INSECTICIDAL EFFECTS OF ACETONE, ETHANOL AND AQUEOUS EXTRACTS OF *Azadirachta indica* (A. Juss), *Citrus aurantium* (L.), *Citrus sinensis* (L.) AND *Eucalyptus camaldulensis* (Dehnh.) AGAINST MEALYBUGS (HEMIPTERA: PSEUDOCOCCIDAE)†

[EFECTO INSECTICIDA DE EXTRACTOS ACETONICO, ETANOLICO Y ACUOSO DE *Azadirachta indica* (A. JUSS), *Citrus aurantium* (L.), *Citrus sinensis* (L.) Y *Eucalyptus camaldulensis* (DEHNH.) CONTRA LA COCHINILLA (HEMIPTERA: PSEUDOCOCCIDAE)]

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SUMMARY

Mealybugs (Hemiptera; Pseudococcidae) are one of the noxious sucking pests infesting ornamental and horticulture crops including citrus. It is emerging as a severe threat to citrus industry in Indo-Pak region. This study determined *in-vitro* toxicity of different botanical extracts *viz*; neem (*Azadirachta indica*), sour orange (*Citrus aurantium*), sweet orange (*Citrus sinensis*) and eucalyptus (*Eucalyptus camaldulensis*) against adult females and 2nd instar nymphs of *Drosicha mangiferae*, a mealybug species regularly infesting citrus crop since last decade. Water, ethanol and acetone were used as extraction solvents. Leaf-dip and twig-dip methods were used for 2nd instar mealybug nymphs and adult female individuals, respectively. Five concentrations (0, 8, 16, 32 and 64%) of botanical extracts were bioassayed with four replications for each. Mortality of insects was observed at 24, 48 and 72 h post-treatment for nymphs and at 24 and 48 h post-treatment for adults. Data was subjected to probit analysis and two-way factorial ANOVA taking time and concentration as factors. Results revealed that the most toxic botanicals with minimum LC_{50} values against citrus mealybug adults were acetone extracts of *A. indica* and *E. camaldulensis* followed by ethanol extracts of *C. sinensis* seeds and *C. aurantium* leaves, while the most effective botanicals against 2nd instar mealybug nymphs were aqueous, ethanol and acetone extracts of *A. indica* and *E. camaldulensis* followed by ethanol extracts of *C. sinensis* peels and *C. aurantium* seeds. As expected, 2nd instar nymphs were found more susceptible to all extracts as compared to adult female individuals, most probably due to reduced penetration of botanical extract in adult insects due to powdery cushion on body. It is concluded that botanical insecticides can play a significant role in the management of insect/mite pests as being substitutes of toxic and hazardous synthetic chemicals. Particularly, neem (*A. indica*) and eucalyptus (*E. camaldulensis*) could be effective options against mealybugs and other hemipterous pests, and should be incorporated in the future pest management programs.

Keywords: Botanical extracts; mealybugs; laboratory evaluation; extraction solvents; *Azadirachta indica*; *Eucalyptus camaldulensis*; *Citrus* spp.

RESUMEN

Las cochinillas (Hemiptera; Pseudococcidae) son una de las plagas chupadoras nocivas que infestan los cultivos ornamentales y de horticultura, incluidos los cítricos. Está surgiendo como una grave amenaza para la industria de los cítricos en la región de Indo-Pak. Este estudio determinó la toxicidad *in vitro* de diferentes extractos botánicos; neem (*Azadirachta indica*), naranja agria (*Citrus aurantium*), naranja dulce (*Citrus sinensis*) y eucalipto (*Eucalyptus*

