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In vitro Safety of Emamectin Benzoate 5 SG to *Chrysoperla carnea* (Stephens)

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Laboratory experiments were conducted at the Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore during 2008-2009 to study the safety of emamectin benzoate 5 SG to the eggs, larvae and adults of *Chrysoperla carnea* (Stephens). Low egg mortality was recorded in emamectin benzoate 5 SG at 7 g (9.67%), 9 g (15.00%) and 11 g a.i. ha^{-1} (15.33%). The standard, Proclaim® 11 g (14.33%), spinosad 45 SC at 75g (19.00%) and endosulfan 35 EC at 350 g a.i. ha^{-1} (34.07%) recorded high mortality. Adult longevity was the highest in untreated check (15.77 days), emamectin benzoate 5 SG at 7 g (10.50 days) and 9g a.i. ha^{-1} (9.00 days). The number of eggs laid per five females was also greater in untreated check (373.67 eggs), 7g (132.33 eggs) and emamectin benzoate 5 SG at 9 g a.i. ha^{-1} (126.67 eggs). At 48 HAT, the lowest larval mortality was recorded in emamectin benzoate 5 SG at 7 g (17.67%) and 9 g a.i. ha^{-1} (21.00%) in larval feeding method. In dry film method at 48 HAT, the lowest per cent mortality of 16.67 per cent in emamectin benzoate 5 SG at 7 g followed by 26.67 per cent in emamectin benzoate 5 SG at 9 g a.i. ha^{-1} . Endosulfan 35 EC at 350 g a.i. ha^{-1} registered the highest larval mortality (43.33%), while untreated check recorded 3.33 per cent mortality.

Key words: Safety, Emamectin benzoate 5 SG, *Chrysoperla carnea*.

Use of natural enemies is an important component of integrated pest management (IPM). Natural enemies are often recommended as the first line IPM progra *m* (Lugojja et al., 2001). *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) as one of the most important natural enemies, has a great role in reducing the use of pesticides and environmental pollution in field crops and vegetables (Dean and Sterling, 1992). It has received much attention as a potential biological control agent because of its geographical distribution and wide prey range including aphids, scale insects, whitefly, mites, eggs and neonate of lepidopteran insects (Reioldi *et al* . 2008). C. *carnea* adult has green cylindrical body, transparent wings with light green veins, long filiform antennae, golden eyes and stalked eggs that offer protection from predation (Pedigo, 1989). Effectiveness of *C. carnea* as a biological control agent has been demonstrated in a field crops, orchards, green house (Hagley and Miles 1987). The insecticidal effect on non-target organisms were categorized as per the recommendations of the International Organisation for Biological Control, West Palaearctic Regional Section (IOBC/WPRS) working group (Nasreen *et al.* 2007) as harmless (< 50% mortality), slightly harmful (50-79% mortality), moderately harmful (80-89% mortality) and harmful (> 90% mortality) when tested at the field recommended dose. The indiscriminate use of insecticide has affected the population of bio control agents as all the recommended insecticide is highly toxic to predators and parasitoids (Dhawan *et al*., 1994). The population of predators declined by 68.4 per cent during the last decades and many of predators and parasitoids have been eliminated from cotton ecosystem (Dhawan and Simwat, 1996). Predatory *C. carnea* with many other beneficial organisms have almost been eliminated from field due to frequent use of non- selective insecticides. Emamectin benzoate, one of the newer compounds is synthesized from the naturally occurring insecticide/acaricide of avermectin family. This was discovered in 1984 as a broad spectrum lepidoptericide.Emamectin benzoate product is a mixture of emamectin benzoate B1a and emamectin benzoate B1b that are extracted from *Streptomyces avermitilis* Burg. It has been reported to possess excellent performance against pests of cotton (Govindan *et al.,* 2010) and is also ecologically sound for effective management of cotton bollworm complex. Emamectin benzoate 5 SG with novel mode of action that is effective even at lower doses and safer to the natural enemies. Hence the present study was undertaken to evaluate the safety of emamectin benzoate 5 SG to predatory green lacewing, *C. carnea* under the laboratory conditions.

Materials and Methods

Laboratory experiments were carried out at Insectary, Department of Agricultural Entomology,

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Tamil Nadu Agricultural University, Coimbatore during 2008 - 2009, to study the toxicity of emamectin benzoate 5 SG to predatory green lace wing, *Chrysoperla carnea*. Safety of emamectin benzoate 5 SG (new formulation) which was compared with an available formulation of emamectin benzoate 5 SG (Proclaim® which is a registered product of Syngenta India Ltd) and other standard insecticides (endosulfan and spinosad).

