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***In vitro* effect of chlorpyrifos 20 EC on coccinellid predators**

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Abstract

In laboratory experiments, chlorpyrifos 20 EC was safer to adults of *Cryptolaemus montrouzieri* Mulsant at 500 and 250 ml/ha and was slightly harmful to *Cheilomenes sexmaculata* Fabricius. Lower doses were comparatively safer to grubs and adults of *C. montrouzieri*. Lower mortality was observed in chlorpyrifos 20 EC 250 ml/ha (33.33%) followed by chlorpyrifos 20 EC 500 ml/ha (36.67%) and Dursban 20 EC 500 ml/ha (40.00%) after 48 hours of treatment. Low adult mortality was observed by chlorpyrifos 20 EC 250 ml/ha (36.67%) followed by chlorpyrifos 20 EC 500 ml/ha (40.00%) and Dursban 20 EC 500 ml/ha (43.33%). Mortality of *C. sexmaculata* grubs and adults were, 56.50 to 80.00 per cent and 61.50 to 82.50 per cent respectively.

Keywords: Chlorpyrifos 20 EC, *Cryptolaemus montrouzieri*, *Cheilomenes sexmaculata* – safety

Introduction

Adverse impact of insecticides on natural enemies can be mitigated through choice of insecticide, dosage or timing of insecticide application. Biological control and selective insecticides are proven compatible tactics in IPM programs (Galvan *et al.*, 2005) [3]. Integrating biological control with selective insecticides can minimize the likelihood of pest resurgence and possibly to reduce the number of insecticide application. The role of coccinellid predators as effective bio agents against many of soft bodied insects including mealy bugs in the orchards is well established. *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is native to Australia commonly known as the mealy bug destroyer. Larvae and adults of this predator are very efficient natural enemies of mealy bugs. The predator has been successfully used for the inoculative release in citrus, grapes, guava and mango orchards to manage mealy bugs (Mani *et al.*, 1995) [8]. Safe and selective chemical pesticides are to be used to conserve the naturally available predators. Safety of chemical insecticides to lady bird predator, *Cheilomenes sexmaculata* Fabricius (Coleoptera: Coccinellidae) has been studied by Sunitha *et al.* (2004) [12].

In the changing scenario of pest management, an integrated approach of exploiting biological control agents as foremost components of IPM with chemical pesticides as adjuncts for efficient control of mealy bugs (Halappa *et al.*, 2013) [4]. Results also recommended that when using conventional pesticides, they must be used with appropriate formulations at right concentration and at the optimum time of intervention following proper application methods to conserve natural enemies.

Materials and Methods

Effect of chlorpyrifos 20 EC on grubs and adults of *Cheilomenes sexmaculata*

Studies were conducted at the Department of Agricultural Entomology, Tamil Nadu Agricultural Entomology, and Coimbatore in completely randomized design with six treatments repeated four times along with a control (water spray).

Treatments

Relative toxicity of insecticides was tested by bioassay method described by McCutchen and Plapp (1988) [9] that was adopted with modifications (Chelladurai, 1999) [2]. Insecticidal concentrations were prepared in acetone:water (80:20). Insecticide concentrations (0.5 ml) were evenly coated in glass scintillation vials of 20 ml capacity and dried thoroughly for 15 min. The third instar grubs were released at the rate of ten per vial and the vial covered with muslin cloth secured by rubber band.

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After an hour of exposure, grubs were transferred to test tubes and field collected aphids, *Aphis gossypii* Glover were given as feed. Observations were made on dead grubs (6, 12, 24 and 48 h after treatment) and mortality was worked out by,

Table 1: The insecticides used in the present investigation and their dosages were as follows.

