Effect of Bio-rational Insecticides on Some Biological Aspects of the Egyptian Cotton Leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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Abstract

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The bio-rational insecticides Dipel 2x, BioFly, Agrin, BioGuard, Spinosad, Neemix, Mectin and Match were tested for their effect on 1st, 3rd and 5th instar larvae and egg masses (24, 48 and 72 h old) of the Egyptian cotton leafworm *Spodoptera littoralis* under laboratory conditions. The doses used were the recommended dose, half of the recommended dose and quarter of the recommended dose. All insecticides caused higher mortality in the 1st than in the 3rd and 5th larval stage, although Match, Mectin and Spinosad at all tested concentrations showed excellent efficacy against the 3rd larval stage of *S. littoralis*. Also, Match resulted in 100% mortality of 5th instar larvae at all tested concentrations. Moreover, these insecticides also strongly affected some biological parameters of treated 3rd and 5th instar larvae of *S. littoralis*. Egg masses of different ages (24, 48 and 72 h old) where dipped into the recommended dose of each insecticide and the mortality rates determined; eggs of different ages were affected similarly, with mortality rates of 83.4, 85.0 and 71.7%, respectively, after treatment with Spinosad compared to the control. In general, eggs 48 and 72 h old were less sensitive than 24 h old ones. A latent effect of the insecticides on egg hatchability of S. *littoralis* was observed only in Match and Neemix, with the average being 55.0% and 51.6%, respectively. Our results suggest that Match, Mectin and Spinosad are potentially potent compounds for control of *S. littoralis*.

Keywords: bio-rational insecticides; *Spodoptera littoralis*

In Egypt, many crops and various vegetables are attacked by numerous insect pests. Among these, the lepidopterous insects in general and the cotton leafworm *Spodoptera littoralis* (Boisd.) in particular are the most damaging. In fact, cotton leafworm is a major limiting factor affecting crop and vegetable production not only in Egypt, but also in many other countries. It is one of the most destructive agricultural lepidopterous pests within its subtropical and tropical range (Hosny *et al*. 1986), where it can attack numerous economically important crops all year round. On cotton, the pest may cause considerable damage by feeding on the leaves, fruiting points, flower buds and, occasionally, also on bolls. When groundnuts are infested, larvae select primarily the young folded leaves for feeding but, in severe attacks, leaves of any age are stripped off. Sometimes, even the ripening kernels in pods in the soil may be attacked. Pods of cowpeas and the seeds they contain are also often badly damaged. In tomatoes, larvae bore into the fruit which is thus rendered unsuitable for consumption. Numerous other crops are attacked, mainly on their leaves.

Chemical control of *S. littoralis* has been extensively reported in relation especially to cotton. In Egypt, *S. littoralis* was held in check by methyl-parathion, but then the pest developed resistance to this compound. Since then, numerous other organophosphorus, synthetic pyrethroid and other insecticides have been used, with appearance of resistance and cross-resistance in many cases (Issa *et al.* 1984a, b; Abo-El-Ghar *et al.* 1986). However, compulsory limitation of the application of synthetic pyrethroids to one per year on cotton in Egypt has stopped the appearance of new resistance (Sawicki 1986). Chemicals used against *S. littoralis* also include insect growth regulators.

Due to the appearance of high resistance to many chemical pesticides and resurgence of chemical pesticides (Forgash 1984; Georghiou 1986) there is growing interest in the use of bioinsecticides such as compounds based on bacteria, fungi, insect growth regulators and botanical pesticides (Rao *et al.* 1990; Ahmad *et al.* 2008; Mourad *et al.* 2008). These groups have modes of action different from those of conventional products (Ascher 1993; Thompson *et al.* 1999); also, their properties may differ considerably from the conventional chemicals with which growers are familiar.

In the present experiments the effectiveness of several such bioinsecticides against the cotton leafworm *S. littoralis* was determined with the intention to find the best compounds for controlling this economic pest in an integrated pest management program.

