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Efficacy of some selected botanical extracts against the Cotton mealybug *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae)

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Abstract- Laboratory studies were carried out to evaluate the efficacy of some locally available botanicals against cotton mealy bug *Phenacoccus solenopsis* on shoe flower plants *Hibiscus rosa-sinensis.* Plants extracts obtained from *Azadirachta indica, Ocimum sanctum, Calotropis gigantea, Nicotina tabacum* and *Alium sativum* using standard methods. Among all these botanicals, extracts were prepared and dilutions were obtained at 0.2, 0.4, 0.6, 0.8, 1.0,1.2,1.5, 2.0 percentage concentrations with the addition of soap solution. Among the treated botanicals, *O.sanctum* was effective significantly ($p<0.05$) at lower concentrations and has the 0.6% concentration as LC_{50}. *O.sanctum* solution of this particular strength was applied in a field trial and was resulted lower adult mortality (39%) and a higher nymphal mortality (72%). Neem, Tobacco, Calotropis and Garlic solutions have LC_{50} values as 0.82, 0.89, 0.95 and 1.15 percentages respectively.

Index Terms- Botanicals, Efficacy, Extracts, *Phenacoccus solenopsis*

I. INTRODUCTION

The mealybug species are widespread throughout the world. The cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has been described as a serious and invasive polyphagous pest with a vast host range by several authors. It has a wide geographical distribution with its origin in Central America (Fuchs et al, 1991) followed by reports of the Caribbean and Ecuador (Ben-Dov, 1994), Chile (Larrain, 2002), Argentina (Granara de Willink, 2003), Brazil (Mark and Gullan 2005). *P. solenopsis* has been described as a serious and invasive pest of shoe flower in Pakistan and India (Hodgson et al. 2008) on *Hibiscus rosa-sinensis* in Nigeria (Akintola and Ande, 2008) Latest report by the authors on the invasiveness of *P. solenopsis* has been from the Eastern region of Sri Lanka (Prishanthini and Vinobaba, 2009) on ornamentals, vegetable crops, and weeds, and in China (Wang *et al*. 2009; Wu and Zhang, 2009) on shoe flower. *P.solenopsis* is identified as one of the major damage causing and fastly spreading invasive mealybugs of Sri Lanka next to Papaya mealybug *Paracoccus marginatus*. Being a polyphagous pest, the *P. solenopsis* has been recorded to feed on a number of cultivated crops including weeds (Patel *et al*. 2009). According to the recent information provided by the authors (Prishanthini and Vinobaba, 2011) *P.solenopsis* has been reported from 28 host plant species comprising 10 families in Sri Lanka. This includes the major field crops of family Malavaceae, Solanaceae and Amaranthaceae. Farmers are currently using some toxic chemical insecticides against cotton mealy on large and small scale cultivations of Okra, Brinjal, Tomato and Amaranthus.

Reliance on synthetic chemicals to control pests has also given rise to a number of problems such as destruction of beneficial non-target organisms (parasitoids and predators) thereby affecting the food chain and impacting on biological diversity. The injudicious use of synthetic pesticides can lead to secondary outbreaks of pests that are normally under natural control resulting in their rapid proliferation. There have also been cases of pests becoming tolerant to insecticides, resulting in the use of double and triple application rates (Stoll, 2000). In addition, due to other problems such as health hazards, undesirable side effects and environmental pollution caused by the continuous use of synthetic chemical pesticides (Nas, 2004), there is renewed interest in the application of botanical pesticides for crop protection. Botanical pesticides are biodegradable (Devlin and Zettel, 1999) and their use in crop protection is a practical sustainable alternative. It maintains biological diversity of predators (Grange and Ahmed, 1988), and reduces environmental contamination and human health hazards. Botanicals are safe for homegardens and green houses. Therefore the present study attempts to evaluate the efficacies of some native botanicals against cotton mealybugs.

II. MATERIALS AND METHODS

The study was conducted under laboratory conditions of Department of Zoology, Eastern University, Sri Lanka during the period from June 2012 to May 2013.

Culture of mealybugs

The laboratory culture of *P.solenopsis* was established from individuals collected from shoe flower plants in home gardens those do not have any previous exposure to pesticides. *P.solenopsis* were reared in on potato sprouts and on potted shoe flower plants under laboratory conditions.