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camaldulensis) contra hembras adultas y ninfas de segundo estadio de *Drosicha mangiferae*, una especie de cochinilla que infecta regularmente cultivos de cítricos desde la última década. Se utilizaron agua, etanol y acetona como disolventes de extracción. Se utilizaron los métodos de inmersión de hojas y de inmersión de ramas para las ninfas de la cochinilla del segundo estadio y las hembras adultas, respectivamente. Se realizaron bioensayos a cinco concentraciones (0, 8, 16, 32 y 64%) de cada extracto con cuatro repeticiones para cada uno. La mortalidad de insectos se observó a las 24, 48 y 72 h post-tratamiento para ninfas y a las 24 y 48 h post-tratamiento para adultos. Los datos se sometieron a análisis probit y ANOVA factorial de dos vías tomando el tiempo y la concentración como factores. Los resultados revelaron que los productos botánicos más tóxicos con valores mínimos de CL_{50} frente a los cítricos adultos fueron los extractos de acetona de *A. indica* y *E. camaldulensis*, seguidos de los extractos de etanol de las semillas de *C. sinensis* y las hojas de *C. aurantium*, mientras que los productos botánicos más efectivos contra el segundo estadio de las ninfas fueron extractos acuosos, de etanol y acetona de *A. indica* y *E. camaldulensis*, seguidos de extractos de etanol de cáscaras de *C. sinensis* y semillas de *C. aurantium*. Como se esperaba, las ninfas del segundo estadio se encontraron más susceptibles a todos los extractos en comparación con las hembras adultas, probablemente debido a la menor penetración del extracto botánico en insectos adultos debido a la presencia de polvo en el cuerpo. Se concluye que los insecticidas botánicos pueden desempeñar un papel importante en el manejo de plagas de insectos / ácaros como sustitutos de químicos sintéticos tóxicos y peligrosos. En particular, el neem (*A. indica*) y el eucalipto (*E. camaldulensis*) podrían ser opciones efectivas contra las cochinillas y otras plagas hemípteras, y deberían incorporarse en los futuros programas de manejo de plagas.

Palabras clave: extractos botánicos; chinches; evaluación de laboratorio; disolventes de extracción; *Azadirachta indica; Eucalyptus camaldulensis; Citrus* spp.

INTRODUCTION

Citrus has a crucial importance among world fruit production. Annual worldwide citrus fruit production has been tremendously increased up to 122 million tons with an area of 8 million hectares under citrus cultivation (FAO, 2014). Most familiar citrus fruits are oranges, tangerines, mandarins, lemons, lime and grapefruits. These fruits are rich in simple sugars, dietary fibers, amino acids, vitamins and minerals and have a major contribution to human diet (Rouseff and Nagy, 1994; Economos and Clay, 1999).

Sweet oranges (*Citrus sinensis*; mosambi and red blood) and mandarins (*Citrus reticulata*; kinnow and feuterll' early) are most widely grown and appraised citrus cultivars around the globe as well as in Pakistan. In Pakistan, citrus is one of the key fruit crops and is grown on an area of about 160,000 hectares with an annual production of about 4.7 million tons (Pakistan Bureau of Statistics, 2015). Pakistan positions among the top kinnow mandarin and orange producing and exporting countries. Most of the citrus production in Pakistan (80%) is contained of mandarins (Kinnow) and feutrell's early (Naz *et al*., 2014; Ahmad *et al*., 2017).

However, per unit citrus production of Pakistan is much less than other citrus producing countries. One of the reasons behind it is that citrus plants are attacked by a number of insect pests and diseases which reduce both quality and quantity of citrus fruits (Mahmood *et al*., 2014). Major insect pests of citrus crop in Punjab (Pakistan) are citrus leafminers (*Phyllocnistis citrella*), citrus psyllids (*Diaphorina citri*), citrus fruit flies (*Bactocera minax* and *B. dorsalis*), citrus whiteflies (*Dialeurodes citri*) and citrus mealybugs (*Drosicha mangiferae* and *Planococcus citri*) (Tahir *et al*., 2015).

Since last decade, *Drosicha* mealybugs have been appearing as regular pests of citrus orchards in Pakistan and cause considerable loss to citrus farmers each year (Mahmood *et al*., 2014; Tahir *et al*., 2015). These insects are usually difficult to control by synthetic chemicals because of the decreased absorption of pesticides into their body due to cushion of waxy scales present on their dorsal body surface. Therefore, there is no operative and effective chemical control option available for mealybugs infesting citrus and other horticultural crops (Mani and Shivaraju, 2016). Consequently, farmers use blind, inadvisable and excessive (sometimes with double and triple application rates) use of synthetic chemicals to control mealybugs infestation which, apart from insufficient control, results in increased risks of phytotoxicity, environmental contamination and human health hazards and other non-target effects such as secondary pest outbreaks, pest resistance, disruption of beneficial fauna (predators and parasitoids) (Desneux *et al*., 2007; Edwards, 2013; Badshah *et al*., 2017).

