EFFECT OF A MACROCYCLIC LACTONE INSECTICIDE, EMAMECTIN BENZOATE ON SOME BIOLOGICAL ASPECTS OF THE COTTON LEAFWORM *SPODOPTERA LITTORALIS* (BOISDUVAL)

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Abstract

Emamectin, the 4”-deoxy-4”-methylamino derivative of abamectin is a novel macrocyclic lactone insecticide, derived from naturally occurring avermectin molecules. It is generally prepared as a salt with benzoic acid "emamectin benzoate" which has potent efficacy against many Lepidoptera species and commercially used in pesticides market under different trade names. In the current study, emamectin benzoate was evaluated for its toxicity on different life stages of *S. littoralis*. The obtained data revealed that emamectin benzoate is highly toxic as a stomach poison and less toxic via contact. Larval stage was highly sensitive and egg stage was the greatest tolerant. The effect of this chemical on the development and the reproductive potential of cotton leafworm was also undertaken to answer the question "what could happen if cotton leafworm larvae exposed to sublethal concentration of this insecticide? Data revealed that feeding 4th instar larvae on emamectin benzoate treated castor bean leaves with the 96hr LC_{25}, negatively affect food consumption and insect development. Larvae consumed less food, gained less weight. In addition, durations of larvae and pupae significantly elongated and emerged adult females significantly laid less eggs (215.0/female) compared to the untreated check (369.0/female). Moreover, some of deposited eggs did not hatch (59.2% egg hatching versus 90.3%in the control). However, this chemical did not affect pupae survival or sex ratio.

Introduction

Macrocyclic lactone pesticides were discovered in the mid-1970's as a direct result of a screening effort for natural products with anthelmintic properties (Lasota and Dybas, 1990). Avermectin group produced by the fermentation of the soil actinomycete, *Streptomyces avermitilis* and have shown low toxicity to non-target beneficial arthropods which has accelerated their acceptance into Integrated Pest Management (IPM) programs for controlling field crop pests (Ishaaya *et al.,* 2002).

Emamectin is a novel macrocyclic lactone insecticide derived from naturally occurring avermectin molecules (Ioriatti *et al.,* 2009), differs from avermectins B1a and B1b by the presence of a hydroxyl group at the 4”-epimethylamino group (McGonigle and Lummis, 2010). It is the 4”-deoxy-4”-methylamino derivative of abamectin (Kaoukhov & Cousin, 2009 and Grossman & Cox, 2010) which has potent efficacy against many species of Lepidoptera pests (Liguori *et al.* 2010).

Emamectin is generally prepared as a salt with benzoic acid "emamectin benzoate" which is a white or faintly yellow powder (Waddy *et al*, 2007). It works, like other avermectins, as a chloride channel activator by binding gamma amino butyric acid (GABA) receptor and glutamate-gated chloride channels disrupting nerve signals within arthropods (Grant, 2002). The compound stimulates the release of GABA from the synapses between nerve cells and additionally increasing GABA’s affinity for its receptor on the post-junction membrane of muscle cells in insects and arthropods. The stronger binding of GABA increases the cells permeability to chloride ions within the cell due to the hypotonic concentration gradient. Neurotransmission is thereby reduced by subsequent hyperpolarisation and the elimination of signal transduction (Rodríguez *et al*, 2007 and Andersch *et al*, 2011).

Emamectin benzoate is acting mainly through ingestion, showing a translaminar activity on leaf surface; therefore the active ingredient breaks down in a very short time to sub-lethal doses which make it safe for most beneficial organisms living on the vegetation. Ishtiaq and Saleem (2011) suggested that emamectin benzoate could be used in pesticide rotation to reduce the development of resistance and to minimize the impacts on environment. Also the residue profile is very favorable, leading to a very low maximum residue level and short pre-harvest interval in all edible crops.

Literature searching confirmed the suitability of this product, as an eco-friendly microbial based insecticide, in integrated pest management strategy against Lepidoptera pests. Field collected populations from Lepidoptera pests exhibited no or very low rate of resistance to emamectin benzoate [Khaliq *et al.* (2007), *Plutella xylostella*; Rao *et al.* (2008), *Spodoptera exigua*; Stanley *et al.* (2009), *Helicoverpa armigera*; Rahman *et al.* (2010), *Plutella xylostella*; Chao *et al.* (2011), *Spodoptera exigua*; Ishtiaq and Saleem (2011), *Spodoptera exigua*; Ishtiaq *et al.* (2012), *Spodoptera exigua*; and Shad *et al.* (2012), *Spodoptera litura*]. This low rate of resistance is probably because the short period of using this product in the field, in addition to its novel mode of action. In contrast, selection pressure with emamectin benzoate dramatically increased emamectin resistance in *Plutella xylostella* (Patil *et al.*, 2011). However, resistance was unstable in *Spodoptera litura*, quickly decreased after stop selection (Rehan *et al.*, 2011).

Emamectin benzoate was found to be safe on beneficial arthropods, did not affect survival or foraging behavior of the biological control agents [Wise *et al.* (2010), *Trichogramma minutum*; Chakraborti and Kanti (2011), *Leucinodes orbonalis*; Kawazu *et al.* (2011), *Cotesia vestalis*; Shimoda *et al.* (2011), *Cotesia vestalis*; and Amor *et al.* (2012), *Amblyseius swirskii* and *Orius laevigatus*]. In contrast, negative impact of ememactin benzoate on beneficial arthropods was reported in few cases of the previous studies [Grundy (2007), *Pristhesancus plagipennis*; and Dilbar *et al.* (2010), *Trichogramma chilonis*; predatory coccinellids, spiders and pollinating bees].

