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# Comparing the effects of crude and refined *Mammea americana* (Mamey apple) leaf extracts on *Doralis symphyti* (aphids) on ochro (*Hibiscus esculentus*) plants

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## Abstract

The aphid, *Doralis symphyti* (Homoptera: Aphididae), a harmful pest of many crops, causes direct damage by consuming all plant parts, reducing plant vigour, and indirect damage by honeydew secretion and transmission of several viruses. Due to the problem of pesticide resistance, alternative techniques for chemical control, such as the use of natural insecticides with minimal effects on the environment and natural enemies also coincide with organic agriculture have been the objective of this research. The effects of Mammea *(Mammey americana*) leaf extracts on the mortality of aphids were evaluated. The mammey extracts (Crude and refined) were tested in the laboratory (bioassays) at 100%, 90%, 80%, 70% and 60% dilution. The 80% was determined to be the most efficient dilution. The refined extract resulted in 100% mortality at 84 hours while the dry leaf and green leaf extracts gave 100% mortality in 96 hours. In the field, the refined extract resulted in 65% mortality, dry leaf crude extract obtained in 43% mortality and the green leaf crude extract gave 25% mortality. The mamey leaf extracts were efficient against the aphid in the laboratory since 100% mortality was obtained for all treatments. The field study found that the green leaf extract obtained 29% mortality, dry leaf extract achieved 47% mortality and the refined extract attained 63% mortality of aphids.

**Keywords:** *Doralis symphyti*; Pesticide; Natural insecticides; Green leaf; Mortality; Mammey

## 1 Introduction

Insects are one of the most diverse species of animals living on earth that can be found in all habitats. However, only about 0.5% of the total number are considered pests with only a few species that pose a serious threat to humans, farm animals, and crops [1]. Providing food for the growing population is a major challenge facing mankind. Insects pests have been competing for food produced by man and are the main cornerstone in this challenge. As such chemical insecticide was primarily used to manage insect pest population and an increase in yields was realized. However, the extensive, unselective, excessive, and incorrect use of these chemicals insecticide have gravely damaged the ecosystem, extending toxicity and pollution in the environment [2].

The current emphasis internationally is to move away from using synthetic insecticides to produce 'safe' wholesome foods. In contrast, many farmers relied on synthetic insecticides as the chief means of controlling pests such as aphids. In addition, these synthetic insecticides not only kill the targeted pest(s) but also natural enemies. As such, the next

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plant cycle will result in pest outbreak, due to an imbalance of the natural enemies to pest ratio in the natural environment. This will lead to higher dosages since the farmers will not be able to control the pest and resistance is likely to build up by these pests. Aphids are harmful pests that attack and destroy many crops, by consuming all plant parts, reducing plant vigour, and indirect damage by honeydew secretion and transmission of several viruses.

*Doralis symphyti* (aphids) are tiny, soft-bodied, pear-shaped insects, ranging from 2-4 mm long. Aphids may be green, yellow, and brown, red, or black depending on the species and the plants they feed on. Aphids suck plant sap with their needle-like mouthparts and usually feed on the underside of leaves, or the growing tip of vines, sucking nutrients from the plant [3]. The foliage may become chlorotic and die prematurely. Their feeding also causes a great deal of distortion and leaf curling. It also results in cupping with the aphid's colony inside the folded leaf, thus, reducing the surface area which subsequently affects the photosynthetic capacity of the plant. In addition, they secrete a great deal of sugary liquid called honeydew which provides a substrate for the growth of sooty mould, so the quality of products may be impaired and the photosynthetic capacity of foliage further hindered. Most aphids multiply quickly, that is, a new generation can be produced within 10-14 days [3]. Aphids are considered polyphagous since they consume many crops and they are also the vectors for many diseases e.g. mosaic viruses.

Mamey apple leaves is an alternative control of aphids without the use of harmful chemical insecticide. Mamey plant has glossy, leathery, dark-green, broadly elliptic leaves, up to 8 inches long and 4 inches wide. Various parts of the mamey tree contain toxic properties which had long been recognized and used for different purposes [4]. *M. americana,* commonly known as mamey apple, is indigenous to the West Indies and Tropical America. Mamey leaf extract had long been used by man to control insects [5]. Infusions of the edible fruits in water were highly toxic to many species of insects [4]. Mamey extracts were historically used as a biopesticide with the active compound mammein [6].