This urges the need to search out new environment-friendly options for controlling insect pests such as indigenous plant extracts (Castillo-Sánchez *et al.*, 2010; Farooq *et al*., 2011; Mamoon-ur-Rashid *et al*., 2016). Plant derived pesticides (botanicals) are relatively safe and environment friendly with no or minimum non-target effects (Isman, 2006; Dubey *et al*., 2010; Kabir *et al.*, 2017). Extracts of many aromatic and medicinal plants have been used as substitutes to chemical insecticides to control different phytophagous insect pests including mealybugs

(Regnault-Roger, 1997; Isman, 2008; Badshah *et al*., 2015; Kabir *et al.*, 2017). Keeping in view of the above cited contemporary issues of synthetic insecticides, this study was aimed to assess the comparative toxicity of different selective indigenous plant extracts against mealybugs (*Drosicha mangiferae*) infesting citrus orchards. Moreover as polarity index of extraction solvent may affect the yield, type and composition of extracted phyto-constituents (Mulla and Su, 1999; Do *et al*., 2014; Iloki-Assanga *et al*., 2015), three extraction solvents with different polarity were also compared for their extraction efficiency against mealybugs.

MATERIALS AND METHODS

Collection of mealybugs

Adult female mealybug individuals were collected into glass petri-dishes from unsprayed infested plants of sweet orange (*Citrus sinensis* L.) orchard located in the vicinity of the College of Agriculture, University of Sargodha (Punjab, Pakistan) with the help of a camel hair brush and were brought to the laboratory of the Department of Entomology under cool conditions. They were maintained in the laboratory till 2nd generation on fresh pumpkin fruits at 26 ± 2ºC and 75 ± 5% relative humidity. Healthy and active 2nd instar nymphs and adult females of 2nd generation of field collected mealybugs were used in all toxicity bioassays.

Extraction of botanicals

Extracts of different parts of four indigenous plants *viz*; neem (*Azadirachta indica* A. Juss; Meliaceae), eucalyptus (*Eucalyptus camaldulensis* Dehnh.; Myrtaceae), sweet orange (*Citrus sinensis* L.) and sour orange (*Citrus aurantium* L.) were used in this study. For this purpose, seeds, leaves and fruit peels of sweet orange and sour orange and seeds plus apical leaves of neem and eucalyptus were collected, washed by clean tap-water and shade-dried for 2 weeks, and then were ground into powder form using an electric blender. Using Soxhlet apparatus (Sigma-Aldrich, Germany), three types of extraction solvents *viz*; acetone (polarity index: 5.1), ethanol (polarity index: 5.3) and water (polarity index: 9.0) were used for the extraction of phytoconstituents from each sample. In brief, 100 g of the pulverized sample was wrapped in a muslin cloth and was put in the thimble of the Soxhlet apparatus and was extracted for 3-5 h with 1 L of the extraction solvent. Extractions were performed in the laboratory of the Department of Food Science and Nutrition, College of Agriculture, University of Sargodha. Excess of extraction solvent was removed from the botanical extracts using a rotary evaporator set at 40°C (Eyela, SB-651, Rikakikai Company Limited, Tokyo, Japan). Extracts were stored in air-tight dark colored glass vials at 4°C in the refrigerator until their utilization in bioassays.

Toxicity bioassay

Separate toxicity bioassays were conducted for 2nd instar nymph and adult female individuals of laboratory reared mealybugs. There were eight plant extracts, each with three types of extraction *i.e.* acetone, ethanol and aqueous extraction. Five concentrations of each plant extract *i.e.* 0.0, 8.0, 16.0, 32.0 and 64.0% were tested against 2nd instar mealybugs with four replications for each treatment. While four concentrations of each plant extract i.e. 0.0, 16.0, 32.0 and 64.0% were tested against adult female mealybugs with four replications for each treatment. Experimental design was completely randomized (CRD). Distilled water was used to prepare serial dilutions of plant extracts. For nymphs, bioassays were performed using leaf dip method, while for adult females, bioassays were performed using freshly cut citrus twigs. Leaf-discs and twigs were dipped in treatment solutions for 30 seconds and were air dried at room temperature (22ºC) on towel paper for 15 min before their transfer on moist filter paper discs in sterilized glass petri-dishes (dia. = 9 cm). Fifteen mealybug nymphs or five adult females were released on the treated citrus leaf discs (and/or twigs) with help of camel-hair brush.

Statistical analysis

Analyses of data were performed using statistical program Statistix® 8.1 (Analytical Software, 2005). Data regarding mortality of test mealybugs were recorded at 24, 48 and 72 h post-treatment and was corrected using Abbot's formula. Toxicity of different types of botanical extracts tested against citrus mealybug adult females and nymphs was determined by calculating median lethal concentration (LC_{50}) values of each type of extract at each observation time by probit analysis using POLO-PC® (LeOra Software 1987) software. Means of treatments with minimum LC_{50} values at 72 h time interval were further compared using factorial analysis of variance followed by Fisher's least significant difference (LSD) test using concentration and time as factors.

