The Evaluation of the efficacy of Bio-pesticides produced from the Leaves of the *Calotropis gigantea* (Madar) Against the *Oebalus poecilus* (Small Rice Stink Bug)

Isaiah Willis[1], Donessa David[1] and Gomathinayagam Subramanian[2*]

[1]Faculty of Earth and Environment Science, University of Guyana, Tukeyne Campus, Guyana.
[2] Division Agriculture, University of Guyana Berbice Campus, Tain, Guyana.

Abstract

The cultivation of rice in Guyana dates to the 1800s. In fact, the climatic conditions in Guyana are ideal for rice cultivation in both summer and spring times. The Agricultural sector, which rice farming contributes heavily to, accounts for approximately a fifth of the nation's Gross Domestic Product (GDP). Farmers have been using synthetic pesticides in the rice industry for over 75 years to maintain and/or improve the yearly crop yield. The most important pest to rice crops was identified as the small rice stinkbug or paddy bug. To combat this pest, most farmers in Guyana use synthetic pesticides which can have a harmful effect on the natural environment. This study was conducted to evaluate how effective a bio-pesticide produced by using Calotropis gigantea against field collected small rice stink bugs (Oebalus poecilus), in hopes to replace synthetic pesticides. The test insects were collected using a net to sweep across the field, as well as hand picking and were then stored in a perforated zip-lock bag. Two bio-pesticides were formulated, an ethanol extract and a water extract. The insecticidal abilities of both extracts were tested by using the film method. This is when a small amount of bio-pesticide is placed in a glass container and swirled around slowly until the entire surface area is coated. Both extracts had 100% mortality, but the difference came with the time taken for effects to be observed. The entire experiment was set for a duration of 24 hours. The ethanol extract accomplished its 100% mortality in under 30 minutes while the water extract gained its 100% mortality in under 8 hours. However, ethanol without the presences of the madar extract also gain 100% mortality, but using distilled water was completely harmless against the small rice stink bugs. Therefore, the madar plant extracts have insecticidal capabilities against the small rice stink bug.

Keywords: Bio-pesticides, *Calotropis gigantean, Oebalus psecilus,* Stink bug/paddy bug, madar, Rice.

Introduction

Guyana, known as the land of many waters, can be found in South America. Guyana has a lot of potential in the agricultural sector with room for explanation, with one of its major agricultural exports being rice. The cultivation of rice in Guyana dates to the 1800s. In fact, the climatic conditions in Guyana are ideal for rice cultivation in both summer and spring times (Gillette & Sakai, 1970). The Agricultural sector, which rice farming contributes heavily to, accounts for approximately a fifth of the nation's Gross Domestic Product (GDP), to maintain or improve the yearly crop yield framers have turned to the use synthetic pesticides (Ansari & Waleema, 2009). Insects can be found in almost every environment on the planet, and they have developed different feeding habits (van der Werf, 1999; Ferial *et al*., 2019). While many are phytophagous consuming seeds, stems, roots or leaves, they also can be carnivorous or bloodsuckers (Bebber *et al.,* 2014; Lage, 2018; van der Werf, 1996).

Phytophagous insects have developed a reputation of being a danger to food security because of their ability to destroy agricultural goods. Dissimilarly to their wild relatives, farm crops like rice have been artificially selected and bred mainly for increased yield and not for pest resistance. Consequently, their predators from the wild have followed them to the farm. The *Oebalus poecilus* (small rice stinkbug or paddy bug) is one of the pests that affects rice quantity and quality in Guyana and other countries in the lower Americas with both adults and nymphs being found to feed on grains of the rice (Krinski & Foerster, 2017; The paddy bug, n.d.). The paddy bug bores into the developing grain extracting the milky substances needed to form the grain. This leads to malformation and discoloration (Krinski & Foerster, 2017; The paddy bug, n.d.).

The Guyana Rice Development Board (GRDB) has suggested the use of Integrated Pest Management (IPM) which is an environmentally sensitive approach to pest management that depends on a combination of "common sense" practices (EPA, 2021; "Agronomy," 2020). The IPM practices which are suggested by the GRDB to aid in controlling the small rice stink bug include preventing the growth of weeds in and around the rice field, removal of alternate host plants, and plants that are heavily infested and if upon inspection by the sweep method the number of stink bugs exceed 1 bug/2 sweeps then the use of the recommended chemicals is needed ("Agronomy," 2020).

