EFFECT OF EMAMECTIN BENZOATE ON FEEDING RESPONSE, MORTALITY AND REPRODUCTION OF ADULT BOLLWORM

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Abstract

Emamectin benzoate was evaluated for its potential as an ingested toxicant and reproduction inhibitor in mixtures with 2.5 M sugar solution as a feeding stimulant on a ppm AI weight:volume basis for bollworm (BW), *Helicoverpa zea* (Boddie), adult control. When emamectin benzoate was mixed with 2.5 M sucrose and ingested by sex pheromone trap-captured males, LC_{90}s (95% Confidence Limits) after 48 and 72 h were 1.3566 (0.8590-6.7833) and 1.3411 (0.7639-8.8511) ppm, respectively; these values were not significantly different based on the presence of overlap in the 95% CLs. Mean lethal times (LTs) in h were 39.3 for 1.3 ppm (1X LC_{90}), 24 for 6.5 ppm (5X), 12.6 for 13 ppm (10X), 10.2 for 32.5 ppm (25X) and 9 for 65 ppm (50X); LTs at 13 ppm and greater were not significantly different from one another and these values were significantly faster than those at 1.3 and 6.5 ppm. Compared to many insecticides evaluated earlier, emamectin benzoate has extremely high oral toxicity and is slow- acting. Compared to 2.5 M sucrose alone, emamectin benzoate at 1.3, 6.5, 13, 32.5 and 65 ppm did not significantly reduce gustatory response of sex pheromone trap-captured males. Similarly, proboscis extension response of sex pheromone trap-captured males to emamectin benzoate at lethal concentrations up to 200 ppm (153.8X LC_{90}) was not significantly different when compared to 2.5 M sucrose alone. These results indicate that emamectin benzoate does not inhibit feeding. When laboratory-reared females were fed emamectin benzoate at concentrations of 0.0125, 0.025, 0.05, 0.075 and 0.1 ppm in 2.5 M sucrose and mated with untreated males, fecundity was not significantly affected, but percent larval hatch of eggs was significantly reduced at concentrations of 0.05 ppm and above during 3 consecutive days when compared to females fed only 2.5 M sucrose alone. When laboratory-reared females were fed emamectin benzoate at concentrations of 0, 0.05, 0.1, 0.2, 0.6 and 1.0 ppm and mated with untreated males, fecundity was significantly reduced at concentrations above 0.6 ppm and percent larval hatch of eggs was also significantly reduced at concentrations of 0.1 ppm and above for all 3 days of evaluations when compared to females fed 2.5 M sucrose alone. Reduction in larval hatch at 0.05 ppm was not significantly different from 2.5 M sucrose alone during all 3 days of evaluations. Mating frequency of laboratory-reared females was not significantly influenced by ingestion of emamectin benzoate. The frequency of larvae hatching from eggs oviposited by treated females found either alive or dead during development to the pupal stage was not independent of emamectin benzoate concentrations and suggests that the larval survival to the pupal stages was significantly influenced by ingestion of emamectin benzoate by laboratory-reared females. Data suggest that emamectin benzoate could be a useful toxicant and reproduction inhibitor in an attracticide formulation at the concentrations tested in this study.

Introduction

Emamectin benzoate belongs to the avermectin group of chemicals produced by the soil-dwelling bacteria, *Streptomyces avermitilisis* and possesses excellent insecticidal potency against several lepidopteran insects with LC_{90} values ranging from 0.001-0.02 µg/ml in ingestion-based foliar spray assays for larvae (White et al. 1997). Dunbar et al. (1998) reported that emamectin benzoate was very effective at controlling tobacco budworm, *Heliothis virescens* (F.) and bollworm (BW), *Helicoverpa zea* (Boddie) larvae at low use rates (0.0075-0.075 lb ai/ac). Jansson and Dybas (1996) reported that emamectin benzoate is stored as a reservoir in plant parenchyma tissues and this accounts for its long residual activity against several phytophagous pest insects. However, no study has been reported on the effect of emamectin benzoate on feeding response, mortality, and reproduction of adult bollworm when provided in a feeding stimulant solution to newly-emerged laboratory-reared females or pheromone trap-captured males. This information is required as part of an overall strategy in evaluating emamectin benzoate as a toxicant and reproduction inhibitor for compatibility and efficacy in the development of an adult control technology for bollworm using attracticides.

Our objectives were to evaluate the effects of emamectin benzoate on proboscis extension response of sex pheromone trap-captured males and gustatory response of both field males and laboratory-reared females when provided in a feeding stimulant solution. Subsequent to determination of gustatory response, females were evaluated for effects on mating frequency, fecundity, fertility, and survival of the progeny. The objective of this study was to determine whether or not emamectin benzoate when ingested by female bollworm will induce mortalities similar to those caused by the compound as a larval contact poison. We also wanted to characterize the effect of emamectin benzoate on pheromone trap-captured males relative to lethal concentration, mean lethal time and proboscis extension response. We chose to use natural populations of

males to study lethal concentration, proboscis extension response and mean lethal time because wild males are readily available throughout the growing season through captures in sex pheromone-baited traps, and there was no significant difference in responses relative to these variables between wild male and female moths (Lopez, unpublished data).

Materials and Methods

Test Insects

Sex pheromone trap-captured BW males were obtained using 75-50 Texas wire cone sex pheromone traps (Hartstack et al. 1979, Hartstack and Witz 1981) baited with laminated plastic Zealure (Hercon Environmental, Emigsville, PA). The traps were operated in farm areas in the Brazos River Valley near College Station, TX. Only males captured the previous night that had been provided deionized water *ad libitum* were used.

Bollworm moths were reared in the laboratory from eggs obtained from the Southern Insect Management Laboratory, USDA-ARS, Stoneville, MS using similar techniques described previously (Lopez and Lingren 1994). Larvae were reared on soybean-wheat germ diet (ARTHROFEEDS "*Manduca* Premix-*Heliothis* Premix", Stonefly Industries, Bryan, TX). Approximately 4 g of the diet was dispensed into a plastic soufflé cup using a caulking gun and an individual larva was placed on the diet and sealed with a lid. About 3 weeks thereafter pupae were harvested, sorted by sex, and male and female pupae were placed separately in 1-gallon jars for moth emergence. All rearing and testing with laboratory-reared moths were conducted during the day in a laboratory maintained at 23.9°C ± 0.38 SE, RH 64.5 % ± 4.6 SE and a photoperiod of 14:10 (L:D) h.

Test Solutions

We formulated 0.1% dilutions (1000 ppm) of emamectin benzoate (MK-0244 5% SG) supplied by Merck Research Laboratories, Hillsborough Road, Three Bridges, NJ 0887 in deionized water. Serial dilutions ranging from 0.0125 to 200 ppm were then prepared from this solution by dilution with 2.5 M sucrose solution (grade II, Sigma Chemical Co., St. Louis, MO). The 2.5 M sucrose solution was prepared with deionized water. The test solutions were stored in a refrigerator, and before each use, the solutions were warmed to laboratory temperature using tap water.