MATERIALS AND METHODS

Insect maintenance. Larvae of the cotton leafworm, *Spodoptera littoralis*, were reared on fresh castor leaves, *Ricinus communis* L., in the Entomology Laboratory, Plant Protection Department, Suez Canal University, at a temperature of 23 ± 2°C and 65 ± 2% R.H. with a photoperiod of 16:8 (L:D).

Compounds tested and bioassay techniques for larval instars. Commercial formulations of the following insecticides were tested against 1st, 3rd and 5th instar larvae of *S. littoralis*: Dipel 2x (*Bacillus thuringiensis* var. *kurstaki* 22000 IU/mg), BioFly (*Beauveria bassiana* 100%, 30 × 10^6 cell), Agrin (*Bacillus thuringiensis* (Bt) 32000 IU/mg), BioGuard (*Bacillus thuringiensis* bacteria 30 million IU/g), Spinosad (spinosyns A and D, *Saccharopolyspora spinosa*, 24% SL), Neemix (4.5% azadirachtin), Match (50% EC lufenuron) and Mectin (*Streptomyces avermitilis*, 80% avermectin B1a and 20% avermectin B1b). Three doses of the tested compounds were prepared in distilled water: the dose recommended by the manufacturer, half of the recommended dose, and a quarter of the recommended dose.

Castor leaf disks (5 cm diameter) were dipped into the test solutions for 15 s with gentle agitation. They were allowed to surface-dry on a paper towel and then placed into Petri dishes containing moistened filter papers to avoid desiccation of leaves. Larvae were transferred onto the leaf disks; either ten 1st instar larvae, five 3rd instar larvae or four 5th instar larvae per Petri dish per replicate. Each treatment was replicated three times, along with an untreated control in a completely randomised design (Ahmad *et al.* 2007). Mortality of 1st instar larvae was recorded 1, 3, 5 and 7 days after application of the insecticides, whereas mortality of the 3rd and 5th instar larvae of *S. littoralis* was accumulatively recorded. Insects were considered dead if they gave no response to stimulation by touch. The pupae obtained were placed separately in Petri dishes. Pupation, pupal weight, deformed pupae, healthy pupae, adult emergence, malformed adult percent were recorded.

Newly emerged adults in all tests with the recommended dose were transferred in pairs to clean jars, provided with honey solution as a source of food and taphla leaves as oviposition sites. Each treatment was replicated five times. Deposited egg masses were collected daily and percent of sterility (latent effect) were recorded (Toppozada *et al.* 1966).

Ovicidal tests. Egg masses of *S. littoralis* 24, 48 and 72 h old were dipped into the recommended dose of all insecticides for 5 seconds. Another group of eggs of the same ages were dipped in water as control. Three egg masses of each age were used and the experiment was repeated three times. After drying the egg masses were transferred to Petri dishes and incubated at 23 ± 2°C until hatching. The percentage of hatchability was recorded.

Statistical analysis. Data obtained were statistically analysed through ANOVA (SAS Institute 1999). When the *F*-test was significant, means were separated using Duncan multiple range test (DMRT) at the 0.05 level of significance.

RESULTS AND DISCUSSION

Data presented in Figure 1 show the effect of three concentrations of all tested insecticides (recommended, half and quarter dose of recommended) on 1st instar larvae of *S. littoralis* at 1, 3, 5 and 7 days after treatment. Spinosad, Mectin and Match caused 100% mortality after treatment of castor leaves with the three concentrations and feeding of larvae on the leaves.

There were significant differences between the tested insecticides at the recommended dose 1 day and 3 days after treatments (dF = 26; $F = 8.78$; $P \leq 0.0000$) and (dF = 26; $F = 15.68$; $P \leq 0.0000$). No significant differences were noticed between insecticides in mortality rate 5 and 7 days after treatment, but it was significant compared with the untreated control (dF = 26; $F = 7.50$; $P \leq 0.0002$) and (dF = 26; $F = 10.44$; $P \leq 0.0000$).

