HAWAII AGRICULTURE RESEARCH CENTER

FORMERLY HAWAIIAN SUGAR PLANTERS' ASSOCIATION
99-193 AIEA HEIGHTS DRIVE, SUITE 300, AIEA, HAWAII 96701-3911
TELEPHONE: (808) 487-5561 FAX: (808) 486-5020
http://www.hawaiiag.org/harc

LK – Lets fund the Papaya not fund neem –

12/27/01

Neem active is well known and sa

Dr. Robert Boesch
Hawaii State Dept. of Agriculture
Pesticide Division,
1481 S. King St.,
Honolulu, HI

Dear Dr. Boesch

Please find enclosed, a final report of the project entitled, "Efficacy and Testing of a Tropical Plant Extract for Control of Golden Apple Snail in Wet Land Taro", funded through purchase order No. 081755.

Following our recent telephone conversation, I have also included two small proposals for the determination of the active compounds in papaya fruit and in neem fruit. I would be most grateful if you would consider these proposals for funding. The potential for effective control of apple snails in taro loi is, in my opinion, very high if active compounds can be identified.

My mailing address is given in the letterhead. Should you wish to communicate further, my telephone number is: 486 5421 and my e-mail address is: mjackson@harc-hspa.com.

Thank you for your consideration and I hope to hear from you soon.

Sincerely,

Mel C. Jackson

Mel C. Jackson, Ph.D.
Chemist

HARC

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Final Report on Apple Snail Research Project **December 20, 2001**

Efficacy Testing of a Tropical Plant Extract for Control of Golden Apple Snail in Wet Land Taro

by
Mel C. Jackson, Ph.D., Chemist
Capre Mtchell, Assistant
Hawaii Agriculture Research Center

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Summary:
Golden Apple snails (***Pomacea canaliculata***) were subjected to the inclusion of plant parts from neem and papaya in their aquarium tanks. Plant parts were either available for feeding on or were placed in containers, such that they were not available for feeding on but were exposed to the rest of the water in the tank, allowing for diffusion of potential active ingredients. These experiments did not show any significant effects on snail mortality, egg laying rate or feeding habits. However, when water extracts of each of the plant parts were made, neem fruit and papaya fruit extracts were potent toxins, causing a 71% mortality rate when neem fruit extract was applied for 18 hours and an 83% mortality rate when papaya fruit extract was used. Similarly, feeding behavior was severely curtailed and egg laying was abolished.

Project Objectives:

1. Determine the efficacy of papaya plant parts in controlling apple snail populations in a test system.
2. Compare the ability of compounds from papaya plant parts to kill apple snails with copper sulfate in the same test system.
3. Determine if control is achieved through ingestion of papaya plant parts or through exposure to leachate from papaya plant parts.

Methods and Results:

In mid July, 2001 Golden apple snails(*Pomacea canaliculata) were p*urchased from Mr. Francis Hun (Bokee Farms), rather than obtaining them from taro loi, because equal numbers of each sex could easily be obtained. By having an equal number of females and males in all tanks, potential effects of each treatment on sex could be observed In addition, equal numbers of males and females ensured approximately the same starting reproductive rate in all tanks prior to initiation of tests.

Neem plant parts were also included in the test, together with papaya plant parts. Hawaii Agriculture Research Center has a small plot containing a number of varieties of neem trees. It thus seemed appropriate to include this plant, already known to contain limnoid compounds documented to have potent insecticidal properties.

After the purchase of 2.5 gallon aquarium tanks and snails, and after consultation with Mr. Hun (Bokee Farms), the following test system was organized:
Each tank contained 7.5 liters of faucet water in which 10 male and 10 female snails were housed. The tanks were each supplied with bubbled room air from a pump connected to a series of airways. Snails were confined to each tank by placing metal mesh over the top. Filtering was not required, provided that the water in each tank was replaced once per week. Initially, all snails were fed washed head lettuce from a local store. However, this proved to be impractical due to the very rapid rotting of uneaten lettuce, with a consequent fouling of the tank water. Therefore, during the first week the lettuce was replaced with pelletized coy food. This was completely consumed. The applied feed rate was one pellet per snail per day (20 pellets per tank). The snails were allowed to acclimate to their environment for 42 days. During this time, an average mortality rate of 10% (2 snails per tank) was observed. This was considered relatively low, and it was therefore concluded that the feed rate was sufficient. Egg cluster (approximately 50 eggs) production per tank was also observed, giving an average cluster production rate of one cluster every three days for individual tanks.

