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Research Article

Safety evaluation of insecticides to the ladybird beetle, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), a major predator of mealybugs

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ABSTRACT: The present experiment was aimed at safety evaluation of different insecticides to *Cryptolaemus montrouzieri* Mulsant, a major predator of mealybugs. In order to understand the impact of chemical insecticides on this predator, different bioassays were carried out. The results of the study showed that the Percent mortality of *C. montrouzieri* at 24 HAT was highest in profenophos 50% EC (49.33%) followed by thiamethoxam (36.00%) and dinotefuran (30.67%). However, azadirachtin, buprofezin and pymetrozine were found comparatively safer insecticide to *C. montrouzieri.* Therefore, both of these insecticides, need to be evaluated further under different agro-climatic zones to confirm their suitability, for mealybug management in cotton ecosystem without adversely affecting the performance of *C. montrouzieri*.

KEY WORDS: Azadirachtin, bioassay, buprofezin, dinotefuran, *Phenacoccuss solenopsis*, profenophos, pymetrozine, thiamethoxa

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INTRODUCTION

The ladybird beetle, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is native to Australia and has been introduced in many countries for biological control of several mealybug species and hence the name, mealybug destroyer. It has been reported to feed on 45 species of mealybugs including *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) (Gosalwad *et al*., 2009), 8 species of scale insect and 2 species of whitefly and aphid in their both larval and adult stages (Mani and Krishnamoorthy, 1997). Both grubs and adults are found to feed voraciously on all stages of *P. solenopsis*. Predation rates are higher for adult females than for males (Jayaraman *et al*., 1988). Inhabitant beetle species such as *C. montrouzieri, Cheilomenes sexmaculata* (Fabricius), *Rodolia fumida* (Mulsant), *Scymnus* sp., *Nephus regularis* Sicard, etc. are present in different ecosystems and feed on naturally occurring insect pests (Sattar *et al.,* 2007). These predators have to be conserved and used for effective pest management so that indiscriminate use of insecticides can be avoided. *Cryptolaemus montrouzieri* can be released, prior to the cotton season, on weeds and perennial trees where mealybug colonies are found. Chemicals meant for insect pest control also affect the natural enemies when they come in contact with the insecticides. Many new generation insecticide molecules with novel modes of action have been registered in India for managing insect pests, particularly sucking pests. These new molecules are not specific in their action, but also are either moderately or slightly toxic to non-target organisms. A few of these molecules have been proved to be most effective on mealybugs. However, *C. montrouzieri* being an insect is known to be very sensitive to insecticides. Under these circumstances, there is a need to understand the effect of new insecticide molecules to the natural enemies of solenopsis mealybug not only to find suitable alternatives to old chemical insecticides, but also to develop effective and sustainable management strategies. Keeping the above points in view, the present investigation was programmed for safety evaluation of different insecticides against *C. montrouzieri*.

MATERIAL AND METHODS

The experiments on safety evaluation of insecticides to the ladybird beetle, *Cryptolaemus montrouzieri* were conducted under laboratory conditions at ICAR-National Bureau of Agricultural Insect Resources, Hebbal, Bengaluru 560024 and Department of Agricultural Entomology, UAS, GKVK, Bengaluru, India.

Rearing of *Phenacoccus solenopsis* and *Cryptolaemus montrouzieri*

Although various procedures and host plants were tried for the mass multiplication of *P. solenopsis*, rearing it on potato sprouts and *Parthenium* was found to be easy and cost effective. About 2 months old potato tubers were procured, washed with clean water, surface sterilized with 5 per cent sodium hypochlorite solution and then treated with 1 ppm solution of Gibberellic acid by making a small incision on the potatoes and immersing the same in the solution for one hour. These potato tubers were air dried under fan for 30 minutes. Tubers were then covered with wet black cloth and kept in a dark humid place for sprouting. The sprouts developed within a week's time. After sprouting, these potatoes were transferred to a plastic tray (of size 15×25×5 cm) or earthen pots (of dia. 0.3 m) containing sand which was watered daily. When the seedlings reached about 10 cm height, five or six mature ovisacs of *P. solenopsis* were released onto the same. In case of *Parthenium*, seedlings were collected from the field and transplanted into pots, allowed to grow for about one week and such plants were also used for maintenance of mealybug culture. Mestha seedlings were also raised in separate pots and mealybugs released on to it for multiplication. This was done in order to ensure sufficient mealybug culture throughout the year for this investigation. Likewise, *C. montrouzieri* can be multiplied directly on the mealybugs cultured (*Maconellicoccus hirsutus* or *Phenacoccus solenopsis*) on the sprouted potato or ripe pumpkin with prominent ridges and grooves similar to mealybug multiplication as per the standard protocol developed by ICAR-NBAIR, Bengaluru.