S. No	Insecticide	Dose (g.a.i./ha)	Product (ml/ha)
1	Chlorpyrifos 20 EC	100	750
2	Chlorpyrifos 20 EC	200	1000
3	Chlorpyrifos 20 EC	300	1500
4	Chlorpyrifos 20 EC	400	2000
5	Dursban 20 EC	200	1000
6	Untreated Check	-	-

$$\text{Per cent grub mortality} = \frac{\text{No. of beetles dead}}{\text{Total number of grubs treated}} \times 100$$

Similarly, beetles were released at the rate of ten per vial and covered with muslin cloth secured by rubber band. After an hour of exposure, the adults were transferred to test tubes and field collected aphids, *Aphis gossypii* Glover were given as feed. Observations were made on the dead adults (6, 12, 24 and 48 h after treatment) and mortality was worked out by,

$$\text{Per cent adult mortality} = \frac{\text{No. of beetles dead}}{\text{Total number of beetles treated}} \times 100$$

Effect of chlorpyrifos 20 EC on Australian lady bird beetle, *Cryptolaemus montrouzieri* Mulsant

Culturing of beetles

Beetles were cultured on mealy bug, *Maconellicoccus hirsutus* Green in the laboratory. Small pumpkin fruit with flat base with several ribs and fruit stalk was selected and the fruit was disinfected by dipping it in 1 per cent carbendazim solution and dried it. Slight bruises were made along the ribs with new razor, so that sap gently oozes out from fruit. Then the fruit was placed in a cage (30 x 30 x 30 cm) used for the production of coccinellids, covered with black cloth on all sides having a glass door in front. Mealy bug crawlers were inoculated on pumpkin fruits using a hair brush continuously for 1 week, till it became adapted to the host. In about 20-30 days, mealy bugs colonized the pumpkin fruit. After colonisation, 50 pairs of *C. montrouzieri* were released from a stock culture. The beetles fed on mealy bug and deposited their eggs singly and in groups of 4-12. Coccinellid grubs were visible in such cages within a week of exposure of beetles. Young grubs fed on eggs and nymphs of mealy bugs and grew fast.

For facilitating pupation of grubs, dried guava leaves or piece of folded papers were kept at the base of each cage. The first beetle started emerging from the cage on 30th day after inoculation. From each cage, about 175 beetles were obtained. Grubs and adults were collected from the cage and the bioassay tests were carried out.

Effect of chlorpyrifos 20 EC on grubs and adults of *Cryptolaemus montrouzieri*

Studies were conducted in a completely randomized design with seven treatments repeated thrice along with a control (water spray) during March 2014.

Treatments

Table 2: The insecticides used in the present investigation and their dosages were as follows.

S. No	Insecticides	Dose (g.a.i./ha)	Product (ml/ha)
1	Chlorpyrifos 20EC	50	250
2	Chlorpyrifos 20EC	100	500
3	Chlorpyrifos 20EC	150	750
4	Chlorpyrifos 20EC	200	1000
5	Dursban 20 EC	100	500
6	Dimethoate 30 EC	100	500
7	Untreated check	-	-

The relative toxicity of insecticides was assessed by bioassay described as per McCutchen and Plapp (1988) ^[9] and Chelladurai (1999) ^[2] for *C. montrouzieri*, as done in previous experiment with *C. sexmaculata*.

Results and Discussion

Effect of chlorpyrifos 20 EC on grubs and adults of *Cheilomenes sexmaculata*

After 48 hours, low mortality was caused by chlorpyrifos 20 EC 750 ml/ha (56.50%) compared to other treatments (Table 1). Chlorpyrifos 20 EC 2000 ml/ha, chlorpyrifos 20 EC 1500 ml/ha, chlorpyrifos 20 EC 1000 ml/ha and Dursban 20 EC 1000 ml/ha registered high grub mortality levels of 80.00, 75.00, 62.50 and 65.00 per cent respectively.