In Guyana, approximately 90% of the farmers use about three to four pesticide each season and some farmers even use insecticide as a preventative measure, which may be costly to the farmers, and be damaging to the aquatic and terrestrial environments (Sutherland *et al*., 2002). In the 1940s, synthetic pesticides became available increasing food production and gained popularity among farmers.

As farmers continued to use pesticides, debates on its benefits and the impact risks on human health and the environment were being held to determine if the risks are worth the reward (Bebber *et al*., 2014; Ferial *et al*., 2019; Lage, 2018; van der Werf, 1996). Polluted surface and ground water, degraded soils and non-target species being affected were found to be some of the effects caused using synthetic pesticides (Fareed et al., 2017; van der Werf, 1996). These effects warranted the search for an alternative to synthetic pesticides. The use of plants for medicinal purposes can be dated to the beginning of human history and certain plant are known to have substances that serve as plant defense mechanisms (Ansari & Waleema, 2009; Mandepudi *et al*., 2012).

Calotropis gigantea (also known as Madar) belong to the Asclepediaceae family, known for its traditional use as a medicinal plant, it is common to the tropical and subtropical regions of Asia (Boomibalag *et al*., 2013; Sutherland *et al*., 2002; Sumathi & Rajasugunasekar, 2017; Prabhu et al., 2020; Parvin *et al*., 2014). The Madar plant has gain popularity over its potential as an environmentally safe bio pesticide. A pesticide is known to have mechanisms that allow it to repel or avoid pests, in the effort for the development an environmentally safe pesticide the Madar plant can be used (Sutherland *et al*., 2002; Sumathi & Rajasugunasekar, 2017; Prabhu *et al*., 2020; Parvin *et al*., 2014).

The most serious pest to the rice industries in Guyana and other South America countries is the small rice stink bug. It causes two types of damages, a loss in yield by the feeding on the endosperm content of the grain and reduction of the product quality of the grain caused by introducing a pathogenic fungus, which causes a discoloration of the kernel known as peck

This study was carried out to find a suitable and effective replacement for synthetic pesticides used by farmers in Guyana to prevent the infestation of small rice stink bugs. This research will utilize the True-Experiment method to investigate if a bio-pesticide created from madar leaves will be effective against the small rice stick bug.

Material and Methods

Work Sites

This project was carried out at two locations, in the field and in the lab. The field work was done at a rice farm located in the village of Black Bush Polder, Corentyne Berbice and the lab activities were carried out at the Johns Campus located in John's village, Corentyne Berbice, Guyana.

Collection of Sample Insects

Insects were collected by random sampling form the rice field. Net pulling and hand picking were used to capture the live insects. The insects were then separated and only the *Oebalus poecilus* (small rice stink bug) were kept in a perforated zip-lock bag.

Collection of Madar Leaves

Fresh and healthy leaves of the *Calotropis gigantea* (madar) were collected from the Tain area in Corentyne Berbice. The healthy leaves were identified by being completely green and lacking black spots which would indicate diseases or viruses. They were then washed and cut into small pieces.

Preparation of Bio-pesticide

Water extract- 100 g of the madar plant's leaves and 300 ml of distilled water were added to a blender. The mixture was then blended to have as small particles as possible. To extract the stock solution, the mixture from the blender was filtered using whatmann No.1 filter paper. The 0.3 g/ml stock solution was then stored in a fridge until further. Ethanol extract- 100 g of the madar plant's leaves were placed into a beaker with 300 ml of ethanol. The mixture will be left overnight and centrifuged at 4000rpm for 25 minutes. The extract was filtered by using Whatmann No.1 filter. The 0.3 g/ml stock solution was the store in a fridge until further.

Experiment with Bio-pesticide Against Small Rice Stink Bugs

To conduct this experiment, the stock solution was diluted to gain the other concentrations of bio-pesticide by using the formula $C1V1=C2V2$ to obtain a volume of 30 ml with concentrations of 0.2 g/ml and 0.1 g/ml from each stock solution, 20 ml and 10 ml were taken from the stock solution and placed into their respective beaker and fill with the desired solvent per extract.