Mamey extract is considered safer than chemical insecticide for pest management because it poses little threat to human health and the environment. This extract has many benefits such as leave little or no residues on the crop, do not kill beneficial insects, break down rapidly in the environment, and has low mammalian toxicity. [6]. Most synthetic insecticides leave high residue on crops. In many cases, beneficial insects are killed. Many of the synthetic insecticides remain long in the environment and are very toxic to humans and other mammals. Mamey leaf (leaf extracts), is an alternative to this problem since it does not affect beneficial organisms and does not leave any residue on the crop. It is a very cheap method of controlling pests such as aphids. This type of pest control is complementary to that of organic farming since safe wholesome foods are produced. Consequently, there is also great export potential for crops cultivated without the use of synthetic insecticides.

Extraction of the leaves and/or flowers with solvents such as ether or acetone also produces oils that are toxic to insects. Many active components can be obtained readily from other botanicals such as basil oil. However, their synthesis would be much less practical commercially at a temperature higher than 35-42$^{o}$ C [6]. Unlike many synthetic chemicals, most botanicals' insecticides decompose quickly in the field [7].

The objective of this study is to find a cheaper alternative to the systemic insecticide that control aphids that is readily available and do not harm humans or the environment.

## 2 Material and methods

This research work was carried out at the Faculty of Agriculture and Forestry at the Agriculture Research station. The University of Guyana Turkeyen Campus, Georgetown Guyana, South America.

## 3 Preparation of leaf extracts

Mamey leaves were selected from disease-free plants for all the extracts.400g green leaves were blended in 1200ml distilled water for 45 minutes. The blended leaves were filtered and the filtrate was collected.

### 3.1 Dry leaf extract

The 400g of green mamey leaves were sun-dried for two days and oven-dried at 40$^{o}$ C for three days. To observed the moisture content was 35%. The dried leaves were ground using an all-purpose mill. The ground leaves were soaked for one day in 1200ml distilled water. It was filtered and the filtrate was collected.

### 3.2 Refined (essential oil) extract

6000ml of distilled water was placed in the extractor and heated at 40° C. 5500g of green leaves were placed in the extractor and steam distillation was done at two bars (2 bars), for three hours (3hrs) at 40° C. 10 grams Sodium Chloride (NaCl) was added to increase the density of the essential oil. 3000ml of the aqueous extract was collected. The essential oil was obtained by placing the aqueous extract in a rotavapor for sixteen hours (16hrs) at 40° C. 800ml of the essential oil and active compounds were collected.

### 3.3 Bioassays

Ochro seeds were planted in the greenhouse. Aphids were reared on ochro plants in the insectary (greenhouse).15 aphids were removed from young leaves and placed in the petri dish on leaves in the laboratory. The aphids were then subjected to 100 %, 90%, 80%, 70% and 60% dilution and the treatments were replicated four times. Mortality counts were made from one to six hours and then twelve hours (12 hrs) intervals up to ninety-six hours (4 days) using a hand microscope. The data were analyzed using Completely Randomized Design (CRD) for the statistical and Least Significant Difference (LSD) to compare the means. The 80% dilution was determined to be the most effective. 80% dilution was used in the field.

**Note:** The bioassays were done to determine the most effective dilution to be used in the field and 100% dilution is the undiluted filtrate.

### 3.4 Field Trials

One hundred and eighty (180) ochro plants were grown in the field. The ochro plants were inoculated with aphids after 28 days. Aphids were allowed to multiply (increase number) on the ochro plants for one week. The number of aphids on the plants was estimated using a hand lens (magnifying glass). A 13ft * 6ft plastic was placed between rows to prevent drift. 800ml of each extract was applied to the ochro plants infested with aphids using a knapsack sprayer. The number of aphids killed was counted using a hand lens. The Randomize Complete Block Design (RCBD) and the Analysis of variance (ANOVA) were used to statistically analyze the data. The Least Significant Difference (LSD) was used to compare the means. There were 4 treatments with 10 plants per treatment and 4 replicates.

## 4 Results and discussion

### 4.1 Bioassays

The control gave 35% mortality of aphids. This treatment resulted in 0% mortality within the first 6hours. Thereafter the percent mortality increased gradually.

