



## Emamectin benzoate: A novel second generation avermectin derivative for management of biotic stress in lac culture through treatment of broodlac

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### ABSTRACT

Experiment was conducted to evaluate the safety of emamectin benzoate, against lac insect [*Kerria lacca* (Kerr)] and bio-efficacy against associated lepidopteran predators in lac culture. Seven concentrations of emamectin benzoate (5% SG) ranging from 0.00025 % a.i. (0.05 g/L) to 0.0030 % a.i. (0.6 g/L) were evaluated by dipping of broodlac (functional seed of lac culture) in insecticidal formulation for 5, 10 and 15 min durations. Non-significant differences amongst various treatments and control on survival of settled second instar larvae and adult female lac insect clearly indicated the safety of insecticide on lac insect. Treatment of broodlac in insecticidal formulations (0.00025, 0.0005, 0.0010, 0.0015, 0.0020, 0.0025 and 0.0030 % a.i.) for 5, 10 and 15 min durations exerted significant reduction in the population of both key lepidopteran predators, *Eublemma amabilis* Moore (Noctuidae) and *Pseudohypatopa pulvereana* Meyr (Blastobasidae) harboring broodlac. The treatment of *rangeeni* broodlac with 0.00025 % a.i. emamectin benzoate for 10-15 min and *kusmi* broodlac with 0.0005 % a.i. for 5-10 min duration provides effective tool for the management of both major lepidopteran predators of lac insects. This novel insecticide can be safely and effectively integrated in IPM programme of lac production system.

**Key words:** Broodlac, *Eublemma amabilis*, *Kerria lacca*, Lac crops, *Pseudohypatopa pulvereana*

The Indian lac insect [*Kerria lacca* (Kerr)] (Hemiptera:Tachardiidae) produces three natural products of commercial importance, the resin (lac), wax and dye. The large scale culture of lac insect is an important source of livelihood for resource constrained farmers inhabiting in forest and sub-forest areas of India, especially in rainfed condition. *Kusmi* and *rangeeni* are two strains of Indian lac insect. These can be distinguished based on their preference to host trees on which it thrives, duration of life cycle, settlement behaviour, quality and quantity of resin it produces. *Rangeeni* strain of lac insect have two crop cycles in a year and *Butea monosperma* is the preferred host tree to culture this strain. Similarly, *Schleichera oleosa* is preferred host tree to culture *kusmi* strain of lac insect and this strain also has two crop cycles in a year. Like other crops, the lac crops (lac insect culture) are also damaged by insect-predators and parasitoids. Two lepidopteran predators, viz. *Eublemma amabilis* (Lepidoptera:

Noctuidae) and *Pseudohypatopa pulvereana* (Lepidoptera: Blastobasidae) are associated with both the strains of lac insect, inflicting extensive damage to lac crops (Malhotra and Katiyar 1975, 1979) and sometimes lead to total crop failure, if not managed properly.

Broodlac is the major source of infestation to new lac crops by lepidopteran predators. Both of these predators are very intimately associated with lac and are host specific, feeding lac insect and lac it produced. Endosulfan, dichlorvos, cartap hydrochloride and ethofenprox are some of the selective insecticides recommended by the earlier workers for the management of lepidopteran and neuropteran predators (Malhotra and Katiyar 1975, 1979; Mishra *et al.* 1995; Bhattacharya *et al.* 2005b; Jaiswal *et al.* 2004, 2007). In recent years, some newly developed molecules, viz. spinosad, indoxacarb, fipronil etc have also been identified to be safe on lac insect and effective against lepidopteran pest of lac culture (Singh *et al.* 2009, 2011, 2014).

Screening and evaluation of insecticides is a continuous process as newer improved molecules are being developed regularly having some merits and superseding old conventional pesticides. Avermectins are a natural product homologues produced by the soil microorganisms, *Streptomyces avermitilis*. Emamectin benzoate [EB] is a semisynthetic derivative of abamectin and has been developed for the purpose of controlling lepidopterous pests on a variety of vegetable crops world wide (Babu 1988,

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Jansson *et al.* 1997, Liguori *et al.* 2010).