Mass culturing of green lacewing, Chrysoperla carnea (Stephens)

The nucleus culture of *C. carnea* was obtained from the Project Directorate of Biological Control (PDBC), Bangalore. Mass rearing of was done with *Corcyra cephalonica* eggs as feed following the method described by Swamiappan (1996).

Larval rearing

Grubs of *C. carnea* were reared in galvanized iron (GI) basins (28 cm dia) at 250 larvae basin-1 covered with ghada cloth. The eggs of *C. cephalonica* were provided as feed for the grubs in the laboratory. About 25cc of *C. cephalonica* eggs basin were provided on alternate days. After five feedings, the larvae pupated into white coloured round silken cocoon. The cocoons were collected and transferred into one litre plastic container with wire mesh window for emergence of adults.

Adult rearing

The adults were collected and transferred to GI troughs (30 cm dia. x 12 cm ht.), wrapped inside with brown sheets for collecting the eggs. The trough was covered with nylon cloth and kept firm with the help of a rubber band. Over the cloth covering, two bits of foam sponge (2.5 cm2) dipped in water were kept, besides an artificial protein rich diet in the form of semi solid paste was smeared. This diet consisted one part of yeast powder, one part of fructose, one part of honey and one part of Protinex. Water was mixed to make it just a paste. The adults laid eggs on the brown sheet wrapped inside the trough. The adults were collected daily and allowed into fresh rearing troughs with fresh feed. From the old troughs the brown paper sheets along with Chrysoperla eggs were removed.

Effect of emamectin benzoate 5 SG on the eggs of C. carnea

Laboratory experiments were conducted to assess the effect of emamectin benzoate 5 SG on egg, grubs and adults of as per the method described by Krishnamoorthy (1985). The insecticidal solution was prepared by mixing of 0.14, 0.18, 0.22, 0.26, 0.30 and 0.44 g of emamectin benzoate 5 SG, supplied by Insecticides India Pvt Ltd, 0.17 ml of spinosad 45 SC, 0.22 g of emamectin benzoate 5 SG (Proclaim®) and 1.00 ml of endosulfan 35 EC dissolved in one -liter of distilled water these concentrations were equivalent to field doses (emamectin benzoate 5 SG, 7, 9, 11, 13, 15 and 22 g a.i. ha^{-1}, endosulfan 35 EC at 350 g a.i. ha^{-1}, Proclaim® at 11 g a.i. ha^{-1} and spinosad 45 SC at 75 g a.i. ha^{-1}) this dilutions used for safety tests. Eight treatments and replicated three times were maintained to assess the safety of emamectin benzoate 5 SG to eggs, grubs and adults of *C. carnea*

Toxicity to eggs

The eggs along with stalk collected on brown paper strips were sprayed with different concentrations of emamectin benzoate 5 SG and standard insecticidal solutions and using an atomizer. Each treatment was replicated three times with 30 eggs per treatment. Untreated check was maintained by spraying distilled water. The number of grubs hatching from each treatment was recorded and per cent hatchability was worked out. Observations were also made on pupation and adult emergence.

Toxicity to larvae

Larval feeding method (Diet contamination method)

Eggs of *C. cephalonica* were exposed to UV radiation of 15W capacity for 15 minutes to kill the embryo and then sprayed with different concentrations of the insecticide with an atomizer. The treated eggs were shade dried for 15 minutes and then transferred to test tubes (1cc test $tube^{-1}$) of 2.0 x 15 cm size. In the control, the eggs were sprayed with distilled water. Second instar grubs of *C. carnea* were transferred to these test tubes at the rate of 10 per test tube. After complete feeding of the treated eggs, the grubs were provided with untreated *C. cephalonica* eggs until pupation. Observations were made on the grub mortality after 24 HAT.

Dry film method

The bioassay method described by McCutchen and Plapp (1988) was adopted with modifications. In glass vials of 20 ml capacity with 1mm thickness were evenly coated with 1ml of acetone solutions of insecticide formulations dried by rolling for few seconds. Second instar predatory lacewing grubs were released into the vials at 10 per vial, covered with muslin cloth and secured with a rubber band. For untreated check only acetone was used. Mortality observations were taken at 12, 24 and 48 HAT. After 24 h exposure of the grubs, 1cc of *C. cephalonica* eggs were given as food to the grubs and mortality of the grubs was worked out

Toxicity to adults

Five pairs of freshly emerged *C. carnea* adults were allowed in separate plastic containers. The adults were fed with 10 per cent sucrose solution containing different concentrations of emamectin benzoate 5 SG formulations. In the untreated check, the adults were fed with 10 per cent sucrose solution alone. The eggs laid in each treatment were collected daily by keeping a brown paper sheet of

21x6 cm size along the inner side of the plastic container. Observations were made on the adult longevity and fecundity per five pairs and per cent mortality of eggs was recorded.

