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The effectiveness of some selected insecticides against cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) and their non-targeted predators

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Abstract
The invasive polyphagous cotton mealybug, *Phenacoccus solenopsis* Tinsley has emerged as a serious sucking pest of cotton. The laboratory and field bioassays of seven selected insecticides belonging to five toxicant groups were evaluated against *P. solenopsis* and their most abundant natural predators, *Chrysoperla carnea* (Steph.) and *Hyperaspis vinciguerrae* Capra. Based on the toxicity index the most toxic group of insecticides under laboratory conditions was esfenvalerate, acetamiprid and dimethoate after 24h and 72h of treatment followed by the oxadiazines metaflumizone, indoxacarb and finally the anti-moulting IGRs, diflubenzuron and chlorfluazuron against the 3rd instar nymphs of *P. solenopsis* using spraying method technique. Of the selected insecticides, dimethoate was significantly superior over the rest of treatments with a highest average reduction percentage in cotton mealybug population (98.26%) under field conditions followed by esfenvalerate (96.72%), indoxacarb (89.66%), metaflumizone (89.43%), acetamiprid (86.28%), diflubenzuron (81.51%), and the least one chlorfluazuron (76.82%). Field experiments recorded that the anti-moulting IGRs were the safer toxicants towards *C. carnea* with average reduction diflubenzuron (45.99%) and chlorfluazuron (25.68%) and towards *H. vinciguerrae*, were diflubenzuron (49.39%) and chlorfluazuron (41.52%).

Keywords: Cotton Mealybug, *Phenacoccus solenopsis,* insecticides, predators

Introduction

Cotton mealy bug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has been described as a serious and invasive pest of cotton and is known to be cryptic in nature with a wide range of variation in morphological characters, biological adaptations and ecological adjustability (Hodgson *et al.*, 2008) [14] (Mostafa *et al*., 2018) [18] (Abel El-Mageed *et al*., 2018) [2] (Eldesouky *et al*., 2023) [8].

The mealy bugs feed on phloem tissue, removing plant sap and causing leaves to distort yellow and dry. They feed on all parts of a plant, particularly new growth. Yellowing of leaves or leaf drop is the symptom of its infestation. They can be observed particularly on growing tips or on leaves that join stems or along leaf veins. Like the aphids, mealy bugs also excrete the honeydew substance over plant surfaces and sometimes a secondary fungus called black sooty mold that grows on it and reduces the quality of the lint (Fand and Suroshe 2015) [10] (Abel El-Mageed *et al*., 2018) [2] (Waqas *et al*., 2021) [29].

Management of cotton mealy bug using intensively chemical insecticides is difficult. It has hard layer of wax that protects against the contacts pesticides also, the habit of cotton mealy bug sap sucking is also a defensive mode of nutrition against chemical contact pesticides in addition to the high reproductive potential and the wide host range. Recently some insect growth regulators (IGRs), organophosphates and bio-pesticides have been recommended for the control of mealy bug (Abel El-Mageed *et al*., 2018) [2].

The rotation of insecticides with diverse modes of action in suppressing *P. solenopsis* is a vital component of effective insecticide resistance management (IRM) strategy. There is an urgently need to develop economically feasible and viable integrated pest management approach to combat the insect pests of cotton. Use of safer chemical insecticides are recommendable for integrated pest management programs aiming at the conservation of the important natural enemies in agroecosystems (Ahmed *et al*., 2014) (Fand and Suroshe 2015).

A successful management program should seek to control *P. solenopsis* without seriously affecting populations of natural enemies.

Coccinellids and Chrysopids are thought to be major predators of *P. solenopsis* (Joshi *et al.*, 2010) [15] (Fand and Suroshe, 2015) [10] (Abel El-Mageed *et al*., 2018) [2].

The present study evaluated the effectiveness of seven insecticides belonging to five different classes to control the cotton mealy bug *P. solenopsis* under both laboratory and field conditions. In addition, studying the effect of these toxicants against the natural enemies associated with the pest under investigation.