Determination of Lethal Concentration and Lethal Time

The LC values were determined to optimize toxicant concentration in the attracticide formulation. Determination of LCs was based upon preliminary feeding studies with several ppm values, which were either increased or decreased by trial and error. Ten sex pheromone trap-captured males were fed for one-half h on each toxicant concentration in each replication, and was replicated 5X. The moths in each replication were placed inside a one-quart bottle, and were examined for mortality at 24, 48 and 72 h thereafter. A male was considered dead when it could not right itself when upside down. To determine mean time to death, each moth was placed individually in a sealed plastic soufflè cup and observed for mortality at 15, 30 and 45 min and every h until a 6 h period when checking was increased to 12, 18, and 24 h. The mid-point of the interval during which the moths were considered dead was used in calculations.

Determination of Gustatory Response

The conduct of feeding studies and the feeding apparatus used here was similar to that described earlier (Lopez and Lingren 1994; Clemens 1996). Only laboratory-reared females which emerged during the previous night were used. To determine the gustatory response of sex pheromone trap-captured males and laboratory-reared female bollworms, moths were mounted individually in the feeding apparatus and were offered the test solutions contained in a disposable polystyrene microcentrifuge tube (0.5 ml). A total of ten moths were tested with each treatment that included a check comprising of 2.5 M sucrose solution. The amount fed was determined from the differences between the before and after feeding weights of the tubes corrected for evaporation loss from tubes not fed.

Determination of Proboscis Extension Response

The ability to elicit proboscis extension is a pre-requisite for feeding and the determination of this response for use of emamectin benzoate as a toxicant in a feeding stimulant/attracticide formulation is essential for the development of this technology for control of BW moths. The methods used to determine proboscis extension response were similar to those described by Lopez et al. (1995). Proboscis extension response was evaluated at night in an insectary under red light using sex pheromone trap-captured males. The test solutions were placed in the wells of porcelain spot plates and moths were allowed to contact their front tarsi with the test solutions. Ten replications each consisting of ten moths for each concentration was conducted. If the proboscis was extended for feeding initiation, a positive response was recorded, and if no proboscis extension was observed, a negative response was recorded. Partial response when the proboscis did not contact the test solution was not considered a positive response.

Determination of Reproduction Inhibition

Each laboratory-reared female was fed until satiated which did not exceed more than one-half h and was paired with a male. The mouths of the glass jars containing test moths were closed with a paper towel. A strip of paper towel was suspended from the mouth of each jar, which provided a substrate for the moths to climb and lay eggs. The moths were fed 10% (wt:vol) sucrose solution in a 25 ml plastic soufflè cup with a lid through which a cotton wick was inserted. Dead males were replaced with live males during the test.

Two tests were conducted to evaluate the reproductive effects of emamectin benzoate at sublethal concentrations. In Test 1, emamectin benzoate concentrations used were 0.0125, 0.025, 0.05, 0.075 ppm and 0.1 and in Test 2, emamectin benzoate concentrations used were 0.05, 0.1, 0.2, 0.6 and 1.0 ppm. Both tests included females fed 2.5 M sucrose alone as control. After the 2nd night, the moths were transferred to clean jars, numbers of eggs in the used jars were counted and a sample of ca. 30 eggs was collected from the paper toweling in each jar for three consecutive days from each treatment. Eggs were set aside in 25 ml plastic soufflé cups sealed with a lid for determining larval hatch which was checked for three consecutive days. Eggs from unmated females were excluded from determination of egg viability. At the end of each test, female moths were dissected under a 30X stereo zoom microscope to determine mating frequency by counting the number of spermatophores in the bursa copulatrix. The male bollworm moth transfers a spermatophore to the female at the time of mating (Callahan 1958). During each egg viability check, a minimum of ten larvae were removed from the cups with the egg samples and reared individually to pupa on a soybean-wheat germ insect diet (ARTHRO FEEDS "*Manduca* Premix-*Heliothis* Premix", Stonefly Industries, Bryan, TX). Approximately three weeks after the placement of larvae on the diet, each soufflè cup was examined for the presence of pupae and their status whether alive or dead during development was determined. Both alive and dead larvae and pupae were counted. Pupae were sexed and sex ratio was calculated as $([1+\text{males}]/[1+\text{females}])^{1/2}$.

Data Analyses

Analyses of variance of the data were conducted using SAS (1988). When F-values for treatment were significant at the 5% level, means were separated using Least Significant Difference (LSD) test at the 5% level of probability. LCs were determined using the POLO Software (LeOra Software 1987). Regression lines were fitted using Microsoft Excel version 7. Analyses of frequency distribution data relative to larval mortality during development to the pupal stage and male to female sex ratios were conducted using the log likelihood ratio test (G-test) for independence and goodness of fit and compared to the χ^2 statistic as the test criterion at the 5% level of probability (Sokal and Rohlf 1969).

Results and Discussion

The dosage mortality equation provided good fit of the mortality data with $\chi^2 = 5.254$ and $\chi^2 = 1.455$ each with 3 df , for 48 and 72 h responses, respectively (Table 1). Emamectin benzoate was toxic to pheromone trap-captured males with LC_{90}s (95% CL) in ppm at 48 and 72 h of 1.3566 (0.8590-6.7833) and 1.3411 (0.7639-8.8511), respectively. The LC_{90} at 48 h was not significantly different from that at 72 h based on the presence of overlap in CLs. If the statistic *g*, which estimates the confidence intervals at any probability level, is >0.5 at any of these probability levels, the value of the lethal dose may lie outside the limits (Robertson and Priesler 1991). Accordingly, LCs for 24 h were not presented because the *g* statistic was >0.5 for all probability levels (0.51441, 0.9407 and 3.1688 for 90, 95 and 99% CLs, respectively). The LC_{10} of 0.1589 ppm at 48 h was not significantly different from 0.0267 ppm at 72 h based on the presence of overlap of the 95% CL values. Similarly, the LC_{50} of 0.4644 ppm at 48 h was not significantly different from 0.1892 ppm at 72 h. These nonsignificant differences in LC values and the inability to get significant data for 24 h suggest that emamectin benzoate is a slow-acting insecticide. Compared to several insecticides tested earlier (Clemens 1996), emamectin benzoate has extremely high oral toxicity. These data indicate that sub-lethal concentrations of emamectin benzoate are below 1 ppm.