Statistical analysis

The data on percentage were transformed in to aresine values and the population number into X+0.5 before statistical analysis. The data obtained from laboratory experiments was analysed in completely randomized design (Gomez and Gomez, 1984). The mean values were separated using Duncan's Multiple Range Test (DMRT) . The corrected per cent mortality was worked out using the formula (Abbott, 1925).

$$\text{Corrected per cent mortality} = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where,

P_0 - Observed mortality in treatment

P_c - Observed mortality in untreated check

Results and Discussion

The results of studies conducted on the safety of emamectin benzoate 5 SG and other standard chemicals to predatory green lace wing *C. carnea* under the laboratory studies and the results are presented in Table 1 to 2.

Toxicity to eggs

The effect of emamectin benzoate 5 SG on the egg hatchability of *C. carnea* was studied under laboratory conditions. The least egg mortality of 9.67 % was observed in the lower dose of emamectin benzoate 5 SG at 7 g a.i. ha^{-1} (Table 1). About 85.00 and 84.67% of larva hatched out from the eggs treated with emamectin benzoate 5 SG at 7 g and 11g a.i. ha^{-1}, respectively. These results are in agreement with Aiswariya; (2010) who observed that emamectin benzoate 5 WSG 7 g a.i. ha^{-1} which recorded 8.33 per cent egg mortality when compared to endosulfan (33.11%) in *C. carnea*. Highest egg mortality in 22 g a.i. ha^{-1} recorded 28.67 which was on par with emamectin benzoate 5 SG at 15 g (28.00%) and emamectin benzoate 5 SG at 13 g a.i. ha^{-1} (22.90%). The present findings are in agreement with the report of Bueno and Freitas; (2004) who reported that *Chrysoperla externa* Hagen egg viability was not affected by abamectin under laboratory conditions and also Badawy and Arnaouty (1999) reported that abamectin was safer than all tested conventional insecticides to *Chrysoperla* eggs at the recommended dose. In the case of standards, egg hatchability emamectin benzoate 5 SG at 11g (82.00%) and spinosad 45 SC at 75 g a.i. ha^{-1}(81.00%).Toxicity of spinosad was intermediately causing short-term effects, but it did not cause any long-term effects to *C. externa* (Schneider *et al.,* 2006). The hatchability was low in endosulfan 35 EC at 350 g a.i. ha^{-1} (62.26 %).

Toxicity to adults

The adult longevity of *C. carnea* revealed that untreated check recorded 15.77 days, emamectin benzoate 5 SG at all used doses were less toxic to *Chrysoperla* adults their live in range of 7.60 to 10.50 days. All the concentrations of emamectin benzoate 5 SG did not affect the fecundity untreated females recorded 373.67 eggs per five females. Emamectin benzoate 5 SG at the lowest dose of 7 g a.i. ha^{-1} registered 132.33 eggs. All the doses of emamectin benzoate 5SG were comparatively safer than other standard chemical endosulfan 35 EC at 350 g a.i. ha^{-1} (Table 1). The above was in accordance with Suganya Kanna *et al.* (2005) who found emamectin benzoate 5 SG all the doses was safe to *C. carnea* eggs.

Toxicity to larvae (Diet contamination method)

All insecticidal treatments significantly little larval mortality, per cent pupation and adult emergence compared to untreated check (Table 2) . The maximum larvae mortality was recorded in the higher dose of emamectin benzoate 5SG (13 g) at 12, 24 and 48 HAT (Hours After Treatment) 11.00, 17.67 and 31.00 %, respectively which was significantly different from all other treatments. The recommended dose of emamectin benzoate 5SG at 11g a.i. ha^{-1} recorded the mortality of 6.68, 14.33 and 24.33 % at 12, 24 and 48 HAT, respectively. The lower dose of emamectin benzoate 5SG at 7 g a.i. ha^{-1} which was on par with 9 g a.i. ha^{-1} recorded 4.33, 11.00 and 17.67 % at 12, 24 and 48 HAT, respectively. The present findings conform to the reports of Amor *et al*. (2012) where emamectin benzoate at the lower dose was not toxic *to C. carnea* under laboratory conditions and under field conditions (Chakraborti and Sarkar, 2011). Emamectin benzoate 5SG at 7 g a.i. ha^{-1} recorded 88.50 % pupation followed by 9g (82.50%), 11 g (79.50%) and 13 g a.i. ha^{-1} (72.20) and the standards, emamectin benzoate 5SG (Proclaim®) at 11 g, spinosad 45 SC at 75 and endosulfan 35 EC at 350 g a.i. ha^{-1} recorded 62.23, 69.30 and 62.33% pupation, respectively, while untreated check registered cent per cent pupation and it was in agreement with the report of Ghosh *et al.,* (2010) reported that spinosad at 73 to 84 g a.i./ha were safer to *Chrysoperla carnea* in field conditions and also Ameta and Bunker (2007) who stated that spinosad @ 25 g a.i.ha^{-1} was less toxic to *C. carnea* However, 94.33 % adult emergence was observed in untreated control. With regard to adult emergence, all the treatments showed cent per cent adult emergence (Table 2).