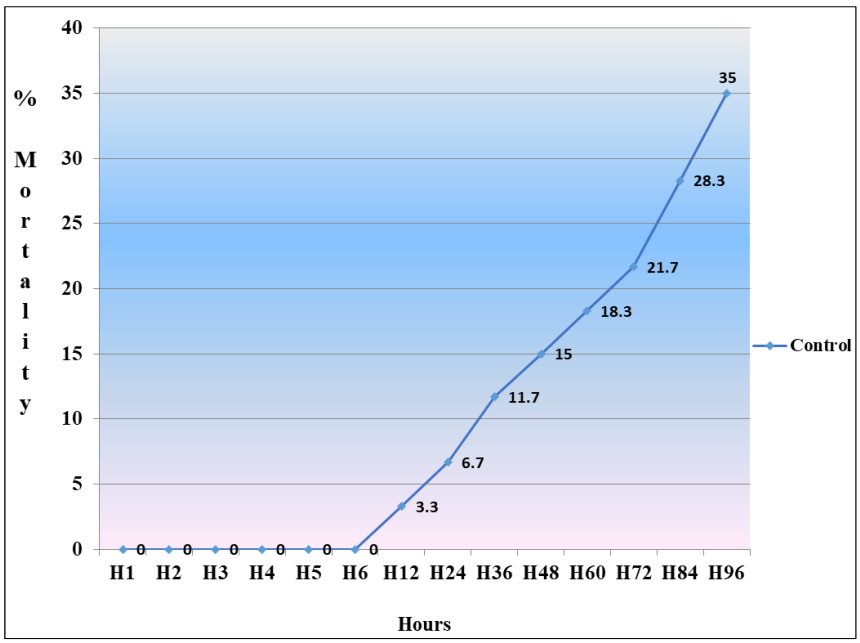

**Figure 1** Percent mortality of aphids for control within 96 hours

### 4.2 Green Leaf Crude Extract

The green leaf crude extract gave 100% mortality of aphids. This extract resulted in more than 60% mortality within 72 hours and less than 7% mortality within 12 hours. The percent mortality increased gradually at first but increased dramatically for the final hour.

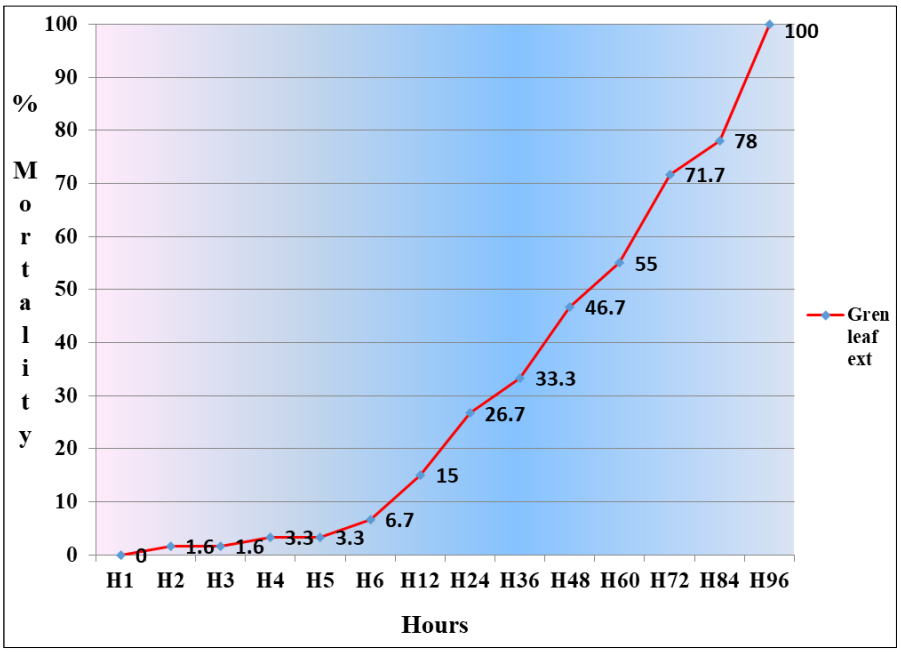


**Figure 2** Percent mortality of aphids for green leaf crude extract within 96 hours

### 4.3 Dry Leaf Crude Extract

The dry leaf crude extract gave 100% mortality of aphids. This extract resulted in more than 80% mortality within 60hours and more than 60% mortality within 48 hours. It resulted in 10% mortality within the first 6 hours. The percent mortality increased gradually for the first six hours but there was a sharp increase afterwards.