Collection of botanicals

The botanicals used in this experiment were, *Ocimum sanctum* L. (Lamiaceae), *Azadirachta indica* A. Juss. (Meliaceae),*Calotropis gigantea* R. Br. (Asclepidaceae), *Nicotina tabacum* Linn. (Solanaceae) and *Alium sativum* Linn. (Amaryllidaceae). Leaves of *Ocimum sanctum* (Tulasi) and *Azadiracta indica* (Neem) and *Calotropis sp.* were collected from the home gardens of Batticaloa. The dried leaves of Tobacco (*Nicotina tabacum*) and Garlic cloves were bought from local market.

The leaves collected were washed with sterile distilled water until the dirt was completely removed and allowed to shade dry for one week. Then the dried materials were finely ground using motor and pestle until the powdered form was obtained. The powders were stored separately in dark bottles for extraction.

Preparation of extracts

A. Extracts of Neem, Tulsi, Calotropis and Tobacco leaves

50 g of powdered botanicals from each was weighed and transferred to a cellulose extraction thimble (Whatman, UK). These materials were extracted using 250 ml ethanol (78 ^{o}c) for 5 hours in a Soxhlet apparatus (250 ml) and the extracts were decanted from the flask separately. Then the volume of each abstract was measured and each was made to a final volume of 200 ml and transferred separately into round bottom flasks. The flasks were fitted with the Rotatory evaporator (Buchi; R-114; Switzerland) individually and evaporated to dryness at a temperature not exceeding 85 ^{o}c. Then the flasks with dried materials were removed and weighed. The weight of the dried extract was calculated by subtracting the weight of the empty flask. Thereafter few milliliters of ethanol were added to each flask to aid the dissolution of the extract with water. Finally 50 ml of distilled water was added to the extract to get 1gml^{-1} (100% w/v) concentration. Further dilutions were made for further experiments.

B. Extract of Garlic

50 g Garlic cloves were taken and ground well using mortar and pestle to obtain a paste form. Then 25 ml of distilled water is added to the paste and shaken well. The mixture was kept for three days; strained using a clean muslin cloth and the volume was made to 50ml by adding further 25 ml of water, shaken well and stored in a dark bottle.

10 ml solution from each of the initially prepared botanical extracts (1gml^{-1}) was transferred into 500ml volumetric flask using a pipette and the final volume was made to 500ml to obtain a solution with a concentration 20 mg/ml (2% w/v). From these solutions different dilutions were obtained by adding certain volume of botanical solution, certain volume of distilled water and 1ml of 1% (w/v) soap solution.

Evaluation of efficacy of different botanicals against *P.solenopsis*

Laboratory studies

Approximately 10-12 cm length terminal portions of the Shoe flower stems (*Hibiscus rosa-sinensis*) infected with the *P.solenopsis* were cut off using a sharp knife. The cut end of each was wrapped with wet paper towel and collected in polythene bags. Then they were brought to the laboratory by keeping them inside a regiform container to prevent from heat and mechanical damages during transport. Then yoghurt cups were taken and filled with tap water. A hole was made on each lid to tightly fix the stem portion. The number of leaves on each plant terminal was reduced to 4 (only the upper terminal leaves were allowed to remain) and all the nymphal stages including crawlers, and egg masses except twenty adult females were removed from the stem by using a hand lens and a camel hair brush. Then the stem portions were fixed with the lid by allowing the cut end to contact with the water inside the yoghurt cup to keep the stem cutting fresh throughout the study. The stem portion fitted with the cup was placed inside a rectangular cubic glass container measuring 10cm x 10cm x 20 cm. Then prepared solution of a particular concentration from each botanical was sprayed over the mealy bugs using a syringe and needle (3 ml of solution) and the container was covered with a muslin cloth. Mealy bug mortality was counted at 24, 48 and 72 hours following initial application. The mortality records for all treatments were obtained in percentage values.