Dry film method

Similar to diet contamination method, all the treatments caused larva mortality, pupation and adult emergence significantly in the dry film method also. Among the emamectin benzoate 5SG treatments, lower dose of emamectin benzoate 5SG at 7g a.i.

Table 1. Effect of emamectin benzoate 5 SG on eggs and adults of *Chrysoperla carnea*

Treatment	Eggs*			Adults**	
	Dose (g a.i.ha^{-1})	Egg hatchability (%)	Egg mortality (%)	Adult longevity (days)	No. of eggs laid per five female
Emamectin benzoate 5SG	7	90.33 (72.95)$_{ab}$	9.67 (17.05)$_{ab}$	10.50 (3.32)$_{b}$	132.33 (11.52)$_{b}$
Emamectin benzoate 5SG	9	85.00 (67.63)$_{ab}$	15.00 (21.37) $_{ab}$	9.00 (3.08)$_{c}$	130.00 (11.37)$_{b}$
Emamectin benzoate 5SG	11	84.67 (67.05)$_{ab}$	15.33 (22.94)$_{ab}$	8.60 (3.02)$_{c}$	126.67 (11.28)$_{b}$
Emamectin benzoate 5SG	13	77.10 (62.81)$_{bc}$	22.90 (26.29))$_{bc}$	7.60 (2.85)$_{d}$	112.00 (10.60)$_{b}$
Emamectin benzoate 5SG	15	72.00 (58.74)$_{bc}$	28.00 (31.18))$_{bc}$	7.60 (2.85)$_{d}$	102.33 (10.10)$_{c}$
Emamectin benzoate 5SG	22	71.33 (58.10)$_{bc}$	28.67 (30.96))$_{bc}$	5.33 (2.41)$_{e}$	95.00 (9.77)$_{d}$
Endosulfan 35 EC	350	62.26 (51.71)$_{d}$	34.07 (36.34)$_{d}$	5.33 (2.41)$_{e}$	75.00 (8.69)$_{e}$
Emamectin benzoate 5SG (Proclaim®)	11	82.00 (65.27)$_{ab}$	14.33 (21.67)$_{ab}$	8.33 (2.96)$_{c}$	126.67 (11.28)$_{b}$
Spinosad 45 SC	75	81.00 (64.01)$_{ab}$	19.00 (21.99)$_{ab}$	8.33 (2.96)$_{c}$	112.00 (10.60)$_{b}$
Untreated control	-	96.33 (81.56)$_{a}$	3.67 (8.44)$_{a}$	15.77 (4.03)$_{a}$	373.67 (19.34)$_{a}$

In a column, means followed by a common letter are not significantly different by DMRT (P = 0.05) # Mean of three replications

* Values in the parentheses are arc $\sqrt{X+0.5}$ sine transformed values

** Values in the parentheses are per cent transformed values

ha^{-1} emerged as the least toxic by registering 3.33, 10.00 and 16.67 % mortality of larva at 12, 24 and 48 HAT, respectively. Emamectin benzoate was relatively safer to all stages of *C. carnea* than thiodicarb and methomyl (Sechser and Ayoub 2003). The recommended dose of emamectin benzoate 5SG at 11 g a.i. ha^{-1} at 12 HAT (7.67 %), 24 HAT (21.00%) and 48 HAT (30.00%), respectively. The results reported by Chakraborti and Sarkar, (2011) who reported that foliar application emamectin benzoate 5 SG at 11 g a.i./ ha brinjal under the field condition. The higher dose of emamectin benzoate 5SG at 13g a.i. ha^{-1} was found to be slightly toxic to the larva of *C. carnea* which recorded 24.34 % at (24 HAT) and 40.00 % at (48 HAT). Emamectin benzoates with novel mode of action is generally more selective and require lower rates than conventional insecticides and has low moderate impact on beneficial insects (Tilman and Mulrooney, 2000). The per cent pupation and adult emergence were found to be 100% in all treatments (Table 2). Fitt *et al*. (2004) reported that bio-rational insecticides are less disruptive to beneficial populations. Selection of a suitable insecticide in an IPM program not only depends on its efficacy against the target pest but also on its toxicity to beneficial insects and its withering and persistence. Emamectin benzoate was not toxic to the predatory green lacewing,