In the case of adults, chlorpyrifos 20 EC 750 ml/ha recorded only 61.50 per cent mortality when compared to other treatments (Table 2). Maximum mortality was observed in chlorpyrifos

20 EC 2000 ml/ha (82.50%) followed by chlorpyrifos 20 EC at 1500 ml/ha, chlorpyrifos 20 EC 1000 ml/ha and Dursban 20 EC 1000 ml/ha which accounted for 75.00, 62.50 and 65.00 per cent respectively. Thus, chlorpyrifos 20 EC and standard check were slightly harmful to the grubs and adults of *Cheilomenes sexmaculata*. Mortality of grubs and adults being 56.50 to 80.00 per cent and 61.50 to 82.50 per cent respectively (Table 1 and 2). However, Suja (2003) ^[11] reported that chlorpyrifos had the highest safety index on the third instar grubs and adults of *C. sexmaculata*

Effect of chlorpyrifos 20 EC on grubs and adults of *Cryptolaemus montrouzieri*

Lower doses of chlorpyrifos 20 EC were comparatively safer to the grubs (Table 3). Low mortality was registered in chlorpyrifos 20 EC 250 ml/ha (33.33%) when compared to other treatments followed by chlorpyrifos 20 EC 500 ml/ha (36.67%) and Dursban 20 EC 500 ml/ha (40.00%) after 48 hours of treatment.

The highest grub mortality was recorded in chlorpyrifos 20 EC 1000 ml/ha (60.00%) followed by chlorpyrifos 20 EC 750 ml/ha (56.67%) and dimethoate 500 ml/ha (50.00%) when compared to control (3.33%).

The results furnished in Table 4 showed that low adult mortality was caused by chlorpyrifos 20 EC 250 ml/ha (36.67%) followed by chlorpyrifos 20 EC 500 ml/ha (40.00%), and Dursban 20 EC 500 ml/ha (43.33%). Chlorpyrifos 20 EC 1000 ml/ha (63.33%) followed by chlorpyrifos 20 EC 750 ml/ha (56.67%) and Dimethoate 500 ml/ha (50.00%) when compared to control (3.33%).

Higher doses of chlorpyrifos and dimethoate were slightly harmful to the grubs and adults of *Cryptolaemus montrouzieri*, the per cent mortality of grubs and adults ranging from 60.00,

56.67, 53.67 and 63.33, 56.67, 50.00 respectively. Lower doses of chlorpyrifos were harmless to the grubs and adults. Mani (1990) [7] reported that dichlorvos (0.2%) and chlorpyrifos (0.05 %) were less harmful to grubs and adults of *C.montrouzieri*. Besides, Harmeet Kaur and Virk (2011) also reported that chlorpyrifos 20 EC (0.40%) and buprofezin 25 EC (0.13%) were comparatively safer to *C.montrouzieri*. According to Anjitha Alexander *et al.* (2013) LC₅₀ for *C. montrouzieri* grubs were; buprofezin (730.94ppm) followed by chlorpyrifos (687.33ppm), profenophos (369.70ppm), spirotetramat (353.13ppm), dimethoate (322.79ppm), carbofuran (170.89ppm) and imidacloprid (163.74ppm).

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) > chlorpyrifos (595.90) based on LC₅₀. However, probit substitution method revealed that only chlorpyrifos and buprofezin were safer to adults of *C.montrouzieri*.

Pokharkar *et al.* (2007) [10] observed that dimethoate (0.08%, 0.10%), oxydemeton methyl (0.08%, 0.10%), fluvalinate (0.02, 0.03%) were moderately toxic while dichlorvos (0.05, 0.10 %) was safe to grubs and adults of *C.montrouzieri*; whereas, Tank *et al.* (2007) [13] reported the most toxic nature of dichlorvos and moderate to highly toxic effect of phosphamidon, monocrotophos and dimethoate against adults of *Cheilomenes sexmaculata* (Fab.). Dimethoate was highly toxic to coccinellid adults, grubs and pupae with 71.3, 76.0 and 72.2% mortality, respectively, at 5 days after spray application (Mahi Imam Mollah *et al.*, 2012) [6].