Then the Film method was used. This technique involves the insecticide solution being deposited on a glass surface such as a petri dish or test tube. In this approach, each test tube was coated with one microliter / 0.1 milliliter and 0.5 microliter / 0.05 milliliter of their respected solution on the inner sides and the solution was then uniformly spread in the test tube by swirling with a gentle motion and then allowing it to dry at room temperature. Then the Film method was used. This technique involves the insecticide solution being deposited on a glass surface such as a petri dish or test tube. In this approach, each test tube was coated with one microliter / 0.1 milliliter and 0.5 microliter / 0.05 milliliter of their respected solution on the inner sides and the solution was then uniformly spread in the test tube by swirling with a gentle motion and then allowing it to dry at room temperature.

Result and Discussion

The water extract had 100% mortality rate against the test insects in 24 hours, whereas at the same exposure time, ethanol extract also had shown 100% mortality rate (Table 1). Table 1 also illustrates the mortality of test insects in distilled water, ethanol and those left the same (control).

Table 1. Insecticidal activities of water extract and ethanol extract of Calotropis gigantea leaves.

Solvent used	Extract (g/ml)	Number of insects used	Number of insects killed at exposure time (hrs.)						Mortality (%)
			0.5	2	4	8	16	24	
Water extract	0.1	6	-	-	-	6	-	-	100%
Ethanol extract	0.1	6	6	-	-	-	-	-	100%
Water extract	0.2	6	-	-	2	4	-	-	100%
Ethanol extract	0.2	6	6	-	-	-	-	-	100%
Water extract	0.3	6	-	-	6	-	-	-	100%
Ethanol extract	0.3	6	6	-	-	-	-	-	100%
Distilled water	-	6	-	-	-	-	-	-	0%
Ethanol	-	6	-	-	-	-	-	-	100%
Control	-	6	-	-	-	-	1	-	16.6%

(-) sign indicates no activity

All the mortalities for the bio-pesticides created using the ethanol extract were observed in the first half hour, while the water extract showed results at a slower rate with its first mortality recorded after two hours. The bio-pesticide created using the ethanol produced results quickly against the paddy bug with its first mortality at 5 minutes and mean mortality time of 13 minutes with extract concentrations ranging from 0.3 g/ml to 0.1 g/ml (Table 2 and Figure 1). The mortality percentage within the first 15 minutes of exposure for the ethanol extract was 100% for the extract containing 0.3 g/ml while 0.2 g/ml and 0.1 g/ml had 83.3% and 16.7% respectively (Table 2).

Table 2. Insecticidal activities of ethanol extract in the 15 minutes after exposure.

Extract (g/ml)	Number of insects used	Number of insects killed at exposure time (15min.)	Mortality (%)
0.1	6	1	16.7%
0.2	6	5	83.3%
0.3	6	6	100%