The efficacy test of the papaya and neem plant parts had the following design:

There were eight treatments in all, including positive and negative controls. All treatments were run in triplicate, with the exception of the positive control which is already known to quickly kill snails and therefore was a single treatment. The treatments were papaya leaf feed and soak, papaya fruit feed and soak, neem leaf feed and soak, copper sulfate (7.5 ppm, upper limit stated in EPA reg. No. 1109 for use of Copper Sulfate Snow Crystals in wet land taro paddies, irrigation and drainage ditches and ponds), and negative controls. Throughout all treatments snails were fed 1 pellet per snail per day, to ensure that any aversion to a particular treatment did not result in starvation.

Papaya and neem leaf and green papaya fruits were collected from HARC's Kunia farm. Pesticides had not been used on any of these trees. At the start of the test, ten grams of papaya leaf (washed and chopped into approximately 2 inch pieces) was put into each of three tanks. Neem leaf was given to snails in another three tanks. Twenty grams of the

peel from green papaya fruit was given to snails in a further three tanks. This constituted the leaf and fruit feed portion of the tests. In similar sets of tanks, the same treatments were applied, but instead of putting the test substances directly into the water, they were placed in glass containers with wire mesh caps, before placing in the tanks. The containers prohibited the snails from eating the test substance but allowed for diffusion of soluble components into the tank water. For the copper sulfate (7.5ppm) treatment, 57 mls of a 1mg/ml aqueous solution of copper sulfate was added to the tank water in a single tank. The negative controls were given no treatment and were fed with the coy pellets as in all of the other tests. Numbers of dead snails per tank and number of egg clusters were counted throughout the test period. First observations were made three days after the initiation of the test and then 6, 12, 19, 20, 31, 50, and 64 days after initiation. Immediately following observations, the tank water and test substance was changed. Test materials were replaced after every water change. The test was completed on October 23, 2001.

Table 1 below shows the egg cluster rate of production for each treatment over the test period:

Treatment	*Number of egg clusters over the 64 day test period*	*Average cluster production rate (egg clusters/day)*
Neem leaf soak	16	0.25
Papaya leaf soak	10	0.16
Papaya fruit soak	24	0.38
Neem leaf feed	20	0.31
Papaya leaf feed	19	0.3
Papaya fruit feed	27	0.42
+ve control	0	0
-ve control	22	0.34

Compared with the negative control, all of the treatments were similar, with the exception of the positive control (all of the snails were dead within the initial three day observation period) which gave no egg clusters, the papaya leaf soak which had a production rate 47% that of the -ve control, and the papaya fruit feed which had a production rate 124% that of the -ve control. No effects were found to be sex specific.

Table 2 shows the mortality data obtained over the test period:

Treatment	*Mortality rate first 20 days (% dead snails per treatment)*	*Mortality rate 20-64 days (% dead snails per treatment)*	*Total number of dead snails/treatment*
Neem leaf soak	11	63	45
Papaya leaf soak	15	52	40
Papaya fruit soak	5	27	19
Neem leaf feed	13	63	29
Papaya leaf feed	18	35	35
Papaya fruit feed	22	33	33
+ve control	100	100	20
-ve control	12	13	20

The mortality data shows that in the first 20 days all of the treatments had similar rates of mortality to that in the negative control, with the exception of the copper sulfate treatment, which had 100% mortality within three days. In addition, the mortality rate for papaya fruit feed was nearly twice that of the negative control. In the following observation period (20-64 days) all leaf and fruit treatment mortality rates were considerably higher than the negative control. None of the effects were found to be sex specific.

