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Challenges in rice (*Oryza sativa*) farming: Towards a biological control of the golden apple snail (*Pomacea sp.*) in Guyana, South America

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Abstract
Rice (*Oryza sativa*) farming is a key economic activity in Guyana that has been progressively increasing over the years in terms of production and export. However, yields are often reduced by pest attacks during different stages of development of the rice plant. Additionally, the desired outcome of controlling pests in an environmentally-friendly manner is not easily achieved. This study investigated the effectiveness of four plant leaf extracts at different concentrations against the golden apple snail (*Pomacea sp.*). Mortality rates over a four-day period were compared with those obtained for traditional chemical control. Two of the four extracts were found to be as effective as the chemical control and are therefore recommended for future consideration as alternative pest control.

Keywords: golden apple snail, pest, rice farming, synthetic pesticides, botanical pesticides

Introduction

Rice was originally documented as a primary subsistence resource around 2,500 B.C. The cultivation of rice began in China 4000 years ago and, as time progressed, it spread to other countries (Weber *et al.* 2010, Yang *et al.* 2015) [22, 23]. Today, rice is still being produced on a large scale in China (197,257,175tonnes). Other countries with high levels of production include India (131,274,000tonnes), Australia (270,000tonnes), Indonesia (64,398,890tonnes), Bangladesh (45,075,000tonnes), Brazil (12,604,782tonnes), Vietnam (38,895,500tonnes), Thailand (31,462,886tonnes), Philippines (16,266,417tonnes), Myanmar (30,500,000tonnes) and Japan (10,592,500tonnes) (Charts Bin Statistics Collector Team, 2011) [5]. In Guyana, rice is mainly produced along the coastal belt where soils are fertile and the low-lying nature of this area allows for growth of wetland varieties via an easily regulated system of natural inundation from the nearby Atlantic Ocean. Production and export have been progressively increasing over the years, with 2014 being a particularly successful year (GRDB 2014) [10].

Rice farming in Guyana is not without its challenges. The rice plant is prone to various pest attacks during different stages of development. The golden apple snail (*Pomacea* sp.), a large gastropod from the phylum Mollusca and the family Ampullaridae (Teo 2002) [19], is a well-known pest of rice in Guyana. Its success in being a pest to the rice industry can be attributed to its high reproductive rate as well as its ability to invade a variety of habitats via several pathways, adapt to unfavorable environmental conditions and have a broad host range with an avid appetite (Massaguni & Latip 2012) [14]. Adult snails have the capability to feed on young seedlings and hamper their maturation (PHA Rice Industry Biosecurity Plan 2009) [17]. They typically occupy swamps, ponds, ditches, rivers and lakes, which are typical conditions of a rice field.

Fentin Acetate 60% is a common means of chemical control for the golden apple snail. Locally, Churaman (2013) [4] investigated the molluscicide action of Super Crekette Powder, Crekette Powder and Snail X Super (all of which contain Fentin Acetate as the primary active ingredient) on the mortality rate of golden apple snails. All showed significant control against the snails. However, Fentin Acetate 60% can result in severe environmental pollution and also affect the well-being of non-target organisms (including aquatic biodiversity) present in the environment if it, like so many other synthetic pesticides, is used abusively (Massaguni & Latip 2012) [14].

This warrants the need for development of more favourable alternatives to help reduce the damage caused by the golden apple snail in rice fields.

The use of biological controls (such as the enhancement of natural predator populations, the use of parasitoids and the use of herbal extracts) can help in the control of pests and simultaneously keep negative environmental impacts at an acceptable level. Moreover, they are often more affordable and can be set up easily. Arunlertaree *et al*. (2003) [3] investigated the effectiveness of crude extracts of cashew nut shell against *P. canaliculata* and found that both ethanolic and liquor extracts had potent molluscicidal activity with less toxicity than that of the reference molluscicide used, Niclosamide. Similarly, Musman (2010) found the dichloromethanic and methanic seed kernel extracts of *Barringtonia racemosa* to be effective controls against *P. canaliculata*.

This study used water extracts of the leaves of four plants: *Nerium oleander* (oleander), *Manihot esculenta* (cassava – bitter variety), *Azadirachta indica* (neem) and *Calotropis gigantea* (madar). These plants were selected based on anecdotal evidence as well as formal descriptions (Herrera 1991, Alves 2002, Girish & Shankara 2008, Alam *et al*. 2009) [11, 2, 8, 1]. that suggest that they may have desirable phytochemical properties.

Issues with golden apple snail infestations in the rice industry need to be urgently addressed in order to remove economic stress from farmers as well as the country. A mixture of control methods can be used to alleviate the impacts of this pest on the rice industry, but the idea behind this study is to help reduce the chemical burden on the environment. It aimed to investigate the effectiveness of biological (plant) extracts at varying concentrations in controlling golden apple snails, using the chemical Fentin Acetate 60% as a reference molluscicide and mortality rate as an indicator of effectiveness.