Safety evaluation of insecticides on grubs and adults of *Cryptolaemus montrouzieri*

A group of 25 freshly moulted fourth instar *C. montrouzieri* grubs and 10 freshly emerged adults were placed in petri dish (100 x 15 mm). Each dish was treated with desired concentrations of insecticides prepared by taking the known quantity on weight or volume basis (Table 1). Each dish was treated with 2 ml spray as a uniform mist over grubs and adults with Potter's spray tower. Mealybugs were provided for grubs and honey-agar mixture (as streaks on paper strips) for adults to meet their food requirements. Each treatment was replicated thrice. The mortality of grubs and adults of *C. montrouzieri* were recorded at 24, 48, 72, 96 and 120 Hours After Treatment (HAT). The per cent mortality of grubs and adults was calculated using the following formula.

a) $$\text{Per cent larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

b) $$\text{Per cent adult mortality} = \frac{\text{Number of dead insects}}{\text{Total number of treated insects}} \times 100$$

Statistical analysis

The data obtained in all the experiments were subjected to Fisher's method of analysis of variance by using Completely Randomized Design. Angular transformations of the data were done and then analysed by using the statistical software, WASP 2 (Web Based Agricultural Statistics Software Package).

Table 1. Safety evaluation of insecticides against grubs of *Crytolaemus montrouzieri*

Treatments	Dosage (g.a.i./ha)	Dosage (per liter of water)	Per cent mortality of *Crytolaemus montrouzieri* grubs at hours after treatment (HAT)				
			24 HAT	48 HAT	72 HAT	96 HAT	120 HAT
Pymetrozine 50% WG	150	0.60 g	13.33 (21.37)[cd]	40.00 (39.18)[cd]	73.33 (59.01)[cd]	89.33 (71.82)[bcd]	94.67 (79.21)[ab]
Flonicamid 50% WG	75	0.30 g	18.67 (25.57)[c]	44.00 (41.54)[c]	70.67 (57.52)[cd]	82.67 (66.02)[cde]	92.00 (77.11)[ab]
Dinotefuran 20% SG	25	0.25 g	30.67 (33.55)[b]	65.33 (54.02)[b]	82.67 (65.61)[bc]	93.33 (77.58)[abc]	98.67 (85.77)[a]
Thiamethoxam 25% WG	50	0.40 g	36.00 (36.85)[b]	73.33 (59.01)[ab]	94.67 (79.21)[a]	97.33 (84.14)[ab]	98.67 (85.77)[a]
Azadirachtin 1% EC	1000	2.00 ml	12.00 (20.09)[d]	26.67 (31.04)[de]	44.00 (41.55)[e]	70.67 (57.28)[e]	78.67 (62.24)[c]
Buprofezin 25% SC	250	2.00 ml	9.33 (17.71)[d]	25.33 (30.12)[e]	54.67 (47.71)[de]	80.00 (63.74)[de]	86.67 (69.44)[bc]
Profenophos 50% EC	500	2.00 ml	49.33 (44.62)[a]	82.67 (66.65)[a]	93.33 (77.58)[ab]	98.67 (85.77)[a]	100.00 (89.43)[a]
Untreated check	--	--	0.00 (0.57)[e]	0.00 (0.57)[f]	1.33 (4.23)[f]	2.67 (7.88)[f]	5.33 (13.17)[d]
F test			*	*	*	*	*
Sem (±)			(1.54)	(2.99)	(4.01)	(4.29)	(4.27)
CD (*p*=0.05)			(4.63)	(8.99)	(12.03)	(12.85)	(12.79)
CV (%)			(10.68)	(12.89)	(12.86)	(11.55)	(10.51)

Figures in parentheses are means of angular transformed values. In each column, means followed by same alphabets are statistically on par by DMRT (*p*=0.05)