Conclusions from this round of experiments

The only treatment to that resulted in a reduction in the rate of egg cluster production was the papaya leaf feed. However, the mortality rate in this treatment was also high and is most likely a contributing factor. Many treatments resulted in very similar rates of egg cluster production to the negative control. While the snails readily ate papaya fruit and leaf, they did not eat the neem leaf. It should be noted that after day 20 large increases in the number of dead snails were observed in the papaya leaf soak and feed. It was observed that with these treatments, the snails were generally killed by what appeared to be bacterial infections, infecting all snails and causing sudden increases in mortality. It was concluded that none of the treatments exhibited effective anti-egg laying or molluscicide activity. The results were confounded by what appear to be bacterial contamination from the leaf and fruit treatments.

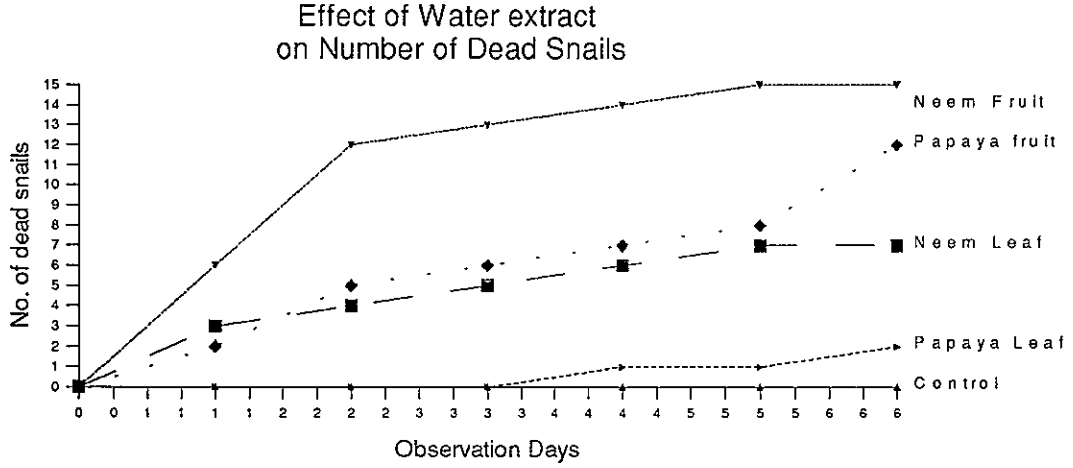
Effects of water Extracts of Neem and Papaya Leaf and Fruit

In order to easily increase the concentration of compounds that the snails were exposed to, (not easily done by increasing the weight of plant material in the tanks, due to volume

and contamination constraints), water extracts of neem and papaya leaf and fruit were made. 1 kilogram of test plant material was homogenized with one liter of water for 3 minutes at full speed using a Waring blender. Extracts were filtered through glass fiber disc filters in a buchner funnel. Ten two and a half gallon tanks were filled with 6 liters of tap water at room temperature. Twelve healthy snails were placed in each tank. Each treatment was run in duplicate (two tanks per treatment). There were a total of five treatments:

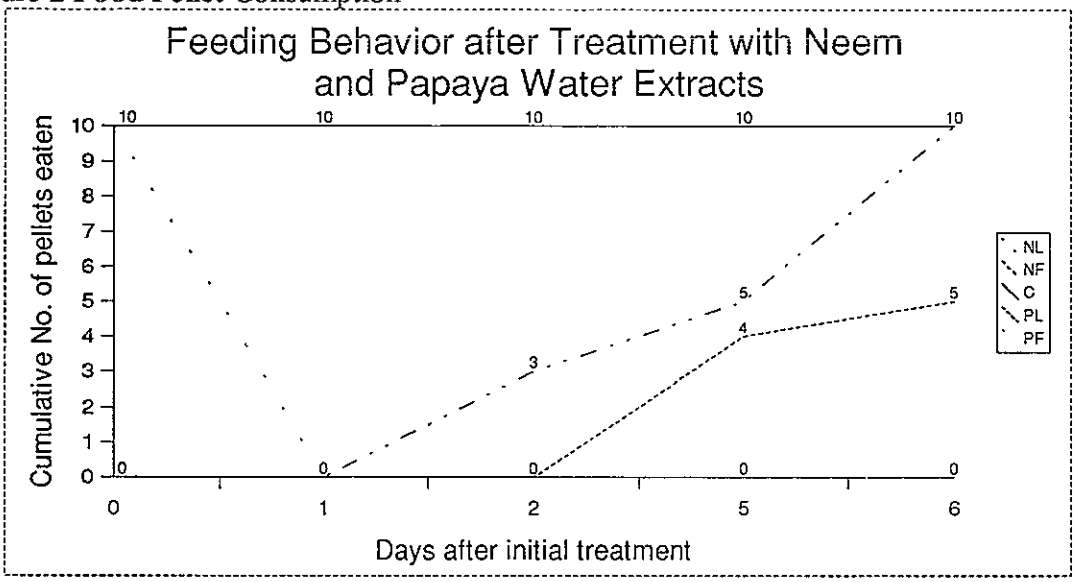
Neem leaf extract
Neem fruit extract
Papaya leaf extract
Papaya fruit extract
negative control (water only)