Figure 1 shows that the lethal time (LT) in h was 39.3 at 1.3 ppm (1X LC_{90}), 24 at 6.5 ppm (5X), 12.6 at 13 ppm (10X), 10.2 at 32.5 ppm (25X) and 9 at 65 ppm (50X). Relationship between LTs and concentrations of emamectin benzoate was inversely related and appears to level off asymptotically. The LTs at 13 ppm and higher were not significantly different and suggests an optimum concentration of 13 ppm for causing the quickest mortality.

Evaluation of gustatory response of sex pheromone trap-captured males to the same concentrations used for LT determinations showed no significant difference between treatments when compared to 2.5 M sucrose alone (Figure 2).

Evaluation of lethal concentrations of emamectin benzoate for effect on proboscis extension response of sex pheromone trap-captured males showed that when compared to 2.5 M sucrose alone there was no significant reduction in response even when the concentration reached 200 ppm (153.8X LC_{90}) (Figure 3). Proboscis extension is a pre-requisite for feeding initiation and the results along with those on gustatory response indicate that emamectin benzoate could be used in the field at relatively high concentrations without interfering with feeding.

Figure 4 shows that in Test 1 there was no significant difference in feeding between laboratory-reared females fed sublethal concentrations of emamectin benzoate and those fed 2.5 M sucrose alone. This indicates that the amount ingested was not a factor in subsequent reproduction inhibition tests. Test 2 in which the range of emamectin benzoate concentrations was broadened to include 0, 0.05, 0.1, 0.2, 0.6 and 1.0 ppm was conducted to better assess the effects of emamectin benzoate concentrations on feeding response and reproduction. Figure 5 shows that in Test 2 there was no consistent difference in feeding response between treatments. Feeding of moths was the lowest on the control sugar (72.1 mg) and moths ingested significantly more emamectin benzoate at 0.1 ppm (106.5 mg) but this value was not significantly different from those at 0.05, 0.2, 0.6 and 1 ppm.

Fecundity of laboratory-reared females in Test 1 did not significantly vary between females fed concentrations of emamectin benzoate and females fed 2.5 M sucrose alone (Fig. 6). In Test 2, the number of eggs oviposited by females fed emamectin benzoate at 0.6 ppm and above was significantly depressed compared to females fed 2.5 M sucrose alone (Fig. 7).

Test 1 result on the effect of emamectin benzoate on percent larval hatch of eggs deposited by females fed concentrations of 0.0125, 0.025, 0.05, 0.075 and 0.1 showed significant reduction compared to eggs deposited by females fed 2.5 M sucrose alone (Fig. 8A). Beginning day 2 of evaluations, significant reduction in larval hatch occurred at concentration as low as 0.0125 ppm. However, with increased concentrations of emamectin benzoate, there was dramatic reduction in larval hatch for all 3 days of evaluations caused by concentrations as low as 0.05 ppm (Fig. 8B).

Results of Test 2 on percent larval hatch of eggs oviposited by treated females showed that significant reduction occurred at 0.1 ppm compared to females fed 2.5 M sucrose alone (Fig. 9A). However, in contrast to Test 1, reduction in percent larval hatch at 0.05 ppm was not significantly different from eggs oviposited by females fed 2.5 M sucrose alone during all 3 days of evaluations. This difference in reduction in percent larval hatch at 0.05 ppm between Test 1 and Test 2 probably reflects the difficulty in mixing the insecticide in a viscous medium such as the 2.5 M sucrose solution using the serial dilution technique. An alternative mixing method in which a stock solution of the insecticide in water is prepared and a minimal amount of the stock solution is added to 2.5 M sucrose solution may reduce this difficulty. Percent reduction in larval hatch compared to control was more apparent at 0.2 ppm and above for all three days of evaluations and exhibited a more consistent dose response relationship (Fig. 9B).

Mating frequency of treated females in Test 1 and Test 2 was not significantly different between laboratory-reared females fed emamectin benzoate and those females fed 2.5 M sugar alone (Figures 10 and 11). The mean number of spermatophores/♀ in Test 1 and Test 2 were 2.1 and 1.9, respectively. Three to four spermatophores/♀ were common in females fed emamectin benzoate.

An assessment of larval mortality during development to pupal stage was conducted only in Test 1. Relationship between larval mortality during development to the pupal stage and emamectin benzoate concentrations in Test 1 is shown in Figure 12. Larval mortality in the control averaged 11.6% but increased to 54.3% at 0.0125 ppm and thereafter showed a decreasing trend in mortality up to 0.05 ppm. Mortality of larvae again increased at 0.075 ppm and then declined slightly at 0.1 ppm. A curvilinear equation of the form, $Y = -2.98X^2 + 25.238X - 1.148$ with $R^2 = 0.5427$, where Y = larval mortality and X = emamectin benzoate concentrations, was obtained which described the relationship between mean percent larval mortality and emamectin benzoate concentrations. The G-test for independence showed that the frequency distribution of the larvae hatching from eggs oviposited by treated females found either alive or dead was not independent of emamectin benzoate concentrations ($\chi^2 = 229.4$ with 5 df; $P < 0.005$). This suggests that emamectin benzoate significantly influenced survival of the larvae to pupal stages. The G-test for independence also showed that the frequency distribution of male and female pupae was independent of emamectin benzoate concentrations ($\chi^2 = 2.2$ with 5 df; $P > 0.05$). Figure 13 shows the relationship between male to female sex ratio and emamectin benzoate and indicates that the sex ratio of the surviving bollworm was not significantly affected by ingestion of emamectin benzoate by females.

Data presented here demonstrate that emamectin benzoate has excellent potential for use in an attracticide/stimulant formulation for areawide suppression of the bollworm. The extremely low amount of emamectin benzoate that suppresses larval hatch of eggs when ingested by laboratory-reared females is a very promising characteristic and this coupled with the lack of gustatory and proboscis extension inhibition makes this chemical an ideal candidate for further research. Furthermore, White et al. (1997) reported that emamectin benzoate provides ecological selectivity to a wide range of beneficial arthropods and is compatible with integrated pest management programs. More studies are needed to determine the effect of emamectin benzoate on male bollworms as well as on field populations of the insect to obtain a more comprehensive knowledge of this novel insecticide.

Conclusion

Emamectin benzoate has potential for use as an adult toxicant because when it is ingested, it kills adults at very low concentrations, although it is slow-acting. It also has potential as reproduction inhibitor at sublethal concentrations in an adult control system using feeding attractants/stimulants because it caused significant reductions of larval hatch of eggs oviposited by females that ingested it at very low concentrations. Furthermore, there was no feeding inhibition exhibited based on both proboscis extension and gustatory responses. More detailed studies in the laboratory as well as in the field are needed for successful implementation of this technology.

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Disclaimer

Mention of a commercial or proprietary product does not constitute an endorsement for its use by the U. S. Department of Agriculture.