Table 1: Effect of chlorpyrifos 20 EC on grubs of *Cheilomenes sexmaculata*

Insecticide	Dose (ml.ha ⁻¹)	After 6h*		After 12 h*		After 24 h*		After 48 h*		Mean mortality (%)
		Mortality (%) (n = 10)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	
Chlorpyrifos 20 EC	750	10.00 (18.43) ^b	10.00	26.50 (30.92) ^b	26.50	41.50 (40.05) ^b	40.00	56.50 (48.90) ^b	54.21	33.63
Chlorpyrifos 20 EC	1000	12.50 (20.47) ^b	12.50	30.00 (33.05) ^{bc}	30.00	47.50 (43.59) ^{bc}	46.15	62.50 (52.56) ^{bc}	60.53	38.13
Chlorpyrifos 20 EC	1500	15.00 (22.50) ^b	15.00	35.00 (36.22) ^{bc}	35.00	52.50 (46.44) ^{bc}	51.28	75.00 (60.11) ^{bc}	73.68	44.38
Chlorpyrifos 20 EC	2000	15.00 (22.50) ^b	15.00	40.00 (39.17) ^c	40.00	60.00 (50.89) ^c	58.97	80.00 (64.18) ^c	78.95	48.75
Dursban 20 EC (standard check)	1000	10.00 (18.43) ^b	10.00	32.50 (34.56) ^b	32.50	45.00 (42.05) ^{bc}	43.59	65.00 (53.78) ^{bc}	63.16	38.13
Untreated check	-	0.00 (0.28) ^a	-	0.00 (0.28) ^a	-	2.50 (4.61) ^a	-	5.00 (6.64) ^a	-	3.75

Mean of four observations * n - No of insects treated Figures in parentheses are arcsine transformed

Per cent mortality in a column followed by the same superscripts are not significantly different according to DMRT (P=0.05)

Table 2: Effect of chlorpyrifos 20 EC on adults of *Cheilomenes sexmaculata*

Insecticide	Dose (ml.ha ⁻¹)	After 6h*		After 12 h*		After 24 h*		After 48 h*		Mean mortality (%)
		Mortality (%) (n = 10)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	
Chlorpyrifos 20 EC	750	7.50 (13.83) ^b	7.50	28.00 (31.86) ^b	28.00	45.50 (42.37) ^b	42.63	61.50 (51.77) ^b	59.47	35.63
Chlorpyrifos 20 EC	1000	10.00 (18.43) ^{bc}	10.00	30.00 (33.21) ^{bc}	30.00	47.50 (43.56) ^b	44.74	62.50 (52.56) ^{bc}	60.53	37.50
Chlorpyrifos 20 EC	1500	12.50 (20.47) ^c	12.50	35.00 (36.16) ^{bc}	35.00	55.00 (47.95) ^b	52.63	75.00 (60.48) ^{bc}	73.68	44.38
Chlorpyrifos 20 EC	2000	12.50 (20.47) ^c	12.50	37.50 (37.66) ^c	37.50	57.50 (49.39) ^b	55.26	82.50 (65.47) ^c	81.58	47.50
Dursban 20 EC (standard check)	1000	10.00 (18.43) ^{bc}	10.00	32.50 (34.72) ^b	32.50	50.00 (45.00) ^b	47.37	65.00 (53.84) ^b	63.16	39.38
Untreated check	-	0.00 (0.28) ^a	-	0.00 (0.28) ^a	-	5.00 (9.22) ^a	-	5.00 (9.22) ^a	-	2.50

Mean of four observations * n - No of insects treated Figures in parentheses are arcsine transformed

Per cent mortality in a column followed by the same superscripts are not significantly different according to DMRT (P=0.05)