Table 2. Effect of emamectin benzoate 5 SG on grubs of *Chrysoperla carnea* by larval feeding method and Dry film method

Treatment	Dose (g a.i.ha^{-1})	Larval feeding method*			Pupation (%)	Adult emergence (%)	Dry film method*			Pupation (%)	Adult emergence (%)
		12 HAT Mortality (%)	24HAT Mortality(%)	48 HAT Mortality(%)			12 HAT Mortality (%)	24HAT Mortality(%)	48 HAT Mortality(%)		
Emamectin benzoate 5SG	7	4.33 (7.15)$_{ab}$	11.00 (16.00)$_{b}$	17.67 (20.93)$_{ab}$	88.50 (70.30)$_{b}$	88.50 (70.30)$_{b}$	3.33 (6.15)$_{a}$	10.00 (15.00)$_{b}$	16.67 (23.86)$_{b}$	89.51 (71.10)$_{b}$	89.51 (71.10)$_{b}$
Emamectin benzoate 5SG	9	7.68 (13.30)$_{b}$	13.34 (21.15)$_{b}$	21.00 (27.07)$_{b}$	82.50 (65.54)$_{c}$	82.50 (65.54)$_{c}$	6.68 (12.29)$_{ab}$	16.67 (23.86)$_{bc}$	26.67 (31.00)$_{c}$	83.21 (65.81)$_{bc}$	83.21 (65.81)$_{bc}$
Emamectin benzoate 5SG	11	6.68 (12.30)$_{b}$	14.33 (22.14)$_{bc}$	24.33 (29.78)$_{b}$	79.50 (63.37)$_{d}$	79.50 (63.37)$_{d}$	7.67 (13.29)$_{b}$	21.00 (2707)$_{c}$	30.00 (33.00)$_{d}$	76.21 (61.48)$_{cd}$	76.21 (61.48)$_{cd}$
Emamectin benzoate 5SG	13	11.00 (16.00)$_{c}$	17.67 (24.86)$_{c}$	31.00 (34.00)$_{bc}$	72.20 (59.18)$_{e}$	72.20 (59.18)e	6.67 (12.29)$_{ab}$	24.34 (29.30)$_{c}$	40.00 (39.15)$_{e}$	74.21 (59.48)$_{cd}$	74.21 (59.48)$_{cd}$
Endosulfan 35 EC	350	14.34 (21.145$_{cd}$	21.00 (27.07)$_{d}$	41.00 (38.85)$_{c}$	62.33 (51.78)$_{bc}$	62.33 (51.78)$_{bc}$	11.00 (16.00)$_{c}$	31.00 (34.00)$_{d}$	43.33 (41.15)$_{ef}$	57.61 (49.378)$_{e}$	57.61 (49.378)$_{e}$
Emamectin benzoate 5SG (Proclaim®)	11	7.67 (13.29)$_{b}$	17.67 (24.86)$_{c}$	31.00 (33.00)$_{bc}$	62.23 (52.49)$_{g}$	62.23 (52.49)$_{g}$	10.00 (15.00)$_{c}$	21.00 (27.07)$_{c}$	30.00 (33.00)$_{d}$	66.67 (54.78)$_{d}$	66.67 (54.78)$_{d}$
Spinosad 45 SC	75	7.67 (13.29)$_{b}$	17.67 (24.86)$_{c}$	30.00 (33.00)$_{bc}$	69.30 (56.35)$_{f}$	69.30 (56.35)$_{f}$	8.67 (14.29)$_{b}$	21.00 (2707)$_{c}$	30.00 (33.00)$_{d}$	61.30 (51.53)$_{d}$	61.30 (51.53)$_{d}$
Untreated control	-	0.00 (0.19)$_{a}$	3.33 (6.15)$_{a}$	6.68 (12.30)$_{a}$	94.33 (78.71)$_{a}$	94.33 (78.71)$_{a}$	0.00 (0.19)$_{a}$	0.00 (0.19)$_{a}$	3.33 (6.15)$_{a}$	98.68 (82.87)$_{a}$	98.68 (82.87)$_{a}$

HAT - Hours after treatment, * Mean of three replications

Figures in parentheses are *arc sine* transformed values;

In a column, means followed by a common letter(s) are not significantly different by DMRT(P=0.05).

C. carnea at the recommended dose, thus can be used in IPM programs.

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