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Table 1. Lethal concentration (LC) (ppm ai wt:vol.) data (48 and 72 h) for the toxicity of emamectin benzoate to pheromone trap-captured male bollworms when fed emamectin benzoate in 2.5 M sucrose solution [a]/ .

Regression Statistics	Number of Hours after Feeding	
	48	72
Slope (±SE)	4.719 ± 0.7291	2.583±0.6318
χ^2 (df)	5.254 (3)	1.455 (3)
LC_{10} (ppm)	0.1589 a	0.0267 a
(95% lower-upper limits)	(0.0115-0.2971)	(0.0002-0.0877)
LC_{50} (ppm)	0.4644 a	0.1892 a
(95% lower-upper limits)	(0.2016-0.7013)	(0.0311-0.3280)
LC_{90} (ppm)	1.3566 a	1.3411 a
(95% lower-upper limits)	(0.8590-6.7833)	(0.7639-8.8511)

[a]/ Based on 250 males for each observation. Calculated using POLO-PC (LeOra Software, 1987). LC values in the same row followed by the same lower case letter are not significantly different based on the presence of overlap in the 95% lower and upper confidence limits.

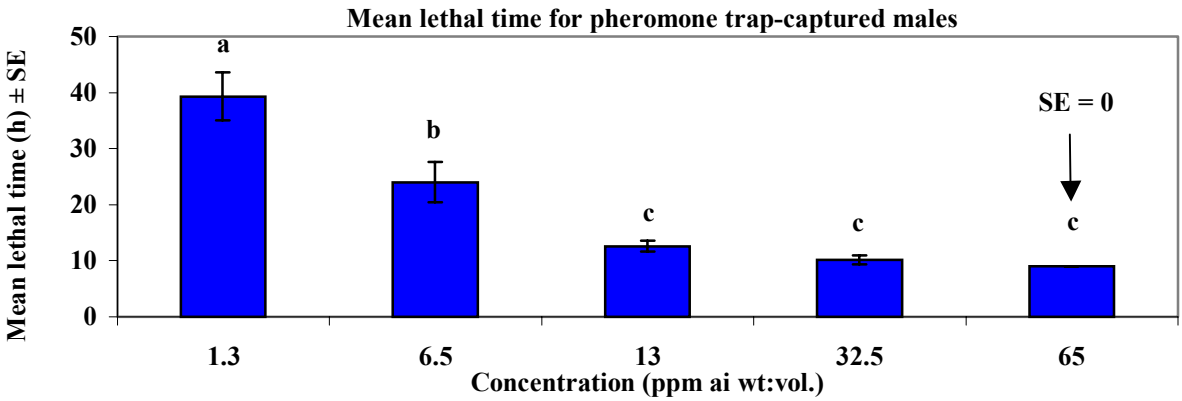

Figure 1. Mean lethal time in hours for pheromone trap captured male bollworms when fed emamectin benzoate mixed with 2.5 M sucrose solution. Means followed by the same lower case letter are not significantly different according to Least Significant Difference Test (P=0.05).

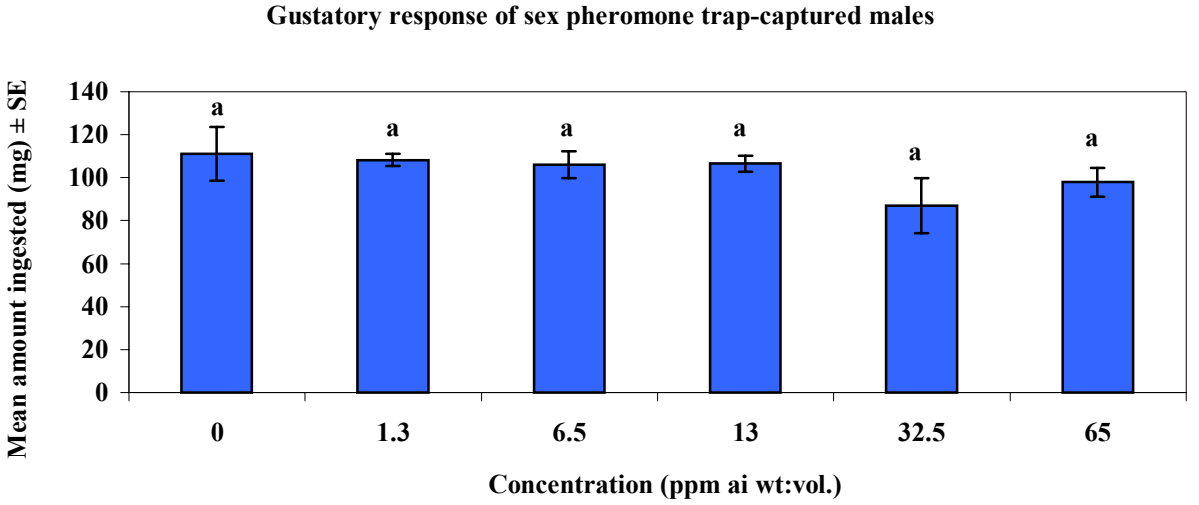

Figure 2. Gustatory response of sex pheromone trap-captured males to emamectin benzoate mixed with 2.5 M sucrose solution. Means followed by the same lower case letter are not significantly different according to Least Significant Difference Test (P=0.05).

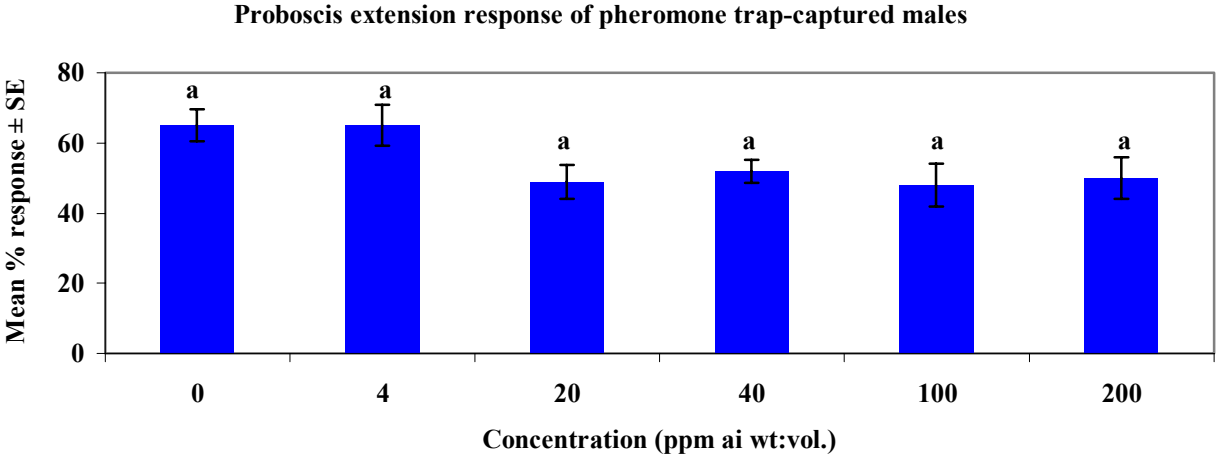

Figure 3. Proboscis extension response of sex pheromone trap-captured males to emamectin benzoate mixed with 2.5 M sucrose solution. Means followed by the same lower case letter are not significantly different according to Least Significant Difference Test (P=0.05).

