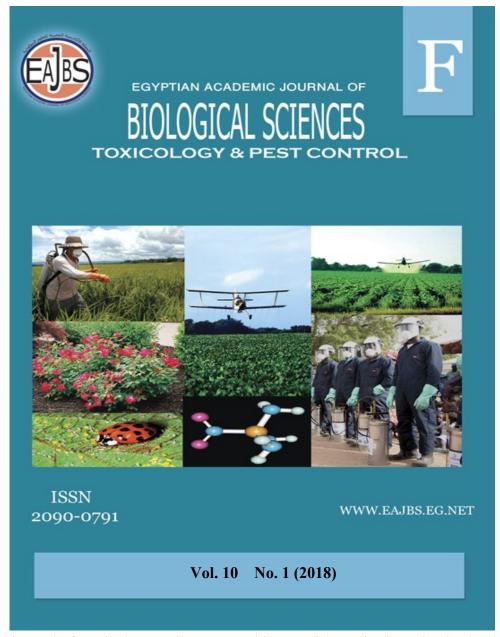
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Appraisal of Positive Pesticides Influence on Pink Bollworm Larvae, Pectinophora gossypiella (Saunders)

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ABSTRACT

Experiments conducted to study the toxic and biological effects of three chemical insecticides (chlorpyrifos, Lambda-Cyhalothrin, and Methomyl), one biochemical insecticide (spinosad), and two bio-insecticides (Bacillus thuringiensis kurstaki Bt and Nucleopolyhedrovirus, NPV) against neonate larvae of the pink bollworm, Pectinophora gossypiella (Saund.), were carried out under laboratory conditions. The highest effect of tested pesticides on larval mortalities was recorded after four days of feeding these larvae on treated diet. Spinosad was the effective toxin (LC₅₀=1.84 ppm) to neonate larvae followed by chlorpyrifos (LC₅₀= 3.06 ppm), Lambda-Cyhalothrin (LC₅₀= 5.99 ppm) then Methomyl (LC₅₀=23.50 ppm) but B.t. $(74.84 \times 10^4 \text{ I.U})$ and NPV $(83.5 \times 10^9 \text{ I.U})$ were the lowest ones. LC₅₀ treatments of neonate larvae with all tested insecticides produced reduction in larval and pupal weights especially Lambda-cyhalothrin treatment, (31.9 and 24.3 %, resp.) compared with untreated insects. The decreasing effect of all treatments observed in fecundity and life span of adult females which reflected in very high percentages of insect sterility with chlorpyrifos (66.7%) and Methomyl (61%) treatments. Insignificant increase of total protein contents and activity of Glutathione-S-transferase enzyme was detected in tissues of LC₅₀ treated larvae compared with untreated larvae, but a significant (23.5%) and highly significant (56.9%) increase in enzyme activity were resulted by Chlorpyrifos and Spinosad treatments. A very high inhibition in activity of Acetylcholinesterase enzyme of larval tissues was recorded with NPV (61.3%), B.t. (58.8%), Lambda-Cyhalothrin (55.6%), Spinosad (54.4%), Chlorpyrifos (48.7%), and Methomyl (37.5%) treatments than untreated ones.

INTRODUCTION

Cotton is one of the most important cash crops in Egypt which plays a dominant role in the industrial and agricultural economy of the country. Cotton yield is

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particularly affected by insect attack because major pests feed preferentially on the fruiting structures which are normally shed after injury (Hearn and Fitt, 1992). The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is one of the most injurious cotton pests, because it is difficult to control with insecticides (Lykouressis *et al.*, 2005). The bolls of cotton are the most susceptible to pink bollworm infestation from 12 to 30 days post bloom. Once a boll is "rock hard" in firmness, it is generally immune to infestation by newly hatched larvae. Insecticidal control is hindered by the larvae being internal feeders; moreover, resistance to insecticides develops making it often more expensive than other methods. Environmental and health problems associated with chemical-based insecticides have prompted the search for ecologically acceptable pesticides. The application of chemical pesticide uses not only aroused increased economic profit but also contributed to a cleaner environment and improved the health status of farmers (Hossain *et al.*, 2004; Kouser and Qaim 2011; Abdullah *et al.*, 2015).

