Research Article

Insecticidal Potential of Some Selected Phytoextracts against Mealybug *Drosicha mangiferae* (Hemiptera: Pseudococcidae) Infesting Citrus Plants in Sargodha

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Abstract | Mealybug *Drosicha mangiferae* (Green) (Hemiptera: Pseudococcidae) is one of the most important polyphagous insect pests of different fruit crops including citrus. A potential strategy to be fitted in integrated management of mealybug would be the use of phytoextracts having insecticidal activity. The main objective of this study was to find out the toxic effect of various plant extracts against *D. mangiferae*. Three concentrations (*i.e.* 6.25, 12.5 and 25.0%) of leaf extracts of neem, *Azadirachta indica* A. Juss (Meliaceae), eucalyptus *Eucalyptus camaldulensis* Dehnh. (Myrtaceae), datura *Datura stramonium* L. (Solanaceae), batho *Chenopodium album* L. (Amaranthaceae) and Indian lemongrass *Cymbopogon citratus* (DC.) Stapf (Poaceae) were tested against 3rd instar nymphs of mealybug. Results showed that mean mortality was higher (76.67%) after the application of *A. indica* extract, followed by *E. camaldulensis* (66.67%) at 72 h post-exposure. The least effective botanicals were *C. citratus* and *C. album* showing 26.67 and 20.0% mean mortality of mealybug, respectively. The mortality rate was increased by increasing the concentration of botanicals up to 25.0%. Furthermore, probit values revealed the highest toxicity of *E. camaldulensis* (12.14%) and *A. indica* (16.96%) after 72 h of post-exposure. Similarly, the LT_{50} values were also lower in the case of *E. camaldulensis* (50.22 h) and *A. indica* (54.39 h) compared to other botanicals at 25% concentration. Among all tested phytoextracts, *A. indica* and *E. camaldulensis* were found to be most effective against nymphal population of *D. mangiferae,* and hence are recommended to the indigenous citrus growers combating mealybug infestations on citrus.

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Introduction

Mealybugs are important insect pests and feed on a various species of fruit and ornamental plants (Wakgari and Giliomee, 2003). About 60 species of mealybugs have been reported feeding on citrus plants worldwide (Franco *et al*., 2004). Mealybugs are widely distributed in Australia, Africa, America, and Asia (Wakgari and Giliomee, 2003; Franco *et al*., 2004; Satar *et al*., 2013).

Mealybug *Drosicha mangiferae* (Green) (Hemiptera: Pseudococcidae) is among the most damaging insect pests of citrus crops and causes considerable losses to citrus orchards as well as nurseries (Uygun and Satar, 2008; Satar *et al*., 2013). Nymphs and female adults cause damage to different parts of the citrus plant by sucking the sap material (Goldasteh *et al*., 2009). They reduce the quantity and quality of fruits due to sucking sap from the base of fruit stalks and leaves. They also secrete honeydew which encourages sooty mold growth on different parts such as fruits and leaves (Cid *et al*., 2010). In case of severe infestation, the photosynthesis stops, and the growth of plants remains stunted (Noureen *et al*., 2016).

It is difficult to control the population of mealybug due to the powdery coating on the body (Kumar, 2016). Synthetic insecticides are being used to manage this insect pest by the farmers (Noureen *et al*., 2016; Erdemir and Erler, 2017). However, the use of chemicals is problematic in terms of resistance development in insects, their non-target effects on natural enemies of insect pests, and harmful effects on humans and the environment (Aktar *et al*., 2009; Nicolopoulou-Stamati *et al*., 2016). So, there is a need for some new concepts to manage the population of insect pests. Botanical pesticides are a good alternative to synthetic chemicals as they are safer for humans and the environment (Nas, 2004; Sithisut *et al*., 2011). More than 2000 species of plants produce metabolites and chemicals which have great importance for the control of insect pests (Stevenson *et al*., 2017). Botanical insecticides control the insect population in different ways that depend on the physiological characteristics of target specie and type of plant material. Botanicals are being classified into different groups such as repellents, feeding deterrents, toxicants, chemosterilants and attractants based on their action (Rajashekar *et al*., 2012). Previously, many researchers have reported the successful results of different botanical insecticides against various insect pests (Green *et al*., 2017; Majeed *et al*., 2018; Isman, 2020; Hanif *et al*., 2021).

