Research Article

Laboratory Evaluation of Selected Differential Chemistry Synthetic Insecticides against some Economically Important Insect Pests

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Abstract | Contemporary non-target effects of conventional synthetic insecticides necessitate evaluating biorational insecticides owing differential chemistry and modes of action than the conventional ones. This laboratory study comparatively evaluated some selected differential chemistry insecticides against some destructive and economically important insect pests *i.e.* mango mealybug (*Drosicha mangiferae* Green), mango leafhopper (*Idioscopus clypealis* Lethierry), Asian citrus psyllid (*Diaphorina citri* Kuwayama) and subterranean termites (*Odontotermes obesus* Rambur). Standard twig- and filter paper-dip bioassay methods were used against sap-feeding pests and termites, respectively. Factorial analyses of variance revealed a significant impact of tested insecticidal formulations and exposure time on the mortality all insect pest individuals. The most effective differential chemistry insecticides against mango leafhopper, citrus psyllids, mango mealybugs and subterranean termites were nitenpyram, clothianidin, thiamethoxam and imidacloprid (causing 74–79% cumulative corrected mortality), thiamethoxam, imidacloprid, clothianidin and spirotetramat (causing 78–85% cumulative mortality), spirotetramat, acetamiprid and thiamethoxam (causing 68–90% cumulative mortality), and chlorantraniliprole, pyriproxyfen and chlorfenapyr (causing 80–85% cumulative mortality), respectively. Overall study findings suggest these above mentioned non-conventional insecticides to be incorporated in the biorational management of these insect pests.

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Introduction

Insect pests have always been a ubiquitous threat to global food production. These invertebrates cause untold qualitative and quantitative damage to various agricultural and horticultural crops all over the world including India and Pakistan (Arif *et al*., 2018; Gundappa and Shukla, 2018). Almost all field and forage, fruit and vegetable and forest and ornamental crops are attacked by different sucking and chewing insect pests. For instance, mango mealybug (*Drosicha mangiferae* Green), mango leafhopper (*Idioscopus clypealis* Lethierry), Asian citrus psyllid (*Diaphorina citri* Kuwayama) and subterranean termites (*Odontotermes* *obesus* Rambur) are among the most destructive insect pest species and are of great economic importance under agro-climatic conditions of Pakistan.

Mango mealybug (*D. mangiferae*) is a polyphagous species attacking a wide range of horticultural and agricultural plants. Since last few decades, this mealybug species has acquired a status of regular pest of many fruit crops including citrus and mango in Pakistan and causes considerable direct and indirect losses to these crops (Tahir *et al*., 2015; Mirbahar *et al*., 2017; Ghafoor *et al*., 2020). Mango leafhopper (*I. clypealis*) is also a major sap-sucking insect pest and poses a great menace to mango crop in the country (Gundappa and Shukla, 2016; Karar and Bakhsh, 2018). Adults and nymphs of mango leafhopper infest gregariously at the flowering stage and cause considerable economic damage to mango crop each year (Jha *et al*., 2017; Karar and Bakhush, 2018).

Similarly, Asian citrus psyllid (*D. citri*) has been the challenging pest of citrus all over the world including Pakistan. Desaping of plants by both adults and nymphs of this species cause severe losses to citrus crop (Mahmood *et al*., 2014). Apart from direct sap-feeding, this pest is a notorious vector of citrus greening, a destructive disease of citrus orchards (Teixeira *et al*., 2005; Razi *et al*., 2014; Canale *et al*., 2017). Similarly, subterranean termites (*O. obesus* and other species) infest a number of agricultural, horticultural and forestry crops and damage wooden works in agricultural and urban settings (Manzoor *et al*., 2012; Majeed, 2012; Ahmed *et al*., 2013). They attack and damage many agricultural and fruit crops including gram, sesame, maize, cotton, wheat, sugarcane, citrus and mango (Iqbal and Saeed, 2013; Rasib *et al*., 2017).

In order to combat and suppress these insect pests' infestations, farmers in Pakistan rely exclusively on highly toxic and persistent conventional synthetic pesticides (Ahmed *et al*., 2006; Akbar *et al*., 2010; Tiwari *et al*., 2011; Manzoor *et al*., 2012; Gulzar *et al*., 2015; Ghafoor *et al*., 2020). Extensive and repeated use of these conventional synthetic insecticides is posing many non-target effects on the human health and environment (Edwards, 2013; Chowański *et al*., 2014; Nicolopoulou-Stamati *et al*., 2016). Moreover, many of the field populations of mango leafhopper, mealybugs, citrus psyllids and subterranean termites have been demonstrated to exhibit resistance against these conventional insecticides (Tiwari *et al*., 2011; Tong *et al*., 2013; Afzal *et al*., 2015; Naeem *et al*., 2016; Venkatesan *et al*., 2016; Mahapatro, 2017).

