



## **Antidesma Bunius (Bignay) Fruit Extract as an Organic Pesticide against Epilachna spp**

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### **Abstract**

Ladybird (Family Coccinellidae) has been regarded as a serious pest causing heavy damage to various agricultural crops. The use of effective and natural pesticide will greatly contribute in the control and management of these pests. This study examined the potential of *Antidesma bunius* (Bignay) fruit extract as an organic pesticide against the *Epilachna spp.*, another species of the family Coccinellidae. A laboratory-scale experiment was done to compare the mortality time lapse (MTL) of the lady bird beetles exposed to three concentrations of crude *A. bunius* fruit extracts (50%, 75% and 100%). A commercial pesticide was used as positive control and water as negative control. Each treatment was sprayed on the testing plates and on the cling wrap cover, and mortality was monitored every 15 minutes for 5 hours. Three trials were conducted with three replicates each. One-way ANOVA showed that there is a significant difference in the exhibited MTLs between and within treatments ( $\alpha$  0.05 and 0.01). The recorded MTL of beetles exposed to 100% *A. bunius* extract was relatively close to those exposed to the commercial pesticide compared to those treated with 50% and 75% *A. bunius* fruit extracts. The commercial pesticide registered an average MTL of 10 min while majority of those treated with pure fruit extract registered a 15 min MTL. Phytochemical analysis of the fruit extract showed the presence of flavonoids and phenols that might contribute to its pesticidal property. Results of this study indicate that *A. bunius* fruit extract can serve as a novel alternative source of organic pesticide and that the pure crude fruit extract was proven effective against the *Epilachna spp.*

**Keywords:** *Antidesma bunius*, *Epilachna spp.*, Lady bird, Bignay, Pesticide.

### **1. Introduction**

Recent studies show that the pest of the Family Coccinellidae, collectively known as lady bird or lady beetle, still continues to be a major source of problem among crop growers. In a study presented in the 8<sup>th</sup> International Cool Climate Symposium in Canada, it was mentioned that in 2001 in Ontario, the insect caused the dumping of 1 million liters of wine because it has tainted the wine with flavors of “peanuts, asparagus, bell pepper and earth, giving it a herbaceous and bitter taste” (Easton, 2012). The author even mentioned that these beetles “are an increasing threat to the global wine industry” and the issues are known to be affecting European vineyards as well.

*Epilachna* spp like the Mexican bean beetle, *Epilachna varivestis* Mulsant is one of the very few beetles that are regarded as notorious garden pest instead of the usual bio control known species. The beetle is about 1/3 to a quarter of an inch long, yellow to copper in color with eight black spots on each wing forming three longitudinal rows. The insect exhibits complete metamorphosis with adults laying about 40-60 eggs in groups. The species is believed to be a native of Southern Mexico but has been found in other areas like Guatemala, Canada, New England, western US and eastern US where it is considered a severe pest. Distribution and damage to crops caused by Mexican bean beetles have been reported and started in the late 1920s and economic injury levels were developed in the early 1970s (Sanchez-Arroyo, 2009).

Another notable *Epilachna* spp. is the spotted leaf beetle or Hadda beetle, *Epilachna vigintioctopunctata* Fab. It is regarded as key pest of the solanaceous and cucurbitaceous plants (Islam, et al., 2011). The pest is widely distributed in Southeast Asian countries, Korea, Australia and India (Kapur, 1950 as cited in Mohanchander, et al., 2013). The 28-spotted lady bird has a roundish, convex shiny body that is reddish brown in color. Mohanchander, et al., (2013), likewise cited in his study a number of cases that proved the considerable economic loss brought about by the infestation of Hadda beetle.

In the case of this study, the lady bird species that was observed to infest the eggplants was close to the description of a Mexican bean beetle that is found in the province of Rizal, Philippines. As observed, it feeds upon the *Solanum* leaves leaving a laced-like skeletonized appearance, similar to the other observed damages to preferred plants as cited in many literatures. The larval form of the beetle is more voracious than the adult and are the ones causing heavy damage on the leaves and the crop as a whole. In the plantation where this study was conducted, the population of adult alone is so numerous that the researchers did not find so much difficulty in getting samples. No single plant is without the adult and the larval forms.

