



## TOXICITY OF SOME SELECTED CONVENTIONAL SYNTHETIC INSECTICIDES AGAINST MEALYBUG *DROSICHA MANGIFERAE* GREEN (HEMIPTERA: PSEUDOCOCCIDAE) INFESTING CITRUS IN DISTRICT SARGODHA

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### ABSTRACT

Mango mealybug *Drosicha mangiferae* (Pseudococcidae: Hemiptera) is one of the most damaging insect pests of a wide range of horticultural crops. This sap-feeding insect pest causes substantial loss to different fruit crops, particularly to citrus. For its management, citrus farmers rely on extensive and irrational applications of synthetic insecticides. Due to which, *D. mangiferae* have attained resistance against most these chemicals. This laboratory study was aimed to determine the toxicity of some selected conventional insecticidal formulations concurrently being used by the farmers against *D. mangiferae* infestation. Using standard twig-dip method, toxicity bioassays were conducted against 2<sup>nd</sup> instar nymphs of *D. mangiferae*. Treatments included nine conventional synthetic insecticides from three insecticidal groups. Results revealed that both treatments *i.e.* insecticidal formulations ( $F_{9,245} = 93.05, P < 0.001$ ) and time intervals ( $F_{4,245} = 158.72, P < 0.01$ ) and their interaction ( $F_{36,245} = 5.73, P < 0.001$ ) had a significant impact on the mortality of 2<sup>nd</sup> instar mealybug nymphs. The most effective synthetic insecticides against *D. mangiferae* individuals were lambda-cyhalothrin, methidathion, profenofos and deltamethrin with mean mortality and  $LT_{50}$  values of  $45.00 \pm 4.57\%$  and 49.01 h,  $44.67 \pm 3.96\%$  and 49.83 h,  $40.00 \pm 4.07\%$  and 58.98 h and  $35.67 \pm 4.11\%$  and 74.86 h, respectively. These insecticides may be incorporated in future pest management programs against *D. mangiferae* mealybugs.

**Keywords:** *Drosicha mangiferae*, Citrus mealybug, Conventional insecticides, Comparative toxicity, Organophosphates, Pyrethroids

### INTRODUCTION

Mango mealybugs (Pseudococcidae: Hemiptera) are one of the most economic insect pests all over the world. These phloem-feeding insect pests infest and suck sap from tender shoots, twigs, leaves, stems, spurs, panicles, aerial roots, trunks and underground roots of a wide range of plants including various agricultural and horticultural crops (Williams and Willink, 1992). In Southeast Asian countries, more than 300 mealybug species belonging to 50 genera have been described infesting agricultural crops including citrus, mango, pineapple, banana, grape, cotton, okra, tomato etc. (Williams and Willink, 1992; Sirisena *et al.*, 2013).

*Drosicha mangiferae* Green, commonly known as mango mealybug, is one of the most damaging cosmopolitan species in Indo-Pak regions (Gundappa *et al.*, 2018). Apart from mango, it has been found infesting many other agricultural and horticultural crops. For instance, citrus is an important

fruit crop of Pakistan (Ahmad *et al.*, 2018) and *D. mangiferae* has attained a regular pest status in citrus orchards in Sargodha (Punjab, Pakistan). This pest has the ability to cause considerable qualitative and quantitative loss to citrus produce (Tahir *et al.*, 2015; Afzal *et al.*, 2018). This pest is usually difficult to control with routine pesticide sprays because of its body being protected by impervious scales cushion and due to its mouthparts concealed underside of body (Chaudhari, 2012; Mani and Shivaraju, 2016). Therefore, farmers mostly rely on a schedule of heavy and repeated sprays of synthetic conventional insecticides (Aheer *et al.*, 2009; Gulzar *et al.*, 2015). These insecticides are persistence and may cause health hazards and also harmful effects such as contamination of soil, air and water resources, eradication of beneficial fauna, pest resistance and resurgence (Edwards, 2013; Nicolopoulou-Stamati *et al.*, 2016). However, synthetic insecticides have been inevitable plant protection tools for ensuring sustained agricultural

production all over the world. Although, *D. mangiferae* has become resistant to various conventional synthetic insecticides (Karar *et al.*, 2010; Saddiq *et al.*, 2014), many insecticides such as methidathion, profenofos, and lambda-cyhalothrin are still effective to control this pest. It is, therefore, essential not only to seek out novel environment-friendly pest control strategies (Majeed *et al.*, 2018) but also to screen out the prevailing effectiveness of available conventional insecticide formulations against *D. mangiferae*. In this regard, the current study was aimed to have a comparative evaluation of some available synthetic insecticidal formulations against 2<sup>nd</sup> instar nymphs of *D. mangiferae* which can be effectively used against this destructive insect pest.

