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Research Article

Toxicity of insecticides to the coccinellid predators, *Cryptolaemus montrouzieri* Mulsant and *Scymnus coccivora* Ayyar of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink

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ABSTRACT: Studies were carried out under laboratory conditions to assess the relative toxicity of insecticides *viz.,* chlorpyriphos, dimethoate, profenophos, carbofuran, buprofezin, imidacloprid, thiamethoxam and spirotetramat against the non-target beneficials of papaya mealybug, *Paracoccus marginatus* (Williams and Granara de Willink) *viz., Cryptolaemus montrouzieri* Mulsant and *Scymnus coccivora* Ayyar. Among the test chemicals, thiamethoxam registered highest toxicity for *C. montrouzieri* grubs (LC_{50} 156.65 ppm) and *S. coccivora* adults (LC_{50} – 27.4968 ppm) while imidacloprid (LC_{50} – 156.07 ppm) to *Cryptolaemus* adults. All the test insecticides *viz.,* chlorpyriphos, dimethoate, profenophos, carbofuran, buprofezin, imidacloprid, thiamethoxam and spirotetramat were found safe to *C. montrouzieri* and *S. coccivora* based on selective toxicity ratio and sequential testing scheme. By probit substitution method, only chlorpyriphos and buprofezin were found safe to *C. montrouzieri,* while the rest of the insecticides were highly toxic to non-target insects.

KEY WORDS: Selective toxicity, *Cryptolaemus montrouzieri*, *Scymnus coccivora*, insecticides

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INTRODUCTION

Insecticides effectively check the insect pests in the event of outbreak, but can be disruptive to natural and biological control by decreasing natural enemy populations (Johnson and Tabashnik, 1999). Hence, integrated pest management (IPM) systems make use of all feasible options to reduce pest populations with priority to non-chemical measures (IOBC, 2008). Accordingly, chemical treatments should not be imposed on a regular, preventive basis, but only when essential. In contrast, use of selective insecticides that are less toxic to natural enemies than to pests should conserve the natural enemy populations and the surviving natural enemies might suppress the pest population, which in turn would reduce the rate of insecticide application (Tanaka *et al*., 2000). Also, the insecticides used in IPM should have minimum effect on beneficial insects and the environment and maximum effect on the target pests.

Incidence of the invasive pest, papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, a native of Mexico and/or Central America (Miller *et al.,* 1999) with a wide host range of over 60 species of plants was noticed on economically important plants such as custard apple, papaya, hibiscus, tapioca and brinjal (Meyerdirk and Kauffman, 2001). Like most invasive insects, it is not clear how it entered Tamil Nadu. Natural enemies of the papaya mealybug include the commercially available mealybug destroyer (*Cryptolaemus montrouzieri* Mulsant), lady beetles, lacewings and hover flies, which are generalist predators that are likely to have a potential impact on mealybug populations (Walker *et al.,* 2003). *C. montrouzieri* has been used successfully to reduce large populations of *Maconellicoccus hirsutus* in India (Karnataka) (Mani and Krishnamoorthy, 2001) and effective control of all prey stages has been recorded in grapevine (Ramesh Babu and Azam, 1987; McComie *et al*., 1997). Field performance of *Scymnus coccivora* Ayyar (Coccinellidae: Coleoptera) has also been assessed for the management of lime mealybug, *Planococcus citri* (Risso). In this paper, we report the results of the experiments undertaken to study the selective toxicity and risk hazards associated with the use of insecticides in relation to predators of *P. marginatus* for their integration in IPM.

MATERIALS AND METHODS

Acute toxicity tests with the test insecticides were conducted on non-target insects like *C. montrouzieri* (grubs and adults) and *S. coccivora* (adults) during 2009-10 at Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore situated at 11°N latitude, 77°E longitude and an altitude of 426.26 m above mean sea level. This data was generated along with baseline toxicity data against test insect, *P. marginatus*. Test insecticides (technical formulation with purity) used for the bioassay *viz.,* imidacloprid (96.6%), thiamethoxam (98.6%), spirotetramat (99.5%), carbofuran (99.3%), chlorpyriphos (99.5%), buprofezin (99.2%), profenophos (95.3%) and dimethoate (90.3%) were obtained from the respective pesticide companies and required concentrations dissolved in analytical grade acetone followed by serial dilution with double distilled water.

