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INVESTIGATIONS ON INSECTICIDAL ACTIVITIES OF SOME PLANTS TO MEALYBUG (*Pseudococcus* sp.) UNDER LABORATORY CONDITIONS

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Abstract

Mealybug *Pseudococcus* is a serious insect pest of many crops. They can damage plant by sap sucking directly or by transmission of many dangerous viruses. The toxicity of six plant methanol and aqueous extracts were investigated for insecticidal and repellent properties against second nymph of mealybug collected from sour sop under laboratory conditions. In bioassay, soursop methanol seed extract (20 % vol/vol concentration) was the most toxic to mealybug (caused 98.33 % mortality at three days after treating), followed by a tobacco methanol leaf extract (75 % mortality) at 10 %, chinaberry methanol leaf extract (61.67 %) at 20 %, wedelia methanol all plant extract (26.67 %) at 20 %, zanzibar gem aqueous leaf extract (25 %) at 20 % then onion aqueous bulb extract (16.67 % mortality) at 20 % concentration. LC₅₀ of methanol extract of soursop seed, tobacco methanol leaf and chinaberry leaf to *Pseudococcus* sp. nymphs was 5.364, 4.436 and 11.809 %, respectively.

Keywords: Insecticidal, mealybug, *Pseudococcus* sp., toxicity, plant extract

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INTRODUCTION

Mealybugs, various species of scales or cochineals and spider mites attack the stems, leaves, flowers and fruits of the host trees in numerous countries and regions. They all are considered to be sucking insect pests and may be considered as economically important, due to their impact when they suck the sap of the young vegetative parts and fruits of the trees.

Mealybug *Pseudococcus* sp. can damage host trees by sap sucking directly or by transmission of many dangerous viruses. Sforza *et al.* (2003) recorded that *Pseudococcus maritimus* (Ehrhorn) was a vector of *Grapevine leafroll associated virus 3* (GRLaV-3) (*Ampelovirus*) on grape. Mekuria *et al.* (2013) showed that *P. maritimus* (Ehrhorn) was a vector of *Little cherry virus 2* (LChV2) on *Prunus avium*. Garau *et al.* (1995) recorded that *Pseudococcus affinis* Mask was a vector of *Grapevine trichovirus A* (GVA) and *Grapevine trichovirus B* (GVB) on grape.

In recent years, increasing use of synthetic insecticides against crop pests has led to several environmental and human health problems. Additionally, extensive use of synthetics leads to outbreak of pest species of secondary importance, development of resistance by target species to insecticides and accumulation of toxic residues in the environment. Exploring alternatives like pesticides of botanical origin to tackle pest species is a viable option. Many plants are known to have diverse array of chemicals with multiple modes of action on insects and are believed to be safe to non-target organisms (Schmutterer, 1995).

To explore the alternatives for conventional insecticides, insecticidal properties of extracts from plants are investigated. In this paper, we report the investigations on insecticidal and repellent activities of some plant extracts against mealybug, *Pseudococcus* sp. under laboratory conditions done by Plant Protection Division of Southern Horticultural Research Institute, Vietnam from June to December, 2012.

MATERIALS AND METHODS

Materials

- Mealybug, *Pseudococcus* sp. were collected from soursop orchards near Southern Horticultural Research Institute.
- Potato, pumpkin used as food for rearing mealybug.
- Methanol.
- Some plants such as tobacco leaf (*Nicotiana tabacum* L.), all plant of wedelia (*Wedelia trilobata* L.), soursop seed (*Annona muricata* L.), chinaberry leaf (*Melia*

azedarach L.), onion bulb (*Allium cepa* L.) and zanzibar gem leaf (*Zamioculcas zamiifolia* Lodd.).

- Plastic boxes, net cages, lens, microscopes.
- And some needed other materials.

Methods

Preparation of plant extracts

Extraction using organic solvent, methanol

Tobacco leaf, all plant of wedelia and chinaberry leaf were cut into small pieces by knife. They were allowed to dry in shade to prevent the possible loss of active compounds. Soursop seed were collected from fruits and seeds were obtained. The seeds were allowed to dry in shade for preventing the possible loss of active compounds. Shade drying was continued till the seeds were dried enough to be powdered. Dried seeds were then ground into coarse powder using a ware blender (Philips-India).

