Research Article

Field Evaluation of Selected Botanical and Synthetic Insecticides against Mealybug *Drosicha mangiferae* Green (Hemiptera: Pseudococcidae) Infesting Citrus Orchards in Pakistan

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Abstract | Mealybug *Drosicha mangiferae* Green (Pseudococcidae: Hemiptera) is a destructive sap-feeding pest of a number of horticultural and agricultural crops in Indo-Pak region. It has been an emerging threat to citrus industry in Pakistan and is considered as hard-to-control pest. This study evaluated some selected pest control options against the infestation of *D. mangiferae* under field conditions. The treatments included some most effective conventional and novel chemistry insecticides, botanicals and cultural control practice (*i.e.* two under-canopy deep hoeings with 15 days interval along with lower-branch pruning) alone or in combination with the entomopathogenic fungus *Beauveria bassiana* (@ $1x10^8$ conidia mL^{-1}). First experiment was conducted in March 2016 and 2nd was performed from November 2016 to March 2017 in a citrus (*Citrus reticulata* cv. kinnow mandarin) orchard. In first year field experiment, there was a significant impact of all treatments on the reduction of mealybug infestation ($F_{9, 39}$ = 39.10, P < 0.001; HSD at α = 0.05) as compared to control plants. Results of 2nd experiment also clearly demonstrated a significant impact of different treatments on the mealybug infestation ($F_{12, 103}$ = 58.75, P < 0.001; HSD at α = 0.05) as compared to control. At both 3 and 7 days post-treatment, maximum reduction of mealybug infestation was recorded in plots treated with spirotetramat (87.75±3.91%) and lambda-cyhalothrin (85.52±4.42%) in combination with EPF followed by these two insecticides and sulfoxaflor alone. Based on overall study results, the insecticidal formulations of spirotetramat, lambda-cyhalothrin and sulfoxaflor in combination with EPF and under-canopy hoeing are recommended to the local citrus growers for an effective control of *D. mangiferae* infestation.

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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 twigs, shoots, stems, leaves, aerial roots, panicles, trunks, spurs and from the 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 different agricultural crops including citrus, mango, banana, grape, tomato, okra and cotton etc. (Williams and Willink, 1992; Sirisena et al., 2013).

Mealybug *Drosicha mangiferae* Green, commonly known as mango mealybug, is among the most damaging and widespread species in Indo-Pak regions (Gundappa et al., 2018). Besides mango, this mealybug attacks on many other horticultural and agricultural crops. For example, citrus is a major fruit crop of Pakistan being primarily produced in Sargodha region (Ahmad et al., 2018) and *D. mangiferae* has become a regular pest status of citrus orchards in Sargodha and cause substantial qualitative and quantitative damage to citrus fruit production (Tahir et al., 2015; Afzal et al., 2018). This pest has been difficult to manage with most of the routine conventional synthetic insecticides because of its obscured mouthparts and impervious scales present on dorsal side of its body (Chaudhari, 2012; Mani and Shivaraju, 2016). Consequently, farmers mostly apply heavy and repeated sprays of insecticides (Aheer et al., 2009; Gulzar et al., 2015) causing environmental contamination and health hazards (Edwards, 2013; Nicolopoulou-Stamati et al., 2016).

In view of aforementioned situation, it is necessary to screen out the most effective synthetic insecticides being currently used by the local farmers against mealybugs and to seek out other biorational pest control strategies such as microbial and botanical pesticides. This study was therefore aimed to comparatively compare different laboratory selected most effective synthetic conventional and novel chemistry insecticidal formulations and different botanical extracts for their effectiveness against *D. mealybug* infestations in citrus orchards under field conditions. Moreover, compatibility of the most effective treatments was also determined along with microbial formulation (*Beauveria bassiana*) and cultural practice (hoeing and branch pruning) which can be effectively used against this destructive insect pest.

Materials and Methods

Experimental site

Two field experiments were conducted to evaluate the effectiveness of different control options against *D. mangiferae* infesting citrus plants. First experiment was conducted in March 2016 and 2[nd] was performed from November 2016 to March 2017. Both field experiments were conducted in a citrus (*Citrus reticulata* cv. kinnow mandarin) orchard located in the vicinity of the College of Agriculture, University of Sargodha (32°08'21"N; 72°40'11"E). The average annual temperature and rainfall of this area is 23.8°C and 410 mm, respectively (Zaka et al., 2004). Citrus is the main fruit crop of Sargodha region and encompasses more than 70% of country citrus production. Soil texture is sandy loam. The citrus orchard selected for the field experiments was severely infested by *D. mangiferae* and was ensured not to receive any pesticidal application for last 6 months prior to the experiment.