Conventional synthetic insecticides are toxic and risky to humans, livestock and other non-target fauna because of their high persistence in the environment. Contrarily, phytoextracts are being considered to be safer and effective to control insect pests. Keeping in view the harmful effects of chemical pesticides, some eco-friendly, economical and safer products are needed to be explored for mealybug control in citrus orchards. Botanicals or phytoextracts might be a better source of controlling insect pests instead of chemical-based insecticides. The present study was conducted for the evaluation of the toxicological effects of some phytoextracts against nymphal populations of mealybug.

Materials and Methods

Insect culture

Nymphal population of mealybug (*D. mangiferae*) was collected from the citrus plants grown in the horticulture farm area of the University of Sargodha, Sargodha. Rearing of mealybug was performed in the laboratory of the Department of Entomology on sour orange (*Citrus aurantium* L.) plants under controlled conditions of 24±5 °C temperature and 60 ± 5% relative humidity. Uniform sized healthy and active nymphs of F_3 generation were used in all bioassays.

Botanical insecticides

Fresh leaves of indigenous plants including *Azadirachta indica* (Meliaceae), eucalyptus, *Eucalyptus camaldulensis* (Myrtaceae), datura, *Datura stramonium* (Solanaceae), batho, *Chenopodium album* (Amaranthaceae) and indian lemon grass, *Cymbopogon citratus* (Poaceae) were collected and dried under shade for one week followed by oven drying at 40 °C for two days. After complete drying, leaves were separately ground to a fine powder. The aqueous solution of each powder was made by mixing 10 g of powder in 100 ml of distilled water. The solution was placed in an electrical shaker for 12 h to homogenize the mixture. After mixing, the solution was filtered and the solvent was evaporated by a rotary vacuum evaporator. The stock solution was stored in glass vials and kept in the refrigerator for further use (Sarwar, 2012). Three concentrations 6.25, 12.5 and 25.0% of each botanical were prepared to test against 3rd instar nymphs of mealybug.

Bioassay

Fresh twigs (7–8 cm) of kinnow mandarin (*Citrus reticulata* Blanco) were clipped from the field and were washed thoroughly with the tap water. Then, these twigs were dipped in each concentration of botanicals for about one minute. Twigs were placed in Petri plates containing the moisten filter paper to maintain humidity. The treatments were replicated three times and in each replication, twenty specimens of the 3rd instar's mealybug were released in each Petri plate (Prishanthini and Vinobaba, 2014). The mortality data were recorded after 24 h, 48 h, and 72 h of application.

Statistical analysis

Mortality data were subjected to two-factor factorial analysis of variance (ANOVA) under CRD design by keeping botanicals and concentration as main factors to check the percent mortality of mealybug at 24, 48, and 72 h of post-treatment. Means were separated by Tukey HSD all pair-wise comparison tests. Probit analyses were also performed to determine the LC_{50} and LT_{50} values of each botanical. All the analyses were performed by using Minitab 16.1 software.