Acute toxicity studies on C. montrouzieri and S. coccivora

Methodology as described by Ramesh Babu and Azam (1987) was followed for mass culturing of *C. montrouzieri* under laboratory conditions, while *Scymnus* adults were obtained from Central Sericulture Research and Training Institute (CSRTI), Mysore. Method No: 007 recommended by the Insecticide Resistance Action Committee (IRAC, year ?) was followed for conducting bioassay on third instar grubs, freshly emerged adult beetles of *C. montrouzieri* and adults of *S. coccivora*. Fresh papaya leaves collected from the field were cut into 5 cm leaf discs and placed in Petriplates of 50 mm diameter polythene Petridishes were filled with agar gel (12 g L^{-1}, 5 ml) to maintain turgidity of leaf. Accurate dilutions of technical grade insecticides (serial dilution with double distilled water) were prepared and leaves were dipped in solution for 5 seconds with gentle agitation. After air drying for 15 – 20 minutes, 10 third instar grubs and adults per replication were transferred to insecticide treated leaves in Petriplates. The test was replicated thrice and mortality was recorded after 48 hours of exposure. Leaves dipped in water were used as the control.

Statistical analysis

The median lethal concentration (LC_{50}) and LC_{95} of the test insecticides used were determined by Finney's probit method (Finney, 1971) with POLO software. The corrected per cent mortality was worked out using the formula (Abbott, 1925).

$$\text{Per cent corrected mortality} = \frac{\text{Percentage test mortality} - \text{Percentage control mortality}}{100 - \text{Percentage control mortality}}$$

Risk assessment to non-target beneficials of P. marginatus

Selectivity ratio

Selectivity ratio was calculated as

$$\text{Selectivity ratio} = \frac{LC_{50} \text{ of } X}{LC_{50} \text{ of } Y}$$

where X = the beneficial species (ppm)

Y = the pest species (ppm)

Selectivity ratio of less than one indicates selectivity favoring the pest and a value of more than one represents selectivity favoring non-target.

Probit substitution

This method was used to determine relative toxicities of beneficial species at particular levels of pest mortality. This method involved substituting the log LC_{90} and its 95 per cent fiducial limits for the pest species, *P. marginatus* into modified probit equations (Finney, 1971) for each insecticide and beneficial species. The equation used is as follows:

$$Y = 5 + m\ (x - [\log LC_{50} \text{ of beneficial species}])$$

Where, Y = Probit value,

M = Slope of the probit line for the beneficial species,

X = Log of the fiducial limits for the LC_{90} of the pest species (*P. marginatus*).

Solving for Y gives a probit value which is then converted to percentage of mortality using a conversion table (Finney, 1971).

Sequential testing scheme

Hazards of insecticides to non-targets were assessed using sequential testing scheme on the lines of Johansen and Mayer (1990).

LC_{50} (µg/g)	> 100 µg/g	Nontoxic product
LC_{50} (µg/g)	11-100 µg/g	Slightly toxic product
LC_{50} (µg/g)	2 to 10.9 µg/g	Moderately toxic product
LC_{50} (µg/g)	< 2 µg/g	Highly toxic product

RESULTS AND DISCUSSION

Acute toxicity studies on *C. montrouzieri* grubs revealed that the highest LC_{50} value (ppm) was obtained for buprofezin (730.94) followed by chlorpyriphos

(687.33), profenophos (396.70), spirotetramat (353.13), dimethoate (322.79), carbofuran (170.89), imidacloprid (163.74), thiamethoxam (156.65) (Table 1). For *C. montrouzieri* adults, the order of toxicity of insecticides was imidacloprid (156.07) > carbofuran (163.69) > thiamethoxam (189.08) > spirotetramat (323.89) > profenophos (357.07) > dimethoate (402.42) > buprofezin (571.74) > chlorpyriphos (595.50) based on LC_{50} (ppm) (Table 2).

Thiamethoxam was found to be highly toxic to *Scymnus* adults with LC_{50} value of 27.4968 ppm followed by dimethoate (73.1500), imidacloprid (74.2854), chlorpyriphos (85.7908), buprofezin (86.2595), carbofuran (98.9545), spirotetramat (121.5557), and profenophos (221.4393) (Table 3).

Risk assessment based on selectivity ratio showed that the ratio was more than one for all the insecticides tested for the non-target organisms based on LC_{50} values and were safe (Table 4). Based on the probit substitution method (Table 5), the doses of chlorpyriphos, dimethoate, profenophos, carbofuran, buprofezin, imidacloprid, thiamethoxam and spirotetramat that would cause 90 per cent mortality to *P. marginatus* would result in 0, 71,77, 100, 0, 62, 86 and 87 per cent mortality to *C. montrouzieri,* respectively. Chlorpyriphos and buprofezin were found safe to *C. Montrouzieri* beetles. For *S. coccivora,* it would cause per cent mortality of 95, 100, 80, 100, 98, 97, 100 and 100, respectively (Table 6). Based on the sequential testing scheme for *C. montrouzieri*, test insecticides *viz.,* chlorpyriphos, dimethoate, profenophos, carbofuran,

Table 1. Acute toxicity of test insecticides to *Cryptolaemus montrouzieri* grubs