RESULTS AND DISCUSSION

Safety of insecticides on grubs of *Cryptolaemus montrouzieri*

The results on safety evaluation of insecticides on grubs are presented in Table 1 and 2. In case of 24 HAT, significantly highest mortality was recorded in the treatment with profenophos (49.33%) followed by thiamethoxam (36.00%) dinotefuran (30.67%), flonicamid (18.67%) and pymetrozine (13.33%). Azadirachtin (12.00%) was on par with pymetrozine and buprofezin with respect to mortality of *C. montrouzieri* grubs. At 24h of exposure, buprofezin and azadirachtin were found to be safer to grubs. The insecticides in the increasing order of their toxicity towards these predatory grubs were buprofezin < azadirachtin < pymetrozine < flonicamid < dinotefuran < thiamethoxam < profenophos. At 120 HAT, cent per cent grub mortality was observed in case of profenophos followed by thiamethoxam and dinotefuran (98.67% each). Pymetrozine (94.67%) and flonicamid (92.00%) were on par with each other. Among all the treatments, azadirachtin (78.67%) was found to inflict significantly least mortality to the grubs hence it was turned out to be the safest insecticide. The insecticides in the increasing order of their toxicity to the predatory grubs were azadirachtin < buprofezin < flonicamid < pymetrozine < dinotefuran ≤ thiamethoxam < profenophos. The survival trend of *C. montrouzieri* grubs, 24 HAT was found to be highest in case of untreated check (25.00), followed by buprofezin (22.67), azadirachtin (22.00), pymetrozine (21.67) and flonicamid (20.33). Lowest survival of *C. montrouzieri* grubs was noticed in case of profenophos (12.67), followed by thiamethoxam (16.00) and dinotefuran (17.33). The present findings are in conformation with the experiments conducted by Halappa, *et al*. (2013) who reported azadirachtin and buprofezin to be the safest insecticides causing minimum grub mortality of *C. montrouzieri*. Similarly, Sinha (2004) reported that neonicotinoid, imidacloprid at 0.089 per cent concentration was highly toxic to grubs of *C. montrouzieri*.

Safety of insecticides on adults

The results on safety evaluation of insecticides on adults are presented in Table 3 and 4. At 24 HAT, no toxicity was observed in untreated check, azadirachtin 1% EC and buprofezin 25 SC. However, highest mortality of the adult beetles was recorded in thiamethoxam 25 WG (76.67%) followed by profenophos 50 EC (56.67%) and dinotefuran 20 SG (36.67%). Pymetrozine 50 WG (6.67%) and flonicamid 50 WG (13.33%) documented relatively low toxic effects on beetles. The insecticides in the increasing order of their toxicity towards the predatory adult beetles were azadirachtin ≤ buprofezin < pymetrozine < flonicamid < dinotefuran < profenophos < thiamethoxam. Cent per cent mortality was seen in thiamethoxam followed by profenophos (96.67%), dinotefuran (93.33%) and flonicamid (83.33%) at 120 HAT. Pymetrozine 50 WG (73.33%) was on par with dinotefuran 20 SG and flonicamid 50 WG with regard to the beetle mortality. There were no drastic changes in the mortality trend during this period due to buprofezin (23.33%) and azadirachtin (30.00%) which were found to be relatively safer by registering the least toxicity among all the insecticides tested. However, the least mortality among all the treatments was recorded in untreated check (3.33%). The insecticides in the increasing order of their toxicity to the predatory adult beetles were buprofezin < azadirachtin < pymetrozine < flonicamid < dinotefuran < profenophos < thiamethoxam. The survival trend of adults of *C. montrouzieri* was found to be highest in case of untreated check 10.00 and 9.33 at 24 and 120 HAT, respectively. Apart from untreated check, azadirachtin (10.00) and buprofezin (10.00) also showed highest survival

Table 2. Mean survival of *Cryptolaemus montrouzieri* grubs at different intervals

Treatments	Dosage (g.a.i./ ha)	Dosage (per liter of water)	Mean number of grubs survived during different Hours After Treatment (HAT)				
			24 HAT	48 HAT	72 HAT	96 HAT	120 HAT
Pymetrozine 50% WG	150	0.60 g	21.67	15.00	6.67	2.67	1.33
Flonicamid 50% WG	75	0.30 g	20.33	14.00	7.33	4.33	2.00
Dinotefuran 20% SG	25	0.25 g	17.33	8.67	4.33	1.67	0.33
Thiamethoxam 25% WG	50	0.40 g	16.00	6.67	1.33	0.67	0.33
Azadirachtin 1% EC	1000	2.00 ml	22.00	18.33	14.00	7.33	5.33
Buprofezin 25% SC	250	2.00 ml	22.67	18.67	11.33	5.00	3.33
Profenophos 50% EC	500	2.00 ml	12.67	4.33	1.67	0.33	0.00
Untreated check	--	--	25.00	25.00	24.67	24.33	23.67

NB: Initial number of grubs maintained = 25 of adults of *C. montrouzieri* during 24 HAT. The least survival rate was recorded in case of thiamethoxam (2.33), followed by profenophos (4.33) and dinotefuran (6.33). The present findings are consistent with that of Anjitha (2010) who reported that buprofezin was found safer at 571.954 ppm and neonicotinoid, imidacloprid was found highly toxic to *C. montrouzieri* adults at 153.79 ppm. Similarly, Ghelani *et al*. (2014) demonstrated that flonicamid showed small amount of repellence which affected the population of coccinellids in *Bt* cotton ecosystem. However, pymetrozine denotes an insecticide type which is known to have reduced impacts on non-target and beneficial insects on crop plants (Wayne *et al*., 2009).