Excellent performance of emamectin benzoate was reported under field conditions with different Lepidoptera pests [Clarke-Harris *et al.* (2004), Lepidoptera pest complex; Mandal *et al.* (2009), *Plutella xylostella*; Mutkule *et al.* (2009), *Spodoptera litura*; Abdu-Allah (2010), *Spodoptera littoralis*; Wankhede and Kale (2010), *Leucinodes orbonalis*; Govindan *et al.* (2011), *Helicoverpa armigera*; Mahendra *et al.* (2011), *Helicoverpa armigera*; and Shivaraju *et al.* (2011), *Maruca testulalis*. However, the unique publication on the inefficiency of emamectin benzoate was reported with *Pectinophora gossypiella* by Rani *et al.* (2010),.probably because larvae fed inside the green bolls and this chemical is less effective against adult and egg stages.

The excellent efficacy of emamectin benzoate against Lepidoptera insects at extremely low concentrations, its short residual activity under field conditions, its safety on beneficial arthropods and the reported slow development of resistance on Lepidoptera key pests encouraged the authors to conduct the current study. This study is a part from a Ph.D. dissertation and the objectives of this paper are to compare the acute toxicity of emamectin benzoate on different life stages of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.). Comparing the susceptibility of the three life stages will help to clarify the mode of entry via stomach or/and via contact. Also, the second part is conducted using the estimated 96hr-LC_{25} with fourth instar larvae to determine the effects of tested insecticide on some biological aspects of cotton leafworm (daily food consumption, daily larval weight, larval and pupal durations, per cent pupation and emergence, sex ratio, adult fecundity and fertility). The second part of this paper is designed to answer the question what could happen if the cotton leafworm larvae received emamectin benzoate at sub-lethal concentrations in the field?

Materials and Methods

Cotton leafworm larvae, adults and eggs were obtained from a laboratory colony reared free from any insecticide exposure for 5 years. The experiments were conducted at constant temperature (25 ± 2^{o}C) in the laboratory of Plant Protection Department, Faculty of Agriculture, Minia University. The insecticide used is commercially named Elbasha EC-1.9% with the common name, emamectin benzoate.

Newly deposited eggs (0-24hr old), newly emerged adults (0-24hr old) and newly molted fourth instar larvae (7day old with average weight 14.0-14.5 mg) was used in this study. Toxicity of emamectin benzoate on the three life stages was compared based on the LC_{50} and LC_{90} values.

For evaluating the ovicidal activity of emamectin benzoate, Tafla plant (*Nerium oleander*) leaves carrying newly deposited egg masses were dipped for 60 seconds in different concentrations of the commercial product diluted in water, then % un-hatching was calculated after five days post treatment and corrected with the corresponding mortality for the untreated check using Abbott's formula (Abbott, 1925). Corrected mortalities were transformed to probit units and plotted versus log concentrations, then subjected to Finney probit analysis (Finney, 1971) to obtain the LC-P line data.

Newly emerged adults were fed on 10% sugar syrup prepared in water containing different concentrations of the toxicant. Adults were allowed to feed on contaminated sugar solution for four days and adult mortality was recorded daily, then corrected with control mortality. The probit analysis data were established as previously mentioned (Finney, 1971).

For studying the toxicity of emamectin benzoate on larval stage; newly molted fourth instar larvae were fed on castor bean leaves treated with different concentrations of emamectin benzoate. The feeding period on treated leaves was for 4 days. Mortality was daily recorded up to four days post treatment. Mortality in chemical treatment was corrected with the corresponding mortality in the untreated check and subjected to Finney probit analysis as mentioned before.

To study the effects of emamectin benzoate on some biological aspects of cotton leafworm, newly molted 4th instar larvae were placed individually in plastic vial provided with a disc of fresh castor bean leaves and covered with a pored lid for refreshing the air. Two groups of 100 larvae, each was set up to conduct this study. One group was served as a control, receiving untreated leaves. Another group was received emamectin benzoate treated castor bean leaves with a concentration corresponding to the 96hr LC_{25} that established from the previous study. Each group was divided to ten replicates of ten individual larvae each, which used to follow the daily effects on food consumption, larval weight, larval mortality and finally on larval duration from fourth instar until pupation. Every day, surviving larvae were individually weighed and food consumption was calculated. The Date of pupation was recorded in each vial and pupae were daily observed for recording the date of emergence and the sex of emerged adults. For chemical and control treatment, 10 pairs of adults were coupled in half kilogram jar provided with Tafla (*Nerium oleander*) branch and a piece of cotton pad soaked in 10% sugar solution and jars were covered with muslin cloth tied with a rubber band. The jars were observed daily for collecting Tafla leaves having egg masses and placed after counting in Petri-dishes. For each replicate, numbers of eggs laid and numbers of un-hatched eggs were counted. Mean number of eggs laid and %un-hatching was used to compare adult fecundity and fertility among control and chemical treatment (unpaired t test).