Insecticides	Regression Equation	Calculated χ^2	LC_{50} (ppm)	Fiducial limits LL	Fiducial limits UL	LC_{95} (ppm)	Fiducial limit LL	Fiducial limit UL
Chlorpyriphos	y = – 12.96 + 6.34x	1.39	687.33	630.36	748.44	1230.36	968.46	1563.09
Dimethoate	y = – 1.81 + 2.73x	0.15	322.79	242.96	389.87	1241.13	684.33	2250.96
Profenophos	y = – 4.03 + 3.50x	0.71	396.70	346.75	458.73	1104.97	716.22	1704.71
Carbofuran	y = – 4.92 + 4.46x	1.64	170.89	133.01	195.73	389.07	297.03	509.62
Buprofezin	y = – 8.60 + 4.76x	0.48	730.94	661.51	852.41	1586.25	1012.36	2485.46
Imidacloprid	y = – 9.06 + 6.34x	0.06	163.74	150.41	180.44	175.69	251.62	356.80
Thiamethoxam	y = – 2.43 + 3.38x	6.65	156.65	106.89	197.55	475.90	308.39	734.60
Spirotetramat	y = – 7.49 + 4.92x	0.39	353.13	330.24	390.03	748.84	450.26	1245.44

Table 2. Acute toxicity of test insecticides to *Cryptolaemus montrouzieri* adults

Insecticides	Regression Equation	Calculated c^2	LC_{50} (ppm)	Fiducial limits LL	Fiducial limits UL	LC_{95} (ppm)	Fiducial limit LL	Fiducial limit UL
Chlorpyriphos	y = – 19.06 + 8.67x	1.05	595.50	548.37	633.44	921.42	806.75	1052.40
Dimethoate	y = – 2.15 + 2.76x	1.56	402.42	346.77	473.36	1519.52	779.57	2961.81
Profenophos	y = – 3.29 + 3.27x	0.17	357.07	286.78	432.66	1083.05	686.83	1707.83
Carbofuran	y = – 3.44 + 3.83x	3.19	163.69	111.23	193.05	428.96	302.43	608.43
Buprofezin	y = – 15.38 + 7.39x	4.57	571.74	507.07	639.59	954.80	804.81	1132.74
Imidacloprid	y = – 5.28 + 4.70x	0.71	156.07	135.99	172.17	344.19	249.86	474.12
Thiamethoxam	y = – 5.27 + 4.54x	1.29	189.08	169.48	210.12	417.31	314.96	552.92
Spirotetramat	y = – 11.33 + 6.49x	0.07	323.89	299.51	353.42	584.12	444.34	767.89

Table 3. Acute toxicity of test insecticides to *Scymnus coccivora* adults

Insecticides	Regression Equation	Calculated χ^2	LC_{50} (ppm)	Fiducial limits LL	Fiducial limits UL	LC_{95} (ppm)	Fiducial limit LL	Fiducial limit UL
Chlorpyriphos	y = 0.47 + 2.34x	5.56	85.79	66.20	111.16	432.02	246.41	757.43
Dimethoate	y = – 0.24 + 2.81x	1.35	73.15	59.87	89.36	281.25	162.71	486.17
Profenophos	y = 0.27 + 2.012x	1.67	221.43	172.26	284.65	1453.67	660.97	3197.03
Carbofuran	y = – 1.10 + 3.06x	3.16	98.95	84.38	116.04	341.03	192.84	603.11
Buprofezin	y = – 1.66 + 3.44x	5.87	86.25	72.74	102.27	259.22	174.87	384.25
Imidacloprid	y = – 0.91 + 3.16x	0.81	74.28	63.00	87.58	246.28	166.88	363.44
Thiamethoxam	y = + 0.90 + 2.84x	1.68	27.49	23.04	32.80	103.96	63.60	169.68
Spirotetramat	y = – 8.54 + 6.49x	2.39	121.55	111.54	132.46	217.73	177.08	267.71

Table 4. Selectivity ratio of test chemicals to non-target insects

LC_{50} of Non-target insects/ LC_{50} of Target pest	Chlorpyri-phos	Profeno-phos	Dimeth-oate	Carbo-furan	Bupro-fezin	Imida-cloprid	Thiame-thoxam	Spiro-tetramat
	LC_{50}	**LC_{50}**	**LC_{50}**	**LC_{50}**	**LC_{50}**	**LC_{50}**	**LC_{50}**	**LC_{50}**
C. montrouzieri/ P. marginatus	43.51	24.82	13.23	4.16	22.17	11.39	34.04	38.67
S. coccivora/ P. marginatus	6.27	16.12	2.50	2.58	2.89	5.50	4.51	14.41