Although synthetic insecticides have been unavoidable plant protection option to ensure sustained agricultural production, it is imperative to search for novel insect pest management approaches which would be biorational and safer than the conventional insecticides. For instance, there are many soft insecticides available in the market since last few decades which have a different mode of action and chemistry than the conventional ones, and are more target specific, less toxic to non-target fauna and are usually quickly biodegradable (Ishaaya and Degheele, 2013; Oberemok *et al*., 2015; Singh *et al*., 2016). This laboratory work determined the comparative effectiveness of some available insecticidal formulations with differential chemistry against above mentioned insect pests using standard laboratory bioassay methods.

Materials and Methods

Culture of insect pests

Mango leafhopper (*I. clypealis*) adults were collected from a mango orchard of Faisalabad (73°4'44.79" E; 31°25'7.37" N) using aerial nets. Adults of female mealybugs (*D. mangiferae*) and Asian citrus psyllids were collected manually and by aspirator, respectively, from a kinnow mandarin orchard (*Citrus reticulata*) situated nearby College of Agriculture, University of Sargodha (72°41'E; 32°08'N). Subterranean termites (*O. obesus*) were collected along with their intact nest portions from the stubbles of sugarcane crop (72°45'E; 32°11'N) situated at the farm area of the College of Agriculture. These collected insects were carried under cool conditions to the laboratory and were maintained and reared up to F_2 or F_3 generations on their respective food sources separately in insect rearing (Bugdorm®) cages (2×3×1.5 ft.) at 27±2°C temperature and 65±5% relative humidity. In all toxicity bioassays, only healthy and active insect specimens were used.

Insecticides procurement

Based on literature and survey of pesticides dealers in the grain markets of district Sargodha (Punjab, Pakistan), six contact and nine systemic synthetic insecticide formulations with differential chemistry (other than conventional insecticide groups) were selected. Commercial formulations of selected synthetic insecticides with differential chemistry were procured from authenticated pesticide retailers. Details regarding the brand and company names, mode of action, chemical group and family and label recommended dose rates of these selected insecticides are given in Table 1.

Bioassays

A total of nine and six synesthetic insecticides (including neonicotinoids, insect growth regulators and others) were evaluated against sucking insect pests and termites, respectively. Standard leaf- and twig-dip bioassay methods were followed for mango leafhopper, citrus psyllids and mealybugs, respectively as described previously (Majeed *et al*., 2020). For termites, standard filter paper-dip method was used. In brief, for sucking insect pests, 5 cm long unsprayed twig tips of citrus (*C. reticulata*) or mango (*Mangifera indica*) plants were collected, rinsed thoroughly using tap-water and then were allowed to be drained at 28°C (prevailing room temperature). For termites, 9 cm filter paper discs were used. These twigs and filter papers were dipped in insecticide solutions prepared according to their label recommended dose rates. Control treatment received clean tap-water which was used for preparing the insecticide solutions. Ten healthy and active insect specimens (nymphs or adults as per insect pest species) were placed on the treated plant twigs or filter paper discs lined in Petri-plates (Ø = 90 mm). These Petri-plates were stacked in plastic laboratory trays and were incubated within a climate chamber set at 65±3% relative humidity, 27±2°C temperature and at 8h: 16h dark–light photoperiod. The mortality data of exposed insect specimens were recorded at regular time intervals *i.e.* at 6, 12, 24, 48 and 72 h post-treatment. Experimental design for all toxicity bioassays was completely randomized (CRD) and each treatment was replicated six times.

Statistical analyses

Statistical interpretation of the bioassays' data was done using Statistix® Version 8.1 (Analytical Software, Tallahassee, FL) was employed for the. Before statistical analysis, percent mortality data of all four insect species were corrected using Abbott's formula. In order to determine the significant effect of tested insecticidal formulations on insect pests, corrected mortality data were analyzed by factorial ANOVA (analysis of variance) taking exposure time and insecticides as factors. Furthermore, Tukey's HSD (honestly significant difference) post-hoc test was used to compare the treatment means at standard probability level ($P \geq 0.05$).