Treatments

Commercial formulations of conventional and novel chemistry insecticides (Table 1) were procured from the registered pesticide dealers of multinational companies from the local grain market of the Sargodha district. Formulation of entomopathogenic fungi (*Beauveria bassiana* RACER 1.15% WP; formulated from naturally occurring soil fungal strains ATCC 26851 and NCIM 1216) was purchased from AgriLife™, Hyderabad, India. Botanical extracts were prepared in the laboratory of Department of Entomology, College of Agriculture, University of Sargodha. In brief, different plant parts as described in Table 1 were collected from the vicinity of the College of Agriculture, University of Sargodha and were washed with clean tap-water and were air-dried at room temperature (26°C) for a week followed by grinding of plant material to course powder form using an electric blender. Extraction of botanicals was done through Soxhlet apparatus (Sigma-Aldrich, Germany) using 1:10 (w/v) methanol as extraction solvent. Plant essential oils were extracted by hydro-distillation using Clevenger-type apparatus.

Experimental protocol

In first field experiment, treatments included botanical extracts of *Azadirachta indica* (neem) and *Nerium indicum* (oleander) applied @ 20% v/v, essential oil of *Datura alba* (Dhatura) applied @ 2.0 % v/v, formulations of spirotetramat, sulfoxaflor, thiamethoxam, methidathion, lambda-cyhalothrin and deltamethrin applied according to their field-recommended dose rates (Table 1). Second field experiment contained the most effective treatments of 1[st] field experiment (*i.e. A. indica*, spirotetramat,

Table 1: *Different botanical extracts and conventional and novel chemistry insecticides evaluated against 2nd instar nymphs of mealybug Drosicha mangiferae Green under field conditions.*

Chemical name (active ingredient)	Chemical family*	Mode of action	Brand name	Company	Does (ha^{-1})
deltamethrin	3A (pyrethroids)	Sodium channel modulator	Decis® 10.5 EC	Bayer crop science	300 ml
lambda-cyhalothrin	3A (pyrethroids)	Sodium channel modulator	Karate® 2.5 EC	Syngenta	625 ml
methidathion	1B (organophosphates)	Acetylcholinesterase (AChE) inhibitor	Supracide® 400 EC	Syngenta	2500 ml
spirotetramat	23 (tetramic acid derivatives)	Acetylcholinesterase (AChE) inhibitor	Movento® 240 SC	Bayer crop science	800 ml
sulfoxaflor	4C (sulfoximines)	Nicotinic acetylcholine receptor (nAChR) allosteric modulator	Closer® 240 SC	Dow agro sciences	400 ml
thiamethoxam	4A (neonicotinoids)	Nicotinic acetylcholine receptor (nAChR) allosteric modulator	Actara® 25 WG	Syngenta	130 g
Botanical name	**Common/ Vernacular name**	**Family**	**Major bioactive constituents**	**Extraction type**	**Plant parts extracted**
Azadirachta indica	Neem	Meliaceae	Azadirechtins and triterpenoids (Benelli *et al.*, 2017)	Botanical extract	Leaves and fruits
Datura alba	Dhatura	Solanaceae	Tropane alkaloids (Moniraand Munan, 2012)	Essential oil	Leaves and seeds
Nerium indicum	Kaner (Oleander)	Apocynaceae	Oleandrin and oleandrigenin (Dodia *et al.*, 2010)	Botanical extract	Leaves

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

sulfoxaflor and lambda-cyhalothrin) and their combination with entomopathogenic fungus (*B. bassiana* @ 5 g L^{-1}) and manual hoeing (*i.e.* two under-canopy deep hoeings of soil with 15 days interval along with lower-branch pruning). The experiment was laid out in a randomized complete block design (RCBD). Each treatment was replicated independently and randomly in three blocks of the experiment. One row of orchard trees was left untreated in between two replication plots as buffer zone. Treatments were applied on 30 cm long infested apical twigs (4 per plant, one on each side of the plant) using knapsack sprayer. Data regarding number of *D. mangiferae* mealybugs (both adults and nymphs) was taken 1 day before and 3 and 7 days post-treatment and percent reduction in mealybug population was calculated.

Statistical analysis

Using Statistix® 8.1 (Analytical Software, 2005), data regarding percent reduction of mealybug population was subjected to factorial analysis of variance. Treatment means were compared using Tukey's honestly significant difference (HSD) test at 95% level of significance.