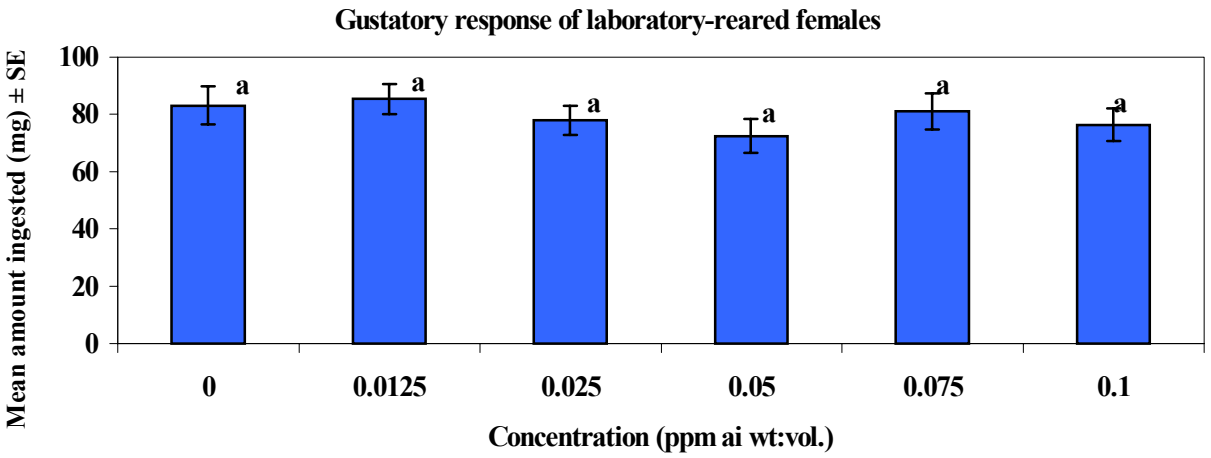

Figure 4. Gustatory response of laboratory-reared females to emamectin benzoate mixed with 2.5 M sucrose solution in Test 1. Means followed by the same lower case letter are not significantly different according to Least Significant Difference Test (P=0.05).

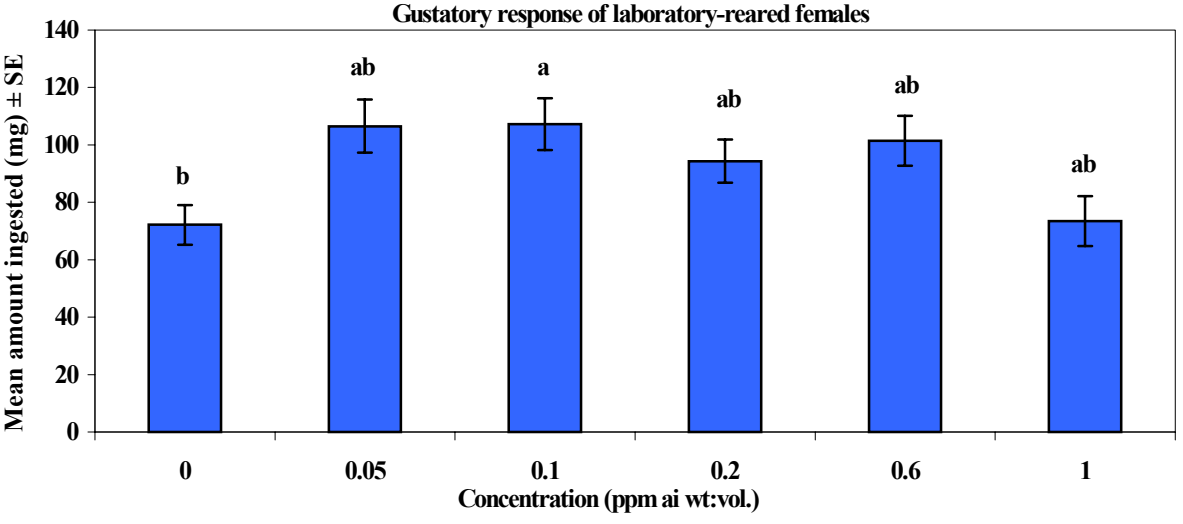

Figure 5. Gustatory response of laboratory-reared females to emamectin benzoate mixed with 2.5 M sucrose solution in Test 2. Means followed by the same lower case letter are not significantly different according to Least Significant Difference Test (P=0.05).

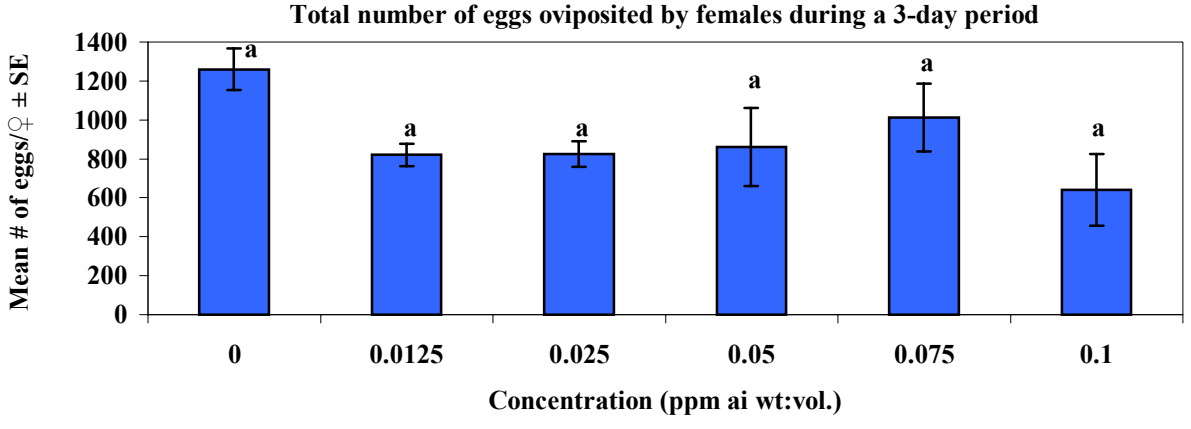

Figure 6. Fecundity of laboratory-reared females when fed emamectin benzoate mixed with 2.5 M sucrose solution in Test 1. Means followed by the same lower case letter are not significantly different according to Least Significant Difference Test (P=0.05).