RESULTS

Response of adult female mealybugs to different botanical extracts

According to results, after 48 h of exposure, most effective botanicals were acetone extracts of *E. camaldulensis* and *A. indica* with minimum LC_{50} values of 21.34 and 22.37%, respectively, followed by aqueous extract of *C. sinensis* seeds (LC_{50}=28.65) and ethanol (LC_{50}=31.87) and aqueous (LC_{50}=32.42) extracts of *E. camaldulensis*. Least effective botanical extracts against adult female mealybugs at 48 h of exposure were ethanol extracts of *C. aurantium* seeds (LC_{50}=144.76) and *C. sinensis* peels (LC_{50}=131.71). At 72 h of exposure, acetone and ethanol extracts of *E. camaldulensis* were the most effective with minimum LC_{50} values of 20.34 and 20.97%, respectively, followed by ethanol and acetone extracts of *A. indica* with LC_{50} values of 21.15 and 21.55%, respectively (Table 1). Least effective botanicals with maximum LC_{50} values at 72 h of exposure were aqueous extracts of *C. sinensis* leaves (LC_{50}=139.93) and *C. aurantium* seeds (LC_{50}=111.26).

Toxicity of botanicals against 2nd instar mealybug nymphs

In case of mealybug nymphs, the most effective botanicals were found to be ethanol extracts *E. camaldulensis* (LC_{50}=12.75), aqueous extract of *E. camaldulensis* (LC_{50}=13.28) and ethanol extract *A. indica* (LC_{50}=23.17), followed by acetone extracts of *E. camaldulensis* (LC_{50}=29.51) and *A. indica* (LC_{50}=42.29). Least effective botanical against mealybug nymphs were aqueous extracts of *C. sinensis* peels (LC_{50}=237.75) and acetone extract of *C. sinensis* leaves (LC_{50}=227.22). At 48 hours of exposure, minimum LC_{50} values were found for ethanol and aqueous extracts of *E. camaldulensis*, *i.e.* 6.30 and 6.48%, respectively, followed by aqueous (LC_{50}=13.25) and acetone (LC_{50}=14.60) extracts of *A. indica*. Botanicals which gave highest LC_{50} values at 48 h of exposure were aqueous (LC_{50}=180.15) and ethanol extracts (159.21) of *C. sinensis* leaves. At 72 h post-exposure, minimum LC_{50} values were obtained for aqueous extract of *E. camaldulensis* (LC_{50}=2.83) and *A. indica* (LC_{50}=4.55), followed by ethanol extract of *A. indica* (LC_{50}=14.66) and acetone extract of *A. indica* (LC_{50}=21.00). Least effective botanicals at 72 h of exposure were aqueous extract (LC_{50}=138.50) of *C. aurantium* followed by its ethanol extract (LC_{50}=113.16).

Effect of extraction solvents

Moreover, mean mortality of 2nd instar mealybug nymphs was higher as compared to adult females. Therefore, means of treatments bioassayed against mealybug nymphs were further compared using analyses of variance. Results revealed that all types of botanicals ($F_{7,\ 215}$ = 19.85; P < 0.001), extraction solvents ($F_{2,\ 215}$ = 5.25; P < 0.01) and their interaction together ($F_{14,\ 215}$ = 1.84; P < 0.05) had a significant effect on mortality of 2nd instar mealybug nymphs (Table 2). According to ANOVA results, on overall basis, all three extraction types, *i.e.* aqueous, ethanol and acetone, of *A. indica* and *E. camaldulensis* exhibited toxicity against 2nd instar mealybug nymphs followed by ethanol extracts of *C. sinensis* peels and *C. aurantium* seeds, while the remaining botanicals exhibited moderate or very low level of toxicity against citrus mealybugs (Figure 1).

DISCUSSION

In order to suppress insecticide resistant pest species, there is a need to screen out alternate options with least non-target effects. Plant derived chemicals and phytoextracts emerge as environment-friendly and safe alternates to hazardous synthetic pesticides in modern era of bio-intensive agriculture (Isman, 2006; Castillo-Sánchez *et al.*, 2010).