The biological control agent, *Bacillus thuringiensis*, has been found to be effective in combination with chemical insecticides in Egypt (Hussein *et al.*, 1990). *B. thuringiensis* produces crystalliferous proteins known as δ-endotoxins or Cry toxins. These toxins display insecticidal activities against many insect species (Tabashnik *et al.*, 2008). The mode of action of Cry toxins has been studied primarily in Lepidoptera insects (Pigott *et al.*, 2007). It is a multi-step process, involving sequential interaction with at least three different receptors including cadherins, aminopeptidase (AP), and alkaline phosphatase (ALP). The proteins between 175-210 kD, belonging to the cadherin superfamily of transmembrane glycoproteins, have been identified as the putative Cry1A toxin-binding receptors on the cell surface of midgut cells in many Lepidoptera species, including *Manduca sexta* (Vadlamudi *et al.*, 1993&1995), *Heliothis virescens* (Gahan *et al.*, 2001; Jurat-Fuentes and Adang 2006), *Helicoverpa armigera* (Wang *et al.*, 2005), and *P. gossypiella* (Morin *et al.*, 2003).

Viruses, particularly those belonging to the family Baculoviridae, are some of the most promising biological insecticides to date. These are characterized by double-stranded circular DNA genomes ranging from 88 to 153 kb (Blissard and Rohrmann, 1990). These viruses occur naturally in insect populations and can cause very high mortality. This family is pathogenic to insects, mostly those in the order Lepidoptera, but it is also reported to infect insects in the orders Coleoptera, Diptera, Hymenoptera, Neuroptera, Siphonaptera, Thysanura, and Trichoptera (Adams and McClintock, 1991). Baculoviruses are insect-pathogenic and insect-specific viruses that have been utilized as biological control agents and vectors for the expression of heterologous proteins in insect cells culture and insect larvae (Moscardi, 1999; Jarvis, 1997). The genus nucleopolyhedrovirus (NPV) produces polyhedra that are proteinaceous crystal bodies occluding many progeny virions in the infected host cell nuclei (Granados and Williams, 1986). BV, generated early in infection when nucleocapsid buds enter through the plasma membrane, is responsible for systemic spread in the insect host and propagation in tissue culture.

This work's interest is to study the toxic and latent effects of three chemicals insecticides (Chlorpyrifos, Lambda-Cyhalothrin, and Methomyl), one biochemical insecticides (Spinosad), and two bio-insecticides (*B. thuringiensis* and Nucleopolyhedrovirus) on neonate larvae of the pink bollworm.

MATERIALS AND METHODS

Insect:

Laboratory strain of the pink bollworm (PBW), *P. gossypiella* (Saund.), was obtained from the Bollworms Research Department, Plant Protection Research Institute and reared for two years without exposure to insecticides, in Central Agricultural Pesticides Laboratory, Agricultural Research Center, on artificial diet according to Rashed and Ammar (1985) under constant conditions ($27\pm2^{\circ}$ C, and 70 ± 5 R.H. and 12 D + 12 L photoperiod).

Insecticides:

- **1-Chlorpyrifos:** (Pestban 48% EC, from Dow AgroSciences company) an organophosphorus compound, Non-systemic with contact and stomach effect which acts as inhibitor of Acetylcholinesterase enzyme in insects (e-pesticides manual V5-2011).
- **2-Methomyl**: (Lannate 90% SP, from DuPont company) a carbamate compound used as a systemic insecticide-nematicide which kills by contact and stomach effect which inhibits the activity of pest Acetylcholinesterase enzyme (e-pesticides manual V5-2011).
- **3-Lambda-Cyhalothrin**: (Pilarmada 2.5% EC, from Sam-Trade company) a synthetic pyrethroid, non-systemic, with contact and stomach effect. This insecticide interacts with Sodium channels of insect nervous system neurons (e-pesticides manual V5-2011).
- **4-Spinosad**: (Traser 24% SC, from Dow Agro-Sciences company) a biochemical insecticide produced from fermentation of the soil actinomycete *Saccharopolyspora spinosa*. It acts as contact and stomach poison which activates the nicotinic Acetylcholine receptor of insect nervous system (e-pesticides manual V5-2011).
- **5-Bacillus thuringiensis**, **sub sp.** *Kurstaki*: (Dipel 2X 6.4% WP,16 Billion International Units per kg from Valent Biosciences company). Anaerobic spore forming Gram-positive rod-shaped bacterium belonging to the family Bacillaceae. At sporulation in addition to spore crystals of proteins, the delta-endotoxin are also formed. The endotoxin binding with its receptor on the brush border membrane of insect's mid-gut.
- **6-Nucleopolyhedrovirus:** (Helicovex 7.5×10¹² I. U /L SC, *Helicoverpa armigra* nucleopoly hedrovirus, from Indramat AG Biocontrol- sewesra company) enveloped viruses that have double-stranded, circular DNA genome, and produce proteinaceous crystal bodies (polyhedra) which occluding many generations in cell nuclei of the insect host.

