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Effect of Selected Botanical Extracts for the Control of Plesispa Beetle (*Plesispa reichei* Coleoptera: Chrysomelidae) in Laboratory Bioassays

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ABSTRACT

Plesispa beetle (*Plesispa reichei* Chapuis) is one of the most serious pests in coconut nurseries and young seedlings. Currently, the growers use chemical pesticides to control the pest. However, continuous and indiscriminate use of chemical pesticides becomes threat to the environment and cause secondary pest outbreaks. Therefore, two botanical extracts, tobacco (*Nicotiana tabacum* L.) and castor (*Ricinus communis* L.) were evaluated for their toxicity against *P. reichei* in laboratory bioassays. Aqueous extracts of tobacco recorded a higher mortality on larvae (83.80%) and adults (100%) of plesispa beetle than that of castor (56.46% and 87.50% on larvae and adults respectively), 72 hours after the application of botanical extracts. Probit analysis revealed that the LC_{50} value for castor on larvae and adult were 4.64×10^4 ppm and 5.15×10^4 ppm respectively whereas for tobacco, it was 2.77×10^4 ppm on larvae. Both botanical extracts resulted over 50% mortality in *P. reichei* larvae and adults. The study did not show any dose dependence of botanical extracts on *P. reichei* larvae and adults. Therefore, extracts of tobacco and castor have potential for use in biological control of *P. reichei* which should be tested under field conditions.

KEYWORDS: Botanicals, Castor, Coconut, *Plesispa reichei*, Tobacco

INTRODUCTION

Coconut is one of the major plantation crops grown in Sri Lanka which accounts for approximately 12% of all agricultural produce in Sri Lanka (Anon, 2015). Total land area under cultivation is 395,000 hectares and about 2,500 million nuts are produced per year (Anon, 2015). Nevertheless, the industry is continued to increase in land extent especially in non-traditional areas in the country. The annual requirement of seedlings for replanting and new planting programmes of coconut is about 1.45 million (Nainanayake *et al*., 2002). These seedlings are produced in the coconut seedling nurseries of the Coconut Research Institute and Coconut Cultivation Board of Sri Lanka.

Pests and diseases are among the major limiting factors that determine the seedlings mortality and rejections in coconut nurseries. Moreover, the control of pests and diseases substantially contributes the cost of production of coconut seedlings. Plesispa beetle (*Plesispa reichei* Chapuis) (Coleoptera: Chrysomelidae) is one of the serious pests in coconut nurseries in Sri Lanka. *P. reichei* was first reported in Sri Lanka in 1997 from Badalgama (Gampaha district). It is now wide spread throughout the coconut triangle (Kurunegala, Puttalam and Gampaha) (Anon, 2006). Both adults and larvae live between the folded blades of leaflets and in unopened young fronds in the bud. The damage is done by larvae and adults feeding on tissues of the young unopened leaflets (Anon, 2006). The damage caused by this pest retards the growth and in a severe outbreak, seedling mortality is obvious. Thus, control of *P. reichei* is very important to obtain quality seedlings with low rejection percentage and to reduce the field casualties. Two insecticides have been recommended to control the pest by the Coconut Research Institute. Though the synthetic insecticides control the pest damage, continuous application of them creates many harmful effects on the environment and health. Moreover, current coconut industry is rapidly moving towards the organic coconut production with high returns. In organic coconut cultivations, growers find it difficult to control *P. reichei* due to unavailability of organic pesticides. With this concern, botanical extracts based on locally available plants have frequently been claimed to be effective in pest management in organic crop production (Shiberu *et al*., 2014).

Organic pesticides for the management of *P. reichei* have not been evaluated in Sri Lanka or elsewhere. However, botanicals have been tested with promising results in China for Brontispa beetle (*Brontispa longissima*) which is a close relative of plesispa beetle (Chaojun *et al*., 2012). Growers in Sri Lanka use different crude extracts of some plants that are locally available, but their efficacy has not been validated.

Therefore, the objective of this study was to determine the effect of selected botanicals on *P. reichei* adults and larvae in the laboratory.

MATERIALS AND METHODS

Experimental Site

This study was conducted at the Crop Protection Division, Coconut Research Institute of Sri Lanka, from January to May 2016.