The effect of half and quarter of the recommended dose on mortality rate was significant with all tested insecticides compared to untreated larvae, i.e. 1 day after treatment with recommended dose control (dF = 26; $F = 11.69$; $P \leq 0.000$); 3 days (dF= 26; $F = 10.26$; $P \leq 0.000$); 5 days (dF = 26; $F = 8.91$; $P \leq 0.0001$); 7 days (dF = 26; $F = 39.20$; $P \leq 0.000$); and 1 day after treatment with a quarter of the recommended dose (dF = 26; $F = 8.78$; $P \leq 0.0001$); 3 days (dF = 26; $F = 7.84$; $P \leq 0.0002$); 5 days (dF= 26; $F = 5.70$; $P \leq 0.0011$); 7 days (dF = 26; $F = 6.81$; $P \leq 0.0004$).

Data presented in Figure 1 clearly indicated that the mortality rate of 1st instar larvae of *S. littoralis* increased gradually with increasing concentrations of the insecticides and time of exposure.

Pineda *et al.* (2004) reported that Spinosad was a potentially effective compound for the control of *S. littoralis*. Aydin and Oktay (2006) stated that Spinosad was very effective in the control of *S. littoralis,* and showed its high efficacy (100% mortality) when applied at the recommended rate. On the other hand, Spinosad was found to have remarkably similar toxicity to all three economically important fruit fly species, i.e. the Mediterranean fruit fly *Ceratitis capitata*; the melon fly *Bactrocera cucurbitae,* and *B. dorsalis* (Stark *et al.* 2004). Miguel *et al.* (2008) stated that Dalneem, *B. bassiana* and the extract of neem leaves at 20% performed best for control of *Spodoptera eridania.*

Data in Tables 1 and 2 show the effects of the insecticides on 3rd and 5th instar larvae and the latent effects on pupation, deformed and healthy pupae, pupal weight, adult emergence and malformed and recovery of adults after feeding of the larvae on castor leaves treated with three insecticide concentrations. The results showed that the mean percent of cumulative mortality of the 3rd and 5th instar larvae of *S. littoralis* varied significantly among the treatments and control. Maximum mortality (100%) of the 3rd instar larvae was observed after treatment with Match at all tested concentrations, and with Mectin at a quarter of the recommended dose only, whereas the mortality achieved by Mectin at the recommended and half the recommended dose was 93.3%. This was followed by Spinosad with 86.6% at half and quarter of the recommended dose, while the mortality was low (66.6%) at the recommended dose. Cumulative mortality at the recommended dose was (dF = 26; $F = 5.87$; $P \leq 0.0009$); at half the recommended dose (dF = 26; $F = 3.20$; $P \leq 0.0190$); and at quarter the recommended dose it was (dF = 26; $F = 6.82$; $P \leq 0.0006$). Maximum mortality (100%) of the 5th instar larvae was observed after treatment with Match at all tested concentrations; at recommended dose (dF = 26; $F = 3.63$; $P \leq 0.0109$); half of recommended dose (dF = 26; $F = 6.61$; $P \leq 0.0004$); quarter of recommended dose (dF = 26; $F = 10.58$; $P \leq 0.0000$).

The emergence of moths was highly affected by all treatments (Table 1) compared to the control. The results obtained in this experiment are similar to those obtained by El-Ghar *et al.* (1995) working with *Bacillus thuringiensis* and Abamectin against cotton leafworm *S. littoralis*, with a pronounced decrease of pupation (36%) after Abamectin treatment, and a high reduction of moth fecundity (87.4%).

The data presented in Table 2 indicate that the levels of pupation and adult recovery were reduced by all tested insecticides and their concentrations as compared to the control.