Materials and Methods

Insecticides

Commercial formulations of Ultraprid (Acetamiprid 20% SP, Shanghai hanfu biotechnology co. ltd, China), Somi-gold KZ (Esfenvalerate 20% EC, Sumitomo Chemical Ltd, Japan), Camvaal (Indoxacarb 15% EC, Jiangsu Flag Chemical Industry Co., Ltd. China), Topron (Chlorfluazuron 5% EC, Agrochem Co., Alexandria, Egypt), Metazone (Metaflumizone 24% SC, Jingbo Agrochemicals Technology Co, Ltd., China), Dimilin (Diflubenzuron 48% SC, Arysta Life Science Benelux SPRL, Belgium) and Dancothoate (Dimethoate 40% EC, Jiangsu Tenglong Biological & Medicinal Co., Ltd., China) were assessed for their toxicity to *P. solenopsis* under laboratory and field conditions and also against the associated predators under field conditions only.

Insecticidal test against *P. solenopsis* under laboratory conditions

Cotton mealybug, *P. solenopsis* was transferred from untreated infested cotton field plants by the authors in Aga district, Dakahalia governorate, Egypt during summer 2022 and identified at Scale Insect Department, Plant Protection Research Institute, Agric. Res. Center, Giza, Egypt as *P. solenopsis.* Cotton mealybug was brought to the laboratory and adult females were separated and inoculated on cotton plants, potted under laboratory conditions of 30±2 °C, 65±5 RH and 13:11 (L: D) photoperiod. Daily examination for the morphological changes were recorded and monitored until adult stage. The laboratory experiments were performed on the newly moulted 3rd instar nymphs (Abel El-Mageed *et al*., 2018; Attia and Ebrahim, 2015 and Mostafa *et al.*, 2018) [2, 5, 18].

Ten reared laboratory 3rd instar nymphs of *P. solenopsis* were released to a cotton leaf, placed in a culture Petri dish and ready for the insecticidal applications. Each treatment was replicated thrice in addition to control. Five diluted aqueous dispersions concentrations of commercial insecticide were assessed using spray method technique (Mostafa *et al*., 2018) [18] (El-Zahi and Farag 2017) [9]. Mortality recorded after 24 h and 72h of treatment and corrected by using Abotts formula (Abotts, 1925) [1] and they are statistically analyzed to estimate LC_{50}, LC_{90} and slope values according to Finney (1971) [11]. Toxicity index was computed for different insecticides by comparing these materials with the most potent one using Sun's equation (Sun, 1950) [27].

Field bioassay of the tested insecticides against *P. solenopsis* and its associated natural predators

Field study was laid out during summer 2022 to assess the effectiveness of seven toxicants against *P. solenopsis* on cotton plants Giza 86 at the field of Aga district, Dakahalia governorate, Egypt. Field experiments was carried out in a randomized complete block design with eight treatments (Seven insecticides + control). Each treatment was replicated three times (42 m^2 each) per plot.

For each replicate twenty cotton plants were randomly selected and tagged for observation to count *P. solenopsis* population. A knapsack sprayer provided with one nozzle delivering 200 l water/feddan was used. Mealybugs were recorded on top apical shoot and presented as number of mealybugs/ ten inches shoot length according to the method described by (Abel El-Mageed *et al*., 2018) [2].

Observation a sufficient number of mealybug population imposed the field application in the experimental blocks. Observations regarding the *P. solenopsis* population were made a day before spray as well as one, three, seven, 14 and 21 days after spray. Data concerning mealybug population reduction in the different treatments was calculated based on Henderson and Tilton (1955) [13].

The impact of the selected insecticides on the related predators, *H. vinciguerrae* and *C. carnea*, which were identified at the Scale Insect Department of the Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt, was also observed day before spraying as well as 3, 7, 14, and 21 days afterwards.

Statistical analysis

The collected data were subjected for one way analysis of variance (ANOVA), and the means separated using Duncan's Multiple Range Test at $p< 0.05$ (Costat, 2004) [7].