Throughout the test period snails were fed at a rate of ten pellets per tank per day. 250 mls of extract or water were added to each of the tanks. After 18 hours, the water in all of the tanks was changed and replaced with tap water. At this time egg sac production, mortality and number of pellets consumed were determined. Observations were then made 2, 5and 6 days after the initial treatment. The results of the test are illustrated in the figures below:
Figure 1 Mortality



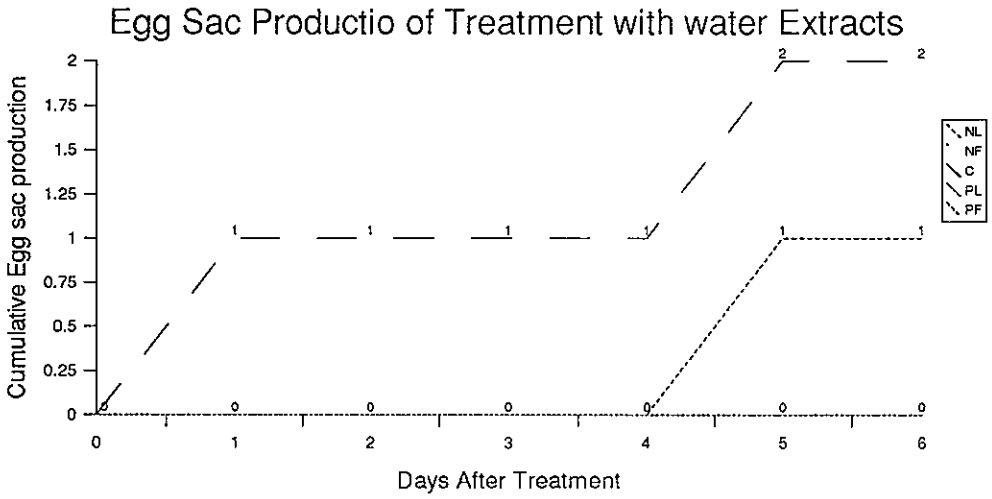
The graph above shows that after 6 days, 15 of 24 snails in the neem fruit test (62.5%) were dead. The initial mortality rate for this treatment was high, with 50% of the snails dead within two days after applying the neem fruit extract. The papaya fruit test showed 50% mortality after the 6 day period. The initial rate of mortality was not as high as that for the neem fruit. Neem leaf gave a 25% mortality rate over the 6 day period. Papaya leaf gave an 8% mortality rate. No deaths in the negative control were recorded during this period. Observations were continued for a total of 14 days. The mortality rate for the neem fruit increased to 71%, for the papaya fruit 83%. The rest of the treatments including the negative control remained unchanged

Figure 2 Food Pellet Consumption



The data above illustrates the feeding behavior of the snails in each tank. The papaya leaf and the negative control both exhibited no decline in the number of pellets eaten during the 6 day period. In contrast, the neem leaf treatment resulted in none of the pellets in the test being eaten after the first day. This steadily increased over the 6 day period. Similarly, the snails in the neem fruit test ate no pellets through the first two days of the treatment., However, the surviving snails steadily regained their appetite and began to eat some of the pellets. Of most significance was the effect of the papaya fruit treatment. While the maintained their appetite during the first day of the test, from then on, pellet consumption was reduced to zero. The surviving snails did not eat for the rest of the 6 day period. In fact over the extended 14 day observation period, the surviving neem fruit test snails did not begin to consume pellets until the twelfth day.