In general the treatments gave higher mortality in the 1st larval stage than in 3rd and 5th stage, although Match, Mectin and Spinosad at all tested concentrations showed excellent efficacy against the 3rd larval stage of *S. littoralis*. Also, Match gave 100% mortality of 5th instar larvae at all tested concentrations. Corbitt *et al.* (1989) obtained similar effects working with cotton leafworm *S. litoranea* and demonstrated that the relative toxicity of Abamectin decreased from the 3rd to the 4th and 5th larval stage.

Table 1. Effect of bio-rational insecticides on mortality and some biological aspects after treatment of 3rd instar larvae of *Spodoptera littoralis*

Insecticide	Concentration	Cumulative mortality of larvae (%)	Pupal stage				Adult stage		
			pupation (%)	weight (mg)	deformed (%)	healthy (%)	emergence (%)	malformed (%)	recovery (%)
Dipel 2x	0.5 g/l	40.0[bc]	60	215	13.8	26.6	33.3	13.3	20
Agrin	0.75 g/l	66.6[ab]	33.3	175	0	33.3	20	0	20
BioGuard	5 g/l	46.6[b]	46.6	183.6	0	46.6	40	0	40
BioFly	1.5 cm/l	26.6[bc]	66.6	167	6.6	60	46.6	6.6	40
Match	0.4 cm/l	100[a]	–	–	–	–	–	–	–
Mectin	0.4 cm/l	93.3[a]	6.6	166	0	6.6	–	–	–
Neemix	1.25 cm/l	53.3[b]	40	181	0	33.3	26.6	6.6	20
Spinosad	0.5 cm/l	66.6[ab]	33.3	196	0	33.3	13.3	6.6	6.6
Control	–	6.6[c]	86.6	242.7	0	86.6	86.6	6.6	80
Dipel 2x	0.25 g/l	46.6[ab]	53.3	191.6	20	33.3	33.3	6.6	26.6
Agrin	0.375 g/l	53.3[ab]	40	138.6	0	40	40	0	40
BioGuard	2.55 g/l	26.6[b]	73.3	187.8	6.6	66.6	60	0	60
BioFly	0.75 cm/l	66.6[ab]	40	142	6.6	33.3	33.3	6.6	26.6
Match	0.2 cm/l	100[a]	–	–	–	–	–	–	–
Mectin	0.2 cm/l	93.3[a]	6.6	154	0	6.6	–	–	–
Neemix	0.625 cm/l	46.6[ab]	53.3	169.7	0	46.6	40	0	40
Spinosad	0.25 cm/l	86.6[a]	13.3	175.5	6.6	6.6	6.6	6.6	–
Control	–	6.6[b]	93.3	263	0	93.3	93.3	0	86.6
Dipel 2x	0.125 g/l	26.6[cd]	73.3	197	33.3	40	33.3	0	33.3
Agrin	0.187g/l	73.3[ab]	26.6	157.5	0	26.6	13.3	0	13.3
BioGuard	1.275 g/l	60.0[abc]	40	169.5	6.6	33.3	33.3	0	33.3
BioFly	0.375 cm/l	66.6[abc]	33.3	138.6	6.6	26.6	20	0	20
Match	0.1 cm/l	100[a]	–	–	–	–	–	–	–
Mectin	0.1 cm/l	100[a]	–	–	–	–	–	–	–
Neemix	0.312 cm/l	46.6[bc]	46.6	157.5	0	46.6	40	0	40
Spinosad	0.125 cm/l	86.6[ab]	13.3	188	0	13.3	13.3	0	13.3
Control	–	6.6[d]	86.6	250	0	86.6	86.6	0	86.6

The obtained data revealed that all tested insecticides had a significant effect on the hatchability of *S. littoralis*, after 24 h (dF = 26; F = 11.06; $P \leq 0.0000$); 48 h (dF = 26; F = 18.28; $P \leq 0.0000$); 72 h (dF = 26; F = 15.26; $P \leq 0.0000$).