In this study, two bioassays were performed in order to determine the insecticidal effect of eight different botanical extracts against citrus mealybug adults and nymphs. Control treatments exhibited no or negligible mortality. As expected, 2nd instar nymphs were found more susceptible than adult female mealybugs most probably due to least penetration of plant extract constituents into insects due to more intricate body integument covered with waxy layer of mealy powder in adult female mealybugs (Mani and Shivaraju, 2016).

Results revealed that the most toxic botanicals with minimum LC_{50} values against citrus mealybug adults were acetone extracts of *A. indica* and *E. camaldulensis* followed by ethanol extracts *C. sinensis* seeds and *C. aurantium* leaves, while the most effective botanicals against 2nd instar mealybug nymphs were aqueous, ethanol and acetone extractions of *A. indica* and *E. camaldulensis* followed by ethanol extracts of *C. sinensis* peels and *C. aurantium* seeds. Our results are in line with those of many previous works. Botanical extracts of many plants such as tobacco (*Nicotiana tabacum*), eucalyptus (*E. camaldulensis*), dhatura (*Dhatura alba*), parthenium (*Tanacetum parthenium*) and neem (*A. indica*) have been found encouraging and useful for pest control and are being employed against a wide range of insect pests (Weathersbee and McKenzie, 2005; Nathan *et al.*, 2007; Isman, 2006 and 2008; Akhtar *et al.*, 2008; Dubey *et al.*, 2010; Ali *et al.*, 2016).

Table 1. Median lethal concentration (LC_{50}) values of botanical extracts bioassayed against adult females of mealybugs (*Drosicha mangiferae*; Hemiptera: Pseudococcidae)

Botanical extracts		Obs. time (h)	LC_{50} (%)	95% FL	Slope	X^2 (DF = 10)	P value
Azadirachta indica	Water	48	52.09	36.52-132.12	2.40	22.38	0.430
(seeds plus leaves)		72	22.86	17.88-25.45	1.66	0.97	0.067
	Ethanol	48	36.92	32.93-41.83	2.12	3.80	0.043
		72	21.15	18.12-23.93	2.14	2.48	0.045
	Acetone	48	22.37	17.54-26.64	1.40	0.62	0.051
		72	21.55	17.70-25.02	1.71	0.56	0.064
Eucalyptus camaldulensis	Water	48	32.42	22.63-47.79	0.73	0.43	0.310
(seeds plus leaves)		72	44.03	38.82-51.33	1.99	2.93	0.049
	Ethanol	48	31.87	28.77-35.30	2.54	1.36	0.033
		72	20.97	16.96-24.54	1.65	0.84	0.069
	Acetone	48	21.34	17.75-24.58	1.83	0.74	0.057
		72	20.34	17.38-24.88	1.67	0.57	0.067
Citrus sinensis (seeds)	Water	48	28.65	19.45-39.03	0.79	0.59	0.260
		72	29.42	20.52-39.92	0.82	0.35	0.250
	Ethanol	48	107.80	70.63-301.84	0.95	0.50	0.200
		72	47.98	41.61-58.68	1.67	1.05	0.067
	Acetone	48	107.80	70.63-301.68	0.95	0.50	0.200
		72	115.93	73.22-382.26	0.90	0.40	0.220
Citrus sinensis (leaves)	Water	48	111.93	70.46-390.20	0.87	1.80	0.240
		72	139.93	82.70-599.50	0.88	0.66	0.240
	Ethanol	48	66.91	35.23-181.09	2.70	30.79	0.670
		72	92.24	62.95-227.28	0.95	1.38	0.200
	Acetone	48	123.93	78.50-612.62	0.84	0.55	0.260
		72	107.80	70.63-301.84	0.95	0.50	0.200
Citrus sinensis (peels)	Water	48	45.06	32.63-87.32	2.21	27.36	0.320
		72	31.71	26.75-98.55	2.49	1.53	0.210
	Ethanol	48	101.71	80.99-59.42	0.94	0.38	0.210
		72	45.63	39.69-54.47	1.81	0.13	0.058
	Acetone	48	49.11	42.70-58.49	1.89	3.33	0.054
		72	47.35	45.65-60.23	1.80	0.67	0.069
Citrus aurantium (seeds)	Water	48	111.26	71.66-336.29	0.93	0.45	0.210
		72	99.45	66.82-255.08	0.96	1.08	0.200
	Ethanol	48	105.98	69.65-294.32	0.95	0.69	0.200
		72	99.38	66.17-267.96	0.93	1.39	0.210
	Acetone	48	102.97	68.10-281.92	0.94	0.83	0.200
		72	107.23	80.93-645.20	0.84	0.37	0.260
Citrus aurantium (leaves)	Water	48	51.21	43.54-64.15	1.64	0.42	0.070
		72	51.54	43.97-64.17	1.69	3.05	0.066
	Ethanol	48	51.54	44.06-63.92	1.71	3.98	0.064
		72	45.63	39.69-54.74	1.81	0.13	0.058
	Acetone	48	44.77	39.11-53.02	1.85	0.14	0.055
		72	54.33	46.100-68.52	1.69	1.93	0.067
Citrus aurantium (peels)	Water	48	45.52	39.73-54.06	1.86	0.28	0.055
		72	55.17	40.77-106.80	1.77	8.64	0.260
	Ethanol	48	144.76	81.34-902.76	0.79	0.84	0.290
		72	45.30	39.35-54.17	1.78	0.79	0.050
	Acetone	48	51.23	43.83-63.44	1.71	2.42	0.064
		72	43.97	38.55-51.68	1.90	0.35	0.053