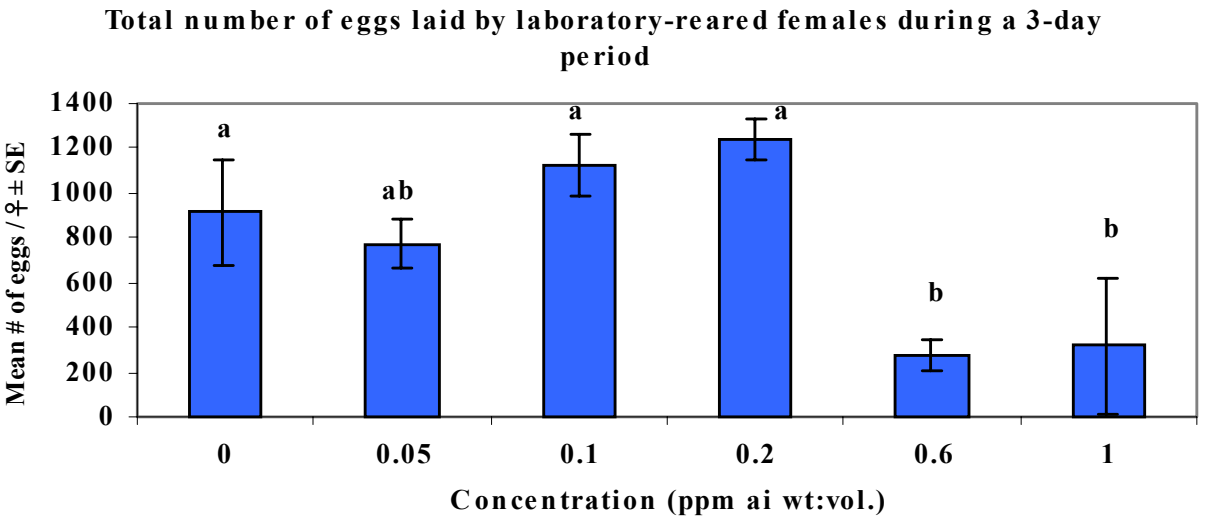

Figure 7. Fecundity of laboratory-reared females when fed emamectin benzoate mixed with 2.5 M sucrose solution in Test 2. Means followed by the same lower case letter are not significantly different according to Least Significant Difference Test (P=0.05).

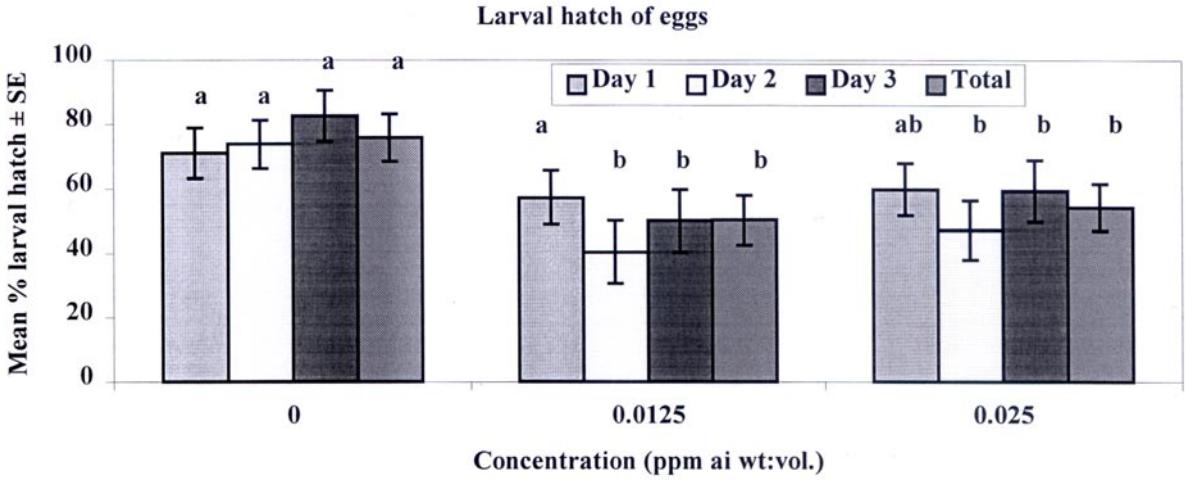

Figure 8A. Percent larval hatch of eggs oviposited by laboratory-reared females fed emamectin benzoate mixed with 2.5 M sucrose solution in Test 1. Means followed by the same lower case letter within each day and total are not significantly different according to Least Significant Difference Test (P=0.05).

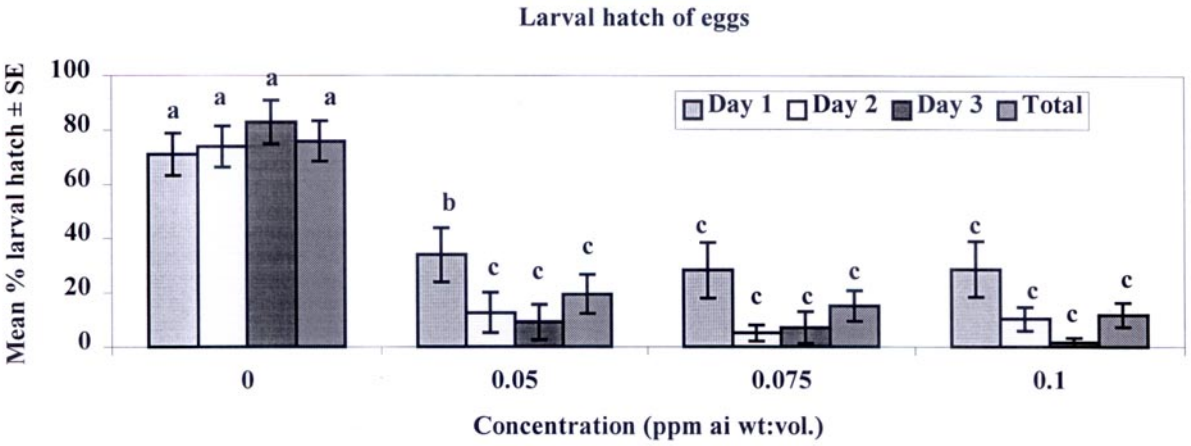

Figure 8B. Percent larval hatch of eggs oviposited by laboratory-reared females fed emamectin benzoate mixed with 2.5 M sucrose solution in Test 1. Means followed by the same lower case letter within each day and total are not significantly different according to Least Significant Difference Test (P=0.05).

