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EFFECTS OF BIO-RATIONAL INSECTICIDES ON SELECTED BIOLOGICAL ASPECTS OF THE EGYPTIAN COTTON LEAFWORM *SPODOPTERA LITTORALIS* (BOISD.) (*LEPIDOPTERA: NOCTUIDAE*)

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Abstract: Effectiveness of 8 bio-rational insecticides, Dipel 2x, BioFly , Agrin, BioGaurd, Spinosad, Neemix, Mectin and Match were tested at recommended dose, half of recommended dose and quarter of recommended dose against 1st, 3rd and 5th instar larvae and the egg masses (24, 48 and 72 h old) of the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) under laboratory conditions.

All the bio-rational insecticides provided higher mortality in the first instar larvae comparing to the third and fifth instar larvae, although Match, Mectin and Spinosad showed also excellent efficacy against third larval stage of *S. littoralis* at all tested concentrations. Also, Match showed 100% mortality of fifth instar larvae at all tested concentrations.

The mortality rates of *S. littoralis* eggs of different ages (24, 48 and 72 h old) when dipped in recommended dose of each insecticide diluted in water were investigated. At recommended dose of all insecticides eggs of different ages were highly affected and the reductions of hatchability were 83.4, 85.0 and 71.7%, respectively in Spinosad compared to the control. In general, eggs 48 and 72 h old were less sensitive than younger eggs 24 h old. The latent effect of bio-rational 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 effective compounds for control of *S. littoralis*

Key words: bio-rational insecticides, *Spodoptera littoralis*

INTRODUCTION

Many crops in Egyptian fields, as well as various vegetables are attacked by numerous insect pests. The lepidopterous insects in general and the cotton leafworm *Spodoptera littoralis* (Boisd.) in particular, are the most dangerous in this respect. In fact, the cotton leaf-worm is a major limiting factor affecting crop and vegetable production, not only in Egypt, but also in many other countries.

S. littoralis is similarly one of the most destructive agricultural lepidopterous pest within its subtropical and tropical range (Hosny *et al*. 1986). It can attack numerous economically important crops all the 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 the 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 become unsuitable for consumption. Numerous other crops are attacked, mainly on their leaves.

The 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 resistance to this compound developed. 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; 1984b; 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 appearance of high resistance to many chemical pesticides and resurgence of chemical pesticides (Forgash 1984; Georghiou 1986) more attentional should be paid to the use of bioinsecticides such as compounds based on bacteria, fungi, insect growth regulators and botanical pesticides (Rao *et al*. 1990). These groups have different mode of action from conventional products (Asher 1993; Thompson *et al*. 1999) and their properties may differ considerably from the conventional agents with which growers are familiar.

The present experiments demonstrate the comparative efficacy of insecticides against the cotton leafworm *S. littoralis*, to find the best chemicals for controlling this economic pest through the integrated pest management program.

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MATERIALS AND METHODS

Insect maintenance

Larvae of the cotton leaf-worm, *Spodoptera littoralis* (Boisd.) were reared on fresh castor leaves *Ricinus communis* in 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).

Control agents tested and bioassay techniques for larval instars

Commercial formulations of the following insecticides were tested against 1st, 3rd and 5th instar larvae of the cotton leafworm *S. littoralis*: dipel 2x (*Bacillus thuringensis* var. *kurstaki* 22 000 IU/mg), BioFly (*Beauvaria bassiana* 100%, 30 ×10^6 cell), Agrin [*Bacillus thuringensis* (Bt) 32 000 IU/mg], BioGaurd (*Bacillus thuringensis* bacteria 30 million IU/g), Spinosad (spinosyns A and D, *Saccharopolyspora spinosa*, 0.24% SL), Neemix (4.5% azadirachtin), Match (50% EC lufenuron), Mectin (*Streptomyces avermitilis*, 80% avermectin B1a and 20% avermectin B1b). Formulations of test compounds were prepared in distilled water as a recommended dose, half of recommended dose and a quarter of recommended dose. Castor leaf disks (5 cm in diameter) were cut and dipped into the test solutions for 15 seconds 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 to the leaf disks by tapping lightly to dispense 10 larvae (1st instar), 5 larvae (3rd instar), and 4 larvae (5th instar) per petri-dish per replicatation. Each treatment was replicated 3 times, along with an untreated control under complete randomized design. Mortality was recorded after 1, 3, 5 and 7 days of insecticide application for the first instar larvae, whereas mortality of 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 obtained pupae were placed separately in Petri-dishes. Pupation, pupal weight, deformation pupate, healthy pupae, adult emergence and malformed adult percent was recorded.