Results and Disussion

This laboratory study determined the comparative toxicity of different contemporary differential chemistry synthetic and contact insecticide formulations against four destructive and economically important insect pests *i.e.* mango leafhopper (*I. clypealis*), Asian citrus psyllid (*D. citri*), mango mealybug (*D. mangiferae*) and subterranean termites (*O. obesus*) using standard *in-vitro* bioassay methods.

Mortality of mango leafhopper by synthetic insecticides

Factorial analysis of variance subjected to mortality percentage of mango leafhopper adults bioassayed against different selected systemic synthetic insecticides showed a significant impact of both insecticidal treatments ($F_{(8, 215)} = 41.64$, $P < 0.001$), time factors ($F_{(3,215)} = 76.56$, $P < 0.01$), and their interaction ($F_{(24, 215)} = 3.99$, $P < 0.001$) on the leafhoppers' mortality response (Table 2). A significant mortality of the exposed leafhopper individuals was recorded for all insecticidal treatments as compared to control treatment. Maximum mortality observed at 48 h post-exposure was exhibited by nitenpyram (78.67%), followed by clothianidin, thiamethoxam, imidacloprid and pymetrozine (74.68% each), while the insecticides buprofezin (24.25%) and acetamiprid (56.67%) were least toxic against leafhoppers (Figure 1).

Mortality response of Asian citrus psyllid against tested insecticides

According to factorial ANOVA and Tukey HSD test, there was a significant effect of both insecticidal treatments ($F_{(8, 215)} = 95.66$, $P < 0.001$) and time ($F_{(3,215)} = 336.86$, $P < 0.05$) factors and their interaction ($F_{(24, 215)} = 10.40$, $P < 0.001$) on the mortality response of psyllids (Table 2). At 48 h of bioassay, the most toxic differential chemistry systemic insecticides against psyllids were thiamethoxam, imidacloprid, clothianidin and spirotetramat causing up to 85.10% mortality. While the insecticides flonicamid, buprofezin and pymetrozine exhibited minimum psyllid mortality (up to 31.67%; Figure 2).

Toxicity of tested insecticides against mango mealybug

In case of toxicity bioassay against 2nd instar mealybug individuals, again both factors *i.e.* insecticidal treatments ($F_{(8, 215)} = 70.88$, $P < 0.001$) and time ($F_{(3, 215)} = 267.76$, $P < 0.05$) and their interaction ($F_{(24, 215)} = 9.61$, $P < 0.001$) showed statistically considerable impact on the mortality response of mealybug

Table 1: *Different selected differential chemistry synthetic insecticides bioassayed against some insect pests of economic importance under laboratory conditions.*

Chemical Name (active ingredient)	Chemical family*	Mode of Action	Brand Name	Company	Label Dose ($acre^{-1}$)
abamectin	6 (avermectins)	Glutamate-gated chloride channel (GluCl) allosteric modulators	Alarm Plus® 3.5 EC	Ali Akbar Chemicals	200 ml
acetamiprid	4A (neonicotinoids)	Nicotinic acetylcholine receptor (nAChR) allosteric modulator	Acelan® 20 SP	FMC	125 g
buprofezin	16 (buprofezin)	Chitin biosynthesis inhibitor (IGR)	Sitara® 25WP	Ali Akbar Chemicals	500 g
chlorantraniliprole	28 (diamides)	Ryanodine receptor modulators	Coragen® 20 SC	FMC	50 ml
chlorfenapyr	13 (pyrroles)	Uncouplers of oxidative phosphorylation via disruption of the proton gradient	Foxal® 36 SC	Tara Imperial Industries	100 ml
clothianidin	4A (neonicotinoids)	Nicotinic acetylcholine receptor (nAChR) allosteric modulator	Clutch® 50 WDG	Joshi AgroChemicals	210 g
flonicamid	29 (flonicamid)	Chordotonal organ Modulators - undefined target site	Ulala® 50 WG	ICI Pakistan	60 g
imidacloprid	4A (neonicotinoids)	Nicotinic acetylcholine receptor (nAChR) competitive modulators	Confidor® 200 SL	Bayer Pakistan	50 ml
indoxacarb	22A (oxadiazines)	Voltage-dependent sodium channel blockers	Steward® 150 EC	FMC	175 ml
nitenpyram	4A (neonicotinoids)	Nicotinic acetylcholine receptor (nAChR) competitive modulators	Hurry Up® 10 AS	Ali Akbar Chemicals	200 ml
pymetrozine	9B (pyridine azomethine derivatives)	Chordotonal organ TRPV channel modulators	Anpon Super 25 WP	Sun Crop Pesticides	300 g
pyriproxyfen	7C (pyriproxyfens)	Juvenile hormone mimics (IGR)	Admiral® 10EC	FMC	75 ml
spinetoram	5 (spinosyns)	Nicotinic acetylcholine receptor (nAChR) allosteric modulators – Site I	Delegate® 25 WG	Ali Akbar Chemcials	60 g
spirotetramat	23 (tetramic acid derivatives)	Acetylcholinesterase (AChE) inhibitor	Movento® 240 SC	Bayer CropScience	800 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 9.4, March 2020.*