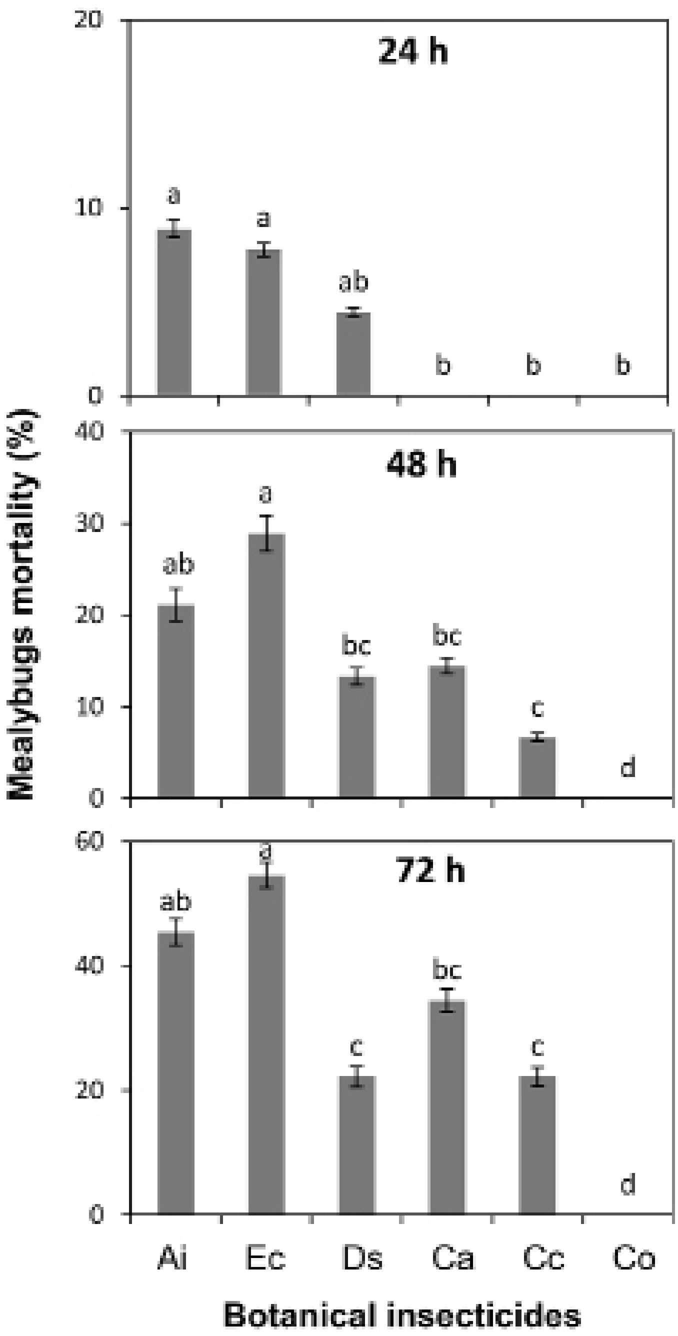

Figure 1: *Percent mortality (mean ± SE) of 3rd instar nymphs of mealybug Drosicha mangiferae after application of phytoextracts at different time intervals, Ai = Azadirachta indica, Ec = Eucalyptus camaldulensis, Ds = Datura stramonium, Ca = Chenopodium album, Cc = Cymbopogon citratus, Co = control. For each time interval, different letters at bar tops indicate statistical significance among the treatments (factorial ANOVA; HSD at α = 0.05).*

Results and Discussion

Mortality of mealybug individuals was significantly different for all botanical insecticides at 24 h (F = 7.10, $P < 0.001$), 48 h (F = 13.1, $P < 0.001$) and 72 h (F = 9.92, $P < 0.001$) of application. Results indicated that *E. camaldulensis* appeared to be the most effective botanical with maximum mortality percentages of 54.5% after 72 h of application and 28.9% after 48 h of application. *Azadirachta indica* was found to be the second-best botanical against mealybug showing 45.4% and 21.1% mortality rates after 72 h and 48 h of application. The least effective botanicals were *D. stramonium* and *C. citratus* that showed only 22.2% mortality at 72 h of post-treatment interval as shown in Figure 1. By increasing the concentration of botanicals, the mortality rate was also increased. The highest concentration (25.0%) exhibited the maximum mortality of 52.7% after 72 h of application as shown in Figure 2.