Field studies

The most effective botanical solution which has the lowest LC_{50} (concentration which cause lethality of 50% of the mealybug population) was used for the field trial. 20 Shoe flower plants of 50- 60 cm height were infected adult female mealybugs. Plant to plant and row to row distances were maintained at 30cm and 75cm respectively. Number of irrigations and all other practices were performed uniformly to all plants. The plants were maintained in this manner for 35 days until the development of sufficient population of mealybugs. Five plants were kept as control without any application of botanical solution. Other plants were sprayed solution was prepared and applied over the mealybug colonies. Number of mealybugs was counted 24 hours before spray and the mealy bug mortality following spray was counted at 24, 48 and 72 hours after initial application. The data was recorded from three randomly selected twigs of 10 cm long in each replication.

Percent corrected mortality was calculated by following formula described by Schneider-Orelli's (1947) and Puntener (1981)

$$\text{Corrected \% mortality} = \frac{\%\, mortality\ in\ treatment - \%\, mortality\ in\ control}{100 - \%\, mortality\ in\ control} X\ 100$$

Statistical analysis

For all the data obtained the differences among the mortality of mealybugs at all treatments were subjected to analysis of variance (one way ANOVA) and differences among means were considered significant at a probability level of five percent ($p \leq 0.05$). Probit analysis was carried out to determine the LC_{50} values of each botanical. Statistical package Minitab 14.0 was used for all these statistical analyses.

III. RESULTS AND DISCUSSION

Efficacy of botanicals under laboratory conditions

The efficacy of few selected botanical pesticides at different concentrations was evaluated against cotton mealybug. The results revealed that the treatments are significantly differing among themselves in causing mortality of *P.solenopsis*. ($p<0.05$) except at 0.2% concentration ($p=0.230$). After an exposure of 24 hours the maximum mortality (100%) was observed in ocimum extract beyond 1.2%, in Neem and tobacco extracts beyond 1.5% and Calotrophis at 2.0%. The mortality caused by each treatment increased gradually with an increase in the exposure interval.Figure 1 shows the comparison of percentage mean mortalities to different botanicals at different concentrations at 24 hrs after initial applications. Mortality rates increased with increasing concentrations for all botanicals.

Table 1: Mean percentage mortalities of *Phenacoccus solenopsis* to different botanical solutions at various concentrations at 24hours after initial application. Values express (Mean ± S.E)

Treatment	*O.sanctum*	*A.indica*	*N.tabacum*	*C.gigantea*	*A.sativum*
Control	0.00	0.00	0.00	0.00	0.00
0.2%	5.00±2.24	3.33±2.11	3.33±2.11	1.67±1.67	0.00
0.4%	16.67±2.11	10.00±2.58	6.67±2.11	5.00±2.24	3.33±2.11
0.6%	50.00±2.58	20.00±2.58	16.67±2.11	13.33±3.33	10.00±2.58
0.8%	76.67±2.11	43.33±2.11	31.67±1.67	25.00±2.24	16.67±2.11
1.0%	91.67±1.67	75.00±2.24	65.00±2.24	60.00±2.58	28.33±1.67
1.2%	100.00	86.67±2.11	83.33±3.33	78.33±1.67	51.67±3.07
1.5%	100.00	100.00	100.00	88.33±3.07	75.00±2.24