Rearing Insects

Adult beetles of *P. reichei* of unknown age were collected from the field and mass reared in incubators (larvae and pupae at 22 °C and adults and eggs at 28 °C, 12:12 photo period and 75% RH). The adults and larvae from these cultures were used for all the bioassays.

Experimental Design

The experiment was arranged in Completely Randomized Design (CRD) with 30 replicates for different concentrations (4.0×10^4, 4.5×10^4, 5.0×10^4 and, 5.5×10^4 ppm) of crude leaf extract of castor (*Ricinus communis* L.; T_1) and different concentrations (1.0×10^4, 1.5×10^4, 2.0×10^4, 2.5×10^4 and 3.0×10^4 ppm) of leaf extracts of tobacco (*Nicotiana tabacum* L.; T_2). Carbosulfan 20% (Marshal 20%; T_3) was used as standard check and distilled water (T_4) was used as the control.

Pre-test of Selected Botanicals

A pre-test was done with six selected plant species based on the past literature (Chaojun *et al.*, 2012). The selected species were *Chromolaena odorata* L., *Mikania mikarantha, Ricinus communis* L., *Catharanthes roseus, Nicotiana tabacum and Calotropis procera.* From the tested species, only *N. tobacum* and *R. communis* L. were selected for further studies as they showed considerable level of mortalities compared to other botanicals that recorded less than 10% mortality.

Preparation of Crude Aqueous Leaf Extracts

Ricinus communis: Fresh leaves were weighed using analytical balance (AB204-S). Weighed leaf samples were crushed using a homogenizer (Type-T 25 B) and 1 L of the crude aqueous extract was prepared by adding distilled water.

Nicotiana tabacum: Dried leaves were weighed using analytical balance (AB204-S). Weighed leaves were soaked in 1 L of boiled water overnight to brew.

Determination of Mortality Rate of Larval and Adult Stages of P. reichei

Immature coconut leaves were cut into pieces (12 cm long) and dipped in crude leaf extracts of the each concentration for 5 min. They were held upright for 2-3 min to remove excess solution and placed in plastic boxes (30 boxes: 140×90 mm). One larva of the L_3 stage was introduced in to each box individually.

Mature coconut leaves were cut into pieces (12 cm long) and dipped in crude leaf extracts of each concentration for 5 min. After draining excess solution, they were placed in transparent plastic bottles (10 bottles: 160 mm height, 80 mm diameter). An adult beetle of one week old was introduced in to each bottle.

The leaf pieces were dipped in 250 mL distilled water (control) or leaf pieces dipped in Carbosulfan 20% (Marshal© 20%) at the rate of 4 mL/L were used as the positive control. After removing excess water and insecticides, leaf pieces were placed in plastic boxes and bottles and larvae and adults were introduced as mentioned in the above section.

Percentage mortality was recorded at 24, 48 and 72 h intervals after introducing the larvae and adults. Insects that showed no response with head movements or body contraction when touched with a fine brush were scored as dead. The larvae that had moulted into pupae were scored as alive.

Larvae and adult percentage mortality were calculated using the following formula.

$$Mortality\ \% = \frac{total\ death}{total\ observed} \times 100\%$$

Statistical Analysis

The data were statistically analyzed by SPSS (version 19) statistical software. Where significant differences were observed, mean separation was done using Tukey post hoc test to determine the statistical significance of the treatments. The LC_{50} value was determined by Probit regression analysis.

RESULTS AND DISCUSSION

Mortality Rate of P. reichei Larvae

The mean mortalities of *P. reichei* larvae exposed to different botanical extracts are shown in Figure 1. After 24 h, the highest mortality of *P. reichei* larvae was observed when the leaflets dipped in Carbosulfan 20% were offered (100.00%) and it was significantly different from the other treatments ($F=359.56$, $df=3$, $P<0.01$;Figure 1). Second highest mortality of *P. reichei* was recorded on leaflets dipped in *N. tabacum* (83.80%) and it was significantly different from the other treatments (Figure 1). The least percentage (56.46%) of morality was showed when the larvae were offered *R. communis* treated leaflets. Negative control treatment showed 1.25% mortality (Figure 1).