EB is highly potent and LC<sub>90</sub> values against a variety of lepidopterous pest range between 0.002-0.89 µg/mL (Cox *et al.* 1995, Dybas 1989, Jansson and Dybas 1996). The efficacy of EB on different crops against several lepidopteran pests have been evaluated and established by various workers (Jansson and Lecrone 1991, Jansson *et al.* 1996, Leabee *et al.* 1995, Ishaaya *et al.* 2002, Loriatti *et al.* 2009, Bengochea *et al.* 2014) but no efforts have been made so far to assess the efficacy of this derivative against insect pests of lac crops. Keeping in mind the novelty of the compound and its efficacy against lepidopterous pests, the bio-efficacy potential of EB was assessed for its safe and effective use in lac pest management programme.

#### MATERIALS AND METHODS

Field experimentation was carried out at Research Farm of ICAR-Indian Institute of Natural Resins and Gums, Ranchi, India (23°23' N longitude, 85°23' E latitude and 650 m above MSL) during 2014 and 2015. *Rangeeni* summer lac crop was raised on *B. monosperma* by inoculating the broodlac in November and mature broodlac was harvested during July, which was utilised for dipping treatment. Similarly, *kusmi* summer crop was raised on *S. oleosa* by inoculating the broodlac during January and harvested during July, around six months after inoculation. The harvested broodlac was treated by dipping both strain of broodlac derived from summer crops, raised on *S. oleosa* and *B. monosperma*, respectively, in insecticidal formulations for 5, 10 and 15 min. After drying of residual film, the *kusmi* broodlac was inoculated on *Ziziphus mauritiana*, whereas *rangeeni* broodlac was inoculated on *B. monosperma*.

The commercial formulation of EB 5 % SG (EM-1) procured from market, was evaluated in the present study. Seven concentrations of the commercial formulation of insecticide, viz. 0.00025, 0.0005, 0.0010, 0.0015, 0.0020, 0.0025, 0.0030 % a.i. equivalent to a quantity ranging from 0.05 g to 0.6 g/L of water have been considered for the experiment. Dipping of broodlac in water was used as a control.

The safety response was assessed on the basis of survival of lac insect after settlement on specific host trees from the treated broodlac. The bio-efficacy potential was assessed on the basis of reduction in population of insect predators from treated broodlac. There were eight treatments including control with six replications for *kusmi* and five replications for *rangeeni* broodlac. Randomly selected broodlac weighing 50 g each bundle was treated and assessed for safety on lac insect as reported by Singh *et al.* (2013). The bags containing *kusmi* broodlac were inoculated on trees of *Z. mauritiana* and *rangeeni* on *B. monosperma*. The quantification of living and dead lac insect was carried out twice. First at second instar, this corresponds to 30 days after inoculation (DAI) and second, at adult stage of female, corresponding to 60 DAI. After 25 days of inoculation, the 60 mesh net bags used as broodlac container were removed from host trees and hanged on iron pipe in the laboratory

under well aerated condition. These bags were opened after two months of treatment and number of adult lepidopteran predators emerged from treated broodlac were quantified. Per cent reduction in emergence over control was calculated for each treatment to assess the bio-efficacy potential of EB on predators of lac insect.

Statistical analysis was carried out using standard analysis of variance (ANOVA) in randomised block design (Gomez and Gomez 1984). Treatment means were compared at  $P < 0.05$  level of significance using least square difference (LSD). Duncan's multiple range test was carried out to calculate the differences between various means using statistical software AGRES.