Reductions of hatchability 24 h after treatment of eggs compared to the control were recorded with Spinosad (–83.4), Mectin (–81.7) and Dipel (–78.4), after 48 h with Match (–88.3), Mectin (–85.0) and Dipel (–73.3), and 72 h after treatment with Spinosad (–71.7) and Mectin (–61.7). Treatment with Agrin gave the lowest reduction of hatchability after 24 h (–28.4) and 48 h (–20.0), respectively; with BioGuard it was found to be (–15.0) at 72 h after treatment (Table 3). Moreover, the level of hatchability decreased with increasing

Table 2. Effect of bio-rational insecticides on mortality and some biological aspects after treatment of 5th instar larvae of *Spodoptera littoralis*

Insecticide	Concentration	Cumulative mortality of larvae (%)	Pupal stage				Adult stage		
			pupation (%)	weight (mg)	deformed (%)	healthy (%)	emergence (%)	malformed (%)	recovery (%)
Dipel 2x	0.5 g/l	46.6^{b}	33.3	254.2	0	33.3	33.3	6.6	26.6
Agrin	0.75 g/l	33.3^{bc}	20	266.6	0	20	13.3	0	13.3
BioGuard	5 g/l	53.3^{b}	26.6	265.5	0	26.6	20	6.6	13.3
BioFly	1.5 cm/l	46.6^{b}	20	224.2	6.6	13.3	13.3	0	13.3
Match	0.4 cm/l	100^{a}	–	–	–	–	–	–	–
Mectin	0.4 cm/l	53.3^{b}	26.6	275	0	26.6	20	6.6	13.3
Neemix	1.25 cm/l	53.3^{b}	20	250	6.6	13.3	13.3	0	13.3
Spinosad	0.5 cm/l	46.6^{b}	33.3	255.7	6.6	26.6	20	0	20
Control	–	0^{c}	86.6	273.2	6.6	80	80	6.6	73.3
Dipel 2x	0.25 g/l	53.3^{b}	26.6	241	0	26.6	20	6.6	13.3
Agrin	0.375 g/l	46.6^{b}	33.3	252.5	0	33.3	20	6.6	13.3
BioGuard	2.5 g/l	60^{b}	20	229.3	0	20	13.3	6.6	6.6
BioFly	0.75 cm/l	60^{b}	20	239	0	20	6.6	0	6.6
Match	0.2 cm/l	100^{a}	–	–	–	–	–	–	–
Mectin	0.2 cm/l	40^{b}	33.3	266	0	33.3	26.6	0	26.6
Neemix	0.625 cm/l	46.6^{b}	40	270.7	6.6	26.6	20	0	20
Spinosad	0.25 cm/l	53.3^{b}	26.6	284.7	6.6	20	13.3	0	13.3
Control	–	0^{c}	86.6	261.5	0	86.6	80	0	80
Dipel 2x	0.125 g/l	73.3^{ab}	13.3	231	0	13.3	13.3	0	13.3
Agrin	0.187 g/l	40^{bc}	26.6	293.7	0	26.6	26.6	6.6	20
BioGuard	1.25 g/l	53.3^{bc}	26.6	237.7	0	26.6	13.3	0	13.3
BioFly	0.375 cm/l	53.3^{bc}	20	250.6	6.6	13.3	13.3	0	13.3
Match	0.1 cm/l	100^{a}	–	–	–	–	–	–	–
Mectin	0.1 cm/l	53.3^{bc}	33.3	260.5	0	33.3	13.3	0	13.3
Neemix	0.312 cm/l	33.3^{cd}	46.6	282	0	40	33.3	0	26.6
Spinosad	0.125 cm/l	26.6^{cd}	53.3	236	20	33.3	20	6.6	13.3
Control	–	6.6^{d}	80	280	0	80	73.3	0	73.3

age of the eggs. Statistical analysis showed that all tested insecticides had a significant effect on hatchability rates (Table 3).