Table 3. Safety evaluation of insecticides on adults of *Cryptolaemus montrouzieri*

Treatments	Dosage (g.a.i./ ha)	Dosage (per litre of water)	Per cent adult mortality at different Hours After Treatment (HAT)				
			24 HAT	48 HAT	72 HAT	96 HAT	120 HAT
Pymetrozine 50% WG	150	0.60 g	6.67 (12.59)^{d}	20.00 (26.07)^{c}	40.00 (39.15)^{d}	56.67 (48.93)^{d}	73.33 (60.00)^{c}
Flonicamid 50% WG	75	0.30 g	13.33 (21.15)^{d}	30.00 (33.00)^{c}	46.67 (43.08)^{cd}	60.00 (50.85)^{cd}	83.33 (69.77)^{bc}
Dinotefuran 20% SG	25	0.25 g	36.67 (37.14)^{c}	53.33 (47.01)^{b}	66.67 (55.78)^{c}	76.67 (61.71)^{abc}	93.33 (89.09)^{abc}
Thiamethoxam 25% WG	50	0.40 g	76.67 (61.22)^{a}	86.67 (68.86)^{a}	93.33 (77.41)^{a}	100.00 (89.09)^{ab}	100.00 (85.77)^{a}
Azadirachtin 1% EC	1000	2.00 ml	0.00 (0.91)^{e}	6.67 (12.59)^{d}	16.67 (23.86)^{e}	26.67 (30.99)^{e}	30.00 (33.00)^{d}
Buprofezin 25% SC	250	2.00 ml	0.00 (0.91)^{e}	6.67 (12.59)^{d}	13.33 (21.15)^{e}	16.67 (23.86)^{e}	23.33 (28.78)^{d}
Profenophos 50% EC	500	2.00 ml	56.67 (48.85)^{b}	70.00 (56.99)^{ab}	80.00 (63.93)^{b}	93.33 (77.41)^{a}	96.67 (83.25)^{ab}
Untreated check	--	--	0.00 (0.91)^{e}	0.00 (0.91)^{d}	0.00 (0.91)^{f}	0.00 (0.91)^{f}	3.33 (6.75)^{e}
F test			*	*	*	*	*
SEm(±)			(2.86)	(4.29)	(4.45)	(3.69)	(5.91)
CD (*p*=0.05)			(8.58)	(12.87)	(13.35)	(11.06)	(17.72)
CV (%)			(21.59)	(23.05)	(18.97)	(13.31)	(18.28)

Figures in parentheses are means of angular transformed values. In each column, means followed by same alphabets are statistically on par by DMRT (*p*=0.05)

Table 4. Mean survival of *Cryptolaemus montrouzieri* adults at different intervals

Treatments	Dosage (g.a.i./ ha)	Dosage (per liter of water)	Mean number of adults survived during different Hours After Treatment (HAT)				
			24 HAT	48 HAT	72 HAT	96 HAT	120 HAT
Pymetrozine 50% WG	150	0.60 g	9.33	8.00	6.00	4.33	2.67
Flonicamid 50% WG	75	0.30 g	8.67	7.00	5.33	4.00	1.67
Dinotefuran 20% SG	25	0.25 g	6.33	4.67	3.33	2.33	0.67
Thiamethoxam 25% WG	50	0.40 g	2.33	1.33	0.67	0.00	0.00
Azadirachtin 1% EC	1000	2.00 ml	10.00	9.33	8.33	7.33	7.00
Buprofezin 25% SC	250	2.00 ml	10.00	9.33	8.67	8.33	7.67
Profenophos 50% EC	500	2.00 ml	4.33	3.00	2.00	0.67	0.33
Untreated check	--	--	10.00	10.00	10.00	10.00	9.33

NB: Initial number of adults maintained = 10 no's

CONCLUSION

In the present experiment, among the insecticides evaluated for its safety to the predator, *Cryptolaemus montrouzieri* revealed that, thiamethoxam and dinotefuran were inflicting highest mortality, hence it should be avoided in the management of mealybugs. However, azadirachtin, buprofezin and pymetrozine exhibited comparatively good results regarding their safety to *C. montrouzieri*, therefore, these can be evaluated further to confirm their suitability for achieving the mealybug management in cotton without adversely affecting the performance of natural enemies.

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