#### RESULTS AND DISCUSSION

##### *Safety evaluation of emamectin benzoate on lac insect*

The survival of second instar *kusmi* lac insect after 30 days of treatment varied from 79.94 to 98.72, 89.04 to 92.91 and 90.91 to 93.34 % for 5, 10 and 15 min dipping of broodlac, respectively in different treatments as compared to 98.35, 91.56 and 91.73 % in control. The mean values amongst the different treatments including control were at par ( $P > 0.05$ ) with all the three durations of dipping treatment. After 60 days of treatment (female adult), the survival percentage in different treatments varied from 91.04 to 93.83, 88.65 to 92.50 and 91.11 to 93.96 for 5, 10 and 15 min dipping, respectively as compared to 93.19, 90.85 and 92.86 % in control. The mean values amongst the different treatments including control were at par ( $P > 0.05$ ) with all the three durations of dipping.

The results on survival of second instar *rangeeni* lac insect after 30 days of treatment varied from 82.82 to 93.53, 85.64 to 94.06 and 84.94 to 90.31 % for 5, 10 and 15 min duration of dipping, respectively in various treatments, as compared to 81.23, 86.18 and 92.64 % in control. The mean values among different treatments including control were at par ( $P > 0.05$ ) with all the three durations of dipping. After 60 days of treatment (female adult), the survival varied from 86.13 to 91.55, 83.96 to 91.73 and 84.39 to 88.47% for 5, 10 and 15 min duration, respectively in different treatments, as compared to 88.11, 92.40 and 85.70 % in control. The mean values amongst the different treatments including control were at par ( $P > 0.05$ ) with all the three duration of dipping.

##### *Response of kusmi broodlac treatment on population of E. amabilis*

The treatment of broodlac with EB was found to be significantly effective in reducing the population of *E. amabilis* in all the treatments as compared to control (Table 1). The mean population varied from 0.00 to 2.17, 0.00 to 1.50 and 0.00 to 0.17 in different treatments of insecticide as compared to 6.83, 6.50 and 6.50 in control for 5, 10 and 15 min dipping durations, respectively. The reduction in population of *E. amabilis* over control with various concentrations tried varied from 68.28 to 100.0, 76.92 to

Table 1 Effect of emamectin benzoate (5% SG) on population of *Eublemma amabilis* due to dipping of kusmi broodlac

Conc. (%)	Duration of broodlac dipping					
	5 min		10 min		15 min	
	Mean number*	% reduction	Mean number*	% reduction	Mean number*	% reduction
0.00025	2.17 (1.58) <sup>c</sup>	68.28	1.50 (1.31) <sup>bc</sup>	76.92	0.17 (0.79) <sup>a</sup>	97.44
0.0005	0.67 (1.03) <sup>ab</sup>	90.24	0.50 (0.97) <sup>ab</sup>	92.31	0.00 (0.71) <sup>a</sup>	100.00
0.0010	0.17 (0.79) <sup>a</sup>	97.56	0.17 (0.79) <sup>a</sup>	97.44	0.00 (0.71) <sup>a</sup>	100.00
0.0015	0.17 (0.79) <sup>a</sup>	97.56	0.00 (0.71) <sup>a</sup>	100.00	0.00 (0.71) <sup>a</sup>	100.00
0.0020	0.17 (0.79) <sup>a</sup>	97.56	0.00 (0.71) <sup>a</sup>	100.00	0.00 (0.71) <sup>a</sup>	100.00
0.0025	0.00 (0.71) <sup>a</sup>	100.00	0.00 (0.71) <sup>a</sup>	100.00	0.00 (0.71) <sup>a</sup>	100.00
0.0030	0.00 (0.71) <sup>a</sup>	100.00	0.00 (0.71) <sup>a</sup>	100.00	0.00 (0.71) <sup>a</sup>	100.00
Control	6.83 (2.66) <sup>d</sup>	0.00	6.50 (2.60) <sup>d</sup>	0.00	6.50 (2.56) <sup>d</sup>	0.00
SEd ±			0.17			
F			28.75			
p			0.0			

\*Mean number in 50 g broodlac. Figures in parentheses are transformed values to  $\sqrt{n+0.5}$ . Means marked with different letters including 5, 10 and 15 min of each dipping time are significantly different ( $P < 0.05$ )

100.0 and 97.44 to 100 % with 5, 10 and 15 min dipping duration, respectively.