Results and Discussion

Acute toxicity of emamectin benzoate on three life stages of *Spodoptera littoralis*

1- Larval stage

Of three life stages tested, larval stage was highly sensitive to emamectin benzoate via ingestion, as time of feeding 4th instar larvae on emamectin benzoate treated castor bean leaves increased, the LC_{50} and LC_{90} values significantly decreased (based on the overlapping of upper and lower confidence limits as cited in Table, 1). The 24hr LC_{50} [0.46 (0.38-0.56)] was about 10 times greater than the 96-hr LC_{50} [0.055 (0.044-0.069)]. The same trend was obtained when the comparisons were based on the LC_{90} values. The 24hr LC_{90} was 1.51 (1.25-1.84) and significantly decreased after 96 hours feeding on treated leaves to 0.276 (0.22-0.35). The great acute toxicity of emamectin benzoate against cotton leafworm larvae that reported in the current study was confirmed in previous studies with the same insect species. El-Aw (2003) found that emamectin benzoate (based on LC_{50} value) was highly toxic against cotton leafworm larvae. Also, Dahi *et al.* (2009) determined the LC_{50} values of methylamine avermectin (Radical 0.5% EC); against 2nd and 4th instar larvae after 48 hours to be 0.005 and 0.008 ppm, respectively. In more recent study, emamectin benzoate (trade name Proclaim®) was much potent than spinetoram and spinosad against *Spodoptera littoralis* larvae; it proved to be better than spinetoram by 31516 fold [Abdu-Allah (2010)]; he also determined the LD_{50} values of emamectin benzoate, chlorpyrifos-methyl, abamectin, spinosad to be 0.0019, 4.00, 59.88, and 558.25 µg/g larvae, respectively. In a field study by Abdu-Allah (2011), emamectin benzoate retains persistence under field conditions against cotton leafworm larvae under Egyptian field conditions and could be a valuable addition in an integrated pest management programme. Abdu-Allah, 2010 & 2011 confirmed that emamectin benzoate is one of the best bio-insecticides in controlling CLW larvae infestations in cotton fields. In the previous studies with emamectin benzoate, LC_{50} values were lower than the obtained values in our study, most likely because using different cotton leafworm strain, different methods of application or/and different formulation, adjuvants play an important role in enhancing the physical properties of insecticide which reflect on its toxicity.

In addition to the concurrence of our results with the previous studies with the same insect species, our results were also coincided with the previous studies with other Lepidoptera pests. The great acute toxicity of emamectin benzoate (based on the probit data) was reviewed in many previous publications. In a study conducted by Ahmad *et al.* (2005), emamectin proved to be the best treatment against *Spodoptera litura* larvae, followed by lufenuron, spinosad and indoxacarb. In more recent study conducted by Charmillot *et al.* (2007), emamectin was found to be

the most effective larvicidal product against *Grapholita lobarzewskii* with an LC_{50} of 0.01 mg kg^{-1}, followed by spinosad, methoxyfenozide and chlorpyrifos-methyl, with LC_{50} values between 0.2 and 0.7 mg kg^{-1}. Also, Rao and Grace (2008) determined the LC_{50} of emamectin benzoate to be 0.0061ppm and it was the most toxic of all the insecticides tested on *Helicoverpa armigera*. Rao *et al.* (2008) determined the LD_{50} of emamectin benzoate on beet armyworm, *Spodoptera exigua*, to be 0.04pg/larva. Stanley *et al.* (2009) tested emamectin benzoate against *Helicoverpa armigera* larvae using topical and feeding techniques, the LD_{50} of emamectin was $3.86x10^{-3}$ µg/larva and the median lethal concentrations (LC_{50}'s) of emamectin and spinosad were found to be 0.09 and 2.94 ppm, respectively. Also, Lavanya *et al.* (2010) found that the median lethal concentrations (LC_{50}) values on *Plutella xylostella* were 0.066 and 2.12 ppm for emamectin and spinosad, respectively. In a semi field study conducted by Khan *et al*. (2011), emamectin benzoate seemed to be more effective against *Spodoptera litura* (Fab.) than chlorpyrifos, lufenuron and methomyl. Massoud *et al.* (2011) confirmed the great toxicity of emamectin benzoate against the newly hatched larvae of *Pectinophora gossypiella* using film residue assay method, the data revealed that emamectin benzoate was a superior potent compound against this insect species with LC_{50} of 0.001 ppm and the cytotoxic effect revealed certain deviations in the ultra structure of the cerebral neurosecretory cells (CNSC) of the treated pink bollworms larvae as compared by the untreated ones. More additional studies on different Lepidoptera pests reconfirmed our results [Khaliq *et al.* (2007), *Plutella xylostella*; Chouraddi *et al.* (2009), *Maruca vitrata*; Firake and Pande (2009), *Spodoptera litura*; Shankarganesh *et al.* (2009), *Spodoptera litura*; Lopez *et al.* (2010), *H. zea*; Sial and Brunner (2010), *Choristoneura rosaceana*; Wankhede and Kale (2010), *Leucinodes orbonalis*; Wise *et al.* (2010), *Acrobasis vaccinii*; Linden *et al.* (2011), *T. absoluta*; Muthusamy *et al.* (2011), *Spilosoma oblique* and XueSong *et al.* (2011), *C. medinalis*].

In our study, abamectin was found to be ineffective up to 300 ppm against larvae of this insect species and as a result the toxicity line could not be established (data not shown), possibly because the high lipophilicity of abamectin makes it more effective via contact, but not as a stomach insecticide. The low efficiency of abamectin on cotton leafworm larvae was confirmed in a previous study by Abdu-Allah (2010) who determined the LD_{50} values of emamectin benzoate and abamectin to be 0.0019 and 59.88 µg/g larvae, respectively.