Results and Discussion

Two sets of field experiments were carried out in citrus (*C. reticulata;* mandarin orange) orchards to determine the effect of selective biorational control options on infestation of mealybug *D. mangiferae* infesting recurrently citrus plants in Sargodha region. Percent reduction in mealybug infestation was assessed to determine the effect of treatments. Results of first year field experiment showed that there was a significant impact of all treatments on the reduction of mealybug infestation ($F_{9,39}$ = 39.10, P < 0.001; HSD at α = 0.05). Infestation reduced significantly on all trees treated with botanical and synthetic insecticides as compared to control trees (Figure 1). In control plots, mealybug infestation increased by 10.96±2.28%. In case of treatments, maximum reduction was observed for spirotetramat (70.54±6.83%), lambda-cyhalothrin (69.01±3.21%) and sulfoxaflor (58.69±6.42%) without any statistical difference (Figure 1). Thiamethoxam, methidathion and deltamethrin showed intermediate response. Among botanical insecticides, the extract of *A. indica* caused maximum reduction (36.23±3.17%) of mealybug infestation followed by the essential oil of *D. alba* (23.89±1.40%), while the extract of *N. indicum* was the least effective with average percent reduction of 10.30±3.16% (Figure 1).

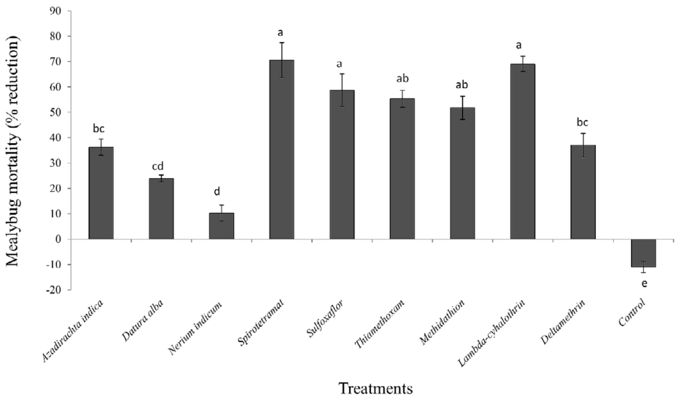

Figure 1: *Percent reduction (mean ± SE) in the population of mealybug Drosicha mangiferae Green on plants of Citrus reticulata (mandarin orange) in response to different botanical and synthetic insecticides observed at 3 days post-treatment. Alphabets at column tops indicate overall significant difference among treatments (GLM; Tukey HSD test at α = 0.05).*

Results of 2nd experiment which was performed using the most effective insecticidal and botanical treatments selected from 1st field experiment in integration with the application of entomopathogenic fungus (*B. bassiana*) and manual hoeing of under-canopy soil. Results of this experiment clearly demonstrated a significant impact of different treatments on the mealybug infestation as compared to control ($F_{12, 103}$ = 58.75, P < 0.001; HSD at α = 0.05). Moreover, observation time ($F_{1, 103}$ = 25.02, P < 0.001) and its interaction with treatments had also a significant effect ($F_{12, 103}$ = 2.39, p = 0.011) on mealybug infestation (Table 1). Nevertheless, at 3 days post-treatment, maximum reduction was exhibited by spirotetramat alone (71.00±4.38%) and in combination with EPF (67.97±6.10%) and by lambda-cyhalothrin plus EPF (70.03±9.79%) without any statistical difference. Minimum reduction was exhibited by the botanical extract of *A. indica* (30.53±2.93%) followed by its combined application with EPF (35.67±2.13%) and with hoeing (37.13±7.05%). Similarly, sulfoxaflor alone and along with EPF and hoeing and lambda-cyhalothrin alone and with hoeing showed intermediate but significant reduction of mealybug infestation as compared to control (Figure 2).

According to data of 2nd observation taken at 7 days post-treatment, maximum infestation reduction was exhibited by plots treated with spirotetramat (87.75±3.91%) and lambda-cyhalothrin (85.52±4.42%) in combination with EPF followed by these two synthetic insecticides alone (Figure 2). The extract of *A. indica* showed minimum infestation reduction (28.92±5.06%) followed by its combined application with hoeing (31.63±3.95%) and with EPF (57.36±2.94%). In control plots, mealybug infestation increased from 7.44% to 16.02% recorded at 3 and 7 days post-treatment, respectively (Figure 2).

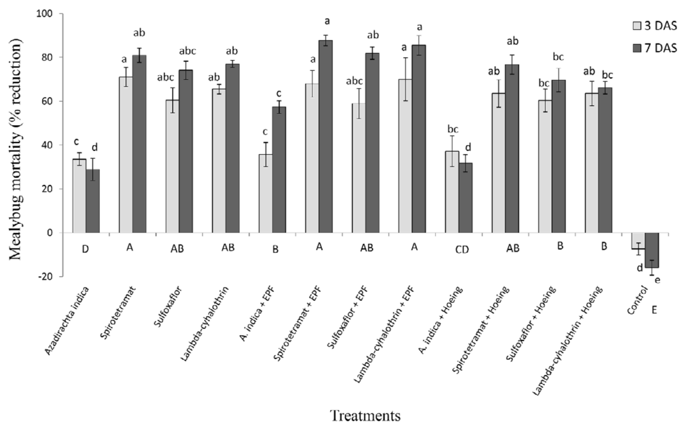

Figure 2: *Percent reduction (mean ± SE) in the population of mealybug Drosicha mangiferae Green on plants of Citrus reticulata (mandarin orange) in response to different pest control treatments observed at 3 days (light-grey columns) and 7 days (dark-grey columns) post-treatment. Small and capital alphabets indicate respectively the significant difference among treatments for each observation time (one-way ANOVA; Tukey HSD test at α = 0.05) and the overall significance among treatments (factorial ANOVA; Tukey HSD test at α = 0.05).*