Newly emerged adults in all tests (recommended dose) were transferred in pairs to clean jars provided with honey solution as a source of food of adults and taphla leaves as oviposition site. Each treatment was replicated 5 times. Deposited egg masses were collected daily and percent of sterility (latent effect) was recorded (Toppozada *et al.* 1966).

Ovicidal tests

Egg masses of *S. littoralis* 24, 48 and 72 h old were dipped at recommended dose of all insecticides for 5 sec. Another group of the same ages were dipped in water as control. Three egg masses of each age were used and the experiment was repeated 3 times. After dryness, 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 analyzed through ANOVA (SAS Institute 1999). When F-test was significant, means were separated using Duncan multiple range (DMRT) at the 0.05 level of significance.

RESULTS AND DISCUSSION

Data presented in figure 1 show effect of three concentrations of all tested insecticides (recommended, half and quarter dose of recommended) on the first instar larvae of *S. littoralis* after 1, 3, 5 and 7 days after treatment. It was evident that Spinosad, Mectin and Match caused 100% mortality after treatments with the three concentrations.

There were significant differences between tested insecticides with recommended dose 1 and 3 days after treatments (dF = 26; F = 8.78; p ≤ 0.0000) and (dF = 26; F = 15.68; p ≤ 0.0000). No significant differences were noticed between insecticides in mortality rate 5 and 7 days after treatment but they were significant when compared with untreated control (dF = 26; F = 7.50; p ≤ 0.0002) and (dF = 26; F = 10.44; p ≤ 0.0000).

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

Data presented in figure 1 clearly indicate that the mortality rate of the first instar larvae of *S. littoralis* increased gradually by increasing the concentrations of biorational insecticides and the time of exposure.

Pineda *et al.* (2004) recorded that spinosad was 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 the experiment results showed a high efficacy of Spinosad (100% mortality) when applied at the recommended rate. Horover, it was found that Spinosad was highly effective against the three economically important fruit fly species, Mediterranean fruit fly, *Ceratitis capitata*; melon fly, *Bactrocera cucurbitae*; *Bactrocera dorsalis* (Stark *et al.* 2004).

Data in table 1 and 2 indicate a good toxic effect of insecticides on the third and fifth instar larvae and the latent effects on pupation, deformation of pupae, pupal weight, adults emergence, malformation and recovery of adult following the 3rd and 5th inastar larvae of *S. littoralis* on oil castor leaves treated with 3 concentrations (recommended and half dose and quarter dose of recommended).

The results (Table 1, 2) showed that mean percent of cumulative mortality of the 3rd and 5th instar larvae of *S. littoralis* varied significantly between the treatments and control.

The maximum mortality of the 3rd instar larvae was observed in Match treatments (100%) at all tested concentrations and Mectin treatments at quarter of the rec-