2 - Adult stage

Mortality of adults fed on contaminated sugar solution with emamectin benzoate did not significantly increase during the 72hrs post treatment, however, significantly increased in the fourth day of continuous exposure to treated sugar solution. The LC_{50} values were not significantly different within the first three days; however start to be significant in the fourth day (Table, 1). The 24hr LC_{50} and LC_{90} values (expressed as mg a.i./l) were 13.59 (10.98-16.82) and 58.77 (47.48 -72.74), respectively. Increasing the feeding period on treated sugar syrup to four days resulted in decreasing the LC_{50} and LC_{90} to be 5.76 (4.77-6.96) and 17.12 (14.18-20.69), respectively. In the present study, adults were 30-fold (based on the 24-hr LC_{50}) to 105-fold (based on the 96-hr LC_{50}) more tolerant to emamectin benzoate than larvae. The larval LC_{50} ranged from 0.055 at 96-hr post treatment to 0.46 ppm at 24-hr post-treatment. However, the corresponding values with adults ranged from 5.76 to 13.59 ppm. In a study conducted by López *et al*, (2010), it seemed that adults of *Helicoverpa zea* were more sensitive to emamectin benzoate than adults of *S. littoralis* used in the present study. In the previous study by López *et al*, (2010), emamectin benzoate was highly toxic to feral *Helicoverpa zea* males with LC_{50} values (95% CL) being 0.718 (0.532-0.878), 0.525 (0.316-0.751), and 0.182 (0.06-0.294) ppm for 24, 48 and 72 h responses, respectively.

3 - Egg stage

Egg stage was the most tolerant with LC_{50} (expressed as ppm) was found to be 311.41 (235.31-412.12) (Table 1). The corresponding LC_{90} value greatly increased to be 32025.84 (3905.4 -5168.6). Literatures regarding the ovicidal activity of emamectin benzoate are very limited. Pineda *et al.* (2004) recorded no ovicidal activity when spinosad (Tracer) and methoxyfenozide (RH-2485) were used against eggs of the noctuid, *Spodoptera littoralis* (Boisduval). Charmillot *et al.* (2007) reported that fenoxycarb and emamectin gave LC_{50} values worthy of note, close to 2 mg /kg, this finding was in dissimilarity with what reported in the current study, most certainly because using different method of application. Murthy *et al.* (2009) studied the ovicidal activity of emamectin benzoate on eggs of *Papilio demoleus*. The greatest percentages of un-hatched eggs (94.44%) were recorded with novaluron (0.01%) followed by emamectin benzoate (0.005%). The great ovicidal activity in the research by Murthy *et al.* (2009) was possibly because using different insect species and different method of application.

Sub lethal effects of emamectin benzoate on some biological aspects of *S. littoralis*

Newly molted fourth instar larvae were fed on castor bean leaves treated with emamectin benzoate at the estimated concentration corresponding to the 96-hr LC_{25}. Latent effects on larval survival, daily food consumption, average weight of larvae, larval and pupal durations, adult fecundity and fertility were undertaken in the present study.

1- Cumulative mortality within larval stage

As shown in Fig. (1), mortality was followed up until pupation. Cumulative mortality increased with time to reach 51% in chemical treatment at the end of larval stage and only 49% of emamectin benzoate treated larvae successively pupated. On the other hand, 86% of untreated larvae pupated in control group. With the exception of the first two days post treatment, mean mortality percentages were significantly greater in emamectin benzoate treatment compared to the untreated check (unpaired t test).

2 - Daily food consumption

Larvae fed on emamectin benzoate treated castor bean leaves consumed significantly less food starting from the second day post treatment (Fig. 2), with the exception of the 24hr post treatment, food consumption was significantly less than that in the untreated check. The maximum consumption of leaves in emamectin treatment was in day 11, but food consumption dramatically decreased in day 12 and day 13 because some larvae stop feeding before entering pre-pupae. On day 14, there was no food consumption because all surviving larvae converted to pre-pupae and pupae. In control treatment, the maximum food consumption was in day 9 and it dramatically decreased in day 10 because most of larvae stop feeding just before entering the pre-pupae stage. Starting from day 11, no food consumption was recorded in control treatment because all surviving larvae converted to pre-pupae and pupae. Our data were confirmed, however with another insect species and another avermectin derivative; Zhu *et al.* (2008) found that sub-lethal concentrations of abamectin (LC_5, LC_{10} and LC_{20}) significantly inhibited the growth and food intake of the larvae of silkworm (*Bombyx mori* L); they found that amylase activity in the midgut of the larvae treated with abamectin decreased significantly. In agreement with our results, however with a different insect species, Sial and Brunner (2010) observed significantly less consumed foliage by obliquebanded leafroller, *C. rosaceana* larvae surviving in the emamectin, chlorantraniliprole, and spinetoram treatments compared with those exposed to untreated foliage. In the current study, total food consumption during the whole period (Starting from the beginning of 4^{th} instar till the end of 6^{th} instar) was calculated (Fig. 3) and compared between control and emamectin treatment. Although of the significant reduction in daily food consumption in emamectin benzoate treatment compared to control treatment; however, total food consumption was not significantly different between the two treatments (Unpaired t test). Moreover, total food consumption was insignificantly greater (1471.42mg/larva) in emamectin benzoate treatment compared to control treatment (1263.03mg/larva). This unexpected results when total food consumption was compared among control and emamectin benzoate treatment could be explained from the point view that food consumption is a summation of daily consumption and period of feeding. larvae took longer time in emamectin benzoate to stop feeding (~14 days); however this period reduced to ~11 days in control treatment. This finding means that leaf damage on the long run will not reduce when larvae exposed to sublethal doses of emamectin benzoate.