Figure 5 Egg Sac Production



The figure above shows that the cumulative control egg sac production was two egg sacs

over the six day test period. This was exactly mirrored by the papaya leaf treatment. The neem leaf treatment resulted in one egg sac produced over the six day test period. In contrast, papaya fruit and neem fruit treatments resulted in no egg production. Over a 14 day extended observation period, the neem leaf and papaya leaf treatments, along with the negative control continued to lay eggs at a rate of about 1 egg sac every three days, whereas the neem fruit treatments and the papaya fruit treatments did not lay any eggs throughout this period.

Titration of water Extracts

Using the same protocol as described in the tests above, snails were exposed to water extracts (250 mls in 6L of tank water) of either neem or papaya with the following dilutions:
Neat
1:10
1:100
1:1000
Four snails per treatment were used. There was also a negative control tank, containing four snails that were not exposed top extract. Snails were exposed to these dilutions for a three day period before replacing the tank contents with fresh water only. Throughout the test and follow up observation period, snails were fed coy pellets at a rate of one pellet per snail per day. During the treatment period and for a total of seven days after initiation of the treatment, snails were monitored for mortality and pellet consumption.

Results:
The table overleaf summarizes the mortality rate and pellet consumption observations for all of the titration tests.

Neem Treatment Mortality									*Neem Treatment Pellet Consumption*							
Day	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Neat	0	1	4	-	n.d	n.d.	-	-	0	0	0	-	n.d.	n.d.	-	-
1 to10	0	0	0	0	n.d	n.d.	0	0	0	2	4	7	n.d.	n.d.	9	10
1 to 100	0	0	0	0	n.d	n.d.	0	0	4	8	12	16	n.d.	n.d.	17	19
1 to 1000	0	0	0	0	n.d	n.d.	0	0	4	8	12	16	n.d.	n.d.	18	20
Control	0	0	0	0	n.d	n.d	0	0	4	8	12	16	n.d	n.d	20	24
Papaya Treatment Mortality									*Papaya Treatment Pellet Consumption*							
Neat	0	0	4	-	n.d	n.d	-	-	4	4	4	-	n.d	n.d	-	-
1 to10	0	0	0	0	n.d	n.d	0	0	2	6	8	12	n.d	n.d	16	20
1 to 100	0	0	0	0	n.d	n.d	0	0	4	8	12	16	n.d	n.d	20	24
1 to 1000	0	0	0	0	n.d	n.d	0	0	0	0	1	5	n.d	n.d	9	13
Control	0	0	0	0	n.d	n.d	0	0	0	0	0	0	n.d	n.d	0	0

n.d. Not determined (weekend)

The table above shows that snails were only affected when neat extracts were used. Further dilution did not result in any mortality. The 1:10 dilutions did show some slight attenuation of appetite for both neem and papaya. As the active ingredients are not known, it is difficult to know what concentrations are effective.

Conclusions:

The experiments described in this report show that water extracts of neem and papaya fruit are toxic to the golden apple snail. In addition the results show that feeding behavior and reproduction were also affected by these treatments. Any one of these three outcomes would have a significant effect on apple snail damage to wetland taro in Hawaii and elsewhere. The fact that the initial treatments with pieces of fruit and leaf rather than a water extract did not result in any effect on the three parameters, suggests that either the overall concentration of active ingredient in the whole food treatments was not high enough, or that simple ingestion is not enough to produce the effect, but rather a systemic exposure is required. Even though some of the snails in the tests were apparently infected by contaminating bacteria when pieces of fruit were present , the progression of the infection was different from the effect seen with the water extracts, where almost immediate behavioral differences and mortality was seen.

Work is now needed to determine the identity of the active ingredients.