Dried leave and seed powder of plants were subjected to extraction in methanol using Soxhlet apparatus unit (Behr Labor Technik-R 106S - Germany). 100 g of each one was filled in whatman filter pouch and kept in the Soxhlet apparatus jar and the jar was filled with 250ml methanol as extractant. The extraction was run for 15-20 hours till the solvent in extraction jar appeared almost clear.

The extract was subjected to evaporation in a vacuum evaporator to remove methanol using Flash Evaporator (IKA-RV 10C - China). After evaporation the obtained semi solid extract was then air dried to remove almost completely the solvent and stored at 8 to 10°C in a refrigerator and used for further studies.

Aqueous extracts

Onion bulb and zanzibar gem leaf were cut into small pieces and then ground into coarse powder using a ware blender (Philips-India). They was soaked in water over night and filtered repeatedly using a fine white muslin cloth. The volume of the filtrate obtained was made up using water to get the desired concentrations (Dodia *et al.*, 2008).

Evaluation of plant extracts to second nymph of mealybug Pseudococcus sp. for insecticidal and repellent properties

Potatoes with sprout (3 - 5 mm) were used to study effect plant extracts on nymphs of mealybug *Pseudococcus* sp. Potatoes were placed into plastic box (30 cm x 30 cm x 10 cm) then they were covered by sand and irrigated by water till to wet enough. They were kept in dark place for 3 - 5 days. Twenty second-

nymphs were released on the sprout potatoes. Potatoes were then sprayed with desired concentrations of test extracts separately using a hand atomizer. Each

treatment was sprayed 4 ml of the extract. The concentrations were used as below:

Sl. no	Extracted plant		Extracted method	Concentration (%)
	Common name	Scientific name		
1	Tobacco	<i>Nicotiana tobacum</i>	methanol	10
2	Wedelia	<i>Wedelia trilobata</i>	methanol	20
3	Soursop	<i>Annona muricata</i>	methanol	20
4	Chinaberry	<i>Melia azedarach</i>	methanol	20
5	Onion	<i>Allium cepa</i>	water	20
6	Zanzibar gem	<i>Zamioculcas zamiifolia</i>	water	20
7	Control	-	-	-

All treatments were added 0.5% dish washing liquid as adhesive. Potatoes sprayed with water + 0.5% dish washing liquid served as controls and three replications were maintained for each of the treatments. One sprout potato was used in each replicate of each treatment. Then they were placed in clean plastic boxes (12 cm in diameter) and covered by fine muslin cloth. These boxes were maintained at room temperature. The mortality of nymphs was recorded at 1, 3 and 5 days after treating and per cent mortality was computed treatment-wise. Mealybugs which did not respond to the probe by a fine camel hair brush or with shrunken body were considered as dead (Huynh Ngoc Hai and Nguyen Van Hoa, 2008).

Data analysis

The mortality data were corrected using Abbott's (1925) formula, considering the mortality in untreated control, if any. The data in percentages were subjected to arc-sine transformation and analysed following the Analysis of Variance technique for Completely Randomized Design (CRD) and the results were interpreted at five per cent level of significance.

Determination of median lethal concentration (LC_{50}) of plant extracts to second nymph mealybug *Pseudococcus* sp.

Median lethal concentration (LC_{50}) of the plant extracts to second nymph mealybug *Pseudococcus* sp. was determined by spray using a hand atomizer method. Each plant extract was tested at appropriate dosages. For *Nicotiana tobacum* methanol extract 4, 6, 8, 10 and 12% vol/vol concentrations were used. For *Melia azedarach* methanol extract 5; 7.5; 10; 15 and 20% vol/vol concentrations were used. For *Annona muricata* methanol extract 2.5; 5; 10; 15 and 20% vol/vol concentrations were used. Potatoes

treated with water + 0.5% dish washing liquid served as corresponding controls and three replications were maintained for each treatment. Potatoes were placed into plastic box (30 cm x 30 cm x 10 cm) then they were covered by sand and irrigated by water till to wet enough. They were kept in dark place for 3 - 5 days to get sprout. Twenty second-nymphs were released on the sprout potatoes. Potatoes were then sprayed with desired concentrations of test extracts by using hand atomizer. Then they were placed in clean plastic boxes (12 cm in diameter) and covered by fine muslin cloth. These boxes were maintained at room temperature. The mortality of nymphs was recorded at 24 hours intervals for 3 days and the per cent mortality was computed treatment-wise. Mealybugs which did not respond to the probe by a fine camel hair brush or with shrunken body were considered as dead.