Bioassay:

Ten serial aqueous concentrations of each pesticide were prepared. Each pesticide concentration was thoroughly mixed with the artificial diet (2 ml/ 100 gm). The treated diet with each insecticide concentration was put in 10 Petri-dishes, (five replicates for each concentration). Another group of 10 Petri dishes was prepared containing the same diet mixed with distilled water only (used as control). Ten newly hatched larvae were placed on the surface of the treated and control Petri-dishes using a soft brush and kept under the laboratory constant conditions. Mortality was recorded after 96 hours of treatment (IRAC, 2012), then corrected with Abbot's formula (Abbot, 1925). LC₅₀ and slope value were determined according to Finney (1971). Toxicity index (TI) was calculated using the equation of Sun (1950).

Biological Aspects:

To study the latent effect of tested insecticides on some biological aspects, the survivors of untreated and LC₅₀ treated of *P. gossypiella* neonates were transferred to new tubes containing untreated diet and kept under laboratory conditions (three replicates for each insecticide and control). The biological aspects as larval and pupal

duration, larval and pupal mortality, larval and pupal weight, and percentage of adult emergence were recorded for treated and untreated insects. After moths' eclosion, 5 pairs of moths (5 males and 5 females) were placed in each cage (three cages for each tested insecticide and control). Laid eggs were counted daily, and the percentage of fecundity, hatchability, and sterility were calculated according to the equation of ToppoZada *et al.*, (1966), also the life span of males and females was estimated, and the percentages of changes were calculated.

Biochemical Assay:

Enzyme Extract:

500 mg of survivor untreated and LC₅₀ treated larvae with all tested insecticides were homogenized in 1 ml sodium phosphate buffer (0.1 M, pH 7) using Teflon glass homogenizer and centrifuged at 10,000 g for 15 min at 4°C (five replicates of each sample). The supernatant was used as a source of protein concentration and activity of Acetylcholinesterase (AchE) and Glutathione-S-transferase enzymes determination. The total protein contents of all larval samples were determined as Biuret reaction kit (Henry, 1964).

Enzyme Activity:

Activity of AChE enzyme was detected according to Ellman *et al.*, (1961). The reaction mixture consists of 50 μl of sample enzyme, 10 μl of 100 mM AChI (acetylthiocholine iodide), 10 μI 9.2 mM DTNB (5,5-dithio-bis 2-nitrobenzoic acid), and potassium phosphate buffer (0.1 M, pH 7.2) up to 1 ml (five replicates for each sample). The increment in absorbance at 405 nm & 25°C was recorded during 5min. The activity was expressed as nanomoles of acetylthiocholine hydrolyzed/mg protein/min. GST activity was measured based on the method of Habig *et al.*, (1974). The assay was conducted to incubating 50 mM of CDNB(1-chloro-2,4-dinitrobenzen) as a substrate with 50 mM GSH (reduced glutathione) and 50 ul of sample enzyme in 0.1 M phosphate buffer (pH7) for 5min at 27°C (five replicates for each sample). The activity monitored at 340 nm and expressed as nmoles of CDNB conjugated/mg protein/min.

Data Analysis:

The biological aspects, total protein contents and enzyme activities of untreated and LC_{50} treated larvae, were statistically analyzed as Means \pm SE (standard error) by using SPSS program V25. Differences were considered by significant of P less than 0.05.

RESULTS

Bioassay:

The toxic effect of Chlorpyrifos, Lambda-Cyhalothrin, Methomyl, Spinosad, Bt, and NPV was shown in Table 1 revealed that Spinosad treatment gave the highest toxic effect on neonate larvae of the pink bollworm (LC₅₀ = 1.84 ppm and Toxicity index, T.I = 100%) then Chlorpyrifos (3.06 ppm and 6.01%), Lambda-Cyhalothrin (5.99 ppm and 30.7%), and Methomyl was the lowest chemical insecticide (23.5 ppm and 7.8%). The bioinsecticides had a very low toxic effect against neonate larvae but B.t. (74.84×10⁴ I.U. and 100%) was more effective than NPV virus (83.5×10⁹ I.U. and 0.1×10^{-3}).