## MATERIALS AND METHODS

### Culture of mealybugs

Third instar female individuals of *D. mangiferae* were collected from citrus (*Citrus reticulata* cv. kinnow mandarin) orchard (32°08'21"N; 72°40'11"E) located in the vicinity of the College of Agriculture, University of Sargodha. Insect collection were carried out during the 1<sup>st</sup> fortnight of January upon emergence of early batch of mealybugs. It was also ensured that no pesticidal application was made in the orchard against these pests yet. These individuals were brought to the laboratory under cool conditions and were reared at 27±2°C and 65±5% relative humidity in 90 × 60 cm plastic cages up to F<sub>2</sub> generation on young seedlings of *C. reticulata*. For bioassays, only healthy and active 2<sup>nd</sup> instar individuals were utilized.

### Insecticides

Based on the field information obtained during a preliminary survey from different areas of district Sargodha, nine synthetic conventional insecticides were selected for this laboratory study (Table 1). These conventional insecticides are being frequently contemporarily used by the local farmers against mealybug infestation on their citrus orchards. Commercial formulations of these selected insecticides were purchased from registered pesticide dealers from the grain market of district Sargodha (Punjab, Pakistan).

### Toxicity bioassays

Treatments included one control and nine conventional insecticide formulations (including three organophosphates, three pyrethroids and three carbamates) were selected for the leaf dip bioassays. These insecticides were used at the field labeled dose rate of each insecticide as mentioned in Table 1. Standard twig-dip bioassays were conducted using 9 cm glass Petri-plates. In brief, unsprayed 5 cm long twig-tips of *C. reticulata* plants were collected and washed with clean tap-water. These twigs were air dried for three days at room temperature (26°C). Their stems were wrapped in moist cotton plug to ensure their freshness for at least three days. Insecticides were applied with recommended labeled dose in CRD design with 6 replications per treatment. Ten healthy and active 2<sup>nd</sup> instar mealybug nymphs were released on treated citrus twigs and Petri-plates were incubated at 27±1°C and 65±2% relative humidity in an environment chamber set at 16:8 h light–dark photoperiod. Data regarding mortality of

mealybug individuals were recorded at 6, 12, 24, 48 and 72 h post-exposure.

### Statistical analysis

Data regarding percent mortality of mealybugs in response to conventional insecticides were corrected using Abbott's formula. Using Statistix<sup>®</sup> 8.1 (Analytical Software, Tallahassee, FL), Analysis of Variance (ANOVA) was used to find out the significant effect of treatment and time factors on the mealybug mortality. Treatments were compared using Tukey's honestly significant difference (HSD) test at 5% probability level. Median lethal time (LT<sub>50</sub>) values were calculated by probit analysis using POLO-PC<sup>®</sup> (LeOra Software 1987).

## RESULTS

Comparative toxicity of nine different conventional insecticides belonging to three different groups of conventional synthetic pesticides was determined under laboratory conditions against 2<sup>nd</sup> instar nymphs of mealybug *D. mangiferae*. Percent mortality of mealybug individuals observed at different post-treatment time intervals was subjected to factorial analysis of variance. The insecticides ( $F_{9,245} = 93.05, P < 0.001$ ) and exposure times ( $F_{4,245} = 158.72, P < 0.01$ ) and their interaction ( $F_{36,245} = 5.73, P < 0.01$ ) had a significant influence on the mean mortality of 2<sup>nd</sup> instar mealybug nymphs (Table 2).

As compared to control treatment, a significant mortality of mealybugs was observed for all insecticides (Table 3). Mean mortality in control treatment varied from 0.00 to 5.00%. According to analysis of variance and all pair-wise comparisons, the most effective conventional synthetic insecticides against *D. mangiferae* mealybugs were methidathion, lambda-cyhalothrin, deltamethrin and profenofos. Carbofuran and methomyl were least effective conventional insecticides, while chlorpyrifos and bifenthrin showed intermediate response against 2<sup>nd</sup> instar mealybug (Table 3).

According to the data analyses at different time intervals, there was no significant mortality of mealybug nymphs by any of the conventional insecticides at 6 h of exposure duration (Table 3). At 12 h of exposure time, lambda-cyhalothrin exhibited maximum mortality of mealybugs (38.33±3.07%), followed by methidathion (35.00±4.28%), profenofos (28.33±4.01%) and deltamethrin (27.13±5.21%), while minimum mealybug mortality was recorded for chlorpyrifos and carbofuran (11.67±3.07%). At 24 h of exposure time, maximum mortality was exhibited by lambda-cyhalothrin (56.67±5.58%) and methidathion (55.00±3.42%) which was similar to profenofos (53.33±4.22%) and deltamethrin (48.33±4.77%). Carbofuran and methomyl caused minimum mortality. Moreover, chlorpyrifos, carbofuran and bifenthrin caused similar level of mortality at 24 h time interval (Table 3). Similar trend of mortality was observed after 48 h of exposure. According to the observation at 72 h post-treatment, again lambda-cyhalothrin and methidathion exhibited maximum mortality (61.67%) of mealybugs followed by profenofos and deltamethrin (Table 3).