Data analysis

Mortality data were corrected using Abbott's (1925) formula, considering the mortality in untreated control, if any. The data were subjected to Probit analysis (Finney, 1971) for computing regression equation for dosage-mortality response and to determine the LC_{50} values.

RESULTS AND DISCUSSION

Evaluation of plant extracts to second nymph mealybug *Pseudococcus* sp. for insecticidal and repellent properties

Data with respect to killing effect of different plant extracts on second nymph mealybug are given in table 1. As observed with second nymph, the effect of extracts was evident from first day after treatment onwards third day. On the first day, the mean nymph mortality of seed soursop methanol extract was the

highest (96.67%) at 20% vol/vol concentration. The corresponding mortalities recorded with another plant extracts were low (Table 1).

Though the killing effect of these extracts was found to continue up to third day after treatment, the corresponding mortality recorded with methanol extract of soursop seed was 98.33% (at 20% vol/vol concentration), tobacco leaf was 75% (at 10% vol/

vol concentration), chinaberry leaf was 61.67% (at 20% vol/vol concentration), all plant of wedelia was 26.67% (at 20% vol/vol concentration); with aqueous extract of zanzibar gem leaf was 25% (at 20% vol/vol concentration), onion bulb 16.67% (at 20% vol/vol concentration) and the treatments were statistically on par. The effect of these plant extract stopped at 3 days after treated.

Table 1. Effect of plant extracts to second nymph of mealybug *Pseudococcus* sp. for insecticidal property

Sl.no	Treatments	Concentrations (% vol/vol)	Extracted methods	Mortality (%)		
				1 DAT	3 DAT	5 DAT
1	Tobacco	10	methanol	23.33 (28.78) ^b	75.00 (60.07) ^b	75.00 (60.07) ^b
2	Wedelia	20	methanol	1.67 (4.31) ^c	26.67 (31.00) ^d	26.67 (31.00) ^d
3	Soursop	20	methanol	96.67 (81.39) ^a	98.33 (85.69) ^a	98.33 (85.69) ^a
4	Chinaberry	20	methanol	11.67 (19.88) ^b	61.67 (51.76) ^c	61.67 (51.76) ^c
5	Onion	20	water	1.67 (4.31) ^c	16.67 (23.86) ^d	16.67 (23.86) ^d
6	Zanzibar gem	20	Water	1.67 (4.31) ^c	25.00 (29.80) ^d	25.00 (29.80) ^d
7	Control	-	-	0.00 (0.00) ^c	0.00 (0.00) ^e	0.00 (0.00) ^e
<i>Significant</i>				**	**	**
<i>CV%</i>				28.88	11.00	11.00

DAT: Days after treatment; Figures in parentheses are angular transformed values;

***: Significant at P=0.01; Means followed by same alphabetical superscript are statistically on par.*

Najmeh *et al.* (2011) recorded that aqueous *Nicotiana tobacum* extract (removed water) caused 77.55% mortality of second instar larvae of *Lycoriella auripila* at concentration of 0.4% after 72 hours. Ukey *et al.* (1999) reported that tobacco decoction at 1% concentration was found equally effective as that of monocrotophos against aphids on cowpea.

Le Minh Tam *et al.* (2003) showed that mineral oil (DC-Tron Plus 98.8 EC) caused 42.20% mortality of mealybug *Pseudococcidae* on pine apple under laboratory conditions.

Relative toxicity of plant methanol extract to second nymph mealybug *Pseudococcus* sp.