3 - Daily weight of larvae

The dynamic changes in mean weight of larvae were graphed in Fig. (4). With the exception of data recorded in day 0 (just before treatment) and day 1 (24hr post treatment), average weight of larvae was significantly less in chemical treatment compared to the untreated check, certainly because of the less intake of food. The maximum gained weight was recorded in day 10 for control treatment and in day 13 for chemical treatment. This means that larvae in chemical treatment grow slowly; they spent almost 13 days to gain the optimum weight that control larvae gained in 10 days. As expected, gained weight is an essential function of food consumption in both control and chemical treatment (Fig. 5). The linear correlation between daily weight of larvae and daily food consumption was established in both treatments. Also the strong and significant correlation (R^2 = 0.965 and 0.988 for emamectin and control treatments, respectively) confirmed that the chemical may not affect digestive enzyme activities and most likely affect the feeding behavior. In incongruity with our finding, Zhu *et al.* (2008) found that amylase activity in the midgut of the larvae treated with abamectin decreased significantly.

Insect development and its reproductive potential

1 - Larval and pupal durations

Data expressed the effects of emamectin benzoate on insect development and the reproductive potential are recorded in Table (2). Duration of larvae was significantly elongated in chemical treatment. Larvae exposed to sub-lethal

concentration of emamectin benzoate grow slowly to reach pupal stage; they took longer time averaged 16.33 days compared to 13.89 days for the untreated check (Table 2). The difference in larval duration among the control and chemical treatment was significant (Unpaired t-test). Pupal duration was 12.52 and 10.25 days in chemical and control treatments, respectively and the difference was also significant.

2 - Percentages of pupation and adult emergence

About 86% of untreated larvae were pupated compared to 49% in chemical treatment and this difference was highly significant (Table 2). All formed pupae in control treatment converted to adult stage compared to 89% of formed pupae in chemical treatment succeeded to develop to adult stage. However, when percentages of adult emergence calculated based on number of treated larvae, the difference was highly significant among chemical and control treatment. This means that larvae that were able to convert to pupae in emamectin benzoate treatment suffered no longer toxic effect during pupal stage. There was no significant difference between the female ratios in chemical and control treatments.

3 - Adult fecundity and fertility

Females emerged from pupae treated in larval stage laid significantly less eggs compared to those emerged in control treatment. Moreover, 59.2% of eggs hatched in chemical treatment compared to 90.3% in control treatment.

Conclusion

In conclusion, emamectin benzoate at sublethal concentration (96hrLC_{25}) negatively affect larval survival, daily food consumption, average weight of larvae, elongated larval and pupal durations and reduced adult fecundity and fertility. In agreement to our finding, El-Aw (2003) fed 4th instar larvae of *S littoralis* on castor bean leaves treated with the estimated LC_{25} value of Proclaim (emamectin benzoate) and found that this product significantly reduced female and male pupal weights, fecundity of emerged adults and egg hatchability. Previous results by Yu *et al.* (2007) with other Lepidoptera pests confirmed that, fecundity of the diamondback moth, *Plutella xylotella* was obviously negatively affected after treated the 3rd instar larvae with sub-lethal concentrations of emamectin. In a study conducted by López et al, (2010), newly emerged corn earworm adults, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) when ingested sub-lethal concentrations of emamectin in 2.5 M sucrose as a feeding stimulant reduced per cent larval hatch of eggs and mating frequency of *H. zea* female. Larval survival to the pupal stage was also significantly reduced.

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Table (1): The LC- P line data established from the toxicity lines of emamectin benzoate when tested against newly molted 4th instar larvae (leaf disc feeding assay), newly emerged adults (sugar solution feeding assay) and 0-24hr old eggs (egg-dipping assay) of cotton leafworm, *Spodoptera littoralis* (Boisd.).

Tested stage	Time post treatment	Toxicity line equation	Calculated Slope ± SE	C. χ^2	T.χ^2	*df*	LC_{50} as ppm (95% CL)	LC_{90} as ppm (95% CL)
Larval stage	24hr	*y = 2.452x+0.915	2.51 ± 0.37	3.78	18.47	4	0.46 (0.38-0.56)a	1.51 (1.25-1.84)
	48hr	*y = 1.714x+2.874	1.74 ± 0.20	6.61	24.32	7	0.18 (0.15-0.22)b	0.79 (0.66 -0.97)
	72hr	*y = 1.837x+3.264	1.79 ± 0.22	2.82	22.46	6	0.088 (0.072-0.11)c	0.44 (0.36 – 0.55)
	96hr	*y = 1.823x+3.652	1.81 ± 0.30	0.25	18.47	4	0.055 (0.044-0.069)d	0.276 (0.22-0.35)
Adult stage	24hr	y = 1.980x+2.775	1.92 ± 0.26	3.62	18.47	4	13.59 (10.98-16.82)a	58.77 (47.48 -72.74)
	48hr	y = 1.556x+3.351	1.44 ± 0.29	6.27	18.47	4	11.38 (8.36-15.49)a	76.04 (55.86 -103.5)
	72hr	y = 2.170x + 2.90	2.17 ± 0.27	1.62	20.52	5	9.16 (7.58-11.06)a	35.94 (29.74 -43.9)
	96hr	y = 2.693x+2.957	2.66 ± 0.51	3.39	16.27	3	5.76 (4.77-6.96)b	17.12 (14.18 -20.69)
Egg stage	120hr	y = 1.164x+ 2.089	1.16 ± 0.13	1.22	24.32	7	311.413 (235.31-412.12)	32025.84 (3905.4 -5168.6)

- ***Because of the low concentrations used to establish toxicity lines with larvae, log concentrations were mostly negative and to solve this problem, a value of 2 was added to each log when establishing the toxicity line, so x in the toxicity equation = log concentration + 2.**