In addition, similar pattern of toxicity of conventional synthetic insecticides against 2<sup>nd</sup> instar *D. mangiferae* mealybugs was manifested by median lethal time (LT<sub>50</sub>) values calculated by probit analysis. According to probit analysis, the most toxic and effective conventional insecticides against mealybug individuals were lambda-cyhalothrin, methidathion, profenofos and deltamethrin with

LT<sub>50</sub> values of 49.01 h (40.46 – 60.59 h), 49.83 h (42.38 – 59.52 h), 58.98 h (49.81 – 72.20 h) and 74.86 h (60.11 – 102.63 h), respectively (Table 4). On the contrary, least effective conventional insecticides with maximum LT<sub>50</sub> values were methomyl, carbofuran, carbosulfan, chlorpyrifos and bifenthrin (Table 4).

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**Table 1**

Different conventional insecticides evaluated under laboratory conditions against 2<sup>nd</sup> instar nymphs of mealybugs *Drosicha mangiferae* Green.

Chemical Name (active ingredient)	Chemical family*	Brand Name	Company	Label Dose (ha <sup>-1</sup> )
bifenthrin	3A (pyrethroids)	Talstar® 10 EC	FMC	625 ml
carbofuran	1A (carbamates)	Carbofuran® 10 GR	Arysta LifeScience	10 Kg (5 g L <sup>-1</sup> )
carbosulfan	1A (carbamates)	Advantage® 20 SC	FMC	1250 ml
chlorpyrifos	1B (organophosphates)	Lorsban® 40 EC	Dow Agro Sciences	2500 ml
deltamethrin	3A (pyrethroids)	Decis® 10.5 EC	Bayer CropScience	300 ml
lambda-cyhalothrin	3A (pyrethroids)	Karate® 2.5 EC	Syngenta	625 ml
methidathion	1B (organophosphates)	Supracide® 400 EC	Syngenta	2500 ml
methomyl	1A (carbamates)	Lannate® 90 SP	DuPont	400 g
profenofos	1B (organophosphates)	Curacron® 50 EC	Syngenta	2000 ml

**Table 2**

Analysis of variance for mean mortality of 2<sup>nd</sup> instar nymphs of mealybug *Drosicha mangiferae* Green exposed to label recommend dose rates of different conventional insecticides under laboratory conditions.

Source	DF	SS	MS	F-value	P-value
Treatment	9	56776	6308.5	93.05	<0.001
Time	4	43045	10761.2	158.72	<0.01
Treatment * Time	36	13982	388.4	5.73	<0.001
Error	245	16611	67.8		
Total	299	132420			
Grand Mean	25.96				
CV	31.71				

$P < 0.001$  (highly significant) and  $P < 0.01$  (significant); one-way factorial ANOVA at  $\alpha = 0.05$

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**Table 3**

Percent mortality of 2<sup>nd</sup> instar nymphs of mealybug *Drosicha mangiferae* Green exposed to label recommended dose rates of different conventional insecticides.

Treatments	Mean mortality at different post-application time intervals				
	6 h	12 h	24 h	48 h	72 h
bifenthrin c	3.33 ± 3.33 a	15.00 ± 2.24 cde	28.33 ± 4.77 b	30.00 ± 4.47 b	30.00 ± 4.47 b
carbofuran d	1.67 ± 1.67 a	11.67 ± 3.07 de	18.33 ± 1.67 bc	18.33 ± 1.67 bc	18.33 ± 1.67 bc
carbosulfan c	5.00 ± 3.42 a	20.00 ± 3.65 bcd	25.00 ± 3.42 b	28.33 ± 3.07 b	28.33 ± 3.07 b
chlorpyrifos c	5.00 ± 3.42 a	11.67 ± 3.07 de	23.33 ± 2.11 b	28.33 ± 3.07 b	28.33 ± 3.07 b
deltamethrin b	5.00 ± 2.24 a	28.33 ± 4.01 abc	48.33 ± 4.77 a	48.33 ± 4.77 a	48.33 ± 4.77 a
lambda-cyhalothrin a	6.67 ± 3.33 a	38.33 ± 3.07 a	56.67 ± 5.58 a	61.67 ± 5.43 a	61.67 ± 5.43 a
methidathion a	10.00 ± 2.58 a	35.00 ± 4.28 ab	55.00 ± 3.42 a	61.67 ± 4.77 a	61.67 ± 4.77 a
methomyl cd	6.67 ± 3.33 a	15.00 ± 2.24 cde	20.00 ± 2.58 b	20.00 ± 2.58 bc	20.00 ± 2.58 bc
profenofos ab	5.00 ± 2.24* a	28.33 ± 4.01 abc	53.33 ± 4.22 a	56.67 ± 4.94 a	56.67 ± 4.94 a
control e	0.00 ± 0.00 a	1.67 ± 1.67 e	1.67 ± 1.67 c	3.33 ± 2.11 c	5.00 ± 2.24 c