Toxicity of methanol extracts to *Pseudococcus* sp. second nymphs was assessed by determining LC₅₀ value. LC₅₀ or median lethal concentration of soursop seed extract to mealybug was 5.364% with the corresponding regression equation of $y = 2.8731x + 2.9041$ (Fig. 1). LC₅₀ of tobacco leaf extract

to mealybug was 4.436% with the corresponding regression equation of $y = 1.8299x + 3.8161$ (Fig. 2) and LC₅₀ of chinaberry leaf extract to mealybug was 11.809% with the corresponding regression equation of $y = 1.4952x + 3.3968$ (Fig. 3).

Comparative methanol extract from tobacco leaf was more toxic with a lower LC₅₀ value of 4.436% against the LC₅₀ value of 5.364 and 11.809% for soursop seed and chinaberry leaf, respectively.

Comparatively methanol extract from *Nicotiana tobacum* leaf was more toxic with a lower LC₅₀ value of 4.436 against the LC₅₀ value of 5.364 and 11.809% for *Annona muricata* seed and *Melia azedarach* leaf, respectively. Toxicity of methanol extract from tobacco leaf in the present study indicated that it was 6.06 times less toxic than aqueous extract (removed water) from *Nicotiana tobacum* (LC₅₀ value of 0.73165%) observed on second instar larvae of *Lycoriella auripila* by Najmeh *et al.* (2011).

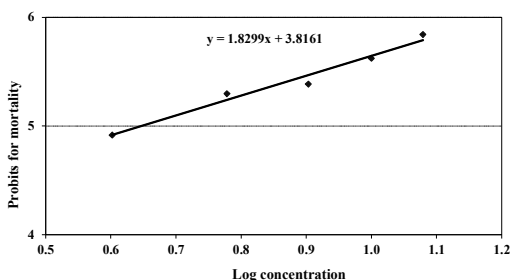


Fig. 1. Toxicity of tobacco leaf methanol extracts to second nymph mealybug *Pseudococcus* sp.

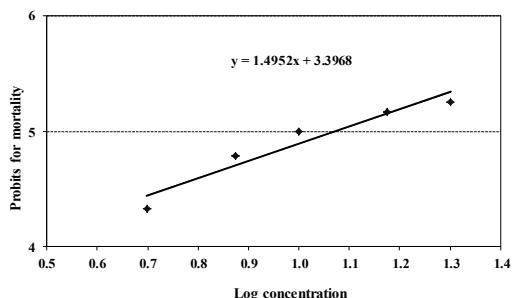


Fig. 2. Toxicity of chinaberry leaf methanol extracts to second nymph mealybug *Pseudococcus* sp.

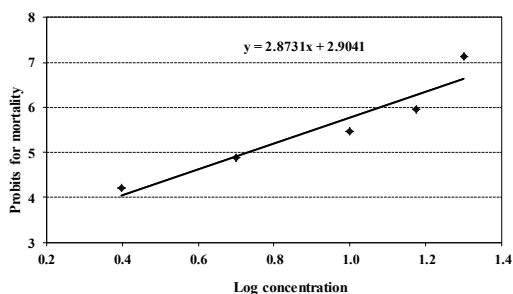


Fig. 3. Toxicity of soursop seed methanol extracts to second nymph mealybug *Pseudococcus* sp.

CONCLUSION

Methanol extract of tobacco leaf at 10% vol/vol concentration, soursop seed and chinaberry leaf at 20% vol/vol concentration killed 75.00; 98.33 and 61.67 %, respectively of exposed second nymphs after 3 days of treatment. The LC_{50} value of tobacco leaf, soursop seed and chinaberry leaf was 4.436; 5.364 and 11.809%, respectively. Mortality of second nymphs mealybug caused due to aqueous (onion bulb, zanzibar gem leaf at 20% vol/vol concentration) or methanol extract (all plant of wedelia at 20% vol/vol concentration) was not significant, as the mortality recorded even after 3 days of treatment did not exceed 30%.