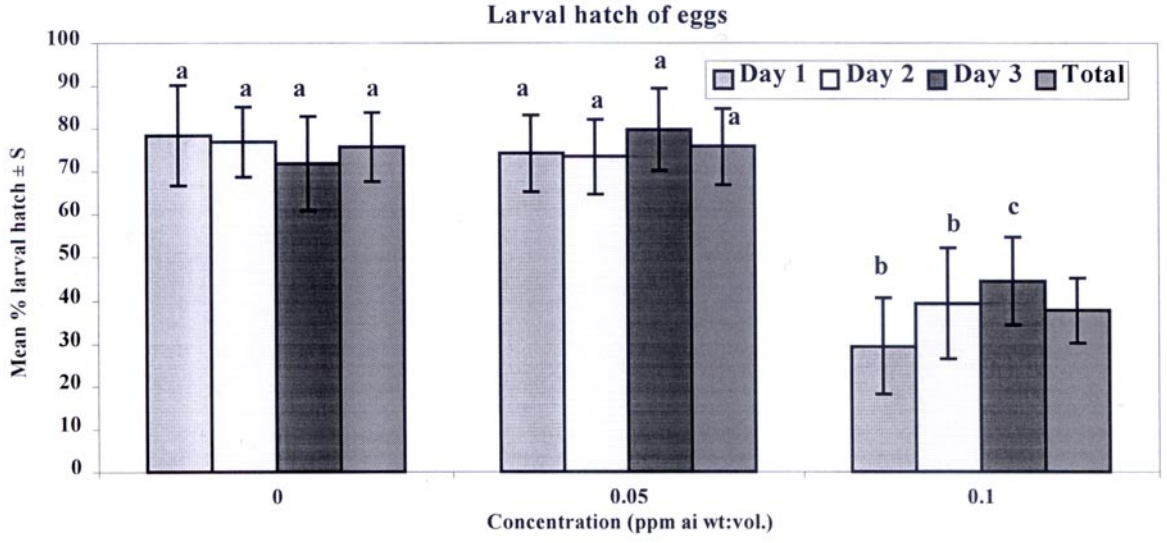

Figure 9A. Percent larval hatch of eggs oviposited by laboratory-reared females fed emamectin benzoate mixed with 2.5 M sucrose solution in Test 1. Means followed by the same lower case letter within each day and total are not significantly different according to Least Significant Difference Test (P=0.05).

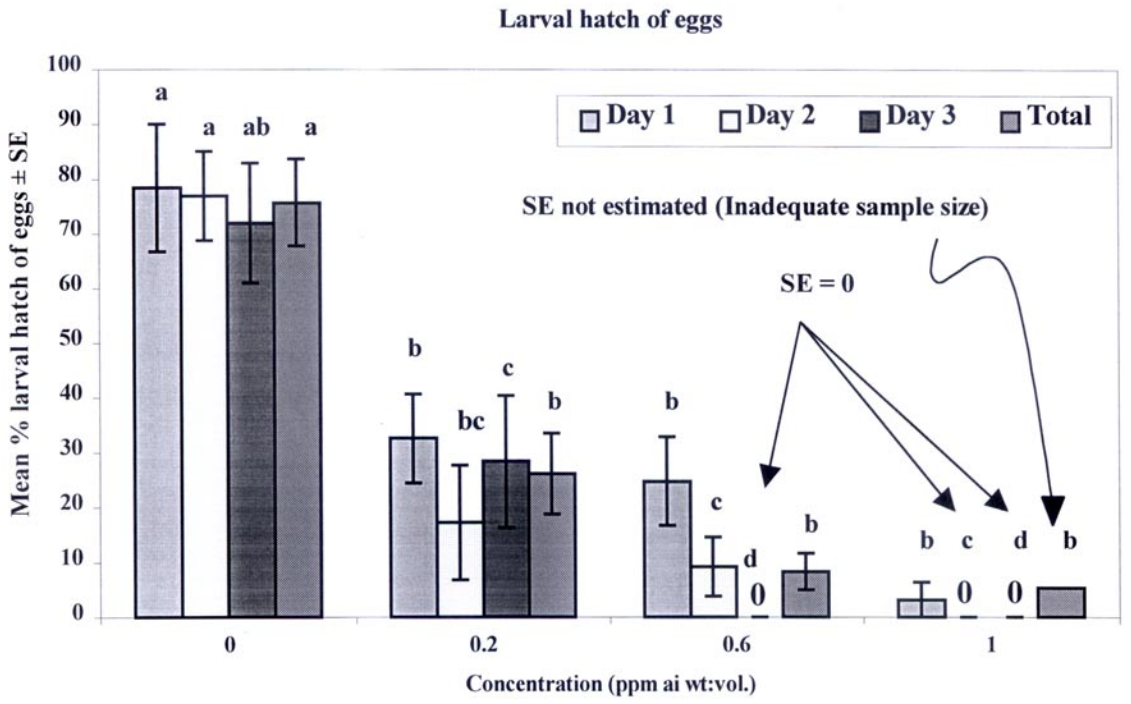

Figure 9B. Percent larval hatch of eggs oviposited by laboratory-reared females fed emamectin benzoate mixed with 2.5 M sucrose solution in Test 2. Means followed by the same lower case letter within each day and total are not significantly different according to Least Significant Difference Test (P=0.05).

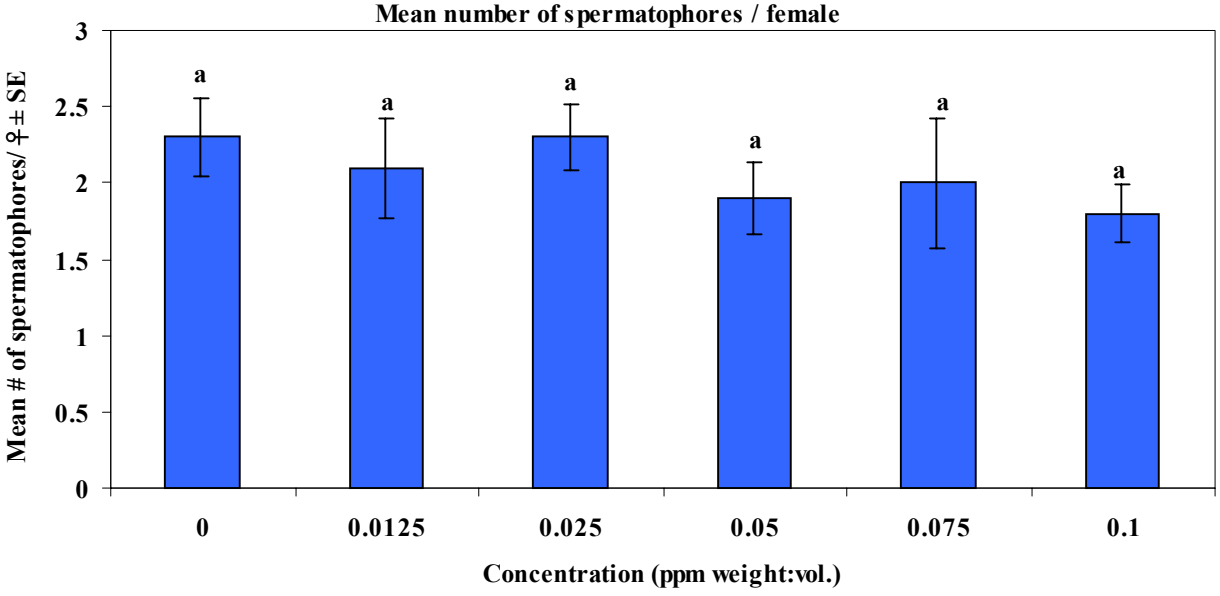

Figure 10. Mean number of spermatophores per female fed emamectin benzoate mixed with 2.5 M sucrose solution in Test 1. Means followed by the same lower case letter are not significantly different according to Least Significant Difference Test (P=0.05).