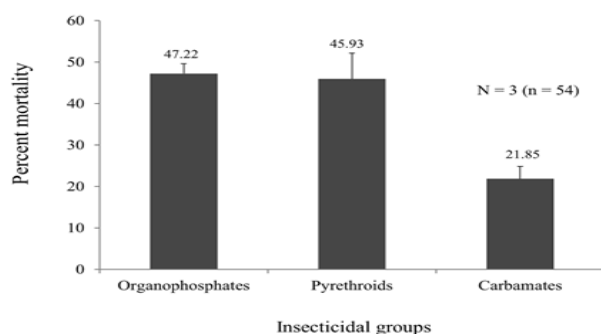
\*values are means of six independent replications for each treatment ± standard errors. Means within a column bearing different letters are significantly different from each other (one-way factorial ANOVA for overall treatments comparison and one-way ANOVA for comparison of treatments at each time interval; Tukey's HSD at  $\alpha = 0.05$ ).

**Table 4**

Median lethal time (LT<sub>50</sub>) values of some conventional insecticides bioassayed against 2<sup>nd</sup> instar nymphs of mealybug *Drosicha mangiferae* Green.

Treatments	LT <sub>50</sub> (hr)	Lower and Upper 95% Fiducial Limits (hr)	X <sup>2</sup> (df = 28)*	P
bifenthrin	189.44	121.50 – 484.74	195.56	< 0.001
carbofuran	553.04	265.98 – 2941.86	105.53	< 0.001
carbosulfan	284.22	155.99 – 1172.64	152.91	< 0.001
chlorpyrifos	237.17	150.21 – 579.18	133.94	< 0.001
deltamethrin	74.86	60.11 – 102.63	203.42	< 0.001
lambda-cyhalothrin	49.01	40.46 – 60.59	239.22	< 0.001
methidathion	49.83	42.38 – 59.52	157.32	< 0.001
methomyl	Incalculable	Incalculable	105.04	< 0.001
profenofos	58.98	49.81 – 72.20	193.70	< 0.001

Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.

**Fig. 1**

Mean percent mortality of 2<sup>nd</sup> instar nymphs of mealybug *Drosicha mangiferae* exposed to different conventional chemistry insecticidal groups. Alphabets over columns indicate statistical difference among treatments (one-way ANOVA; LSD at  $\alpha = 0.05$ ).

## DISCUSSION

Periodic evaluation of the effectiveness of prevailing insecticidal formulations being used by the indigenous farming community is necessary for the sustainable management of insect pests (Miller *et al.*, 2010). This laboratory study evaluated some currently used conventional synthetic insecticides against mealybugs *D. mangiferae*. The most effective conventional insecticides against 2<sup>nd</sup> instar mealybug nymphs found in this study were methidathion and lambda-cyhalothrin followed by deltamethrin and profenofos. Carbamates and chlorpyrifos were the least effective. These findings are in line with many previous studies (Saeed *et al.*, 2007; Aheer *et al.*, 2009; Gulzar *et al.*, 2015; Barbosa *et al.*, 2018).

However, if we compare the mean mortality of mealybugs caused by different groups of conventional insecticides (Fig. 1), we can see that organophosphate and pyrethroid

formulations caused similar level of toxicity without any statistical difference. On the other hand, mean mealybug mortality exhibited by carbamates was two times less than organophosphates and pyrethroids. These results are in line with the findings of Saeed *et al.* (2007), where they found that the organophosphate insecticides are more effective against mealybugs than carbamates.

It was found that no single insecticide caused 100% mortality of 2<sup>nd</sup> instar mealybug nymphs till the last observation at 72 h post-exposure. This again corroborates the fact that most of the synthetic insecticides are ineffective against coccid pests particularly against mealybugs due to their reduced penetration in their bodies and due to the resistance developed in pests by the repeated use of same insecticides with similar mode of action (Saddiq *et al.*, 2014; Venkatesan *et al.*, 2016).

#### Authors' contributions

HAG and MZM conceived the idea and planned the experiment. HAG performed experiments and wrote first draft of the manuscript. MZM and MAK performed statistical analyses and technically revised the manuscript. MA provided technical assistance and proofread the manuscript.

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