In the New Jersey Department of Agriculture (NJDA, 2007) annual report, it was reflected that in terms of pesticide cost, about 1.2 million dollars is allotted to address Mexican bean beetle infestation alone making it rank 3 in the list. However, because of the decision of the state of New Jersey to invest on biological control research and utilize its findings, they no longer have to spend so much for pesticide but instead, beginning 2007 was able to annually save as much as 100 million dollars.

To biologically control Mexican bean beetle (*Epilachna varivestis*) at least 17 species of predators have been identified to feed on bean beetle eggs, larvae and pupa. Parasitoids like tachinid fly *Paradexodes epilachnae* and the eulopid wasp *Pediobius faveolatus* from India are the ones capable of suppressing the number of beetles (Sanchez-Arroyo, 2009). In fact, *P. faveolatus* has been used by New Jersey since 1980 to effectively kill the beetle by rearing them in the laboratory each winter and release them into the soybean fields during summer (NJDA, 2007). In the University of California, the Agriculture and Natural Resources (2013) suggests the use of reflective mulches like aluminum foil, or anything that has reflective silver paint to invading insect population. Anderson (2013) enumerated several other natural ways of controlling Mexican bean beetle. These include companion planting by interspersing bean plants with plants like marigold and rosemary which repels the insects; introducing parasitic wasps or attracting them by planting flowering plants such as daisies; manual handpicking of adults and pinching of larvae; and plant crop trapping.

Biological control is very helpful in cases where it is impractical to spend for very expensive and environmentally hazardous chemical pesticides like carbaryl, cyhalothrin and malathion, which are found most effective against *E. varivestis*. Bio control provides long term benefits and economic advantage. Pyrethrin, insecticidal soap, and neem oil are so far the only known natural organic insecticides which are recommended to be used (Williamson, 2009). This study therefore would like to promote bio control methods by considering the potential of another natural organic pesticide, in this case *A. buniis* fruit extract, that may effectively eradicate the Mexican bean beetle.

*Antidesma buniis* (Linn) Spreng is more popularly known in the Philippines as “bignay”. The plant is a small, smooth, dioecious tree, 4 to 10 m high. Leaves are shiny, oblong, 8 to 20 cm long. The fruit is fleshy, ovoid in shape transforming its color from green to pale yellow then red to blackish color as it ripens. The edible fruit is about 8 mm long with only one seed and grow in clusters like grapes (Benerjee, 2013). *A. buniis* is widespread in the Philippines but is also claimed to be common in the wild (from the lower Himalayas in India, Ceylon and southeast Asia and northern Australia), grown in Indonesia, and cultivated in Malaya (Morton, 1987). The entire plant is of medicinal value

acting as anti-dysenteric, antioxidative, anticancer and antidiabetic and gives sudorific effects. The fruit is highly edible and nutrient –rich and is prepared either as a healthy juice drink or cooked with fish and other foods. Phytochemical analysis of the different species of *Antidesma* has confirmed presence of varying amounts of phenolic acids, flavonoids of catechin, procyanidins B1 and B2 and anthocyanins (Lim, 2012). In the study conducted by Butkhu & Samappito (2008), an analysis of 15 cultivars of *A. bunius* grown in northeast Thailand have shown that all cultivars contain three major flavonoids namely catechin, procyanidin B1 and procyanidin B2. The very presence of these phytochemicals as plants natural defense motivated the researchers to look into the possibility of finding a strong insecticidal property in *A. bunius* fruit.