The mean populations of *E. amabilis* differ significantly with control even at lowest concentration (0.00025 % a.i.) for 5, 10 and 15 min dipping duration ( $P < 0.05$ ). With this lowest concentrations, the mean values for 5 and 10 min dipping duration was at par but significant difference was observed between 10 and 15 min dipping duration ( $P < 0.05$ ). The mean values were 1.50 and 0.17 per 50 g broodlac, respectively. Similar observations have been recorded with 0.0005% a.i. and the mean values were 0.50 and 0.0. There was also significant difference in mean values between 0.00025% and 0.0005% a.i. for 5 and 10 min duration of dipping. The latter concentration caused 90.24 and 92.31% reduction in population against 68.28% and 76.92% recorded with 0.00025% a.i. for 5 and 10 min dipping duration, respectively. Contemplating to select a lowest concentration which caused maximum significant reduction in population of *E. amabilis* 0.00025% a.i. (0.05g/L of water) will be suitable for 15 min dipping and 0.0005% a.i. (0.1 g/L of water) for 5-10 min duration.

Due to very low natural incidence of *P. pulvereae* in kusmi broodlac, the response of EB on *P. pulvereae* couldn't be conclusively drawn.

#### Response of rangeeni broodlac treatment on population of *E. amabilis*

The mean population of *E. amabilis* emerging from 50 g treated broodlac was significantly low in comparison to control (Table 2). The mean values in different treated broodlac varied from 0.10 to 1.60, 0.10 to 0.70 and 0.1 to 0.60 as compared to 9.00, 9.20 and 8.40 in control with 5, 10 and 15 min dipping durations, respectively. The reduction in population of *E. amabilis* varied from 82.22 to 98.89, 92.39 to 98.91 and 92.86 to 98.81 % for 5, 10 and 15 min dipping durations, respectively over control. The mean

populations of *E. amabilis* differ significantly with control even at lowest concentration (0.00025 % a.i.) for 5, 10 and 15 min dipping duration ( $P < 0.05$ ). A lowest concentration which caused maximum significant population reduction was 0.00025 % a.i. for 10-15 min dipping duration. Though, same results may be obtained with 0.0015 % a.i. with only 5 min dipping duration.

#### Response of rangeeni broodlac treatment on population of *P. pulvereae*

The mean number of adult emergence of *P. pulvereae* was significantly higher in control as compared to treated broodlac ( $P < 0.05$ ). The mean population of *P. pulvereae* varied from 0.40 to 1.70, 0.30 to 0.80 and 0.20 to 0.80 from 50 g broodlac dipped for 5, 10 and 15 min durations, respectively. The mean population in control was recorded as 9.00, 7.80 and 8.80 for the same duration, respectively (Table 3).

The reduction in population due to dipping of broodlac for 5, 10 and 15 min varied from 81.11 to 95.56, 89.74 to 96.15 and 90.91 to 97.73 %, respectively. The mean populations differ significantly with control even with lowest concentration (0.00025 % a.i.) for 5, 10 and 15 min dipping duration ( $P < 0.05$ ). With this concentration the mean values for 5, 10 and 15 min dipping duration was at par with each other. The lowest concentration (0.00025 % a.i.) tried in the present experiment is sufficient enough to cause maximum significant reduction in the population of *P. pulvereae* with 10 and 15 min dipping duration. The similar result can be obtained with 0.0005 % a.i. for 5 min dipping.