Materials and methods

Preparation of extracts

Aqueous extracts were prepared using the leaves of *N. oleander*, *M. esculenta*, *A. indica* and *C. gigantea*. Leaves were dried at 60ºC for 4 days and subsequently crushed into fine pieces. 100 grams of each type of dried, crushed leaf was soaked in 500 mL of hot water and allowed to sit for 4 hours. The extracts obtained were in the form of a 20% concentrated tea and they were stored in labelled, air-tight containers. The Fentin Acetate 60% (snail powder) extract was prepared by adding 4.5 g of snail powder to 500 mL of water at room temperature and mixed well. Then, 20 mL of this solution was added to 180 mL of water and mixed.

Three different concentrations (0.25%, 0.50% and 0.75%) were derived from each of the extract previously prepared. After the extract of each plant and snail powder (Fentin Acetate 60%) were obtained, the varying concentrations were made out as follows: 800 mL of distilled water was added to 10 mL, 20 mL and 31 mL of each plant extract to obtain concentrations of 0.25%, 0.5% and 0.75%, respectively. Additions of this nature provided the dissimilar concentration needed from each plant extract. The Fentin Acetate 60% extract was applied to the snails at the same concentrations as the plant extracts.

Gathering of snails

A total of 480 golden apple snails were used in this experiment. This included triplicates for each concentration in each treatment. Snails were handpicked from their natural environment, for example, drainage and irrigation canals nearby rice fields. The snails were acclimatized for 4 days in the imitated conditions. The drainage water was changed every 24 hours in order to get rid of excess food, faeces and dead snails before extract was added. This changing of water at prescribed intervals avoided toxic build up that may hamper survival.

The snails were placed into separate experimental units/containers to facilitate treatment with varying concentrations of the dissimilar extracts. The volume of each unit was 5.4 L and the snail's natural environment was mimicked by lining the unit's base with 5 cm of mud, along with 800 mL of water from their natural habitat. A fine cloth mesh was placed over each unit to provide aeration, protection from predators and prevent escape. Buckets were placed in a slightly shaded area in order to prevent desiccation of artificial habitats and of snails.

Application of extracts

Each experimental unit contained 10 snails in their mimicked environment. The respective extracts were added and observations were made every 24 hours for 4 days subsequent to application of the treatments. Mortality was assessed by examining response after inserting a stainless steel needle into the operculum.

Statistical Analyses

Microsoft Excel and Statistix were used for two-factor ANOVA with replication and LSD pairwise comparison, respectively. ANOVA was used to test the significance of distinct parameters while LSD pairwise comparison was used to identify specific differences that occurred. Differences were considered to be significant when $p < 0.05$.

Results

Overall effect of treatments at different concentrations on snail mortality

Differences between the rates of each treatment and also between the concentrations of each treatment were significant, according to two-way ANOVA with replication (Table 1). Table 2 is a key to the abbreviations of the different treatments represented in Figure 1.

Based on the homogenous groups portrayed in Figure 1, it is evident that snail mortality experienced at 0.25% for T1 was significantly different from that of T2 and T5 but not different from that of T3 and T4 at the same concentration and the control. Mortality at 0.50% and 0.75% for T1 was significantly different from that of T2, T4 and T5 but not different from that of T3 at the same concentration and the control. Snail mortality experienced at 0.25% and 0.50% for T2 was significantly different from that of T1, T3, T5 and the control but not different from T4 at the same concentration. Mortality at 0.75% for T2 was significantly different from that of T1, T3 and the control but not different from that of T4 and T5 at the same concentration. Snail mortality experienced at 0.25%, 0.5% and 0.75% for T3 was significantly different from that of T2, T4 and T5 at the same concentration. Snail mortality experienced at 0.25% for T4 was significantly different from that of T3 and T5 at the same concentration as well as the control. Mortality at 0.50% for T4 was significantly different from that of T1, T3 and T5 at the same concentration as well as the control. Mortality at 0.75% for T4 was not significantly different from that of T2 and T5 at the same concentration. Generally, as the concentration increased the mortality rate increased for all treatments.