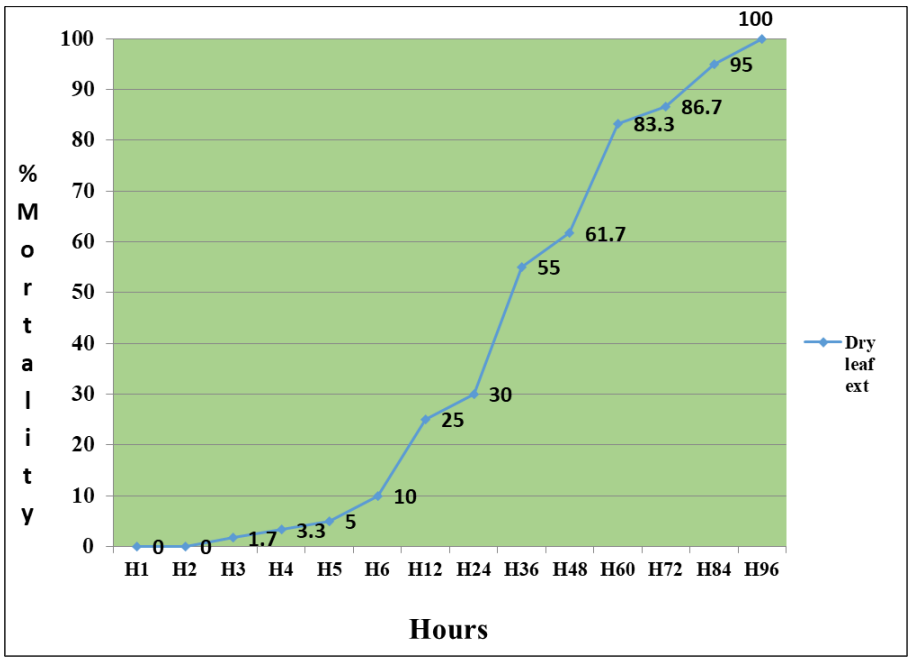

**Figure 3** Percent mortality of aphids for dry leaf crude extract within 96 hours

### 4.4 Refined Extract

The refined extract gave 100% mortality of aphids within 84 hours. This extract resulted in more than 80% mortality within 60 hours and more than 60 % mortality within 48 hours. The per cent mortality increased gradually for the first six hours but there was a dramatic increase afterwards.

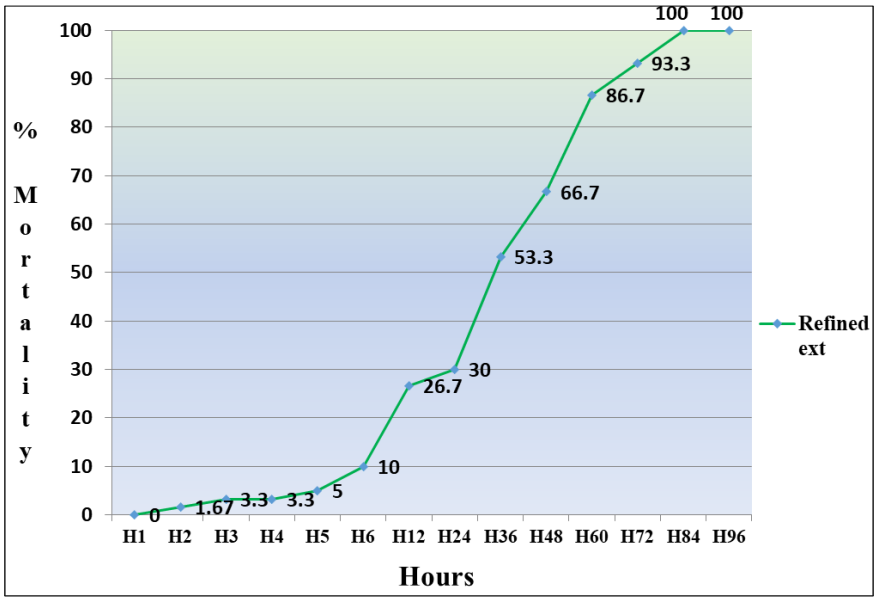


**Figure 4** Percent mortality of aphids for the refined extract within 96 hours

### 4.5 Comparing the treatments

The refined extract and the dry leaf crude extract gave more than 80% mortality of aphids within 60 hours. All the treatments except the control gave more than 70% mortality within 72 hours. All the extracts gave 100% mortality of aphids but the refined extract gave 100% mortality for the shortest time. All the treatments gave less than 20% mortality of aphids within the first six hours. The control gave the least per cent mortality. There was a gradual increase in the per cent mortality at first for all the treatments but the last few hours gave a sharp increase for all the treatments except the control.