Table 5. Predicted mortality of *Cryptolaemus montrouzieri* at the dose resulting in 90 per cent mortality of *Paracoccus marginatus* (Probit substitution method)

Insecticides	LC_{50} of *C. montrouzieri* (ppm)	LC_{90} of *P. marginatus* (ppm)	Probit value (Y)*	Predicted mortality of *C. montrouzieri*
Chlorpyriphos	595.34	426.77 (124.05–1468.20)	3.75 (–0.90–8.39)	0
Dimethoate	386.22	612.24 (155.50–2410.58)	5.55 (3.91–7.19)	71 (14–98)
Profenophos	340.93	574.20 (119.15–2767.18)	5.74 (3.50–7.98)	77 (7–100)
Carbofuran	159.63	727.26 (216.87–2438.79)	7.52 (5.51–9.54)	100
Buprofezin	571.95	372.61 (102.00–1361.23)	3.63 (–0.53–7.79)	0
Imidacloprid	153.79	178.69 (63.70–501.28)	5.31 (3.19–7.41)	62 (3.5–100)
Thiamethoxam	181.49	729.52 (102.00–5217.52)	6.09 (2.21–9.99)	86 (0–100)
Spirotetramat	326.13	489.90 (96.69–2482.28)	6.15 (1.57–7.19)	87 (0–98)

* $Y = 5 + m(x - [\log LD_{50}$ of beneficial species])

Table 6. Predicted mortality of *Scymnus coccivora* at the dose resulting in 90 per cent mortality of *Paracoccus marginatus* (Probit substitution method)

Insecticides	LC_{50} of *S. coccivora* (ppm)	LC_{90} of *P. marginatus* (ppm)	Probit value (Y)*	Predicted mortality of *S. coccivora*
Chlorpyriphos	85.79	426.77 (124.05–1468.20)	6.64 (5.38–7.89)	95 (65–100)
Dimethoate	73.15	612.24 (155.50–2410.58)	7.59 (5.92–9.27)	100
Profenophos	221.43	574.20 (119.15–2767.18)	5.83 (4.45–7.20)	80 (29–99)
Carbofuran	98.95	727.26 (216.87–2438.79)	7.66 (6.04–9.26)	100
Buprofezin	86.25	372.61 (102.00–1361.23)	7.19 (5.25–9.12)	98 (60–100)
Imidacloprid	74.28	178.69 (63.70–501.28)	6.19 (4.78–7.61)	97 (41–100)
Thiamethoxam	27.49	729.52 (102.00–5217.52)	9.05 (6.62–11.49)	100
Spirotetramat	121.55	489.90 (96.69–2482.28)	8.93 (4.35–13.51)	100

* $Y = 5 + m(x - [\log LD_{50}$ of beneficial species$])$

buprofezin, imidacloprid, thiamethoxam and spirotetramat were considered to be non toxic while, chlorpyriphos (85.7908 ppm), dimethoate (73.1500 ppm), carbofuran (98.9545 ppm), buprofezin (86.2595 ppm), imidacloprid (74.2854 ppm), thiamethoxam (27.4968 ppm) were slightly toxic to *S. coccivora* and profenophos (221.4393 ppm) and spirotetramat (121.5557 ppm) were non toxic.

From the acute toxicity results for *C. montrouzieri*, imidacloprid was the most toxic chemical and chlorpyriphos was the least toxic for the adult beetles. These results were in corroboration with earlier findings that neonicotinoids cause toxic and sub-lethal effects while IGR's seemed not to affect the mortality and fecundity of the adults of the predator *C. montrouzieri* (Mullin *et al.*, 2005; Papachristos and Milonas, 2008). The selectivity ratio for the non-target beneficials *viz., C. montrouzieri* and *S. coccivora* at LC_{50} was more than one for all the test insecticides which indicated that the test molecules were favouring the non-target insects and can be included in the IPM. All the insecticides based on probit substitution method were found highly toxic except for chlorpyriphos and buprofezin to *C. montrouzieri* adults (zero % mortality). Sequential testing scheme revealed that all the test insecticides were non-toxic to *C. montrouzieri* (LC_{50} values >100 ppm) while chlorpyriphos, dimethoate, carbofuran, buprofezin, imidacloprid and thiamethoxam were slightly toxic to *S. coccivora* (LC_{50} value between 11 and 100 ppm).

In the present laboratory study, the test insecticides *viz.,* chlorpyriphos, dimethoate, profenophos, carbofuran, buprofezin, imidacloprid, thiamethoxam and spirotetramat were found safe to the non-target beneficials based on selective toxicity ratio and sequential testing scheme. However, probit substitution method revealed that only chlorpyriphos and buprofezin were found safe to *C. montrouzieri,* whereas all the test insecticides were found toxic for *S. coccivora* .

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