On Table 4 the latent effect of tested bioinsecticides on egg hatchability of *S. littoralis* (dF = 26; $F = 5.42$; $P \leq 0.0014$) is compared. The average of egg hatchability of untreated larvae was 90.0%. Significant reduction in egg hatching was recorded only after treatment with Match and Neemix, with the average being 55.0% and 51.6%, respectively. Match is highly active with other insects such as *Ceratitis capitata* (Wied.) when administered at 1.000 ppm on females and 5.000 ppm on males (Casana *et al.* 1999). Ascher (1993) stated that effects which may be exhibited by one or more compounds present in Neem seed extracts (azadirachtin) include: oviposition repellency, egg sterility, longevity, fitness and inhibition of chitin biosynthesis.

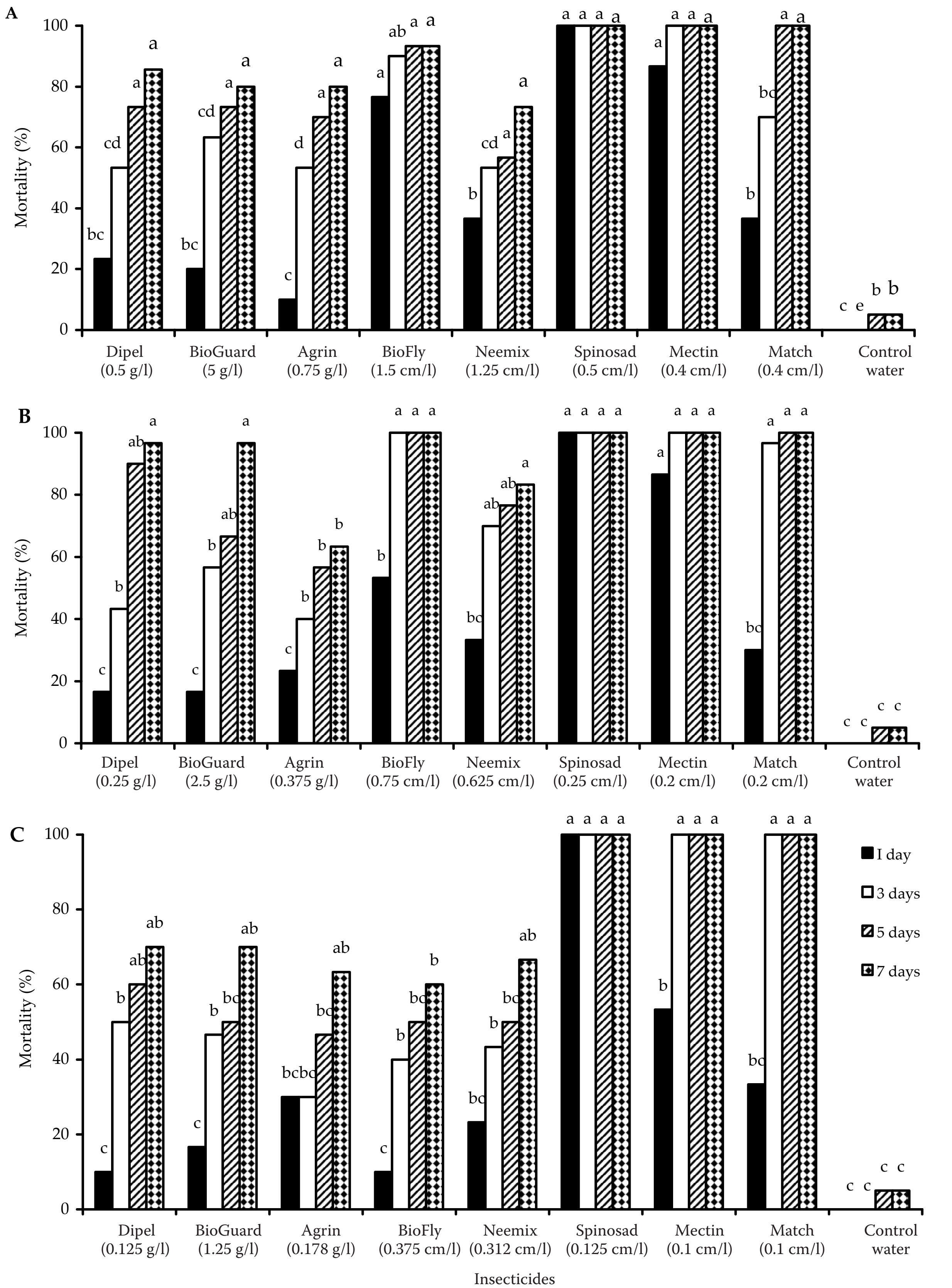

Figure 1. Toxicity to 1st instar larvae of *Spodoptera littoralis* that had been fed on castor leaves treated with three doses of bio-rational insecticides (A – recommended dose; B – half of recommended dose; C – quarter of recommended dose)

Table 3. Effect of bioinsecticides (recommended dose) on hatchability of *Spodoptera littoralis* eggs

Insecticide	Concentration	Hatchability (%) of eggs aged					
		24 h		48 h		72 h	
Dipel 2x	0.5 g/l	11.6[d]	(–78.4)	20.0[de]	(–73.3)	48.3[cd]	(–46.7)
Agrin	0.75 g/l	61.6[b]	(–28.4)	73.3[ab]	(–20.0)	75.0[ab]	(–20.0)
BioGuard	5 g/l	40.0[bc]	(–50.0)	70.0[b]	(–23.3)	80.0[ab]	(–15.0)
BioFly	1.5 cm/l	25.0[cd]	(–65.0)	35.0[cd]	(–58.3)	65.0[bc]	(–30.0)
Match	0.4 cm/l	18.3[d]	(–71.7)	5.0[de]	(–88.3)	33.3[d]	(–61.7)
Mectin	0.4 cm/l	8.3[d]	(–81.7)	8.3[e]	(–85.0)	46.6[cd]	(–48.4)