Mortality Rate of P. reichei Adults

The mean mortalities of *P. reichei* adults exposed to different botanical extracts are shown in Figure 2. After 24 h, 100% mortality of adults was observed when the leaflets that were treated either with Carbosulfan 20% or *N. tabacum* were offered. They were significantly different from the negative control ($F=175.49$, $df=3$, $P<0.01$) but were not significantly different from the mortality observed when *P. reichei* adults were offered with leaflets dipped in *R. communis* (87.50%; Figure 2). The lowest mortality was observed from the negative control treatment (0.00%; Figure 2).

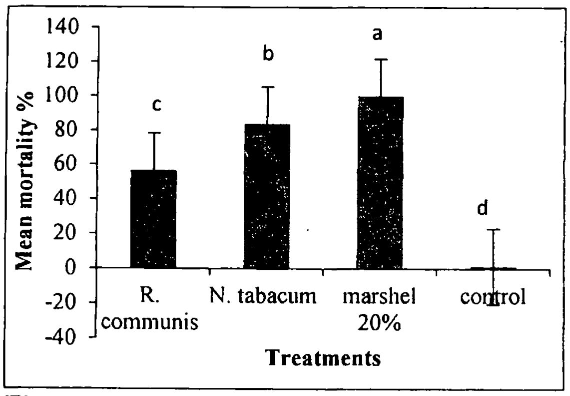

Figure 1. Mean percentage mortality of *Plesispa reichei* larvae

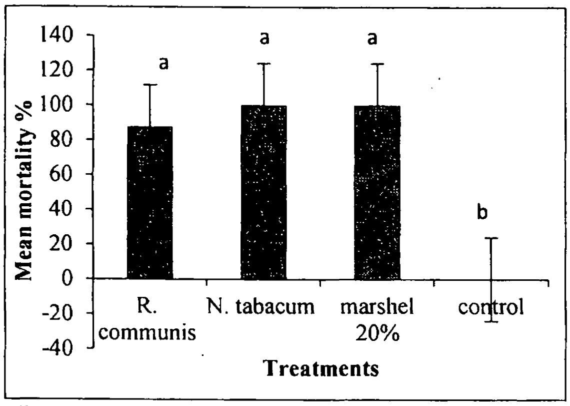

Figure 2. Mean percentage of mortality of *Plesispa reichei* adults

Dose Dependence of Botanical Extracts

There was no significant difference in the dose dependence of both larvae and adults (Table 1) on *R. communis* or on *N. tabacum* (Table 2).

Table 1. Mortality percentage under different doses of *Ricinus communis*

Doses ($\times 10^3$ ppm)	Mortality of larvae (%)	Mortality of adults (%)
55	65.52	77.77
50	61.53	90.00
45	62.00	90.00
40	42.85	90.00

Table 2. Mortality % under different doses of *Nicotiana tabacum*

Doses ($\times 10^3$ ppm)	Mortality of larvae (%)	Mortality of adults (%)
30	100.00	100.00
25	100.00	100.00
20	96.00	100.00
15	88.00	100.00

Determination of LC_{50}

The LC_{50} value for *R. communis* against *P. reichei* larvae was 4.64×10^4 ppm (Figure 3) whilst that was 5.15×10^4 ppm on *P. reichei* adults (Figure 4). The LC_{50} value for *N. tabacum* against *P. reichei* larvae was recorded as 2.77×10^4 ppm (Figure 5). However, Probit analysis could not be conducted for *N. tabacum* treated adults, as all the doses showed significantly similar percentages of mortalities.

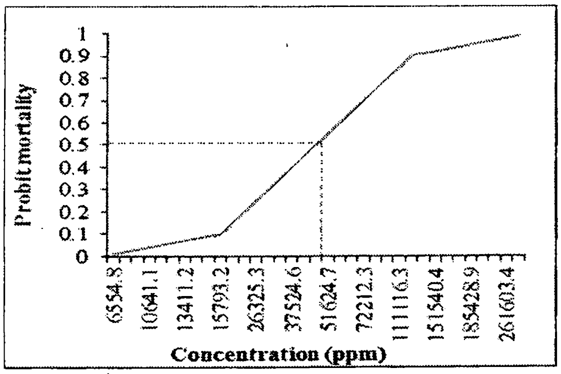

Figure 3. Probit mortality curve for *Ricinus communis* against *Plesispa reichei* larvae