Table 2: *Analysis of variance comparison table for mean reduction of mealybug Drosicha mangiferae Green population in response to different botanical and synthetic insecticides.*

Source	DF	SS	MS	F-value	P-value
DAS	1	2277.8	2277.76	25.02	<0.001
Treatments	12	64194.6	5349.55	58.75	<0.001
DAS * treatments	12	2613.3	217.78	2.39	0.0113
Error	75	6828.9	91.05		
Total	103	76423.4			
Grand Mean	56.99				
CV	16.74				

P < 0.001 (highly significant) and P < 0.01 (significant); two-way factorial ANOVA at α: 0.05. DAS: days after spray.

Mealybugs *D. mangiferae* is posing a severe threat to horticultural industry in Indo-Pak region. Farmers rely on repeated and over-dosed applications of conventional insecticides with unsatisfactory control of this pest. Although many previous studies have evaluated the toxicity of available synthetic, botanical and microbial insecticidal compounds against mealybugs (Karar et al., 2010; Kulkarni and Patil, 2013; Arshad et al., 2015; Dwivedi et al., 2018; Majeed et al., 2018), no study so far determined the field efficacy of these insecticidal compounds against *D. mangiferae* mealybug infestation. This study evaluated some most effective conventional and novel chemistry synthetic insecticides and botanicals selected from the preliminary laboratory bioassays First field trial was carried out with three most effective conventional, novel chemistry and botanical pesticides, while second trial incorporated the most effective treatments of first experiment alone or in combination with microbial formulation of *B. bassiana* and cultural (hoeing and pruning) practices.

Results of both field experiments revealed that there was a significant reduction in mealybug infestation by all treatments as compared to control. In first field trial, regarding synthetic insecticides, maximum mealybug infestation reduction was observed for spirotetramat and lambda-cyhalothrin, followed by sulfoxaflor, methidathion and thiamethoxam. Among botanical treatments, extract of *A. indica* was the most effective causing maximum and significant reduction of mealybug infestation followed by the essential oil of *D. alba*. In the second field trial, treatments and time intervals (days) exhibited a significant effect on the reduction of mealybug infestation. Most effective treatments in this trial were spirotetramat and lambda-cyhalothrin along with entomopathogenic fungi application, followed by these two synthetic insecticides alone.

Our results are consistent with those of Mansour et al. (2010) and Seni and Sahoo (2015) demonstrating that spirotetramat and lambda-cyhalothrin are effective insecticidal formulations against different mealybug infestations. Similarly, our results corroborate the findings of Majeed et al. (2018) who showed that extract of *A. indica* is most effective botanical against mealybug *D. mangiferae*. In addition, our results showed a synergistic effect of these synthetic insecticides along with *B. bassiana* formulation and cultural practices. Many previous studies have shown laboratory and field efficiency of *B. bassiana* against *D. mangiferae* individuals alone (Masarrat et al., 1998; Haseeb and Srivastava, 2003) or in combination with different synthetic insecticides (Andaló et al., 2004; Tanwar et al., 2007). Likewise, cultural operations such as deep soil hoeing/ploughing and pruning of tree lower braches appeared to be very effective for the integrated management of mealybug *D. mangiferae* infestations (Karar et al., 2010b; Bhau, 2012).

Conclusions and Recommendation

Based on overall findings of this field study, the insecticidal formulations of spirotetramat, lambda-cyhalothrin and sulfoxaflor in combination with EPF and under-canopy hoeing are recommended to the local citrus growers for an effective control of *D. mangiferae* infestation.

Author's Contributions

Muhammad Afzal and Muhammad Zeeshan Majeed conceived and designed the experimental protocols. Hafiz Abdul Ghafoor performed the experiments. Muhammad Luqman performed the statistical analyses. Hafiz Abdul Ghafoor prepared the first draft of manuscript. Muhammad Arshad Javed and Syed Wasim Hasan provided technical assistance in the field experiments and during data collection. Muhammad Zeeshan Majeed technically revised the manuscript. Muhammad Afzal provided technical assistance in the experimentation.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this research work.

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