Table 3: Effect of chlorpyrifos 20 EC on grubs of *Cryptolaemus montrouzieri*

Insecticide	Dose (ml.ha ⁻¹)	After 6h*		After 12 h*		After 24 h*		After 48 h*		Mean mortality (%)
		Mortality (%) (n = 10)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	
Chlorpyrifos 20 EC	250	6.67 (12.29) ^b	6.67	10.00 (15.00) ^{ab}	6.90	23.33 (28.29) ^b	20.69	33.33 (34.93) ^b	31.03	17.58
Chlorpyrifos 20 EC	500	10.00 (18.43) ^b	10.00	20.00 (26.57) ^{bc}	17.24	26.67 (31.00) ^{bc}	24.14	36.67 (36.93) ^{bc}	34.49	23.34
Chlorpyrifos 20 EC	750	13.33 (21.14) ^b	13.33	26.67 (31.00) ^c	24.14	40.00 (39.15) ^{bc}	37.93	56.67 (48.85) ^{cd}	55.18	34.17
Chlorpyrifos 20 EC	1000	16.67 (23.86) ^{bc}	16.67	30.00 (33.00) ^c	27.59	43.33 (41.15) ^c	41.38	60.00 (50.85) ^d	58.62	37.50
Dursban 20 EC (standard check)	500	10.00 (18.43) ^b	10.00	20.00 (26.07) ^{bc}	17.24	30.00 (33.29) ^{bc}	27.59	40.00 (39.15) ^{bc}	37.93	25.00
Dimethoate 30 EC	500	10.00 (18.43) ^{bc}	10.00	23.33 (28.29) ^{bc}	20.69	35.00 (36.24) ^{bc}	32.76	50.00 (45.00) ^{bcd}	48.28	29.58
Untreated check	-	0.00 (0.28) ^a	-	3.33 (6.14) ^a	-	3.33 (6.14) ^a	-	3.33 (6.14) ^a	-	2.50

Mean of three observations * n - No of insects treated Figures in parentheses are arcsine transformed

Per cent mortality in a column followed by the same superscripts are not significantly different according to DMRT (P=0.05).

Table 4: Effect of chlorpyrifos 20 EC on adults of *Cryptolaemus montrouzieri*

Insecticide	Dose (ml.ha ⁻¹)	After 6h*		After 12 h*		After 24 h*		After 48 h*		Mean mortality (%)
		Mortality (%) (n = 10)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)	
Chlorpyrifos 20 EC	250	6.67 (12.29) ^b	6.67	13.33 (21.14) ^b	10.34	26.67 (31.00) ^b	24.14	36.67 (39.15) ^b	34.49	20.83
Chlorpyrifos 20 EC	500	10.00 (18.43) ^b	10.00	20.00 (26.57) ^{bc}	17.24	30.00 (33.21) ^b	27.59	40.00 (39.15) ^{bc}	37.93	25.00
Chlorpyrifos 20 EC	750	13.33 (21.14) ^b	13.33	26.67 (31.00) ^{bc}	24.14	36.67 (37.22) ^{bc}	34.49	56.67 (48.85) ^{cd}	55.18	33.33
Chlorpyrifos 20 EC	1000	16.67 (23.86) ^{bc}	16.67	30.00 (33.00) ^c	27.59	46.67 (43.08) ^c	44.83	63.33 (52.78) ^d	62.07	39.17
Dursban 20 EC (standard check)	500	10.00 (18.43) ^b	10.00	23.33 (28.78) ^{bc}	20.69	33.33 (35.22) ^b	31.03	43.33 (41.15) ^{bc}	41.38	27.50
Dimethoate 30 EC	500	10.00 (18.43) ^{bc}	10.00	26.67 (31.00) ^c	24.14	30.00 (33.21) ^{bc}	27.59	50.00 (45.00) ^{bcd}	48.28	29.17
Untreated check	-	0.00 (0.28) ^a	-	3.33 (6.14) ^a	-	3.33 (6.14) ^a	-	3.33 (6.14) ^a	-	2.50

Mean of three observations * n - No of insects treated Figures in parentheses are arcsine transformed

Per cent mortality in a column followed by the same superscripts are not significantly different according to DMRT (P=0.05).

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