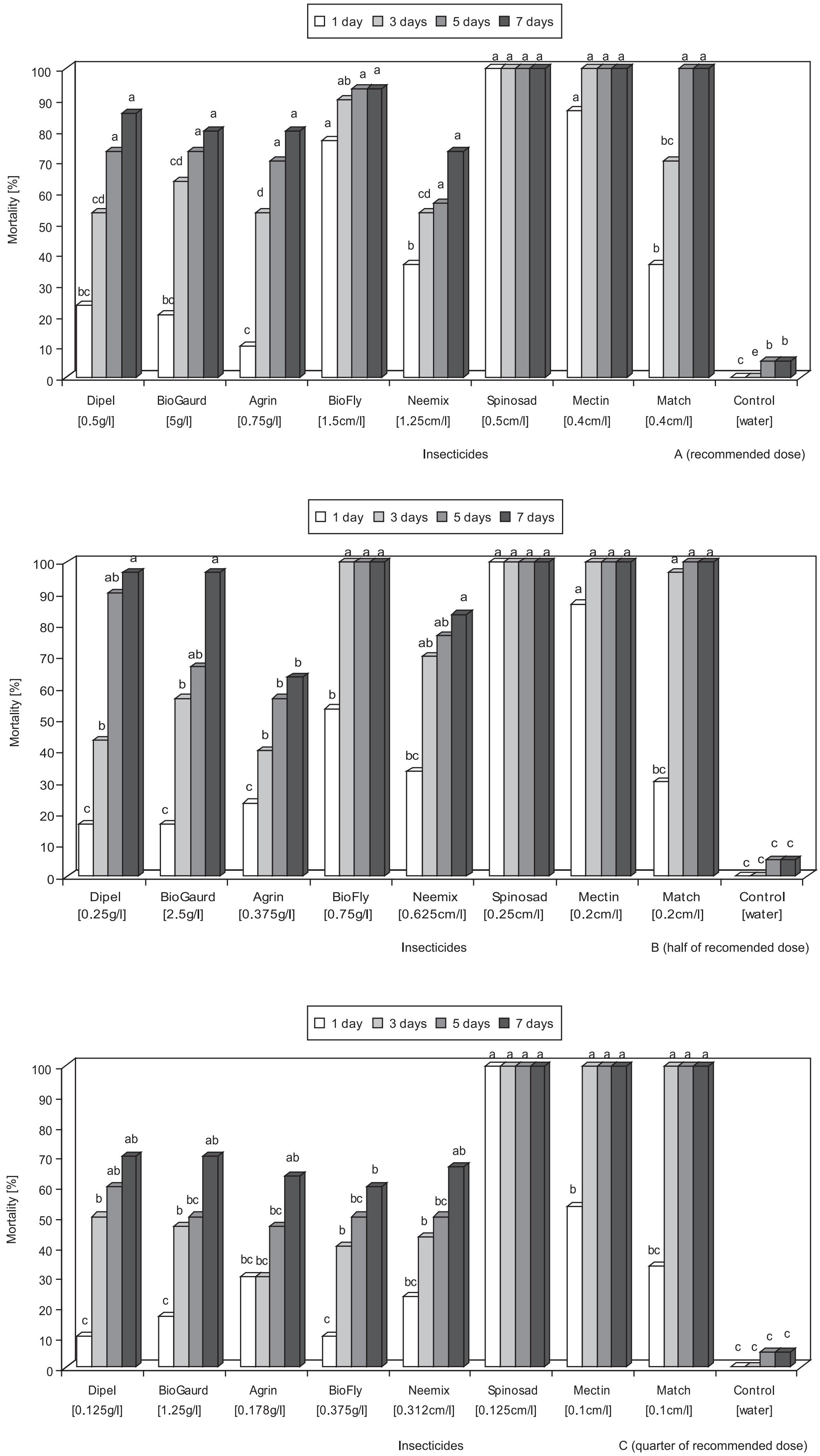

Fig. 1. Toxicity of three doses of bio-rational insecticides against first instar larvae of *S. littoralis*

Table 1. Effect of bio-rational insecticides on mortality % and some biological aspects oof the 3rd instar larvae of *S. littoralis*

Insecticides	Concentrations	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	60.0	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^3/l	26.6 bc	66.6	167	6.6	60	46.6	6.6	40
Match	0.4 cm^3/l	100 a	–	–	–	–	–	–	–
Mectin	0.4 cm^3/l	93.3 a	6.6	166	0	6.6	–	–	–
Neemix	1.25 cm^3/l	53.3 b	40.0	181	0	33.3	26.6	6.6	20
Spinosad	0.5c m^3/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	40.0	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^3/l	66.6 ab	40.0	142	6.6	33.3	33.3	6.6	26.6
Match	0.2 cm^3/l	100 a	–	–	–	–	–	–	–
Mectin	0.2 cm^3/l	93.3 a	6.6	154	0	6.6	–	–	–
Neemix	0.625 cm^3/l	46.6 ab	53.3	169.7	0	46.6	40	0	40
Spinosad	0.25 cm^3/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	0.187 g/l	73.3 ab	26.6	157.5	0	26.6	13.3	0	13.3
BioGuard	1.275 g/l	60.0 abc	40.0	169.5	6.6	33.3	33.3	0	33.3
BioFly	0.375 cm^3/l	66.6 abc	33.3	138.6	6.6	26.6	20	0	20
Match	0.1 cm^3/l	100 a	–	–	–	–	–	–	–
Mectin	0.1 cm^3/l	100 a	–	–	–	–	–	–	–
Neemix	0.312 cm^3/l	46.6 bc	46.6	157.5	0	46.6	40	0	40
Spinosad	0.125 cm^3/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

