Research Article

Comparative Toxicity of some Selected Novel Chemistry Insecticides against Mealybug *Drosicha mangiferae* (Hemiptera: Pseudococcidae) Infesting Citrus Orchards in Pakistan

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Abstract | One of the emerging threats to citrus industry in Pakistan is the infestation of mealybug *Drosicha mangiferae* (Pseudococcidae: Hemiptera), a damaging insect pest of a large number of agricultural and horticultural crops in South East Asia. In spite of extensive applications of conventional synthetic pesticides, citrus farmers are unable to get rid of this pest. In order to find out alternate biorational pesticides, this laboratory study was carried out to evaluate few selected novel chemistry insecticidal formulations against 2nd instar nymphs of *D. mangiferae* using standard twig-dip bioassay method according to Completely Randomized Design. Results showed that both factors *i.e.* insecticidal treatments ($F_{9, 245}$ = 146.90, P < 0.001) and time ($F_{4, 245}$ = 445.75, P < 0.01) and their interaction ($F_{36, 245}$ = 9.20, P < 0.001) had a significant effect on the mortality of mealybug nymphs. The most effective novel chemistry insecticides against *D. mangiferae* mealybugs were sulfoxaflor, spirotetramat, thiamethoxam and pyriproxyfen with mean mortality and LT_{50} values of 64.00±3.50% and 31.67 h, 62.67±2.64% and 34.42 h, 53.01±4.10% and 45.84 h, and 51.00±3.97% and 48.10 h, respectively. Based on these results, the above mentioned novel chemistry insecticides are recommended to be incorporated in future pest management programs against *D. mangiferae* mealybugs.

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Introduction

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 many agricultural and horticultural crops (Williams and Willink, 1992). In Southeast Asian countries, more than 300 mealybug species belonging to 50 genera have been described so far infesting various agricultural crops including citrus, mango, pineapple, banana, grape, cotton, okra, tomato etc. (Williams and Willink, 1992; Sirisena et al., 2013).

Drosicha mangiferae, 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 voraciously feeding on 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) and 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 its body (Chaudhari, 2012; Mani and Shivaraju, 2016). Therefore, to control this pest, indigenous farmers mostly rely on a schedule of heavy and repeated sprays of synthetic conventional insecticides (Aheer et al., 2009; Gulzar et al., 2015), most of which very harmful and persistent and cause many ill-effects such as contamination of soil, air and water, eradication of beneficial fauna, pest resistance and resurgence and health hazards to citrus consumer community (Edwards, 2013; Nicolopoulou-Stamati et al., 2016).

Although synthetic insecticides are inevitable plant protection tools for ensuring sustained agricultural production all over the world, it is essential to find out new pest control options safer and biorational than conventional insecticidal formulations. Novel chemistry insecticides, for instance, would be important alternate options in this case. These insecticides have a differential chemistry than conventional ones and are usually more target specific, quickly biodegradable and less toxic to non-target fauna (Ishaaya and Degheele, 2013; Singh et al., 2016). In this regard, this study was aimed to screen out some available novel chemistry insecticidal formulations against 2nd instar nymphs of *D. mangiferae* which can be effectively used against this destructive insect pest.

Materials and Methods

Culture of mealybugs

Mealybug *D. mangiferae* was collected from different citrus orchards (*cv.* kinnow mandarin; *Citrus reticulata*) located in the surroundings of the College of Agriculture, University of Sargodha (32°08'21"N; 72°40'11"E). Third instar female mealybug individuals were collected in the month of February, when early batches of mealybugs emerge from egg masses. Collection was done from the orchards where no insecticidal application had been made yet against mealybugs or other pests for last 6 weeks. These collected mealybug individuals were carried under cool conditions to the laboratory of entomology of the College of Agriculture and were raised up to F_2 generation on young *C. reticulata* seedlings at 27±2°C and 65±5% relative humidity in plastic cages (2 × 3 ft). Only active and healthy 2nd instar mealybug individuals were used in toxicity bioassays.

Insecticides

Based on a preliminary market survey of the district Sargodha, nine promising novel chemistry insecticides were selected to be evaluated against *D. mangiferae* in this laboratory study. These insecticides with a differential mode of action and chemistry than the conventional ones have been entered into indigenous pesticide market since last few years and are usually recommended against different sucking insect pests (Saddiq et al., 2015). Commercial formulations of these selected novel chemistry insecticides were purchased from the registered pesticide dealers from the grain market of district Sargodha (Punjab, Pakistan).