2.0%	100.00	100.00	100.00	100.00	95.00±2.24

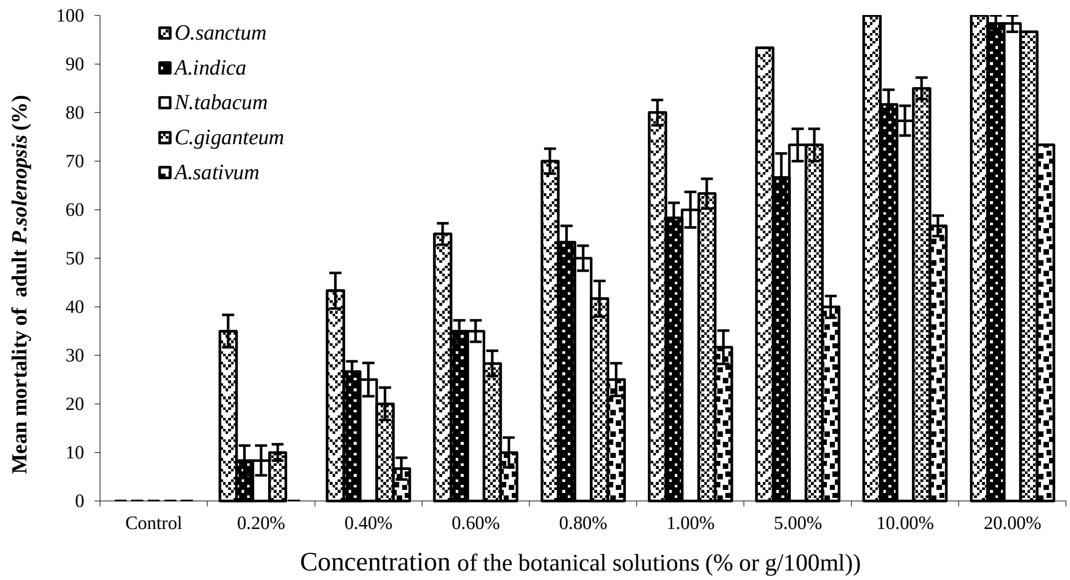

Figure 1: Comparison of percentage mean mortalities of *Phenacoccus solenopsis* to different botanical solutions of various concentrations at 24 hours after initial applications.

LC_{50} values obtained from probit analysis for mortality values after 24 hours of each botanical applied are given in the table 1. According to the results of probit analysis the botanical *O.sanctum* has the lowest value i.e.0.60 % solution. Among all, the *Ocimum sanctum* and soap mixed solution was found to be more effective against P. *solenopsis* with the lowest LC_{50} value. Next to Osmium, Neem Tobacco and Calotropis extracts were efficient with 0.82 0.89 and 0.95 percentages as LC_{50} values. Garlic has the highest LC50 value that refers it is the least effective among all against cotton mealybug.

Table 2: LC_{50} values (after 24 hrs) for the botanicals obtained from Probit Analysis

Name of Botanical	LC_{50}
O.sanctum	0.60
N.tabacum	0.89
C.gigantea	0.95
A.sativum	1.15
A.indica	0.82

The above results indicated that mortality of mealy bug *P.solenopsis* on shoe flower treated with different botanical at different concentrations increased with an increased concentration and with increased exposure.

Field studies

In the field experiment the 0.6% solution of *Ocimum sanctum* which is the LC_{50} value obtained from the laboratory experiments was field applied on healthy shoe flower plants of same age and height. In this trial the 0.6% Tulasi, *Ocimum sanctum* solution caused 39.42% adult morality and 72.21% nymphal mortality to *P.solenopsis* after 24 hours of application.. Adult mortality under field conditions was lower than that of under laboratory conditions. Use of Tulasi for pest control has long history and has both repellent and herbicidal properties. The essential oils from the species of this genus contain linalool, linalol, linoleic acid, *p*-cymene, estragosol, eucalyptol, eugenol, citral, thujone, ocimene, camphor, methyl chavicol, oleic acid, and many other terpenes as active ingredients, all of which are effective repellents (Moore and Lenglet, 2004). Generally contact pesticides are less effective against mealybugs because of their cryptic habitats in plants and the water proof waxy layer over the body (Tanwar *et al*, 2007).The soap solution added at low concentration increases the effectiveness of the botanical solution. Soap facilitates the solubility of the active ingredient and acts as a sticking agent (Nhachi and Kasilo, 1996), breaks down the protective wax cover and also acts as a surfactant.

Based on these results development of new formulations with the combinations of these botanicals which can be produced and applied using simple methods applicable to local public will be very useful. Moreover, analyzing new botanicals from different plant origins and least toxic chemicals for their efficacy against the mealybugs are also necessary to reduce use of the toxic chemical insecticides. More research on the active ingredients, pesticide preparations, application rates and environmental impact of botanical pesticides are a prerequisite for sustainable agriculture.

IV. CONCLUSION

In conclusion it can be stated that *O.sanctum* was effective significantly at lower concentrations and 0.6% concentration of the *O.sanctum* solution was resulted a significant nymphal mortality in the field conditions. The botanicals used in this study such as *A.indica*, *O.sanctum*, *C.gigantea*, *N.tabacum* and *A.sativum* were showed different levels of insecticidal activities. These findings of the present study suggest that Osmium extract can be used as a botanical spray to get better and safe control of cotton mealybug *P.solenopsis*.

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The preferred spelling of the word “acknowledgment” in American English is without an “e” after the “g.” Use the singular heading even if you have many acknowledgments.

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