Table 2: *Analysis of variance comparison of the mortality response of different insect pests bioassayed against some selected differential chemistry insecticides.*

		Leafhoppers			Psyllids			Mealybugs			Termites			
Source	DF	MS	F-value	P-value	MS	F-value	P-value	MS	F-value	P-value	DF	MS	F-value	P-value
Treatment	8	5573.0	41.64	≥ 0.001	6084.8	95.66	≥ 0.001	5421.3	70.88	≥ 0.001	5	8479.6	135.79	≥ 0.001
Time	3	10246.3	76.56	≥ 0.01	21428.0	336.86	≥ 0.05	20478.6	267.76	≥ 0.05	4	17244.9	276.16	≥ 0.05
Treatment × Time	24	534.5	3.99	≥ 0.001	661.8	10.40	≥ 0.001	735.0	9.61	≥ 0.001	20	394.6	6.32	≥ 0.001
Error	180	134.8			63.6			76.5			150	62.4		
Total	215										179			
GM / CV		51.86 / 22.31			39.21 / 20.34			28.29 / 30.92				34.22 / 23.09		

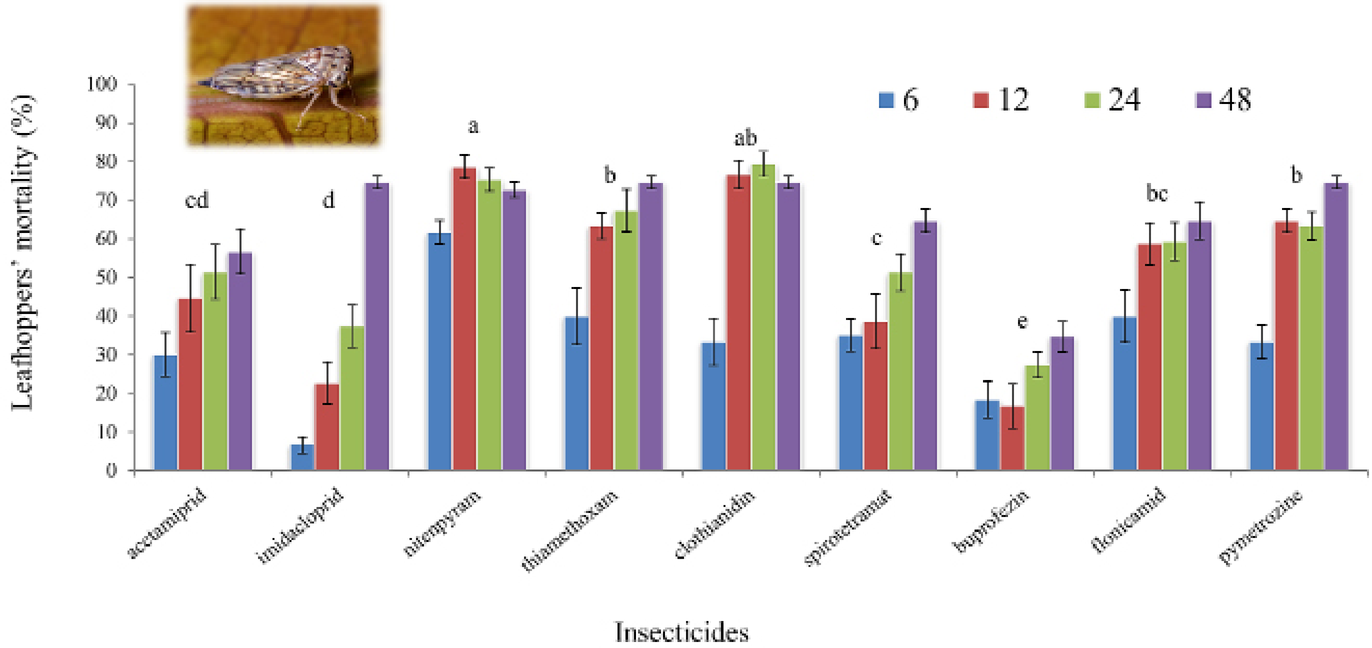

Figure 1: *Percent corrected mortality (mean ± S.E.) of adult individuals of mango leafhopper (Idioscopus clypealis) bioassayed against different differential-chemistry synthetic insecticides at their label recommended dose