Results and Discussion

The toxicity effect of seven insecticides belonging to five different chemical groups was evaluated against the 3rd instar nymphs of *P. solenopsis* under laboratory condition using spraying method technique (Table 1). Of the tested insecticides, esfenvalerate (Pyrethroids) recorded the highest degree of effectiveness after one day of application on the basis of toxicity index followed by acetamiprid (Neonicotinoids), dimethoate (Organophosphates), metaflumizone (Semicarbazone oxadiazines), indoxacarb (Oxadiazines) and the benzoylurea IGRs chlorfluazuron and diflubenzuron (Chitin synthesis inhibitors). LC_{50} values were 4.24, 6.59, 15.04, 45.54, 53.44, 457.60 and 629.91ppm, respectively. After three days of treatment little variation in the order of effectiveness was only recorded, esfenvalerate was consistently the most potent followed by acetamiprid, dimethoate, indoxacarb, metaflumizone, diflubenzuron and chlorfluazuron. LC_{50} values were 1.97, 2.13, 6.85, 7.72, 21.48, 271.43 and 306.58 ppm, respectively.

The study revealed drastic variations between the five tested chemical groups in their degree of toxicity against *P. solenopsis*. Our results revealed that esfenvalerate, acetamiprid and dimethoate found to be the most toxic group in laboratory. According to Saeed *et al*., (2007) [24], the 3rd instar nymphs of *P. gossypiphilous* was susceptible to esfenvalerate using leaf dip method and the LC_{50} values were (27.7 and 24.2 ppm) after 24 h and 48 h in laboratory. Padaliya *et al*., (2022) [21] showed the effectiveness of acetamiprid 20% SP at 0.004% which recorded 80.77% mortality of *P. solenopsis* after 72h of exposure. Saminathan and Jayaraj, (2001) [25] evaluated dimethoate efficacy using leaf dip method, the mortality percentages were 63.33% at 48 h, and 66.67% at 72 h against the mealybug, *Ferrisia virgata* Cockrell on cotton. While, indoxacarb and metaflumizone were the modertly active toxicants, indoxacarb showed LC_{50} value 21.04 ppm against the 2nd instar nymphs of *P. solenopsis* after 72 h of exposure (Saddiq *et al*., 2017) [23]. Metaflumizone is a semicarbazone oxadiazine insecticide which are voltage-dependent sodium channel blockers on nerve axons Kuhar and McCullough (2022) [16]. Mohamed and Bakry (2019) [17] recommended the anti moulting compounds like diflubenzuron for controlling mealybug infested guava trees *Icerya seychellarum* and *Ferrisia virgate*.

Management of cotton mealybug *P. solenopsis* using some selected insecticides under field conditions

The effect of some selected insecticides on the cotton mealybug population in the field experiment was conducted and presented in tables 2. Observations on mealybug population were made on a day before, one, three, seven, fourteen and twenty one days after initial application.

The pretreatment population of *P. solenopsis* ranged from 271.67 to 404.67 per 10 inches apical shoot. Difference in the mealybug population among the plots was not statistically significant on a day before the treatment (Table 2).

One day after spray esfenvalerate was significantly superior over the rest of treatments with a reduction percentage (97.45%) followed by dimethoate (95.80%), indoxacarb (95.27%), metaflumizone (80.52%), chlorfluazuron (77.65%), acetamiprid (77.09%) and diflubenzuron (75.02%). The superiority of dimethoate was significantly recorded after three days of treatment with a population reduction (99.51%) and up to twenty one days to reach (99.63%). The highest average reduction percentage was recorded for dimethoate (98.26%) followed by esfenvalerate (96.72%), indoxacarb (89.66%), metaflumizone (89.43%), acetamiprid (86.28%), diflubenzuron (81.51%), and chlorfluazuron (76.82%).