## 2. Materials and Methods

### 2.1. Plant Material

The *Antidesma bunius* ('Bignay') fruits were harvested in Manuel S. Enverga University Foundation, Lucena City, Quezon Province. Plant materials were submitted to a plant taxonomist from the National Museum and confirmed the species as *Antidesma bunius* (Linn) Spreng. From 2 kilos of *A. bunius* fruits, about 500 ml pure extract was obtained by squeezing out the juice with a cheese cloth. Aliquot of pure *A. bunius* fruit extract was submitted to the Phytochemical Analysis Division of University of the Philippines, Diliman, Quezon City for phytochemical analysis. Using water as the solvent, three concentrations (100 ml each) were prepared, i.e. 50%, 75%, and 100%. Commercial pesticide served as positive control and distilled water as negative control. All solutions were transferred to plastic spray bottles of the same size and type.

### 2.2. Insect Collection

Adult lady birds were collected from eggplant plantation in Bgy. Macamot, Binangonan, Rizal Philippines. Beetles were identified by entomologist from the National Museum as *Epilachna spp.* The insects were randomly collected from the leaves of each egg plant by manually picking or sliding them down into the collecting jars. Regardless of size and relative age (which can be inspected through color difference), about 200 beetles were captured and then brought into the testing site. Beetles were transferred to clean plastic petri dishes for 30 min to acclimatize and somehow relax them from being trapped in the jars. After which, three insects of same size and color (regardless of sex) were distributed to each testing plate. All plates were covered with punctured cling wrap to prevent the insects from flying and to allow them to respire normally. Insects were allowed to acclimatize in the testing plates for another 30 min just before the mortality test.

### 2.3. Mortality Test

Different concentrations of the plant extract and the controls were sprayed on the test plates. Around 2 ml of solutions was applied in each treatment. Initial time of spraying was noted and insect mortality was observed every 15 min. This time interval (15 min) was set based from the initial experiment using the commercial pesticide. Mortality time lapse (MTL) in each treatment was recorded for up to 5 hrs. Three trials were conducted with three replicates per trial.

### 2.4. Data Analyses

Average time of mortality time lapse (MTL) were determined in each treatment. Data were subjected to one-way analysis of variance (ANOVA) for both 0.05 and 0.01 level of significance. Differences between treatments were then compared using F-test.

## 3. Results and Discussion

Table 1 shows the mortality time lapse of test beetles after treatment with *A. bunius* extracts and controls. The recorded MTL of beetles exposed to 100% *A. bunius* extract was relatively close to those exposed to the commercial pesticide compared to those treated with 50% and 75% *A. bunius* fruit extracts. The commercial pesticide registered an average MTL of 10 min while majority of those treated with pure *A. bunius* fruit extract registered a 15 min MTL (Table 1). Both 50% and 70% fruit extracts showed much longer MTL with an average of 106 min and 111 min, respectively. One-way ANOVA showed that there is a significant difference in the exhibited MTLs between and within

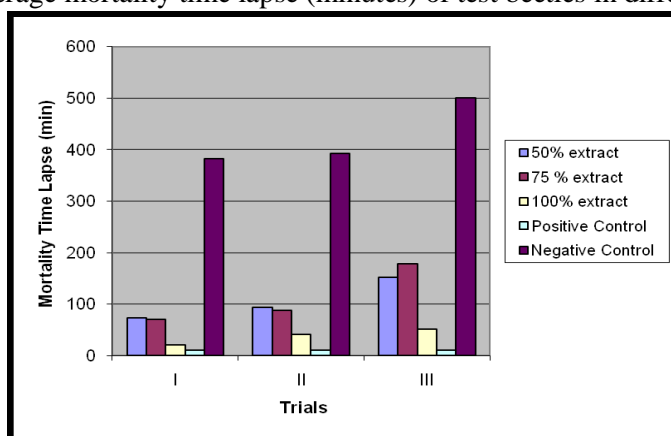
treatments ( $\alpha$  0.05 and 0.01). The observed F value for the group data was 31.43  $\gg$  F tabulated values of 2.44 and 3.47 at  $\alpha$  level .05 and .01, respectively. Based from this experiment, test beetles are less likely to die of drowning or soaking since their body, like most insects, are covered and protected by a hard exoskeleton that is usually water repellent. Therefore, death of beetles treated with relatively the same amount of *A. bunius* extract may be attributed to the effect of the treatment and not by drowning.