rates. Different letters indicate overall significant difference among the insecticidal treatments (two-factor factorial analysis of variance followed by HSD test at P ≥ 0.05).*

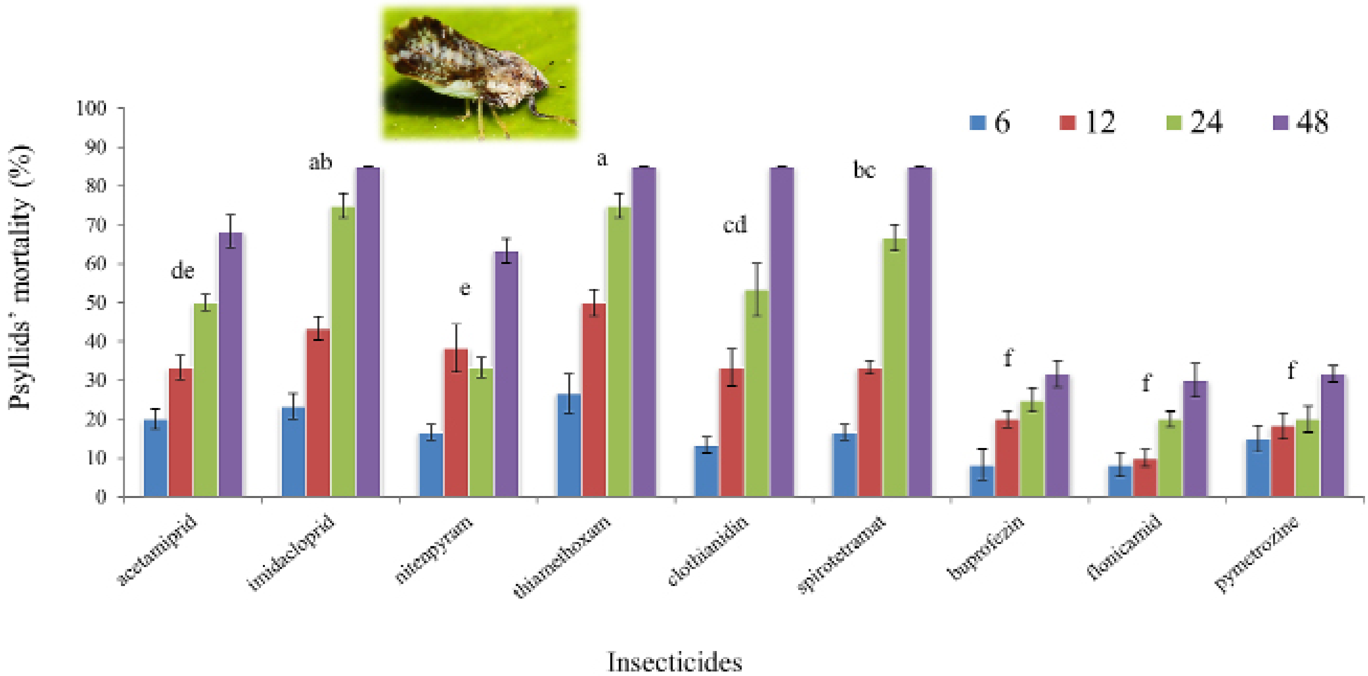

Figure 2: *Percent corrected mortality (mean ± S.E.) of adult individuals of Asian citrus psyllid (Diaphorina citri) bioassayed against different differential-chemistry synthetic insecticides at their label recommended dose rates. Different letters indicate overall significant difference among the insecticidal treatments (two-factor factorial analysis of variance followed by HSD test at P ≥ 0.05).*

individuals (Table 2). Maximum mealybug mortality was caused by the insecticides spirotetramat (90.00%) and acetamiprid (86.67%), followed by thiamethoxam (68.33%) and imidacloprid (58.33%), while insecticides flonicamid, buprofezin, nitenpyram and pymetrozine were the least effective insecticides against mealybug individuals causing 17 – 42% mortality recorded at 72 h post-exposure (Figure 3).