Table 1: Comparison of the different rates of each treatment for snails ($p < 0.05$ = significance)

Comparison	*p*-value	F value	F critical	Significance
Between concentrations	8.41E-16	68.400673	2.838745	Significant
Between rates of treatments	6.54E-18	73.696969	2.605975	Significant

Table 2: Key to different treatments

Key	
T1	Bitter Cassave
T2	Madar
T3	Neem
T4	Oleander
T5	Fentin Acetate 60%

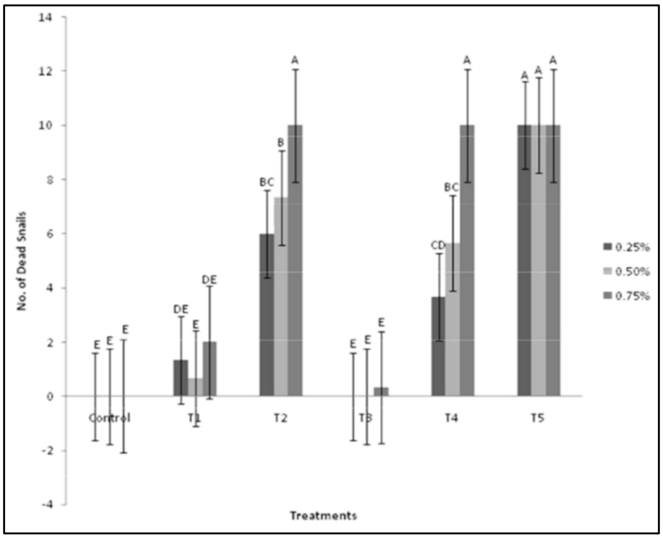

Fig 1: Total death of snails after application of treatments. Bars represent mean ± SE. Those that do not have common letters are significantly different according to Fisher's LSD test

Time taken to achieve total snail mortality

The line graphs represented in Figure 2 show that T5 (Fentin Acetate 60%) produced the greatest amount of deaths at the various concentrations while the control and T3 (neem extract) had the least amount of deaths. The highest mortality rate was achieved at 0.75% while the lowest occurred at 0.25% for the various treatments used.

Although T5 showed the highest mortality rate, significant mortality rates were also achieved with T2 (madar extract) and T4 (oleander extract) over an extended period of time. Further, at 0.75%, these two biological treatments were not significantly different from the chemical treatment in attaining snail mortality. At the lower concentration (0.25%), death rate increased as time progressed while at the higher concentration (0.75%), death occurred more rapidly. At the various concentrations used, T5 achieved mortality end point after 72 hours while the remaining treatments achieved mortality end point after 96 hours.

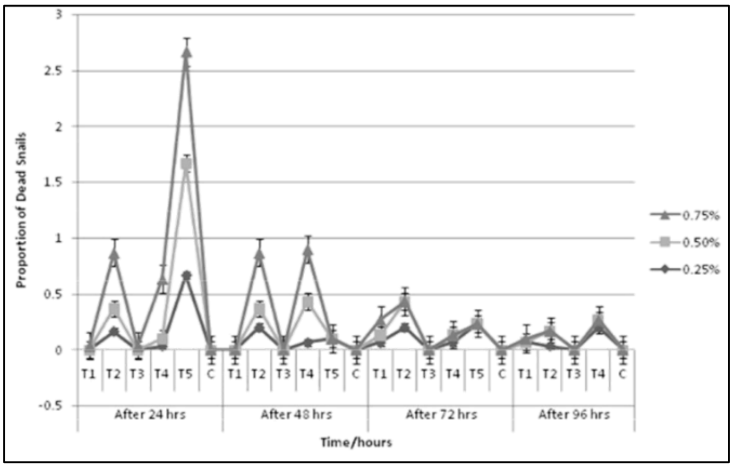

Fig 2: Proportion of dead snails at various concentrations after 96 hours

Discussion

Botanical pesticides can provide farmers with an alternative to chemical pesticides, many of which are hazardous to the environment. Botanical preparations comprise of many traits that make them compatible for use in control efforts, for example, most are non-toxic to beneficial species, non-persistent and have very low environmental cost in terms of use and production (Pluke *et al*. 1999, Isman 2008) [18, 13]. Synthetic chemicals can have quite detrimental effects on biodiversity, human health and the environment as a whole, even when administered or released in small amounts (Isman 2008) [13]. For example, concentrations of drugs (such as antidepressants) that are lower than expected in water have been found to affect the behaviour and biological make-up of creatures such as molluscs and crustaceans and, in some cases, even more than in higher concentrations. The presence of these drugs at low concentrations has proven to alter the reproduction of freshwater snails and also result in some of them losing their ability to attach to surfaces (Fong & Ford 2014) [7].

According to the results gathered from the two factor ANOVA in this study, it is evident that there were significant differences among treatments and concentrations indicating that the different concentrations of plant extracts used had an effect on snail mortality rate. All three concentrations of Fentin Acetate 60% resulted in the highest mortality rates when compared to the other treatments. This was due to its high potency which resulted in rapid death of these snails at various concentrations. Madar and oleander extracts were also able to produce a significant amount of deaths but these occurred over an extended period of time as compared to the chemical treatment. This was so since these biological treatments are less potent than the chemical treatment. Herbal extracts such as these tend to have less undesirable side effects on other biodiversity as well as the environment. This finding therefore has important implications for the development of a biopesticide to control golden apple snail populations in rice farming and other agricultural systems.