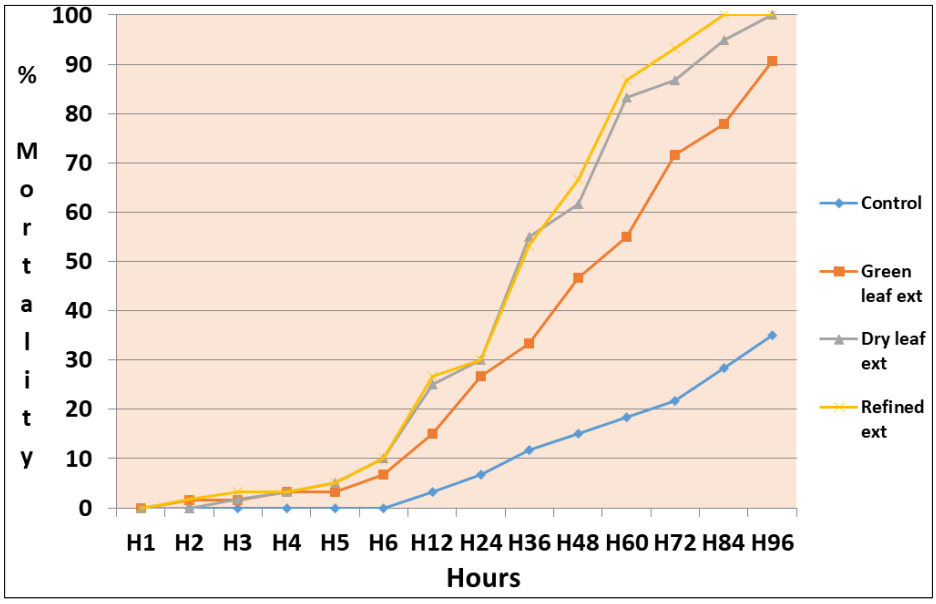


**Figure 5** Percent mortality of aphids for crude (green & dry leaf) and refined extract

### 4.6 Comparing the overall treatments means

Means of the refined extract and the dry leaf extract gave no significant difference but resulted in a significant difference for the means of the green leaf crude extract and the control. There was a significant difference between the means of the green leaf crude extract and the control.

**Note**: Means with the same letter indicate no significant difference.

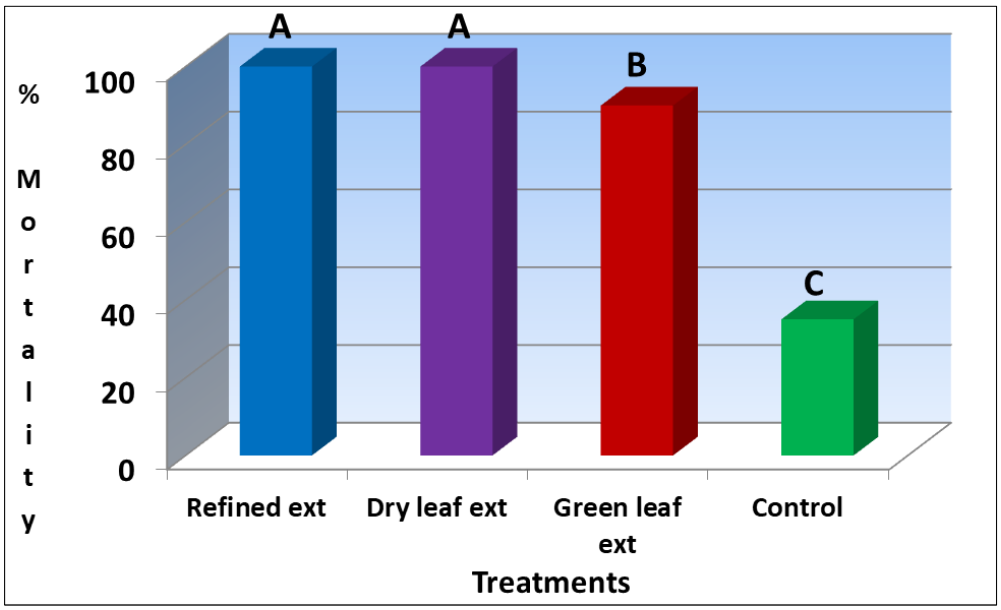


**Figure 6** Comparing the overall treatment means for crude extracts (green leaf and dry leaf), refined extract and control

## 5 Conclusion

The mortality of aphids increased when exposed to mamey leaf extracts and was dependent upon concentration and a linear relationship occurred between these variables. The refined extracts had more toxic compounds which showed statistically significantly higher mortality when compared to other treatments.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

The authors declare that there is no conflict of interest.

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