Neemix	1.25 cm/l	31.6[cd]	(–58.4)	46.6[c]	(–46.7)	75.0[ab]	(–20.0)
Spinosad	0.5 cm/l	6.6[d]	(–83.4)	8.3[e]	(–85.0)	23.3[d]	(–71.7)
Control	–	90.0[a]		93.3[a]		95.0[a]	

Numbers between brackets represent percentage decrease relative to control
Means in column with the same letter are not significantly different at 0.05% level (Duncan's multiple range test)

CONCLUSION

The information based on these results will help in precise calculation of the dosage of candidate insecticides for effective control of *S. littoralis,* and consequently help in avoiding economic losses due to miscalculations of insecticide dosage. Furthermore, these data would facilitate better integration of candidate insecticides into an IPM program for the control of target pests in Egypt. Yet field tests have to confirm the results and fully investigate their field efficacy and side effects to determine their field performance and IPM compatibility.

Table 4. Hatchability of eggs produced after feeding of 5th instar larvae of *Spodoptera littoralis* on castor leaves treated with bioinsecticides (recommended dose)

Insecticide	Concentration	Hatchability (%)	
Dipel 2x	0.5 g/l	88.3[a]	(–1.7)
Agrin	0.75 g/l	83.3[a]	(–6.7)
BioGuard	5 g/l	85.0[a]	(–5.0)
BioFly	1.5 cm/l	80.0[a]	(–10.0)
Match	0.4 cm/l	55.0[b]	(–35.0)
Mectin	0.4 cm/l	83.3[a]	(–6.7)
Neemix	1.25 cm/l	51.6[b]	(–38.4)
Spinosad	0.5 cm/l	81.6[a]	(–8.4)
Control	–	90.0[a]	

Numbers between brackets represent percentage decrease relative to control
Means in column with the same letter are not significantly different at 0.05% level (Duncan's multiple range test)

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