Effect of insecticides on subterranean termites

Bioassay conducted against *O. obesus* termite workers revealed a similar trend of insecticidal toxicity. According to ANOVA, both factors *i.e.* insecticides ($F_{(5, 179)}$ = 135.79, P < 0.001) and time ($F_{(4, 179)}$ = 276.16, P < 0.05), and the interaction of both these factors ($F_{(20, 179)}$ = 6.32, P < 0.001) exerted a significant impact on the average percent mortality of termite individuals

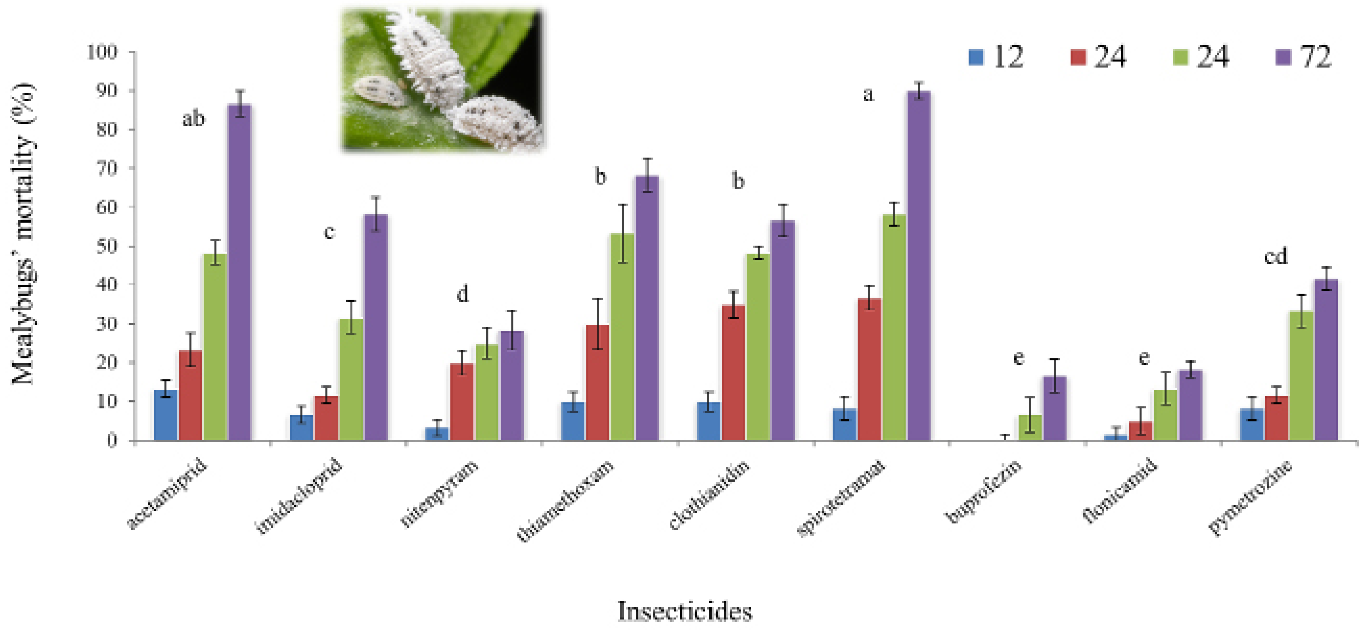

Figure 3: *Percent corrected mortality (mean ± S.E.) of 3rd instar female individuals of mango mealybug (Drosicha mangiferae) bioassayed against different differential-chemistry synthetic insecticides at their label recommended dose rates. Different letters indicate overall significant difference among the insecticidal treatments (two-factor factorial analysis of variance followed by HSD test at P ≥ 0.05).*