Bioassays

Treatments included one control and nine novel chemistry insecticide formulations (including primarily insect growth regulators and neonicotinoids) as detailed in Table 1. Control treatment was consisted of clean tap-water used for preparing insecticidal solutions. Standard twig-dip bioassays were conducted using 9 cm Petri plates. In brief, 5 cm long unsprayed twig-tips of *C. reticulata* (*cv.* kinnow mandarin) plants were collected from young citrus orchard, washed with clean tap-water and were air-dried at room temperature (28°C). Their stems were wrapped with moist cotton plug to keep them fresh for at least three days of bioassay. These twigs were treated with the insecticides according to their label-recommended dose rates according to CRD design with 6 replications per treatment. Ten healthy and active 2nd instar mealybug nymphs were released on treated citrus twigs and Petri plates were incubated at 27±2°C and 65±3% relative humidity in an environment chamber set with 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 novel chemistry insecticides were corrected using Abbott's formula. Using Statistix® Version 8.1 (Analytical Software, Tallahassee, FL), factorial

Table 1: *Different novel chemistry insecticides evaluated under laboratory conditions against 2nd instar nymphs of mealybug Drosicha mangiferae Green.*

Chemical Name (active ingredient)	Chemical family*	Mode of Action	Brand Name	Company	Label Dose (ha^{-1})
acetamiprid	4A (neonicotinoids)	Nicotinic acetylcholine receptor (nAChR) allosteric modulator	Acelan® 20 SP	FMC	100 g
buprofezin	16 (buprofezin)	Chitin biosynthesis inhibitor (IGR)	Sitara® 25WP	Ali Akbar Chemicals	750 g
clothianidin	4A (neonicotinoids)	Nicotinic acetylcholine receptor (nAChR) allosteric modulator	Clutch® 50 WDG	Joshi Agro-Chemicals	210 g
fenoxycarb	7B (fenoxycarbs)	Juvenile hormone analogue (IGR)	Insegar® 25WP	Syngenta	400 g
pyrifluquinazon	9B (pyridine derivatives)	Chordotonal organ TRPV channel modulator	Pyrifluquinazon® 20 SC	Nichino America	250 ml
pyriproxyfen	7C (pyriproxyfens)	Juvenile hormone mimics (IGR)	Admiral® 10EC	FMC	75 ml
spirotetramat	23 (tetramic acid derivatives)	Acetylcholinesterase (AChE) inhibitor	Movento® 240 SC	Bayer CropScience	800 ml
sulfoxaflor	4C (sulfoximines)	Nicotinic acetylcholine receptor (nAChR) allosteric modulator	Closer® 240 SC	DowAgroSciences	400 ml
thiamethoxam	4A (neonicotinoids)	Nicotinic acetylcholine receptor (nAChR) allosteric modulator	Actara® 25 WG	Syngenta	130 g

**according to Insecticide Resistance Action Committee* (www.irac-online.org) *IRAC MoA Classification Version 8.3, July 2018.*

analysis of variance (ANOVA) was performed to find out the significant effect of treatments (insecticidal formulations) and time factor on the mealybug mortality and treatment means were compared using Tukey's honestly significant difference (HSD) test at 5% probability level. Median lethal time (LT_{50}) values were calculated by probit analysis using POLO-PC® (LeOra Software, 1987).

Results and Discussion

In this laboratory study, the toxicity of nine insecticides having differential chemistry and modes of action than conventional ones was assessed against 2nd instar nymphs of mealybug *D. mangiferae*. Percent mortality of mealybugs recorded at different post-exposure time intervals was subjected to factorial analysis of variance which showed that both treatments *i.e.* insecticides ($F_{9, 245}$ = 146.90, $P < 0.001$) and time ($F_{4, 245}$ = 445.75, $P < 0.01$) factors and their interaction ($F_{36, 245}$ = 9.20, $P < 0.001$) had a significant effect on the mortality of mealybug nymphs (Table 2).

As compared to control treatment, a significant mortality was observed for all insecticides (Table 3). Average mortality in control treatments was 5.0±1.42% varying from 0.00% at 6 h to 11.67% at 72 h. According to factorial ANOVA and Tukey HSD test, the most effective novel chemistry insecticides against *D. mangiferae* mealybugs were sulfoxaflor, spirotetramat, thiamethoxam, pyriproxyfen and buprofezin, while clothianidin, fenoxycarb and pyrifluquinazon appeared to be the least effective formulations against mealybugs (Table 3). At 6 h of exposure, sulfoxaflor and spirotetramat exhibited maximum mortality of mealybugs (*i.e.* 28.33±7.03 and 21.67±4.01%, respectively), followed by buprofezin (16.67±2.11%) and thiamethoxam (16.67±6.15%), while clothianidin exhibited minimum mortality (1.67±1.67%; Table 3). According to the observation at 12 h of exposure, again sulfoxaflor (43.33±4.22%)

Table 2: *Analysis of variance comparison table for mean mortality of 2nd instar nymphs of mealybug Drosicha mangiferae green exposed to label recommended dose rates of different novel chemistry insecticides under laboratory conditions.*