As for the proximity of average MTL of those treated with 100% bignay extract with that of the positive control results, data suggest that pure *A. bunius* extract is quite as strong and effective as the commercial pesticide. As shown in Figure 1, MTLs of beetles exposed to the 100% concentrated *A. bunius* fruit extract is near to those of the commercial pesticide treatment. MTL of beetles treated with pure extract is significantly different with those treated with 75% and 50% fruit extract (F-test). This indicates that the recorded MTLs of 100% *A. bunius* fruit extract are considerably shorter than those of the 75% and 50% treatments. The MTLs were both high in beetles treated with 75% and 50% fruit extract (Figure 1). No significant difference (F-test) was observed in their MTL which means that the MTL exhibited by the insects treated with 50% and 75% fruit extract is almost of the same length.

The broad spectrum commercial pesticide used in the study contains chlorpyrifos, a cholinesterase-inhibiting insecticide (Thatcher et al., 1980). An anti-cholinesterase activity can result to neurotransmitter hyperactivity with potential symptoms of twitching, trembling, paralyzed breathing, convulsions and death (Kamrin, 1997). Upon spraying with the commercial pesticide and pure *A. bunius* extract, it was observed that treated beetles exhibited very erratic behavior from rapid movement, tumbling upside down, and spreading of wings prior to death. The same conditions were observed in test beetles sprayed with 50% and 75% fruit extract but the behavior was not sustained compared to those treated with pure extract and commercial pesticide.

Phytochemical analysis of the 100% *A. bunius* fruit extract confirmed the presence of flavonoids and phenols which may be responsible for its pesticidal activity. Flavonoids, a type of phenolics, are cytotoxic and defend plants against various biotic and abiotic stresses including UV radiation, pathogens and insect pests (Treutter, 2006). Phenolics are aromatic benzene ring compounds produced by plants mainly for protection against stress like insect attack, UV radiation and wounding, and to repel or kill many microorganisms and some pathogens (Klepacka et al., 2011). Procyanidins, a flavonoid category of phenolics also known as proanthocyanidins (condensed tannins) are oligomeric compounds. They are related to tissue and cultivar resistance to pests (Bell et al., 1992). Direct defenses on the part of the plants may be exhibited on the surface of the organism (e.g., hairs, trichomes, thorns, spines, and thicker leaves) or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinones) that either kill or retard the development of the herbivores (Hanley et al., 2007). A recent study done by Tavares et al. (2012) on the bioactive compounds of endemic plants in Southwest Portugal showed the presence of high phenolic contents and with anti-oxidant capacities. The plant extracts also demonstrated effective acetyl cholinesterase (AChE) inhibitory activity that could be associated to polyphenols. With the presence of such natural compounds in *A. bunius*, the possibility of its fruit extract having pesticidal properties is not remote.

**Figure-1.** Average mortality time lapse (minutes) of test beetles in different treatments



**Table-1.** Mortality Time Lapses ( minutes) of *Epilachna* spp. in various treatments

Treatments	Trials			Average
	I	II	III	
A. bunius extract				
50%	80-70-70	110-70-100	190-70-195	106
75%	70-70-70	80-110-70	223-185-125	111
100%	33-15-15	75-15-33	57-15-80	38
Positive Control (commercial pesticide)	10-10-10	10-10-10	10-10-10	10
Negative Control (water)	197-500-450	348-450-378	500-500-500	425

#### 4. Conclusion and Recommendations

This study indicates that *Antidesma bunius* fruit extract is a potential organic pesticide and may prove lethal to *Epilachna* spp. if used in concentrated amounts. Further studies on the isolation and purification of its bioactive components are recommended to identify the main compounds responsible for its pesticidal property. Moreover, an actual field testing should also be conducted to further confirm the efficacy of *A. bunius* fruit extract against *Epilachna* species. Lastly, it would be more reliable to consider accurate identification of *Epilachna* spp., gender and other extraneous variables in selection of samples to explain resistance and prolonged mortality time lapses.

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