LC_{50}: lethal concentration (%) of tested botanical that killed 50% of exposed mealybugs (Probit analysis) , FL: 95% Fiducial (confidence) limits, DF: degree of freedom

Table 2. Median lethal concentration (LC_{50}) values (%) of botanical extracts bioassayed against 2nd instar mealybugs (*Drosicha mangiferae*; Hemiptera: Pseudococcidae)

Botanical extracts		Obs. time (h)	LC_{50} (%)	95% FL	Slope	X^2 (DF = 13)	P value
Azadirachta indica (seeds plus leaves)	Water	24	42.29	35.27-65.54	1.47	9.47	0.094
		48	13.25	8.76-17.47	0.76	1.32	0.051
		72	4.55	2.59-6.44	1.16	3.87	0.050
	Ethanol	24	23.17	18.71-28.80	0.98	1.12	0.093
		48	20.12	15.71-25.25	0.90	0.82	0.085
		72	14.66	10.51-18.60	0.87	0.61	0.093
	Acetone	24	46.41	33.12-60.75	0.90	0.77	0.087
		48	14.60	10.51-18.68	0.86	0.55	0.053
		72	21.00	16.54-26.33	0.91	1.14	0.083
Eucalyptus camaldulensis (seeds plus leaves)	Water	24	13.28	9.61-16.77	0.93	0.52	0.083
		48	6.48	2.68-9.68	0.67	0.62	0.150
		72	2.83	0.66-5.37	0.68	1.16	0.160
	Ethanol	24	12.75	5.06-9.69	1.15	13.21	0.500
		48	6.30	3.38-9.01	0.88	0.54	0.099
		72	37.33	28.18-56.71	0.74	0.34	0.120
	Acetone	24	29.51	24.54-36.53	1.11	5.51	0.058
		48	84.68	61.08-144.4	1.01	0.97	0.080
		72	48.12	35.68-78.53	0.77	0.40	0.110
Citrus sinensis (seeds)	Water	24	67.31	50.63-105.13	1.02	2.50	0.074
		48	60.97	47.25-89.31	1.09	3.20	0.066
		72	67.79	50.22-109.55	0.96	5.04	0.083
	Ethanol	24	87.09	64.68-137.99	1.17	5.05	0.064
		48	52.99	32.95-190.12	1.16	23.02	0.340
		72	39.40	32.29-51.16	1.41	6.02	0.062
	Acetone	24	49.36	39.91-66.33	1.17	3.75	0.056
		48	73.81	48.78-177.13	1.22	12.03	0.180
		72	98.28	65.24-208.33	0.84	3.55	0.110
Citrus sinensis (leaves)	Water	24	131.03	79.69-385.84	1.32	9.45	0.160
		48	180.15	99.90-616.03	0.77	0.75	0.130
		72	46.19	34.21-75.42	0.75	5.46	0.120
	Ethanol	24	176.60	102.46-556.64	1.26	7.05	0.140
		48	159.21	93.12-462.33	0.81	1.09	0.120
		72	52.48	37.37-96.33	0.70	5.38	0.140
	Acetone	24	227.22	122.07-804.22	0.84	4.38	0.120
		48	102.90	63.56-320.56	1.40	13.91	0.200
		72	106.66	62.96-349.16	0.63	1.41	0.180
Citrus sinensis (peels)	Water	24	237.75	128.21-824.69	0.88	3.18	0.120
		48	51.09	32.63-153.42	1.07	17.72	0.300
		72	64.07	42.33-155.70	0.90	8.24	0.190
	Ethanol	24	48.89	38.2-70.35	0.98	1.02	0.076
		48	80.90	55.59-158.75	0.82	0.56	0.110
		72	56.43	35.41-153.32	0.89	4.48	0.210
	Acetone	24	132.42	85.99-285.46	0.97	5.54	0.090
		48	113.32	63.23-529.17	0.96	10.40	0.240
		72	81.31	57.61-145.97	0.92	2.62	0.95
Citrus aurantium (seeds)	Water	24	62.38	39.94-189.49	1.34	21.39	.044
		48	41.23	32.67-5688	0.97	2.09	.0706
		72	45.38	27.85-173.11	1.25	31.43	0.41
	Ethanol	24	50.73	37.80-81.82	1.70	20.29	0.11
		48	45.91	35.63-66.95	0.92	2.46	0.085
		72	51.08	37.46-86.16	1.29	10.59	0.13
	Acetone	24	67.49	45.23-153.6	1.12	10.52	0.17
		48	30.82	24.47-41.09	1.13	8.10	0.092
		72	41.61	34.43-52.47	1.32	4.54	0.044
Citrus aurantium (leaves)	Water	24	116.12	7019-333.66	0.71	0.33	0.14
		48	105.00	67.81-240.55	0.81	3.36	0.11
		72	138.50	85.75-342.30	0.86	0.58	0.11
	Ethanol	24	138.50	86.75-345.30	0.90	1.51	0.12
		48	79.13	53.10-169.90	0.74	1.09	0.13
		72	80.037	54.14-165.65	0.77	0.74	0.12