Selection of suitable pesticides for management of harmful predators and parasitoid species of lac insect appears to be a tough task. Various species of insect associated with lac belong to different orders and families. The lac insect belong to family tachardiidae of order hemiptera; major insect-predators, viz *E. amabilis* and *P. pulvereae* of

Table 2 Effect of emamectin benzoate (5% SG) on population of *Eublemma. amabilis* due to dipping of *rangeeni* broodlac

Conc. (%)	Duration of broodlac dipping					
	5 min		10 min		15 min	
	Mean number*	% reduction	Mean number*	% reduction	Mean number*	% reduction
0.00025	1.60 (1.28) <sup>d</sup>	82.22	0.70 (1.04) <sup>abcd</sup>	92.39	0.60 (0.99) <sup>abcd</sup>	92.86
0.0005	1.30 (1.25) <sup>cd</sup>	85.56	0.70 (1.02) <sup>abcd</sup>	92.39	0.50 (0.93) <sup>abcd</sup>	94.05
0.0010	1.00 (1.19) <sup>bcd</sup>	88.89	0.50 (0.95) <sup>abcd</sup>	94.57	0.40 (0.91) <sup>abcd</sup>	95.24
0.0015	0.50 (0.95) <sup>abcd</sup>	94.44	0.40 (0.90) <sup>abc</sup>	95.65	0.20 (0.79) <sup>a</sup>	97.62
0.0020	0.30 (0.86) <sup>ab</sup>	96.67	0.20 (0.81) <sup>a</sup>	97.83	0.10a (0.76) <sup>a</sup>	98.81
0.0025	0.20 (0.81) <sup>a</sup>	97.78	0.20a (0.81) <sup>a</sup>	97.83	0.10a (0.76) <sup>a</sup>	98.81
0.0030	0.10 (0.76) <sup>a</sup>	98.89	0.10 (0.76) <sup>a</sup>	98.91	0.10 (0.76) <sup>a</sup>	98.81
Control	9.00 (3.03) <sup>e</sup>	0.00	9.20 (3.00) <sup>e</sup>	0.00	8.40 (2.86) <sup>e</sup>	0.00
SEd ±			0.19			
F			28.32			
p			0.0			

\*Mean number in 50 g broodlac. Figures in parentheses are transformed values to  $\sqrt{n+0.5}$ . Means marked with different letters including 5, 10 and 15 minutes of each dipping time are significantly different ( $P < 0.05$ ).

family noctuidae and blastobasidae of order lepidoptera, respectively; *Chrysopa* spp, of order neuroptera. Similarly, around 30 parasitoid species belong to family aphelinidae, encyrtidae and eulophidae of hymenoptera (Sharma *et al.* 2006). Safety of insecticide on lac insect is of prime importance prior to assess effect on the associated fauna. A few broad spectrum insecticides have been already recommended for management of lepidopteran predators (*E. amabilis* and *P. pulverea*) and hymenopteran parasitoids (*Eupelmus tachardiae* and *Tachardiaephagus tachardiae*) by dipping of broodlac (Singh *et al.* 2013). However, pest species specific insecticide is a better option in order to avoid possible resistance. In view of this, the EB which is safe on lac insect and highly effective on lepidopteran may be more useful for lac ecosystem. Non-significant differences

amongst various treatments and control in respect of survival of lac insect clearly indicate that the EB is safe to lac insect. Earlier studies on safety of insecticides towards lac insect revealed endosulfan (Malhotra and Katiyar 1979); cartap hydrochloride (Bhattacharya *et al.* 2005b); ethofenprox (Jaiswal *et al.* 2004); spinosad, indoxacarb, fipronil (Singh *et al.* 2011) and flubendiamide (Jaiswal and Singh 2016) to be safe.

Significant suppression in population of lepidopteran predators, *E. amabilis* and *P. pulverea* was recorded with treatment of broodlac. Both key lepidopteran predators feed on lac insect as well as on lac. *P. pulverea* also multiply under storage condition of lac. While treating broodlac, both topical and oral action takes place. The ingested lac larvae and lac cause larval mortality of predators. Dipping of broodlac in

Table 3 Effect of emamectin benzoate (5% SG) on population of *Pseudohypatopa pulverea* due to dipping of *rangeeni* broodlac