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DETERMINATION OF ARTIFICIAL DIETS FOR REARING *Corcyra cephalonica* (LEPIDOPTERA: PYRALIDAE) FOR EGG SUPPLY FOR INDIGENOUS PARASITOID WASPS

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Abstract

Evaluation of artificial diets for rearing *Corcyra cephalonica* (Lepidoptera: Pyralidae) for egg supply for indigenous parasitoid wasps to determine the best mixing formulas of artificial diet made from suitable and reasonable local material resources for development of rice moth, spawning more to provide eggs for indigenous parasitoid wasps was conducted in Plant Protection Division of Southern Horticultural Research Institute, Vietnam. The experiment was arranged in a completely randomized design, with 8 treatments and 3 replications, each replication was one box with 100 rice moth eggs and added 25 mg of Streptomyces sulfate for each treatment to prevent bacteria. The results showed that the male moth could survive for 15 days and have longer life span than those of female moth at diet with 125 g of broken rice and 5 g of powdered peanuts. The female could survive for 10 days on a diet consisted of 125 g broken rice, 5 g powdered peanut and 5 g of powdered milk. In addition, the weight of the female moth and the number of eggs laid by a female moth were highest on a diet consisted of 75 g of broken rice, 50 g of rice bran, 5 g of powdered peanut and 5 g of powdered milk. These values were 29.08 mg/female moth and 330 eggs/female moth, respectively. Therefore, the best artificial diet for rearing *C. cephalonica* was 75 g of broken rice, 50 g of rice bran, 5 g of powdered peanut and 5 g of powdered milk.

Key words: *Corcyra cephalonica*, rice moth, artificial diets

INTRODUCTION

The biological control by using parasitoid wasp for control insect pests is proved to give a very effective way to management of agricultural pests. Among indigenous parasitoid wasp, *Trichogramma* sp. is the most promising bio-agent). From the 1920s *Trichogramma* sp. was used to control the sugarcane borer in the United States and by 1930s the method of producing wasp by rice moth eggs was conducted (Tran Hue Hoa Huien Huien, 2005). In 1982, Plant Protection Research Institute (Hanoi, Vietnam) conducted the research programme for building processes of mass rearing and using 3 wasp species to control corn borer (*Ostrinia nublalis*, recently was corrected as *Ostrinia furnacalis*), jute green looper worm (*Anomis flava*), earworm fruit borer (*Helicoverpa armigera*) and caterpillars piny (*Dendrolimus punctatus*) (Nguyen Van Dinh, 2004).

Parra (1997) reported that eggs of rice moth *C. cephalonica* was one of the artificial host used to produce *Trichogramma* spp. in many countries around the world. Bernardi *et al.* (2000) studied on six artificial diets for *C. cephalonica*. They were a) whole wheat flour (48.5 %), ground rice (48.5 %) and sugar (3 %); b) ground rice (97 %) and sugar (3 %); c) whole wheat flour (48.5 %), rice flour (48.5 %) and sugar (3 %); d) whole wheat flour (97 %) and yeast (3 %); e) wheat germ (97 %) and yeast (3 %); f) rice bran (94 %), sugar (3 %) and yeast (3 %); f) rice bran (94

%), sugar (3 %) and yeast (3 %). They recorded that all of the diets studied permitted the development of *C. cephalonica* although the diets with wheat germ and yeast and that consisting of rice bran, sugar and yeast proved to be the most adequate for rearing the moth. These diets reduced the total (egg-adult) cycle, shortened the egg laying period, and produced heavier adults.

However, studies on diet for mass rearing of *C. cephalonica* were limited and the materials used in these works are not suitable for local conditions in Mekong delta region. Therefore, the experiment was carried out based on the study on biology of *C. cephalonica* to determine the diet formulas that is most appropriate for the mass rearing of rice moth under laboratory conditions. In this paper, we report the investigations on impact of different artificial diets on rice moth conducted at Plant Protection Division of Southern Horticultural Research Institute (SOFRI), Vietnam in the year 2015.

MATERIALS AND METHODS

Materials

Rice moth resource: Larvae of rice moth *C. cephalonica* (Tran Van Hai *et al.*, 2008) collected from the barn in An Huu commune, Cai Be district, Tien Giang province were reared on rice bran + broken rice in the laboratory conditions for collecting their

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