Botanical extracts		Obs. time (h)	LC_{50} (%)	95% FL	Slope	X^2 (DF = 13)	P value
	Acetone	24	143.97	87.49-370.94	0.85	0.48	0.11
		48	50.83	37.19-86.13	0.76	0.35	0.12
		72	57.09	40.52-105.35	0.73	0.45	0.13
Citrus aurantium (peels)	Water	24	106.58	73.31-165.54	1.20	2.76	0.92
		48	80.12	56.88-143.29	0.92	2.87	0.95
		72	72.88	54.74-113.36	1.08	1.02	0.069
	Ethanol	24	113.39	80.79-136.09	1.20	2.09	0.067
		48	113.52	75.12-232.52	0.93	0.63	0.094
		72	113.16	75.24-232.74	0.93	1.19	0.094
	Acetone	24	164.83	99.52-426.31	0.91	4.64	0.10
		48	118.42	84.45-203.95	1.24	0.97	0.065
		72	96.24	67.03-177.79	0.97	1.24	0.086

LC_{50}: lethal concentration (%) of tested botanical that killed 50% of exposed mealybugs, FL: 95% Fiducial (confidence) limits, DF: degree of freedom

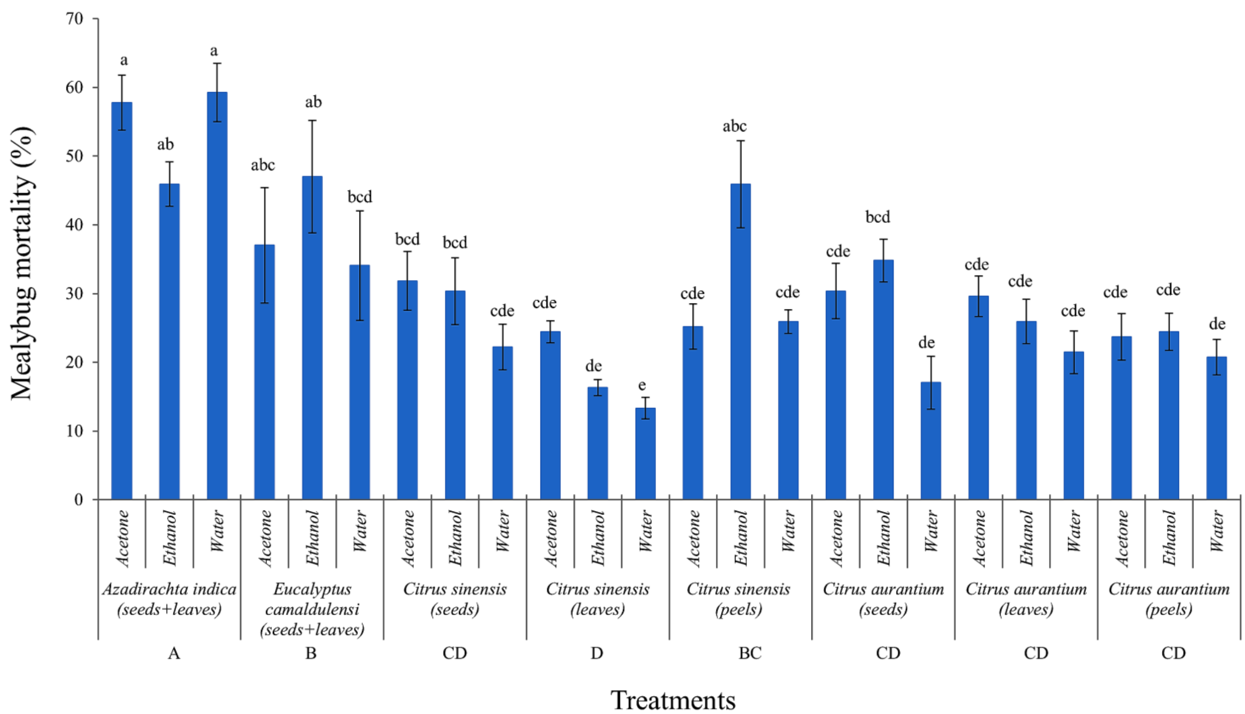

Figure 1. Mean mortality (%) of mealybugs exposed to botanical extracts prepared by different extraction solvents. Columns represent mean percent mortality of 2nd instar mealybugs ± standard error (n = 4). Different small letters over bars signify statistical difference among all treatments (ANOVA; $P \leq 0.05$), and capital letters below x-axis represent overall statistical difference among different botanical extracts (two factor ANOVA; Tukey's HSD at $\alpha = 0.05$).

Botanical extracts of different plant parts of neem (*A. indica*) have been used since ancient times for the management of insect and mite pests of agricultural crops and in stored food products. It has been found with revolting effects against homopterous insect pests including mealybugs (Schmutterer, 1990; Mourier, 1997; Isman, 2008). It is being utilized as efficient botanical insecticide, antifeedant, anti-ovipositing agent and repellent on different fruits and vegetable plants (Weathersbee and McKenzie, 2005; Maheswaran and Ignacimuthu, 2015; Mamoon-Ur-Rashid *et al*., 2016). We found that aqueous extract of neem seeds was most effective against mealybugs, followed by ethanolic extract. Many studies have shown that aqueous extract of neem seed kernels (NSKE) is most