(-) sign indicates no activity

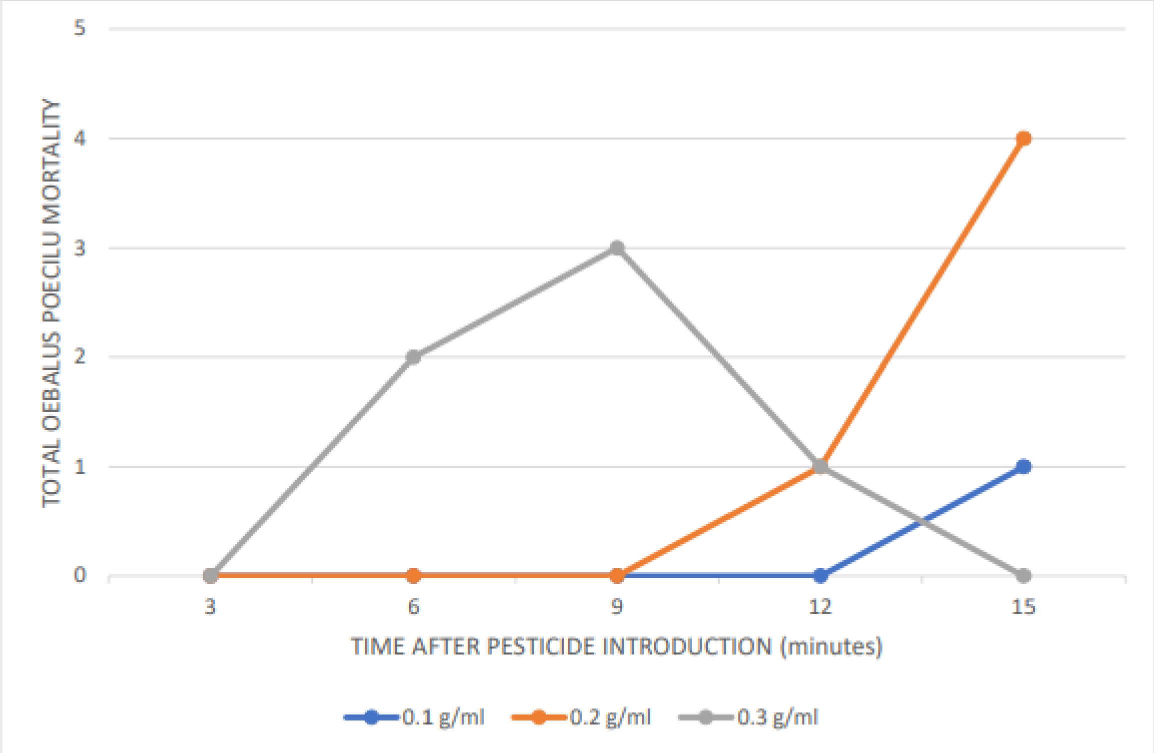

Figure 8. Insecticidal activities of ethanol extract in the 15 minutes after exposure.

Figure 8 illustrates the rate at which test insects die when exposed to the different concentrations ethanol extract for the first fifteen minutes of the experiment. 0.3 g/ml ethanol extract's first mortality was recorded between 3 to 6 minutes with two insects dying while concentrations of 0.2 and 0.1 g/ml recorded their first mortalities between 9 to 12 minutes and 12 to 15 minutes respectfully.

The findings of this research show a bio-pesticide created from the leaves of the madar as one of its main components has insecticidal capabilities against the small rice stink bug. The results recorded in this research indicate that the presence of the madar leaves in both types of extract have a significant effect against the mortality rate of the paddy bug, similarly to other studies which used the bio- pesticide extracts of madar against other insects (Parvin *et al.*, 2015; Prabhu *et al.*, 2020; Sumathi *et al.*, 2017). However, trends were displayed by the insects that were exposed to the bio-pesticides and those placed in other media, for the insects exposed to the ethanol and ethanol extracts an immediate reactions of distress were displayed and for insects exposed to water extracts signs of distress were delayed, only occurring after 2 to 4 hours depending on the concentration, with the higher concentrations providing reactions faster, and the insects exposed to water and in the control having no reactions of distress. Somewhat surprising, both extracts displayed their insecticidal abilities faster than expected, especially when compared to other research that were reviewed like Parvin *et al*. (2014) which had a mortality percentage of 23% after 24 hours of experiment. The rate of mortality for the ethanolic extracts occurred faster than expected, further solidifying itself as a contact kill insecticide. This phenomenon can not only be attributed to the presence of the madar plant in the bio-pesticide but to the use of ethanol as its solvent because ethanol also had similar results against the small rice stink bugs. Conversely, the insecticidal abilities of the water extract must be solely accredited to the presence of madar because distilled water recorded 0 mortality during the time frame of the experiment (24 hours)

Conclusion

The findings of this study highlight the effectiveness of *Calotropis gigantean* as a biopesticide against *Oebalus poecilus*. In this study, two extracts were created using the madar plant, both achieved 100% mortality, However, the difference was in the time taken to achieve the deaths of the test insects. The insects exposed to the ethanol extract showed fast reactions towards the pesticide and dying in less than 30 mins, but this was also seen for insects exposed to ethanol only. The insects exposed to only water had no reaction within the 24 hours of experiment while the water extract had a slower reaction rate in comparison to the ethanol extract. It was also effective as a biopesticide under controlled conditions. However, this study did not observe the anti-feeding abilities of this bio-pesticide and further research should be done.

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