Our findings were in accordance with Ghanim and Elgohary (2015) [12], who demonestrsted that imidacloprid and dimethoate were the most potent insecticides under field conditions against the citrus mealybug. Saeed *et al*., (2007) [24] recorded that methomyl gave the highest control of the mealybug *Phenacoccus gossypiphilous* followed by esfenvalerate and thiodicarb after 24 h of treatment. Neonicotinoid insecticides thiamethoxam, imidacloprid and acetamiprid are highly toxic insecticides against *P. solenopsis* (Waqas *et al*., 2021) [29]. Indoxacarb's field activity against some Homoptera and Hemiptera, while potent, is less than that observed in Lepidoptera, in part due to lower inherent sensitivity, slower bioactivation, and also due to physical characteristics that are less favorable to sucking insect oral uptake (Wing *et al*., 2000) [30]. Application of the non-systemic insect growth regulator diflubenzuron as a foliar spray reduced the striped mealybugs *Ferrisia virgate* (Cockerel) by about half with no phytotoxicity (Price 1979) [22]. Chlorfluazuron is one of potent benzoylphenyl ureas (BPUs) that have been developed to be significantly more effective than diflubenzuron in reducing insect pests of cotton and vegetable crops (Amiard-Triquet *et al*., 2011) [4].

One of the main features that enabled the establishment of the IPM program was the conservation of natural enemies. The inclusion of selective insecticides targeted *P. solenopsis* and enabled the conservation of arthropod predators to give the chance for their biological control called integrated control (Stern *et al*., 1959) [26] (Naranjo and Ellsworth 2009) [19]. Toxicological effects of the candidate insecticides on the associated predators, ladybird beetles *H. vinciguerrae* and green lacewing *C. carnea* were evaluated under field conditions after 3, 7, 14 and 21 days of post treatment (Table 3).

Field bioassays with dimethoate revealed slightly harmful effects towards *C. carnea* with average reduction (64.82%) followed by esfenvalerate (57.35%), indoxacarb (49.74%), metaflumizone (47.27%), acetamiprid (46.12%), diflubenzuron (45.99%) and finally chlorfluazuron (25.68%). While against the second associated predacious *H. vinciguerrae*, dimethoate recorded the highest average reduction (74.64%) followed by esfenvalerate (68.52%), acetamiprid (61.11%), metaflumizone (58.46%), indoxacarb (50.05%), diflubenzuron (49.39%) and the least toxic chlorfluazuron (41.52%).

Results of the present study on effect of insecticides against the natural enzymes associated with cotton mealybugs were on par with those reported by Abel El-Mageed *et al*., (2018) [2] who recorded the tolerance of the most common predator in agroecosystem *C. carnea* against insecticides compared with ladybird beetles *H. vinciguerrae*.

Our data are closely parallel to those presented by Bayoun *et al*, (1995) [6], dimethoate and esfenvalerate were similarly toxic to natural enemies. The IGRs insecticides were found to be safer against both *C. carnea* and *H. vinciguerrae* (Abel El-Mageed *et al*., (2018) [2]. Predator densities were reduced in the acetamiprid plots compared with the IGR plots, the activity including predatory beetles, green lacewing, predaceous and omnivorous bugs and predatory flies (Naranjo and Akey 2005) [20]. Several studies reported the higher mortality of metaflumizone than indoxacarb on the Hemiptera predators and this may be due to the different chemical structure (Wanumen *et al*., 2016) [28].

Table 1: Susceptibility of *P. solenopsis* 3rd instar nymphs to some selected insecticides using spraying method under laboratory conditions