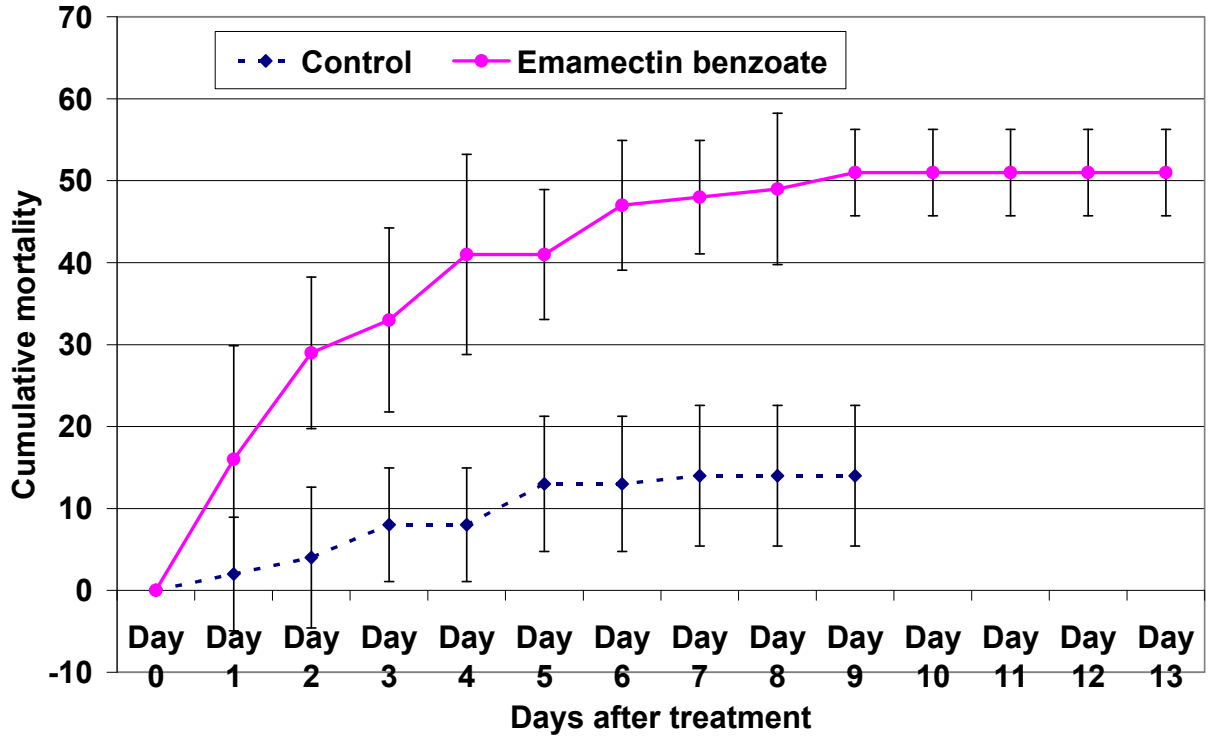

Fig. (1): Mean percentages of cumulative mortality when the newly molted 4th instar larvae of cotton leafworm were fed on treated castor bean leaves with emamectin benzoate at a concentration corresponding to the 96hr LC_{25}.

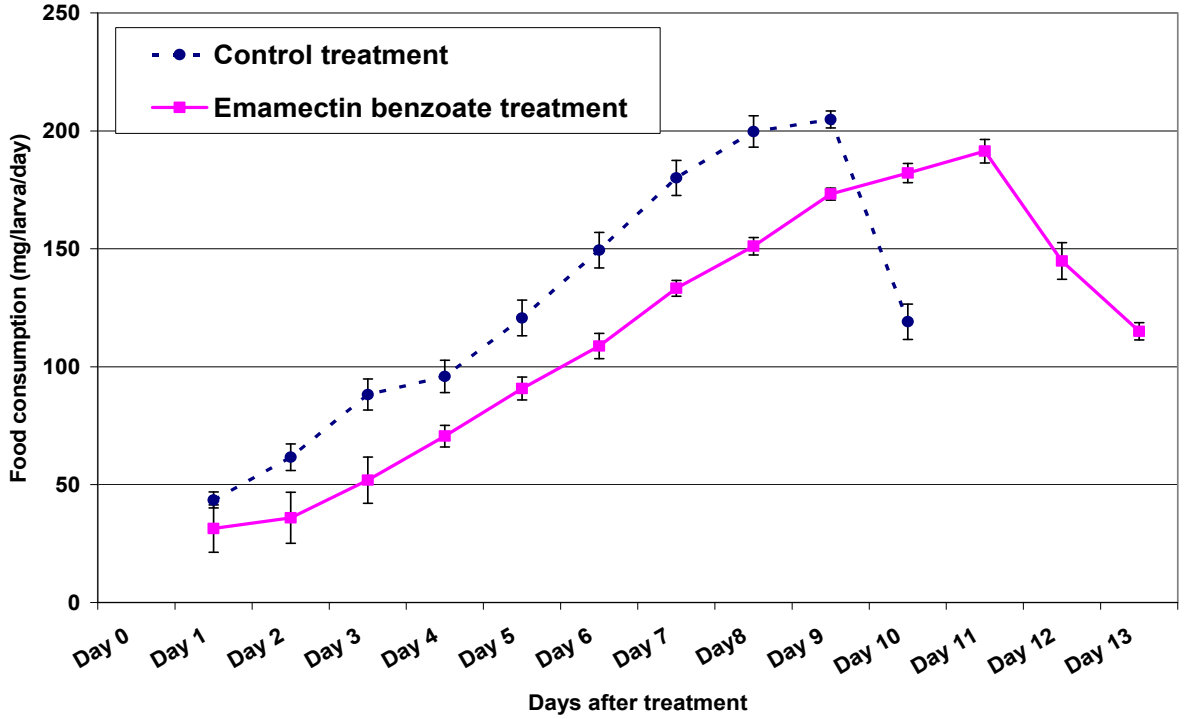

Figure (2): Daily weight of consumed food (Mean ± SD) when newly molted 4th instar larvae of cotton leafworm were fed on treated castor bean leaves that was treated with emamectin benzoate at a concentration corresponding to the 96-LC_{25}.