Source	DF	SS	MS	F-value	P-value
Treatment	9	97434	10826.0	146.90	<0.001
Time	4	131395	32848.8	445.75	<0.01
Treatment * Time	36	24398	677.7	9.20	<0.001
Error	245	18055	73.7		
Total	299	272244			
Grand Mean	38.77				
CV	22.14				

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

Table 3: *Percent mortality of 2nd instar nymphs of mealybug Drosicha mangiferae Green exposed to label-recommended dose rates of different novel chemistry insecticides.*

Treatments	6h	12h	24h	48h	72h
acetamiprid cd	11.67±3.07* abc	18.33±3.07 cd	25.00±3.42 cd	45.00±4.28 d	66.67±4.22 b
buprofezin c	16.67±2.11 abc	30.00±2.58 abc	35.00±2.24 bc	46.67±3.33 d	58.33±8.72 b
clothianidin e	1.67±1.67 c	8.33±3.07 de	16.67±3.33 de	35.00±2.24 d	50.00±3.65 b
fenoxycarb d	3.33±2.11 c	16.67±2.11 cde	21.67±3.07 cd	35.00±2.24 d	61.67±4.77 b
pyrifluquinazon cd	10.00±3.65 bc	20.00±2.58 bcd	23.33±2.11 cd	38.33±3.07 d	60.00±3.65 b
pyriproxyfen b	13.33±3.33* bc	35.00±4.28 ab	45.00±5.00 ab	63.33±5.58 c	98.33±1.67 a
spirotetramat a	21.67±4.01 ab	40.00±5.16 a	51.67±4.01 a	100.00±0.00 a	nd
sulfoxaflor a	28.33±7.03 a	43.33±4.94 a	53.33±2.11 a	100.00±0.00 a	nd
thiamethoxam b	16.67±6.15 abc	31.67±3.07 abc	43.33±4.22 ab	81.67±3.07 b	91.67±4.01 a
control f	0.00±0.00 c	1.67±1.67 e	3.33±2.11 e	8.33±1.67 e	11.67±1.67 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 treatment comparison and one-way ANOVA for comparison of treatments at each time interval; Tukey's HSD at α = 0.05). nd = not determined.*

Table 4: *Median lethal time (LT_{50}) values of selective novel chemistry insecticides bioassayed against 2nd instar nymphs of mealybug Drosicha mangiferae Green.*

Treatment	LT_{50} (hr)	Lower and Upper 95% Fiducial Limits (hr)	X^2 (df = 28)*	P
acetamiprid	76.78	70.39 – 84.79	122.36	< 0.001
buprofezin	78.22	68.13 – 93.21	146.48	< 0.001
clothianidin	93.08	85.80 – 102.85	130.60	< 0.001
fenoxycarb	84.86	78.12 – 93.58	127.70	< 0.001
pyrifluquinazon	85.28	77.71 – 95.40	108.51	< 0.001
pyriproxyfen	48.10	42.13 – 54.12	264.76	< 0.001
spirotetramat	34.42	29.75 – 38.97	255.02	< 0.001
sulfoxaflor	31.67	26.08 – 36.82	293.94	< 0.001
thiamethoxam	45.84	40.23 – 51.38	232.17	< 0.001

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

and spirotetramat (40.00±5.16%) caused maximum mortality of mealybug individuals, followed by pyriproxyfen (35.00±4.28%), while clothianidin (8.33±3.07%) caused minimum mortality followed by acetamiprid (18.33±3.07%). Similar trend of mortality was observed after 24 h of exposure. According to the observation at 48 h, again sulfoxaflor and spirotetramat exhibited 100% mortality of mealybugs, followed by thiamethoxam, pyriproxyfen and buprofezin. Similarly, pyriproxyfen and thiamethoxam caused maximum mortality at 72 h post-exposure followed by acetamiprid and fenoxycarb (Table 3).

Moreover, median lethal time (LT_{50}) values showed similar trend of effectiveness of novel chemistry insecticides against 2nd instar *D. mangiferae* mealybugs. Probit analysis revealed that most effective insecticides were sulfoxaflor, spirotetramat, thiamethoxam and pyriproxyfen with LT_{50} values of 31.67 h (26.08–36.82), 34.42 h (29.75–38.97), 45.84 h (40.23–51.38) and 48.10 h (42.13–54.12), respectively (Table 4). On the contrary, maximum LT_{50} values were recorded for clothianidin (93.08 h), pyrifluquinazon (85.28 h) and fenoxycarb (84.86 h; Table 4).