Tested Compounds	After 24h of treatment					After 72 h of treatment				
	LC_{50} (ppm) and confidence limits at 95%	LC_{90} (ppm) and confidence limits at 95%	Slope ± SE	X^2	Toxicity index*	LC_{50} (ppm) and confidence limits at 95%	LC_{90} (ppm) and confidence limits at 95%	Slope ± SE	X^2	Toxicity index*
Esfenvalerate	4.24 (0.75 8.16)	60.14 (34.47 229.94)	1.112±.0.29	0. 76	100.0	1.97 (0.12 4.28)	18.70 (11.84 46.91)	1.313±0.40	0.72	100.0
Acetamiprid	6.59 (2.76 10.71)	126.61 (51.50 1627.55)	0.999±0.26	0.33	64.28	2.13 (0.49 3.84)	20.63 (12.80 57.57	1.299±0.32	0.97	92.76
Dimethoate	15.04 (12.20 18.56)	41.99 (31.73 64.37)	2.875±0.38	0.58	28.16	6.85 (4.14 9.53)	39.63 (23.59 135.26)	1.681±0.38	0.63	28.81
Indoxacarb	53.44 (27.19 413.67)	2019.5863 (308.40 2891655.76)	0.813±0.25	0.25	7.93	7.72 (2.42 14.36)	510.32 (113.45 209200.06)	0.704±0.22	0.72	25.57
Metaflumizone	45.54 (16.44 86.71)	1996.06 (479.68 741971.83)	0.781±0.25	2.85	9.30	21.48 (5.13 38.12)	536.02 (213.58 10384.45)	0.917±0.26	0.49	9.19
Chlorfluazuron	457.60 (236.32 1361.66)	6598.42 (1976.73 75673.36)	1.106±0.20	0.22	0.93	306.58 (114.05 2464.01)	53570.81 (4986.21 21360286.34)	0.572±0.13	0.05	0.64
Diflubenzuron	629.91 (253.14	22412.01 (3596.12	0.826±0.18	0.40	0.67	271.43 (146.44	4920.81 (1369.73	1.019±0.19	0.35	0.73

	4149.10)	1677145.90)				824.11)	70584.88)			

*Toxicity index = LC_{50} of the most effective compound/ LC_{50} of the tested compound × 100

Table 2: Effectiveness of some selected insecticides against *P. solenopsis* population under field conditions

Insecticide	Field recommended rate*	Pre-spray	Mean number per plant and percent reduction of *P. solenopsis* Days after insecticide treatment										Overall Mean	
			1		3		7		14		21			
			Mean No.	% Reduc.	Mean No.	% Reduc.	Mean No.	% Reduc.	Mean No.	% Reduc.	Mean No.	% Reduc.	Mean No.	% Reduc.
Dimethoate	150 cm^3/100L	280.00^{a}	16.33^{c}	95.80	2.00^{e}	99.51	2.00^{d}	99.06	6.33^{e}	97.29	1.00^{d}	99.63	5.53	98.26
Esfenvalerate	150 cm^3/fed	404.67^{a}	14.33^{c}	97.45	25.00d^{e}	95.74	4.00^{d}	98.70	15.00^{e}	95.56	15.67^{cd}	96.14	14.80	96.72
Indoxacarb	100 cm^3/fed	339.33^{a}	22.33^{c}	95.27	41.33^{cd}	91.59	14.33^{cd}	94.45	31.67^{d}	88.81	74.33^{b}	78.17	36.80	89.66
Metaflumizone	300 cm^3/fed	380.33^{a}	103.00^{b}	80.52	74.00^{c}	86.57	9.33^{d}	96.78	31.67^{d}	90.02	25.67^{c}	93.28	48.73	89.43
Acetamiprid	25 g/100L	283.67^{a}	90.33^{b}	77.09	116.67^{b}	71.62	17.67^{cd}	91.81	8.00^{e}	96.62	16.33^{cd}	94.26	49.80	86.28
Chlorfluazuron	400 cm^3/ fed	285.33^{a}	88.67^{b}	77.65	59.67^{cd}	85.57	52.33^{b}	75.89	78.00^{b}	67.24	63.67^{b}	77.77	68.47	76.82
Diflubenzuron	125 cm^3/fed	294.67^{a}	102.33^{b}	75.02	62.33^{c}	85.40	42.00^{bc}	81.26	55.33^{c}	77.50	34.33^{c}	88.39	59.26	81.51
Control		271.67^{a}	377.67^{a}		393.67^{a}		206.67^{a}		226.67^{a}		272.66^{a}		295.47	
LSD $_{0.05}$		169.77	56.37		37.06		31.02		15.55		21.42			