effective against mealybugs including citrus mealybugs (Mourier, 1997; Sathyaseelan and Bhaskaran, 2010). Similarly, our results regarding eucalyptus (*Eucalyptus* spp.) extracts are in accordance with those of Govindaiah *et al*. (2006) and Ahmadi *et al*. (2012) who found the highest mortality of citrus mealybugs with eucalyptus extracts. Similarly, our results are in line with the findings of Singh *et al*. (2012), Roonjho *et al*. (2013) and Prishanthini and Vinobaba (2014) who found maximum control of cotton mealybugs (*P. solenopsis*) with eucalyptus extracts.

Nevertheless, one of the study objectives was also to compare the effect of three different extraction solvents, *i.e.* acetone, ethanol and water, on the toxicity of botanical extracts against mealybugs. It was found that extraction solvents had a significant ($F_{2, 213} = 3.14$; $P = 0.04$) effect on mealybug mortality. According to overall results, extracts prepared by organic solvents *i.e.* acetone and ethanol were most effective without any significant difference and were statistically different from water-based plant extracts. These findings corroborate more polar nature of major plant bio-constituents such as phenols, flavonoids and terpenoids towards organic solvents than aqueous extraction media (Do *et al*., 2014). Many previous studies have demonstrated higher yield of total phenolic and flavonoid contents by acetone and ethanol extraction solvents with enhanced biological activities as compared to aqueous extracts (Patra *et al*., 2011; Do *et al*., 2014; Iloki-Assanga *et al*., 2015).

CONCLUSION

From overall study results, it is concluded that botanical pesticides can play a significant role in management of insect/mite pests as being substitutes of toxic and hazardous synthetic chemicals. Particularly, neem (*A. indica*) and eucalyptus (*E. camaldulensis*) could be effective biorational options against mealybugs and other homopterous pests, and should be incorporated in the future pest management programs.

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