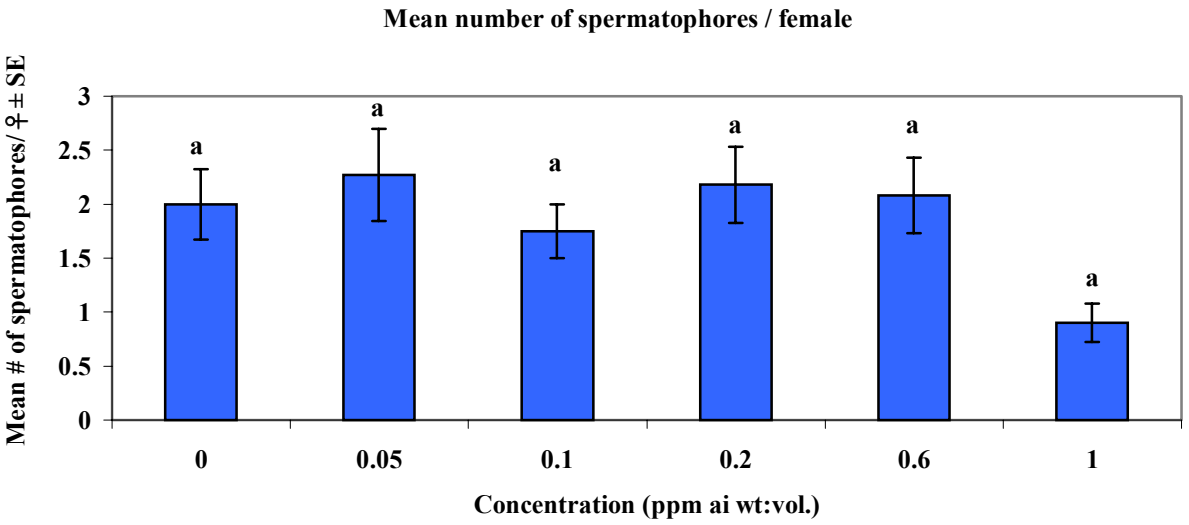

Figure 11. Mean number of spermatophores per female fed emamectin benzoate mixed with 2.5 M sucrose solution in Test 2. Means followed by the same lower case letter within each day and total are not significantly different according to Least Significant Difference Test (P=0.05).

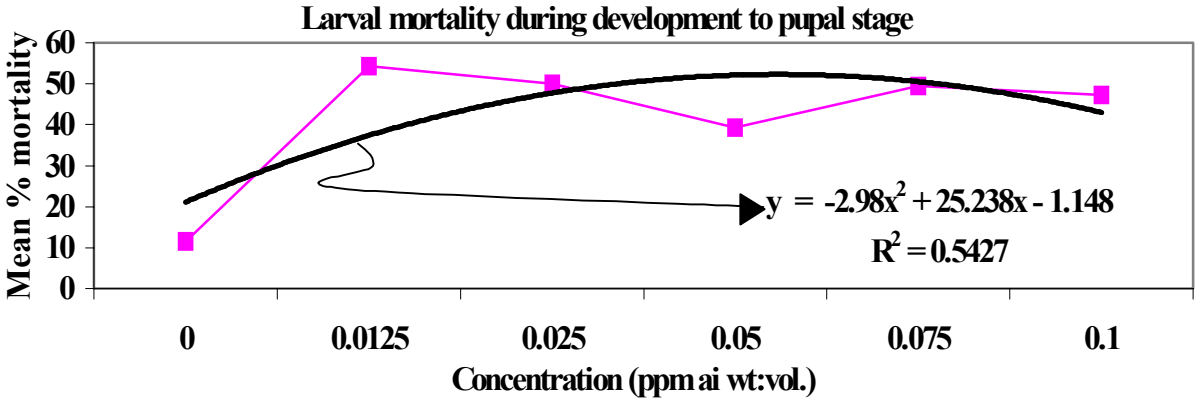

Figure 12. Relationship between larval mortality during development to the pupal stage and concentrations of emamectin benzoate fed laboratory-reared females in Test 1. Regression equation was: $Y = -2.98X^2 + 25.238X - 1.148$, where Y = percent larval mortality and X = emamectin benzoate concentrations and $R^2 = 0.5427$.

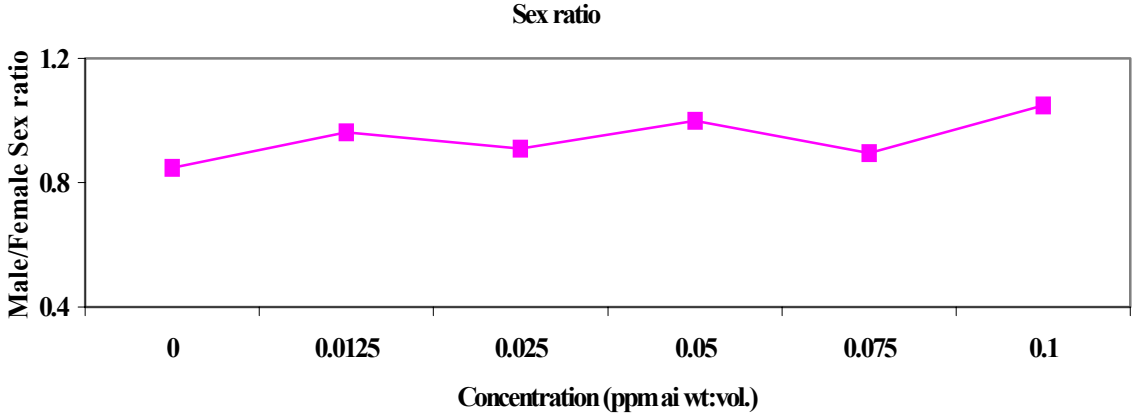

Figure 13. Male/female sex ratio of pupae in Test 1 in which laboratory-reared females were fed emamectin benzoate and paired with unmated males.