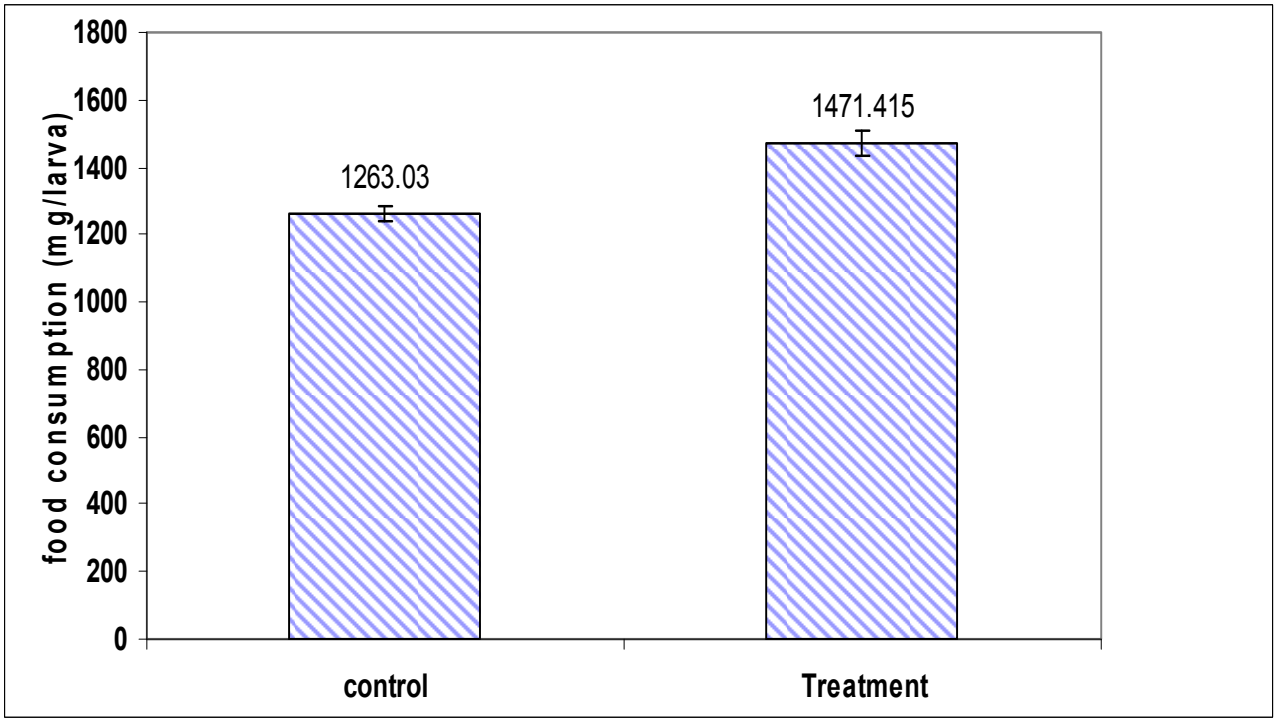

Fig (3): Total food consumption (Mean ± SD) from the beginning of 4th instar larvae until pupation as a result of feeding 4th instar larvae on untreated and emamectin benzoate treated leaves with the estimated 96hr LC_{25}.