Table 1: *Median lethal concentration (LC_{50}) values of different phytoextracts determined for different post-exposure time intervals against 3rd instar nymphs of mealybug Drosicha mangiferae.*

Plants	Time intervals (h)	LC_{50} (%)	SD	95% Fiducial CI Lower- Upper
Azadirachta indica	24	37.21	14.628	26.39-48.03
	48	29.38	16.244	23.04-35.72
	72	16.96	19.595	11.64-22.27
Eucalyptus camaldulensis	24	38.31	14.628	26.96-49.66
	48	24.64	16.244	19.25-30.03
	72	12.14	19.595	6.83-17.446
Datura stramonium	24	42.94	14.628	29.36-56.51
	48	34.58	16.244	26.87-42.30
	72	30.78	19.595	23.35-38.20
Chenopodium album	24	115.6	14.628	75.00-180.0
	48	33.58	16.244	26.15-41.01
	72	22.98	19.595	17.08-28.88
Cymbopogon citratus	24	115.6	14.628	75.00-180.0
	48	41.74	16.244	31.83-51.64
	72	30.28	19.595	22.95-37.61

SD: *Standard deviation;* **CI:** *Confidence interval.*

Probit analysis showed the following trend of LC_{50} value; *A. indica* (37.21%), *E. camaldulensis* (38.31%), *D. stramonium* (42.94%) and *C. album* (115.67%) after 24 h of post treatment. The LC_{50} values of *E. camaldulensis* and *A. indica* were lowest (24.6 and 29.38%, respectively) after 48 h and 72 h (16.96 and 12.14%, respectively) of application as given in Table 1. Probit analysis showed following the trend of lowest values at 6.25% concentration; *E. camaldulensis* (85.02%), *C. album* (93.08%), *C. citratus* (96.10%), *D. stramonium* (96.95%) and *A. indica* (101.82%). When 12.5% concentration was used, LT_{50} values of *E. camaldulensis*, *A. indica*, *C. album*, *D. stramonium* and *C. citratus* were 66.35, 69.84, 85.21, 95.95 and 97.21 h, respectively. Similarly, *E. camaldulensis* and *A. indica* appeared as the most effective insecticidal extracts at maximum concentration (25.0%) with LT_{50} values of 50.22 and 54.39 h, respectively (Table 2).