Conc. (%)	Duration of broodlac dipping					
	5 min		10 min		15 min	
	Mean number*	% reduction	Mean number*	% reduction	Mean number*	% reduction
0.00025	1.70 (1.70) <sup>b</sup>	81.11	0.80 (0.80) <sup>ab</sup>	89.74	0.80 (0.80) <sup>ab</sup>	90.91
0.0005	0.80 (0.80) <sup>ab</sup>	91.11	0.60 (0.60) <sup>ab</sup>	92.31	0.60 (0.60) <sup>ab</sup>	93.18
0.0010	0.70 (0.70) <sup>ab</sup>	92.22	0.60 (0.60) <sup>ab</sup>	92.31	0.50 (0.50) <sup>ab</sup>	94.32
0.0015	0.60 (0.60) <sup>ab</sup>	93.33	0.60 (0.60) <sup>ab</sup>	92.31	0.30 (0.30) <sup>a</sup>	96.59
0.0020	0.40 (0.40) <sup>a</sup>	95.56	0.30 (0.30) <sup>a</sup>	96.15	0.20 (0.20) <sup>a</sup>	97.73
0.0025	0.40 (0.40) <sup>a</sup>	95.56	0.30 (0.30) <sup>a</sup>	96.15	0.20 (0.20) <sup>a</sup>	97.73
0.0030	0.40 (0.40) <sup>a</sup>	95.56	0.30 (0.30) <sup>a</sup>	96.15	0.20 (0.20) <sup>a</sup>	97.73
Control	9.00 (9.00) <sup>c</sup>	0.00	7.80 (7.80) <sup>c</sup>	0.00	8.80 (8.80) <sup>c</sup>	0.00
SEd ±			0.19			
F			23.73			
p			0.0			

\*Mean number in 50 g broodlac. Figures in parentheses are transformed values to  $\sqrt{n+0.5}$ . Means marked with different letters including 5, 10 and 15 minutes of each dipping time are significantly different ( $P < 0.05$ ).

insecticidal formulations for suppression of *E. amabilis* and *P. pulvereae* harbouring broodlac has been recommended by treatment of endosulfan (Malhotra and Bhattacharya 1988, Bhattacharya *et al.* 1994), ethofenprox, profenfos (Bhattacharya 2005a), indoxacarb, fipronil, spinosad and ethofenprox without any adverse effect on lac insect (Singh *et al.* 2013) and recently with flubendiamide (Jaiswal and Singh 2016). In the present study, it has been established that there is no adverse effect on lac insect if broodlac is dipped up to 15 min in EB formulation, even at higher concentration (0.0030 % a.i.). Significant suppression of population of *E. amabilis* and *P. pulvereae* could be achieved even with a very low concentration (0.00025 % a.i.). With due consideration to rotate the insecticides having different mode of action, EB is a new class of insecticide for the management of insect-pest of lac. The earlier recommended insecticides with their mode of actions are endosulfan (presently banned), and fipronil (GABA-gated chloride channel antagonists); cartap hydrochloride (nicotinic acetyl-choline receptor channel blockers); dichlorvos (acetylcholinesterase inhibitors); ethofenprox (sodium channel modulators); indoxacarb (voltage dependent sodium channel blockers); spinosad (nicotinic acetylcholine receptor allosteric activator); flubendiamide (ryanodine receptor modulator) and *Bacillus thuringiensis* sub sp *kurstaki* (microbial disruptors of insect midgut membranes) [IRAC 2009].

The treatment of *rangeeni* broodlac with 0.00025 % a.i. (0.05 g/L water of 5 % SG formulation) for 10-15 min and *kusmi* broodlac at 0.0005 % a.i. (0.1 g/L of water) for 5-10 min duration provides an effective tool for the management of both major lepidopteran predators of lac insects. This insecticide can play a crucial role as a rotational insecticide to manage insecticide resistance in the target pest populations as its mode of action (chloride channel activator on nerve and muscle action) differ from earlier recommended insecticides. This evaluation report of EB on lac insect-pest ecosystem can be safely and effectively integrated in IPM programme formulated for the management of lepidopteran pest.

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