Table 2. Effect of bio-rational insecticides on mortality % and some biological aspects of the 5th instar larvae of *S. littoralis*

Insecticides	Concentrations	Cumulative mortality of larvae [%]	Pupal stage				Adult stage		
			pupation [%]	weight [mg]	peformed [%]	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	20.0	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^3/l	46.6 b	20	224.2	6.6	13.3	13.3	0	13.3
Match	0.4 cm^3/l	100 a	–	–	–	–	–	–	–
Mectin	0.4 cm^3/l	53.3 b	26.6	275	0	26.6	20	6.6	13.3
Neemix	1.25 cm^3/l	53.3 b	20	250	6.6	13.3	13.3	0	13.3
Spinosad	0.5 cm^3/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^3/l	60 b	20	239	0	20	6.6	0	6.6
Match	0.2 cm^3/l	100 a	–	–	–	–	–	–	–
Mectin	0.2 cm^3/l	40 b	33.3	266	0	33.3	26.6	0	26.6
Neemix	0.625 cm^3/l 0.25	46.6 b	40	270.7	6.6	26.6	20	0	20
Spinosad	cm^3/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^3/l	53.3 bc	20	250.6	6.6	13.3	13.3	0	13.3
Match	0.1 cm^3/l	100 a	–	–	–	–	–	–	–
Mectin	0.1 cm^3/l	53.3 bc	33.3	260.5	0	33.3	13.3	0	13.3
Neemix	0.312 cm^3/l	33.3 cd	46.6	282	0	40	33.3	0	26.6
Spinosad	0.125 cm^3/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

ommended dose only, whereas the mortality cased by Mectin at recommended and half of recommended dose was 93.3%, followed by Spinosad which gave 86.6% mortality at half and quarter of recommended dose, whereas the mortality was low (66.6%) at the recommended dose. Cumulative mortality at recommended dose (dF = 26; F = 5.87; p ≤ 0.0009); half of recommended dose (dF = 26; F = 3.20; p ≤ 0.0190); quarter of recommended dose (dF = 26; F = 6.82; p ≤ 0.0006). And the maximum mortality 5th instar larvae was observed in Match (100%) at all tested concentrations, at recommended dose (dF = 26; F = 3.63; p ≤ 0.0109); half of recommended dose (dF = 26; F = 6.61; p ≤ 0.0004); quarter of recommended dose (dF = 26; F = 10.58; p ≤ 0.0000).

Emergence of moths was highly affected in all treatments (Table 1) compared to the control. The effect of Mectin and Spinosad on pupation was significant, as well as the pupal weight of slightly decreased Comparing to untreated check probably due to tested concentrations of all insecticides female moth fecundity (Table 1). The results obtained in this experiment are similar to the ones obtained by El-Ghar *et al.* (1995) working with *Bacillus thuringiensis* and Abamectin against cotton leafworm *S. littoralis*, with pronounced decrease of pupation (36%) after Abamectin treatment, and great reduction on moth fecundity (87.4%).

The data presented in Table 2 indicated that there was a reduction in pupation % and adult recovery % in all the tested insecticides at different concentrations compared with the control. Moreover, the data revealed that pupal weight decreased or increased when compared to control.

In general the treatments provided higher mortality of the first stage than the third and fifth larval stages, although Match, Mectin and Spinosad tested at all concentrations showed excellent efficacy against third larval stage of *S. littoralis*. In addition, Match caused 100% mortality of fifth instar larvae at all tested concentrations. Corbitt *et al*. (1989) obtained similar effect working with cotton leafworm *S. litoranea* and demonstrated that the relative toxicity of Abamectin decreased from the third to the fourth and fifth larval stage.

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 ≤ 0.0000); 48 h (dF = 26; F = 18.28; p ≤ 0.0000); 72 h (dF = 26; F = 15.26; p ≤ 0.0000).