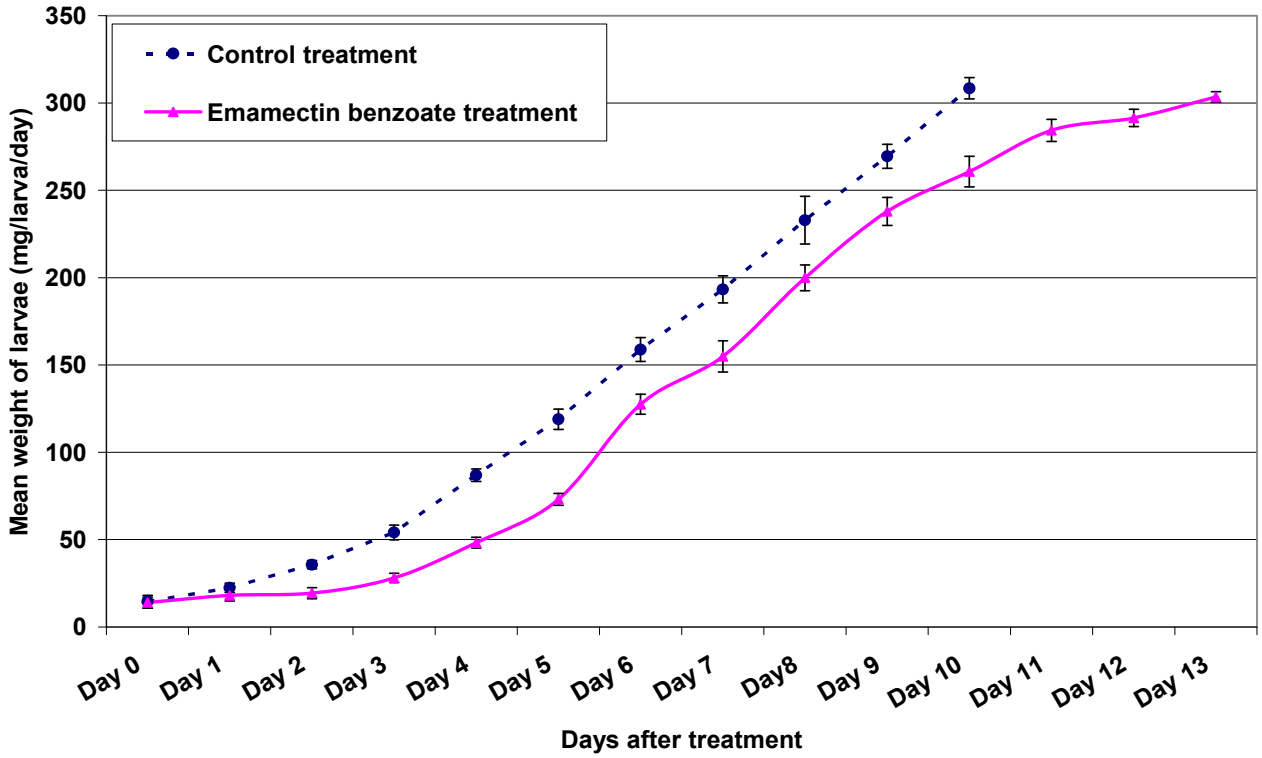

Fig. (4): Daily weight of larvae (Mean ± SD) when newly molted fourth instar larvae of cotton leaf worm were fed on castor bean leaves treated with emamectin benzoate at a concentration corresponding to the 96hr LC_{25}.

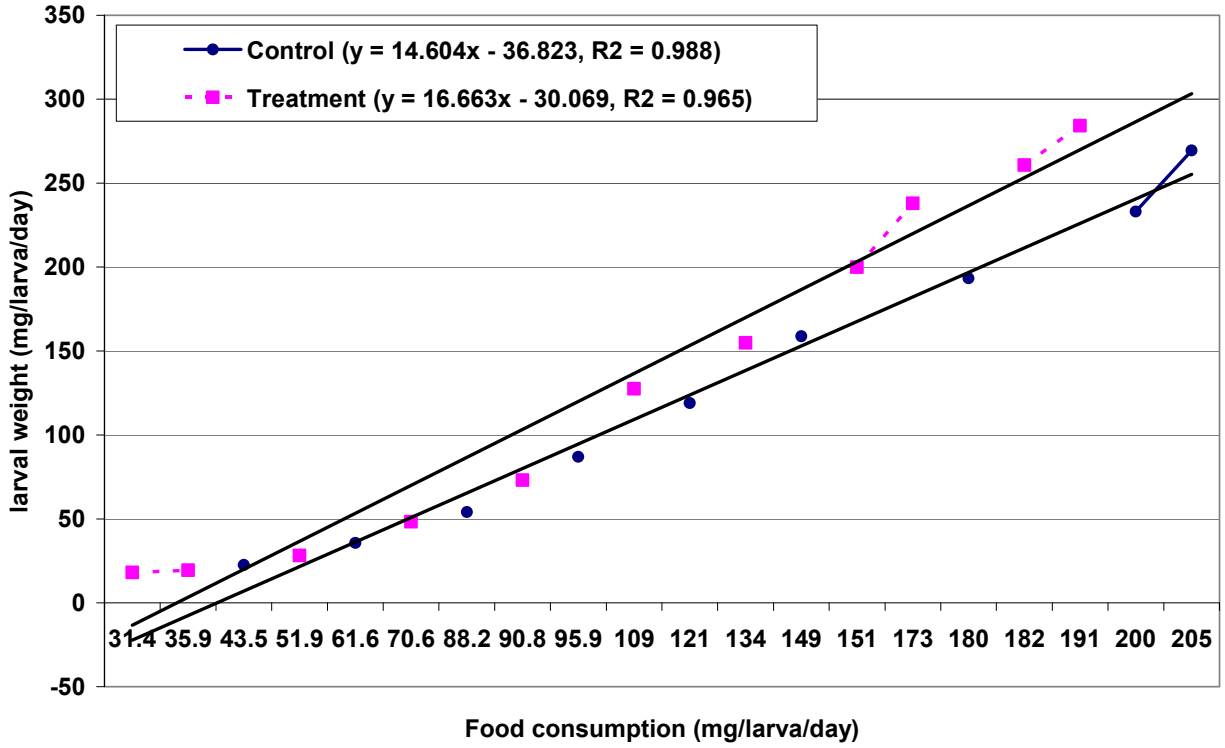

Fig (5): Mean weight of larvae in relation to daily food consumption when *Spodoptera littoralis* (Biosd.) 4th instar larvae fed on untreated or emamectin benzoate treated castor bean leaves with the estimated 96hr LC_{25}.

Table (2): Development and reproductive potential of *S. littoralis* when newly molted 4th instar larvae were fed on treated castor bean leaves with emamectin benzoate at concentration corresponding to the 96 hr LC_{25}.

Measured parameter	Mean ± SD		Un-paired T-test (P-value)
	Control	Emamectin benzoate	
Larval duration	13.89 ± 0.21	16.33 ± 0.12	0.0432*
%Pupation	86.0 ± 5.16	49.0 ± 3.16	0.0064**
Pupal duration	10.25 ± 0.41	12.52 ± 0.17	0.0478*
%Emergence#	100 ± 0.0	89.50 ± 11.17	0.764 NS
%Emergence##	86.0 ± 5.16	44.0 ± 6.99	0.0097**
%Female	51.25 ± 15.83	49.26 ± 21.28	0.732NS
No. of eggs/female	369.0 ± 60.9	215.0 ± 27.7	0.0078**
% egg hatching	90.3 ± 2.4	59.2 ± 5.3	0.0084**

#related to number of formed pupae
Related to number of treated larvae