45% and (C) 15% composition. Types of extract or spray chosen was extract of Annona Squamosa, extract of Annona Muricata and mixture of extract (Annona Squamosa and Annona Muricata). Based on the results, number of mealworms killed after 1-3 days were proportional to the concentrations of the extracts: 15%, 45% and 90% respectively. When 90% of aqueous extracts of Annona Squamosa, Annona Muricata and mixture of extract (Annona Squamosa and Annona Muricata) were used, mortalities of mealworms increased compared to 45% and 15% concentration. For the three concentrations 15%, 45% and 90%, the mortalities of mealworms of Annona Squamosa after 3 days were respectively 1±1, 1±1 and 5±1, and 2±1, 2±1 and 6±1 for the aqueous extracts of Annona Muricata, respectively and for the mixture of Annona Squamosa and Annona Muricata, the mortalities of mealworms after 3 days were respectively 6±1, 7±1 and 8±1. For all extracts and at all concentrations tested, the mixture of Annona Squamosa and Annona Muricata has better performance compare to others. Therefore, this research support research conducted by [13]. The extracts of Annona Squamosa and Annona Muricata mixtures showed higher insecticidal properties compared to extracts of Annona Muricata and extracts of Annona Squamosa. Furthermore, research by [12] stated the potential of mixture sour sop (Annona Muricata) and sugar apple (Annona Squamosa) as a plant-based pesticides in controlling pests and diseases of plants. Phytochemical screening found the
secondary metabolite products in the seed extracts of Soursop (*Annona Muricata*) and sugar apple (*Annona Squamosa*) that is: alkaloids, flavonoids, polyphenols, terpenoids, coumarin, steroids, lactones, anthraquinone, tannins, glycosides, cyanadin, and saponins.

![Figure 2](image-url)  

**Figure 2.** Average number of mealworms killed per day at different concentration of extract (A) 15% composition; (B) 45% composition; (C) 90% composition.
3.2. Effectiveness of *Annona Squamosa*, *Annona Muricata* and mixture of *Annona Squamosa*, *Annona Muricata* extracts in controlling crickets.

Figure 3 explains how many crickets killed by three different types of sprays; extract of *Annona Squamosa*, extract of *Annona Muricata* and mixture of extract (*Annona Squamosa* and *Annona Muricata*) extract at different concentration; 15%, 45% and 90%. For three concentrations 15%, 45% and 90%, the mortalities of crickets of *Annona Squamosa* after 3 days were respectively 0, 3±1 and 3±1, and 2±1, 4±1 and 4±1 for the aqueous extracts of *Annona Muricata*, respectively and for the mixture of *Annona Squamosa* and *Annona Muricata*, the mortalities of mealworms after 3 days were respectively 4±1, 5±1 and 6±1. From the results, *Annona Squamosa* has the least performance rather than *Annona Muricata* and mixture of *Annona Squamosa* and *Annona Muricata*. The similar result was observed by the analysis using mealworms in figure 2 stated that mixture of *Annona Squamosa* and *Annona Muricata* showed the highest efficacy in killing mealworms. Based on the percent composition of the extract, the percent composition at 90% showed the highest number of crickets killed. Therefore, the optimum condition to kill the crickets is at 90% concentration using mixture of *Annona Squamosa* and *Annona Muricata*. According to [13], chemical identifications showed that these extracts contain alkaloids and flavonoids compounds that probably confer their biological insecticidal properties.

![Figure 3](image_url)

*Figure 3. Average number of crickets killed per day at different concentration of extract (A) 15% composition; (B) 45% composition; (C) 90 % composition.*
4. Conclusion

The overall findings of the study indicated the effectiveness of soursop seed extract (Annona Muricata) and custard apple (Annona Squamosa) seed extract on mealworms and cricket’s mortality. It is concluded that the extracts of Annona Squamosa and Annona Muricata both showed insecticidal properties though at varying levels of composition. Extracts of Annona muricata displayed higher insecticidal potential compared to extracts of Annona Squamosa but among them, mixture of Annona Squamosa and Annona Muricata showed the best performance on insecticidal potential. The most effective concentration of soursop and custard apple extract (Annona Squamosa and Annona Muricata) in killing leaf mealworms and crickets is the extract of 90% concentration. Therefore, if used properly at an efficient dose, these extracts of Annona Squamosa and Annona Muricata were revealed to be a low cost and powerful environmentally friendly pesticides.

Some recommendations that can be applied to improve this research are by testing the other Annona family fruit because all Annona fruit contain acetogenin in their seed. Based on the experiment, we used water based with high water composition where the next experiment can be improved such as instead of using high water composition, a high oil extracted composition more efficient. Next, the research can be made by investigating the sustainability of the extract since the bio-pesticide is made up from organic matter that is easily rotten. Besides, the best preservative also needed to be identified to keep the bio-pesticide longer.

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