In comparision to control the highest reduction of hatchability was stated in the case of insecticides such as: Spinosad (–83.4), Mectin (–81.7) and Dipel 2x (–78.4) after 24 hours. Effectiveness of insecticides Match, Mectin and Dipel 2x ranged from (–88.3) to (–73.3) after 48 hours. The lowest effectiveness was shown by Agrin (–28.4), (–20.0) respectively after 24 and 48 hours. On the basis of obtained results it was evident that percentage of hatchability decreased corresponding with the increase of egg age.

Statistical analysis has shown that all tested insecticides have a significant effect on the hatchability rates (Table 3).

Table 4 compares the latent effect of tested bioinsecticides on egg hatchability of *S. littoralis* (dF = 26; F = 5.42; p ≤ 0.0014). The average of egg hatchability in the untreated larvae was 90.0%. Significant reduction in egg hatching was recorded only in 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 administrated at 1 000 ppm for females and 5 000 ppm for males (Casana *et al*. 1999).

(Ascher 1993) stated that one or more compounds presented in Neem seed extracts (azadirachtin) may influence: oviposition repellency, egg sterility, longevity, fitness and inhibition of chitin biosynthesis.

Table 3. Effect of insecticides on hatchability of *S. littoralis* eggs

Insecticides	Concentrations	[%] Hatchability egg age [in hours]		
		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 increase or decrease comparing to control
*Means in column with the same letter are not significantly different at 0.05% level (Duncan's multiple range test)

Table 4. Effect of insecticides on egg hatching of treated 5th instar larvae of *S. littoralis*

Insecticides	Concentrations	[%] 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 4.5%	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 increase or decrease than control
*Means in column with the same letter are not significantly different at 0.05% level (Duncan's multiple range test)

CONCLUSION

Results of laboratory studies revealed that most of tested insecticides could be included into the IPM program for control of target pests in Egypt. Additional field experiments should be performed to confirm the preliminary evaluation of presented insecticides, in order to recommend them for practical use.

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POLISH SUMMARY

WPŁYW BIOINSEKTYCYDÓW NA WYBRANE BIOLOGICZNE STADIA ROZWOJOWE EGIPSKIEJ SÓWKI BAWEŁNÓWKI *SPODOPTERA LITTORALIS* (BOISD.) (*LEPIDOPTERA: NOCTUIDAE*)

W warunkach laboratoryjnych badano wpływ ośmiu bioinsektycydów: Dipel 2x, BioFly, Agrin, BioGaurd, Spinosad, Neemix, Mectin i Match zastosowanych w zalecanej dawce, połowie oraz jednej czwartej zalecanej dawki na stadia larwalne 1, 2 i 5 oraz złoża jaj (24, 48 i 72 godzinne) egipskiej sówki bawełnówki *Spodoptera littoralis*.

Wszystkie testowane bioinsektycydy powodowały wyższą śmiertelność pierwszego stadium larwalnego w porównaniu do pozostałych stadiów 3 i 5. Preparaty Match, Mectin i Spinosad wykazały bardzo wysoką skuteczność w stosunku do trzeciego stadium *S. littoralis* przy zastosowaniu wszystkich badanych koncentracji, a preparat Match powodował 100% śmiertelności piątego stadium larwalnego.

Jednocześnie badano wpływ bioinsektycydów na rozwój jaj (24-, 48- i 72-godzinnych) *S. littoralis*. Złoża jaj zanurzano w wodnym roztworze każdego z testowanych preparatów uwzględniając zalecaną dawkę. Wszystkie testowane preparaty okazały się wysoce skuteczne i ograniczały zdolności jaj do wylęgu larw. W przypadku bioinsektycydu Match skuteczność była najbardziej widoczna i wynosiła odpowiednio dla poszczególnych grup wiekowych jaj 83.4, 85.0 i 71.7% w porównaniu do kontroli. Na ogół jaja 48- i 72-godzinne były mniej wrażliwe na działanie preparatów niż młodsze 24-godzinne jaja. Tylko w przypadku dwóch bioinsektycydów Match i Neemix stwierdzono długotrwały wpływ na rozwój jaj *S. littoralis*, a średnie wielkości procentowe wynosiły odpowiednio 55.0 i 51.6%.

Wyniki przedstawionych badań dowodzą, że biopreparaty Match, Mectin i Spinosad charakteryzują się wysoką skutecznością zwalczania *S. littoralis*.