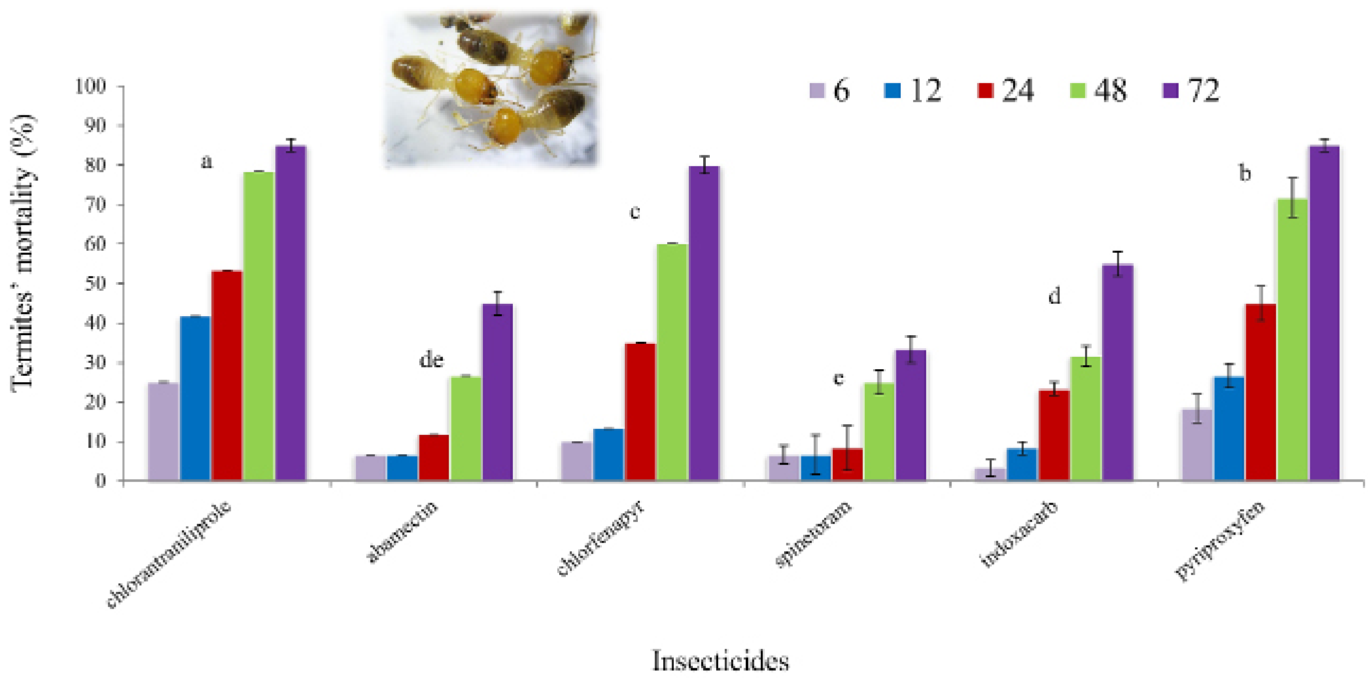

Figure 4: *Percent corrected mortality (mean ± S.E.) of worker individuals of subterranean termites (Odontotermes obesus) bioassayed against different differential-chemistry synthetic insecticides at their label recommended dose rates. Different letters indicate overall significant difference among the insecticidal treatments (two-factor factorial analysis of variance followed by HSD test at P ≥ 0.05).*

(Table 2). Most effective and toxic synthetic insecticides appeared against *O. obesus* termites were chlorantraniliprole and pyriproxyfen each causing 85.00% average mortality observed at 72 h of bioassay, followed by chlorfenapyr (80%). While the insecticides spinetoram (33.33%) and abamectin (45.20%) were least effective against *O. obesus* termite workers (Figure 4).

This laboratory work compared the effectiveness of nine contemporary differential chemistry systemic insecticides against mango leafhopper (*I. clypealis*), mealybugs (*D. mangiferae*) and citrus psyllids (*D. citri*) and six contact insecticides against subterranean termites (*O. obesus*). Bioassay results revealed that the insecticides nitenpyram, clothianidin, thiamethoxam and imidacloprid were most effective against mango

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leafhopper (*I. clypealis*) which exhibited about 74 – 79% cumulative mortality of leafhopper adults within 48 h of exposure. These findings substantiate the findings of Qureshi *et al.* (2011), Kaushik *et al.* (2014) and Kumar *et al.* (2019) showing the effectiveness of imidacloprid and thiamethoxam against leafhoppers and other sap-sucking insect pests of mango trees. Neonicotinoids are systemic insecticides which have always been effective chemical tools against different species of sap-feeding insect pests including *I. clypealis* (Elbert *et al.*, 2008).