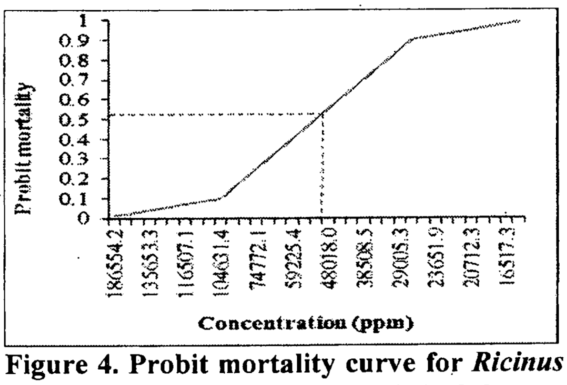

Figure 4. Probit mortality curve for *Ricinus communis* against *Plesispa reichei* adults

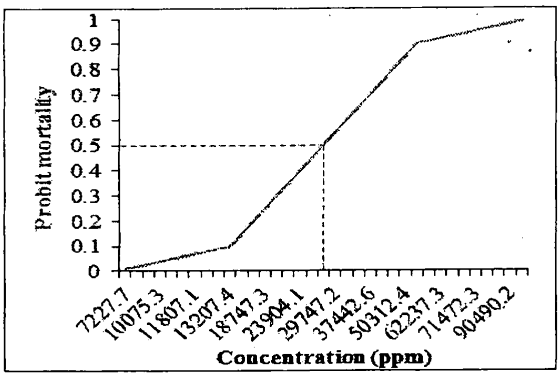

Figure 5. Probit mortality curve for *Nicotiana tabacum* against *Ricinus reichei* larvae

In this study, *N. tabacum* showed significantly higher percentages of mortalities for both larvae and adults of *P. reichei* after 24 hours. Similar results was reported by Sohail *et al.* (2012) using tea aphid (*Toxoptera aurantii),* where 2% tobacco extract caused 98% of mortality, 24 h after treatment.

Further studies on tobacco revealed that nicotine causes symptoms of poisoning similar to those seen in organophosphate and carbonate insecticides and that nicotine is the main constituent of tobacco which induces high insecticidal effects as they are synaptic poisons that mimic the neurotransmitter acetylcholine (Ogbalu *et al.*, 2014).

Current study showed over 50% mortality in *R. communis* of larvae of *P. reichei* (56.46%) and adults (87.50%). However, study conducted by Chaojun *et al.* (2012) on *B. longissima* which is a close relative species of *P. reichei, R. commun is* showed a significantly lower toxicity for L_3 larvae (LC_{50} 1371.39 mg/mL) while LC_{50} for *P. reichei* L_3 stage larvae was 4.64×10^4 ppm that is equivalent to 46.44 mg/mL which showed a higher toxicity. This might be due to different solvents used for the extraction and the variability of degree of resistance shown by *B. longissma* and *P. reichei* to the botanical extract.

Samuel *et al.* (2011) reported that the hydraulic extract of *R. communis* caused 100% mortality of 5^{th}-7^{th} instar larvae of *Tribolium castaneum* at 1 day after treatment (DAT), whereas, 100% larval mortality was only observed at 3 DAT. The study done by Ramos-Lopez *et al.* (2010) demonstrated that the castor oil and ricine are active ingredients of *R. communis.*

Both botanical extracts used caused over 50% mortality in *P. reichei* larvae and adults. Moreover, botanical extracts tested in this study were more toxic to *P. reichei* adults than to the larvae. This could be due to physiological difference and differences in metabolism rates among them. It could also be due to the fact that the adults are more mobile than the larvae and therefore get exposed more to the botanical extracts.

However, this study did not show any dose dependence of botanical extracts on *P. reichei* larvae and adults. Therefore, any tested dose could be used in the laboratory. However, further field testing is required for recommendation of the botanical extracts.

CONCLUSIONS

It is concluded that the aqueous leaf extracts of *N. tabacum* and *R. communis* have the potential to be used as an eco-friendly approach for the control of *P. reichei* under laboratory conditions. Further studies should be conducted to find the potential of use of these botanical extracts in field conditions.

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