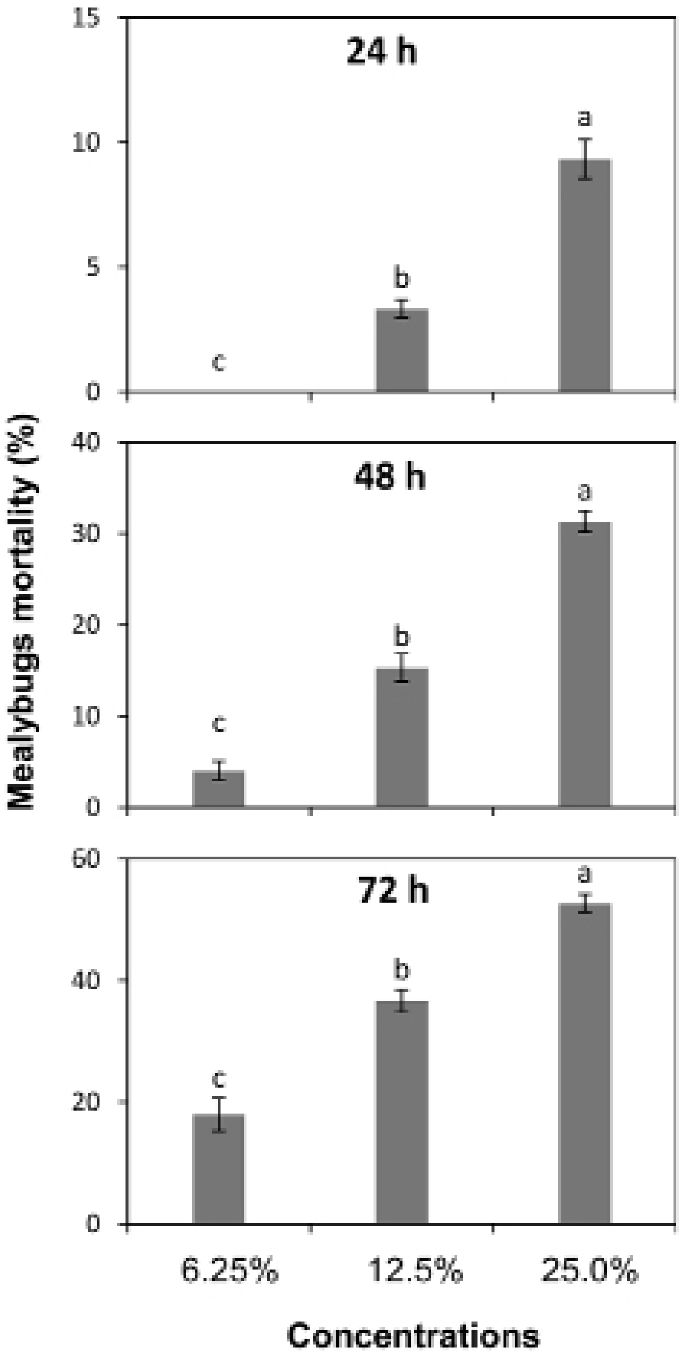

Figure 2: *Percent cumulative mortality (mean ± SE) of 3rd instar nymphs of mealybug Drosicha mangiferae by different concentrations of phytoextracts. For each time interval, different letters at bar tops indicate statistical significance among the treatments (factorial ANOVA; HSD at α = 0.05).*

Five botanicals at three concentrations viz., 6%, 12.5% and 25% were tested against 3rd instar nymphs of mealybug. A significant effect of botanicals was found on mealybug after 24, 48, and 72 h of post-treatment; percent mortality of mealybugs was increased with the increase in time. Maximum mortality was observed after 72 h of post-treatment. *E. camaldulensis* and *A. indica* showed the highest mortality at 25.0% concentration. Previously, researchers have also reported the successful use of botanicals against mealybug (Govindaiah *et al*., 2006; Mamoon-ur-Rashid *et al*., 2012; Lanjar *et al*., 2015; Bharathi and Muthukrishnan, 2017). Majeed *et al*. (2018) also reported that *A. indica* is an effective botanical against mealybug *D. mangiferae*.

Table 2: *Median lethal time (LT_{50}) values of different phytoextracts determined for different concentrations against 3rd instar nymphs of mealybug Drosicha mangiferae.*

Plants	Concentrations (%)	LT_{50} (h)	SD	95% Fiducial CI Lower-Upper
Azadirachta indica	6.25	101.8	24.59	83.19-120.4
	12.5	69.84	30.46	59.76-79.92
	25.0	54.39	32.20	45.42-63.36
Eucalyptus camaldulensis	6.25	85.02	24.59	72.15-97.89
	12.5	66.35	30.46	56.72-75.98
	25.0	50.22	32.20	41.25-59.19
Datura stramonium	6.25	96.95	24.59	79.86-114.0
	12.5	95.95	30.46	80.48-111.4
	25.0	72.59	32.20	62.03-83.16
Chenopodium album	6.25	93.08	24.59	77.71-108.4
	12.5	85.21	30.46	72.52-97.89
	25.0	73.84	32.20	63.04-84.64
Cymbopogon citratus	6.25	96.10	24.59	79.80-112.4
	12.5	97.21	30.46	81.58-112.8
	25.0	90.39	32.20	76.71-104.1