Similarly, most effective differential chemistry insecticides against Asian citrus psyllids (*D. citri*) were thiamethoxam, imidacloprid, clothianidin and spirotetramat causing 78 – 85% mortality of psyllids in 48 h of bioassay. These results are consistent with the findings of some previous works (Stansly and Qureshi, 2007; Khan *et al.*, 2013; Byrne *et al.*, 2017; Fiaz *et al.*, 2018a). These insecticides have been shown effective under field conditions against *D. citri* and many other sap-feeding insect pests (Nazir *et al.*, 2017; Fiaz *et al.*, 2018b; Ghafoor *et al.*, 2019; Iqbal *et al.*, 2020).

In mealybug bioassay, insecticides spirotetramat, acetamiprid and thiamethoxam were found as the most effective against 2nd instar mealybug nymphs causing 68 – 90% average mortality in 72 h. These results are consistent with the those of Ghafoor *et al.* (2019) which demonstrated about 62 and 53% mortality of 2nd instar mealybug nymphs by spirotetramat and thiamethoxam, respectively. These insecticides appeared also effective against mealybugs under the field conditions (Ghafoor *et al.*, 2020). Apart from *D. mangiferae*, spirotetramat insecticide was found effective against other mealybug species such as against pink hibiscus mealybug (*Maconellicoccus hirsutus*) and cotton mealybug (*Phenacoccus solenopsis*) under field and laboratory settings (Dhawan *et al.*, 2009; Ganjisaffar *et al.*, 2019; Sequeira *et al.*, 2020).

Against subterranean termites (*O. obesus*), chlorantraniliprole, pyriproxyfen and chlorfenapyr were the most effective insecticides causing 80 – 85% mortality of termite workers in 72 h of exposure. These results are consistent with some previous studies showing effectiveness of chlorantraniliprole, chlorfenapyr and pyriproxyfen against different species of subterranean termites (Spomer *et al.*, 2009; Mao *et al.*, 2011; Manzoor *et al.*, 2012; Neoh *et al.*, 2012; Ma and Sui, 2013; Du *et al.*, 2020). Moreover, our study findings substantiate the outcomes of a research work done by Akbar *et al.* (2019) which demonstrated 100% mortality of *O. obesus* termite workers by chlorantraniliprole, chlorfenapyr and pyriproxyfen within 48 h of exposure. Moreover, these three differential chemistry insecticides were also found comparatively effective against subterranean termite infestations under field conditions (Jones *et al.*, 2017; Du *et al.*, 2020; Akbar *et al.*, 2021).

Conclusions and Recommendations

It is concluded from the overall study results that neonicotinoid insecticides thiamethoxam, imidacloprid, clothianidin, nitenpyram, and tetramic acid-based insecticide spirotetramat were the most effective systemic insecticides against mango leafhoppers (*I. clypealis*), mealybugs (*D. mangiferae*) and citrus psyllids (*D. citri*), while the insecticides chlorantraniliprole, pyriproxyfen and chlorfenapyr were most effective against subterranean termites (*O. obesus*). Hence, these above mentioned biorational non-conventional and differential chemistry insecticides are recommended to the local farmers combating infestations of these insect pests.

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Novelty Statement

Among prevailing non-conventional differential chemistry synthetic insecticides, neonicotinoids (thiamethoxam, imidacloprid, clothianidin, nitenpyram) and spirotetramat were effective against mango leafhoppers, mealybugs and citrus psyllids, while the insecticides chlorantraniliprole, pyriproxyfen and chlorfenapyr were most effective against subterranean termites, and hence are recommended to the local farmers combating infestations of these insect pests in their crops.

Author's Contributions

Muhammad Zeeshan Majeed: Conceived the reseach idea, designed the experiments, prepared results and wrote the initial manuscript draft.

Muhammad Qasim, Gulfam Yousaf, Hamza Latif and Muhammad Zeeshan: Conducted the bioassays and recorded data.

Muhammad Luqman and Muhammad Irfan Ullah: Did the statistical analyses of data.

Dilbar Hussain: Revised and proofread the final draft.

Muhammad Zeeshan Majeed and Muhammad Irfan Ullah: Gave the technical support for experiments.

Conflict of interest

The authors have declared no conflict of interest.

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