Mealybug *D. mangiferae* is one of the economic insect pests of citrus, mango and other horticultural and agricultural crops. It has been a hard-to-control pest because of its body covering with impervious powdery material and mouthparts obscured on the ventral side of cephalothorax region (Chaudhari, 2012; Mani and Shivaraju, 2016). Consequently, farmers practice excessive and irrational sprays of highly toxic and persistent insecticides with unsatisfactory control (Saeed et al., 2007; Arshad et al., 2015). The present study was aimed to evaluate the toxicity of some

novel insecticides having differential chemistry than conventional insecticidal groups against *D. mangiferae*.

Study results revealed that the most effective novel chemistry insecticides against 2nd instar mealybug nymphs were sulfoxaflor and spirotetramat, followed by pyriproxyfen, an insect growth regulator and thiamethoxam, a neonicotinoid. Both former formulations caused 100% mortality of mealybug nymphs till 48 h or application. Our results are in line with those of previous studies (Afzal et al., 2018). Sulfoxaflor and spirotetramat have been shown to exhibit significant mortality of different mealybug species such as against pink hibiscus mealybug *Maconellicoccus hirsutus* (Ganjisaffar et al., 2019) under laboratory conditions and against cotton mealybug *Phenacoccus solenopsis* under semi-field and field conditions (Dhawan et al., 2009; Lysandrou et al., 2012). In addition, some patent studies have recommended the application of sulfoxaflor in combination with chlorpyrifos (Yadav et al., 2014; Satar et al., 2018) and spirotetramat in combination with imidacloprid (Rizvi et al., 2017) against most of the mealybug species of economic importance. Moreover, our results are consistent with the findings of some recent studies who demonstrated the efficacy of spirotetramat against Asian citrus psyllids, *Diaphorina citri* under laboratory (Fiaz et al., 2017) and field conditions (Fiaz et al., 2018).

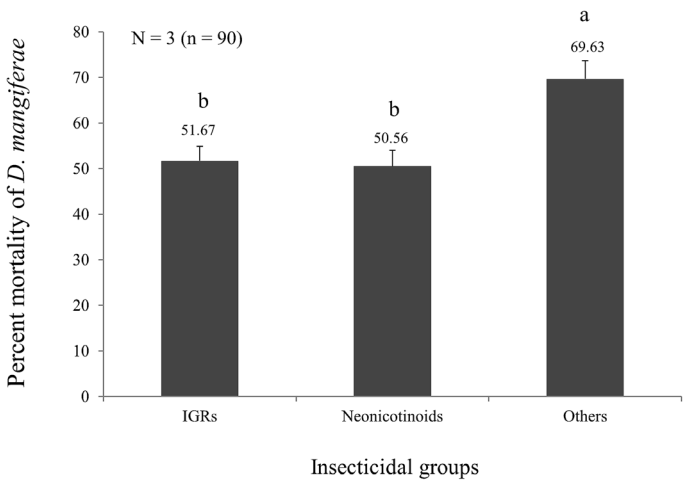

Figure 1: *Mean percent mortality of 2nd instar nymphs of mealybug Drosicha mangiferae exposed to different novel chemistry insecticidal groups. Alphabets over columns indicate statistical difference among treatments (one-way ANOVA; LSD at α = 0.05).*

Nevertheless, if we have a look on the average mortality data pooled for different common groups of novel chemistry insecticides (Figure 1), it can be seen that insect growth regulators (IGR) and neonicotinoid formulations exhibited similar level of toxicity significantly lower ($F_{2,267} = 8.63$, $P = 0.002$) than the average mealybug mortality showed by other three insecticides combined (*i.e.* sulfoxaflor, spirotetramat and pyrifluquinazon). It might be due to more recent entry of sulfoxaflor, spirotetramat and pyrifluquinazon formulations in Pakistan against sucking insect pests than later chemicals. Reduced mortality response of *D. mangiferae* mealybug nymphs to IGR and neonicotinoid formulations would be due to field evolved resistance detected in indigenous populations of *D. mangiferae* mealybugs as manifested by cotton *P. solenopsis* mealybug populations (Afzal et al., 2015; Saddiq et al., 2015; Venkatesan et al., 2016).

Conclusions and Recommendations

Based on the findings of this laboratory evaluation, novel chemistry insecticidal formulations of sulfoxaflor and spirotetramat are recommended to the indigenous citrus growers for an effective chemical control of *D. mangiferae*. However, do these novel chemistry insecticides exhibit lethal or sublethal effects on the prevailing natural enemies of mealybugs, as manifested by Mansour et al. (2011) in vineyards and Sahito et al. (2011) in cotton crop, are yet to be investigated and may comprise future perspective of this study.

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Author's Contributions

HAG and MZM conceived and designed the experimental protocol. HAG performed the experiments. MZM and ML performed statistical analyses. HAG prepared the first draft of manuscript. MZM techincally revised the manuscript. MA provided technical assistance in the experimentation.

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