SD: *Standard deviation;* **CI:** *Confidence interval.*

Our findings showed that *A. indica* and *E. camaldulensis* were found to be the most effective botanicals as compared to others. Probit analysis also showed higher toxicity of these two botanicals with lower values compared to other botanical insecticide. The better results of these botanicals may be due to ability of plants having antifeedant properties and these deterrent activities of plant extracts have already been reported (Gaffari *et al*., 2016; Ilyas *et al*., 2017). The effectiveness of *A. indica* plant extracts have been reported earlier against various species of insect pests including *Bemisia tabaci* Gennadius (Hemiptera: *Aleyrodidae*), *Aphis gossypii* Glover (Hemiptera: *Aphididae*), *Amrasca biguttula* (Shiraki) (Hemiptera: *Cicadellidae*), *Phenacoccus solenopsis* (Tinsley) (Hemiptera: *Pseudococcidae*), *Drosicha mangiferae*, *Thrips tabaci* Lindeman (Thysanoptera: *Thripidae*), *Dysdercus koenigii* Walk (Hemiptera: *Pyrrhocoridae*) and *Oxycarenus hyalinipennis* Costa (Lygaeidae: *Hemiptera*) (Prishanthini and Vinobaba, 2014; Majeed *et al*., 2016; 2018). This plant affects the insects in different ways such as insect growth regulator, antifeedant and sterilant. Azadirachtin compound in this plant has many traits like rapid degradation, systemic activity in plants, and safer to natural enemies of insect pests present in the field (Arshad *et al*., 2019).

Further, *E. camaldulensis* have also good insecticidal properties and have been used to control diverse species of insect pests (Hamdi *et al*., 2015; Andrade-Ochoa *et al*., 2018). Ebadollahi and Setzer (2020) reported that the essential oil of *E. camaldulensis* is very effective to control *Oryzaephilus surinamensis* (Coleoptera: *Silvanidae*) and *Sitophilus oryzae* (Coleoptera: *Curculionidae*) due to the presence of an insecticidal component known as terpenes. Similarly, Izakmehri *et al*. (2013) concluded that *E. camaldulensis* can be considered as a component of integrated management of *Callosobruchus maculatus* F. (Coleoptera: *Bruchidae*) in storage system. Our findings are similar to the results of Govindaiah *et al*. (2006) who reported that *E. globulus* reduce the egg hatching rate and survival of *Maconellicoccus hirsutus*. Similarly, many researchers reported that maximum control of mealybugs, *P. solenopsis* can be achieved by using eucalyptus extracts (Prishanthini and Vinobaba, 2014). Lanjar *et al*. (2015) reported that leaf extracts of *A. indica* reduced the population of mealybug (*D. mangiferae*) up to 78.1%, while eucalyptus reduced it up to 71.1%. Our findings illustrated that the extracts of *A. indica* and *E. camaldulensis* can manage the population of citrus mealybug. The toxicity of these plants against mealybug could be due to fact that their leaves are rich in metabolic compounds that have insecticidal properties as reported previously by Eid *et al*. (2017).

Conclusions and Recommendations

Our findings showed that the extracts of *E. camaldulensis* and *A. indica* are effective biorational insecticides against mealybug *D. mangiferae*. The use of these eco-friendly insecticides will be helpful to reduce the side effects of synthetic insecticides that are harmful to the environment and human health. Further studies should be conducted to isolate pure components from these plants and to evaluate their toxicity against different insect pests.

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Novelty Statement

In the present study, the extracts of *E. camaldulensis* and *A. indica* were proved to be effective biorational insecticides to control mealybug *D. mangiferae* infestation on citrus plants. These botanicals can be part of the future integrated management program of mealybugs on citrus and other horticultural crops.

Author's Contribution

Muhammad Rizwan: Conducted the experiments, recorded the data and prepared the initial draft of manuscript.
Abu Bakar Muhammad Raza: Conceived the idea, designed the experimental protocol and prepared the manuscript.
Muhammad Zeeshan Majeed: Provided the technical support during research work and technically revised the manuscript.
Muhammad Arshad: Helped in data analysis and preparation of results and technically proofread the manuscript.

Conflict of Interest
The authors have declared no conflict of interest regarding the publication of this article.

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