The LSD pairwise comparison indicated that higher snail mortality rates occurred at higher concentrations when compared to lower concentrations. This may be attributed to the high level of potency of the different concentrations and treatments used. Madar and oleander, at the three concentrations tested, showed the highest mortality rates indicating that these plant species may possess useful molluscicidal properties. According to Meena & Rao (2011) [15], the principal active ingredient in *Calotropis* (used to make madar extract) is mudarine; this and other substances such as a bitter yellow acid, resin and toxic glycodies (caltropin, calotoxin and uscharin) possibly made the extract toxic to the snails. Similarly, *N. oleander* (used to make oleander extract) contains oleandrine and cardenolides. Oleandrine has apoptosis properties which can result in failure of the mitochondria and, as a result, disruption of energy transfers and production processes (Ghrabi 2003) [9].

The other botanical treatments (*M. esculenta* - bitter cassava and *A. indica* - neem) proved to be unsatisfactory as it relates to snail deaths. They were able to kill a small number of snails due to toxins present but this occurred at a significantly slower rate. While the active ingredient of neem, azardirachtin ($C35H44O16$), may be toxic and a retardant of reproduction and growth, these may not have been present in high enough concentrations in the aqueous extract of neem leaf. This is possibly why there was low mortality rate when snails were exposed to this treatment. An alcohol/ethanol-based neem extract tends to be more effective as a molluscicide (Ufele *et al*. 2013) [21], as well as higher concentrations of extract (Massaguni & Latip 2012) [14]. However, this will ultimately require more time and preparation on the farmer's part, which is outside the scope of this study's aim to promote the use of readily-available, low-cost and low-tech solutions to pest management. Bitter cassava contains a bitter toxin known aslinamarin, which is a cyanogenic glycoside that can inhibit the life cycle of organisms. When ingested, linamarin cyanide is liberated in the gut during digestion and this results in death through poisoning (Food and Agriculture Organization of the United Nations, 1990) [6]. A small number of deaths occurred in this treatment, but the toxin was not present long enough and in a high enough concentration to facilitate greater mortality rates. The botanical treatments that resulted in low mortality rates (neem and bitter cassava extracts) were more successful at the higher concentrations (0.75%) as compared to the lower concentrations (0.25%). While a possible conclusion may be to increase the concentration of extracts to facilitate higher mortality rate, this cannot be made without further research to confirm that increasing extract concentrations will not adversely affect non-target organisms.

Fentin Acetate 60% contains organotins, which brings about effects upon contact. These organotins can inhibit mitochondrial oxidative phosphorylation, which affects ATP production, Ca^{2+} homeostasis and DNA and protein synthesis in the cell. Immunosuppression and programmed cell death subsequently arise in both vertebrates and invertebrates and photosynthesis-inhibiting properties are also present (Hollingworth 2001) [12]. Even though Fentin Acetate 60% was able to kill all snails treated in a short period of time, it may still be harmful to non-target organisms and the environment. According to Isman (2008) [13], farmers in developing countries often use highly toxic synthetic chemicals for pest control without adequate knowledge and understanding of harmful side effects and their implications. This occurs for one reason or another but can make the use of botanical pesticides more appealing when the impacts of synthetic pesticides on human health are considered. Madar and oleander extracts can be safer alternatives for rice farmers in developing Guyana as they result in similar mortality rates as the more commonly used Fentin Acetate 60%. Additionally, knowledge about the biology of these snails can assist in devising appropriate cultural controls to work in combination with biological treatments and this, in turn, can help in moving away from synthetic chemical controls.

Conclusion

Golden apple snails are a major pest in the rice industry of Guyana and are primarily controlled chemically. Two of the herbal extracts (madar and oleander extracts) in this study showed significant control against these snails, statistically similar to the chemical treatment and significantly different from the control treatment. At the higher concentrations, greater mortality was achieved when compared to the lower concentrations which showed little difference from the control treatments. After 24 hours of application, the madar and oleander extracts at a concentration of 0.75% and Fentin Acetate 60% at concentrations of 0.50% and 0.75% produced the highest snail mortality rates over the 4-day experimental period. At the various concentrations, neem and bitter cassava extracts showed no significant differences from the control as it relates to snail death.

Madar and oleander extracts can be employed as effective biological treatments against the golden apple snail since their ability to induce mortality is statistically similar to that of Fentin Acetate 60%. This can help to address issues with yield and food security in Guyana's rice industry.

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