*The used concentrations were determined based on the recommendations of Egyptian Ministry of Agriculture. The figures superscripted with same alphabets in the same columns do not significantly differ from each other as per Duncan's multiple range test

Table 3: Toxicity of applied insecticides to adults of *C. carnea* and *H. vinciguerrae*

Insecticide	Pre-spray	Mean population per plant and percent reduction of associated predators Days after insecticide treatment								Overall Mean	
		3		7		14		21			
		Mean No.	% Reduc.	Mean No.	% Reduc.	Mean No.	% Reduc.	Mean No.	% Reduc.	Mean No.	% Reduc.
Chrysoperla carnea											
Dimethoate	7.67^{a}	3.00^{c}	67.98	6.33^{b}	52.06	4.00^{bcd}	62.44	2.67^{d}	76.79	4.00^{cd}	64.82
Esfenvalerate	5.33^{abc}	2.33^{c}	64.22	4.00^{cd}	56.41	3.67^{cd}	50.40	3.33^{cd}	58.35	3.33^{d}	57.35
Acetamiprid	6.67^{ab}	4.67^{b}	42.69	7.00^{b}	39.04	5.00^{bc}	46.01	4.33^{bc}	56.72	5.25^{bc}	46.12
Indoxacarb	7.33^{a}	5.00^{b}	44.16	7.50^{b}	40.57	5.33^{b}	47.62	3.67^{cd}	66.62	5.38^{bc}	49.74
Metaflumizone	7.67^{a}	7.00^{a}	25.29	5.67^{bc}	57.06	5.00^{bc}	53.05	5.33^{b}	53.67	5.75^{b}	47.27
Diflubenzuron	4.33^{bc}	3.67^{bc}	30.62	3.00^{d}	59.76	3.00^{d}	50.10	3.67^{cd}	43.49	3.34^{d}	45.99
Chlorfluazuron	3.67^{c}	3.67^{bc}	18.14	5.33^{bc}	15.64	2.67^{d}	47.60	4.33^{bc}	21.34	4.00^{cd}	25.68
Control	6.00^{abc}	7.33^{a}		10.33^{a}		8.33^{a}		9.00^{a}		8.75^{a}	
LSD $_{0.05}$	2.69	1.58		2.65		1.66		1.12		1.71	
Hyperaspis vinciguerrae											
Dimethoate	5.33^{bc}	1.33^{e}	78.45	1.67^{d}	74.14	2.67^{d}	73.58	3.33^{b}	72.40	2.25^{d}	74.64
Esfenvalerate	5.00^{c}	1.67^{e}	71.16	2.00^{d}	66.99	3.00^{cd}	68.35	3.67^{b}	67.58	2.59^{cd}	68.52
Acetamiprid	5.67^{bc}	3.00^{d}	54.31	3.00^{cd}	56.33	4.33^{bcd}	59.72	3.33^{b}	74.06	3.42^{bcd}	61.11
Indoxacarb	6.00^{bc}	5.33^{b}	23.29	4.00^{bc}	44.98	4.67^{bc}	58.94	3.67^{b}	72.98	4.50^{bc}	50.05
Metaflumizone	7.33^{a}	4.67^{bc}	44.98	4.00^{bc}	54.96	5.00^{c}	64.02	5.00^{b}	69.87	4.67^{b}	58.46
Chlorfluazuron	5.00^{c}	3.67^{cd}	36.61	4.67^{b}	22.92	4.67^{bc}	50.73	5.00^{b}	55.83	4.50^{bc}	41.52
Diflubenzuron	5.67^{bc}	4.00^{cd}	39.08	4.00^{bc}	41.78	5.33^{b}	50.41	4.33^{b}	66.27	4.42^{bc}	49.39
Control	6.33^{ab}	7.33^{a}		7.67^{a}		12.00^{a}		14.33^{a}		10.33^{a}	
LSD $_{0.05}$	1.17	1.22		1.37		2.00		2.87		2.00	

The figures superscripted with same alphabets in the same columns do not significantly differ from each other as per Duncan's multiple range test

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