Table 1. Toxic effect of tested insecticides on neonate larvae of the pink bollworm,

P. gossypiella.

Insecticide	LC ₅₀ (ppm)	Slope ± S. E	T. I
	confidence interval	•	
Spinosad	1.84	0.45	
•	0.99 - 2.93	± 0.067	100
Chlorpyrifos	3.06	0.76	
	1.85 - 4.36	± 0.107	60.1
Lambda-	5.99	0.96	
cyhalothrin	4.65 -7.99	± 0.108	30.7
Methomyl	23.50	0.755	
-	13.91-34.70	± 0.19	7.8
Bio insecticide	LC ₅₀ I.U.	Slope ± S. E	T. I
	confidence interval		
B. T	74.84×10^4	0.603	
	$(46 - 118 \times 10^4)$	± 0.134	100
NPV	83.5×10 ⁹	0.79	
	$(58 - 113 \times 10^9)$	± 0.105	0.1×10^{-3}

Biological Aspects:

Effect of insecticides LC₅₀ treatment on biological aspects of the pink bollworm P. gossypiella which treated as neonate larvae was presented in Tables 2-4. Data in Table 2 show the percentage of larva, pupa, total mortality, emergency of adult, female fecundity, egg hatchability, and sterility of treated and untreated insects. The high mortality percentage of larvae (67%) was presented in NPV LC₅₀ treatment, which reflected in high total mortality (74%) and low adult emergency (26%), followed by Bt (64, 73, and 27%), Spinosad (62, 72, and 28%), Chlorpyrifos (58, 69, and 31), Lambda-Cyhalothrin (55, 64, and 36%), and Methomyl (54, 66, and 34%), respectively, compared with those of untreated insect (5, 8, and 92%). The highest pupal mortality (12%) was recorded with Methomyl treatment. The reduction in number of eggs laid by resulted females was observed in all treatments compared with untreated females, but this effect was highly pronounced with Methomyl and Bt (38.3) and 27.8%) treatments. Obtained data revealed that the high decrease in egg hatchability and sterility of adults was correlated with Chlorpyrifos (56.7 and 66.7%) and Methomyl (55.2 and 61%) treatments than those of Lambda-Cyhalothrin (38 and 51.5%), NPV (37.5 and 50.7%), Spinosad (24.4 and 38.3%), and Bt (18.8 and 31.3%), respectively, in comparison with untreated insects. Data in Table 3 showed the effect of insecticides LC₅₀ treatment on larval and pupal weight of the pink bollworm treated as neonate larvae. Weight of all treated larvae (fifteen-days old) and resulted pupae was lighter than untreated ones except Spinosad treatment, which cause a slight increase (0.6 % and 2.6 % resp.) in weight compared with untreated insects. A significant decrease in larval weight was presented in Lambda-Cyhalothrin treatment (31.9%), NPV (29.6%), Methomyl (27.1%), and Bt (24.8%), while Chlorpyrifos treatment produced a low decrease (3.8%). Also, Lambda-Cyhalothrin and NPV treatments caused the significant decreasing effect (24.3 and 20.4%) on pupal weight than other treatments. Table 4 revealed the effect of tested insecticides at LC₅₀ concentration on duration of larva, pupa, and adult of the pink bollworm, treated as neonate larvae. The recorded data show that Lambda-Cyhalothrin treatment caused insignificant prolongation to duration of larvae (4.9%) and pupae (14.3%), followed by Methomyl (4.6 and 10.1%), Chlorpyrifos (3.7 and 4.6%), Spinosad (3.1 and 6.2%), Bt (1.7 and 5%), and NPV (0.9 and 4.2%), respectively, compared with untreated insects.

On the other hand, the same treatments caused insignificant shortened in resulted female and male life span than untreated insects. Chlorpyrifos and Methomyl had moderate effect (10.6 and 9.8%) on female life span, but NPV, Bt, Lambda-Cyhalothrin, and Spinosad gave low effect (7.7, 7.1, 6.3, and 5.9%, resp.). This effect is negligible on male life span with all treatments.

Table 2. Effect of insecticides LC_{50} treatment on some biological aspects of the pink

bollworm, P. gossypiella treated as neonate larvae.

Treatment	ent Mortality %			Emergency %			Hatchability		Sterility %
	Larvae	Pupae	Total mortality %		No. of egg/ female ± S. E	Change	%	Change	
Spinosad	62	10	72	28	214.2 ± 14.6	(-16.4)	67.8	(-24.4^*)	38.3*
Chlorpyrifos	58	11	69	31	201.4 ± 8.5	(-21.2^*)	38.8	(-56.7^{**})	66.7**
Lambda- Cyhalothrin	55	9	64	36	226.2 ± 21.2	(-11.5)	55.6	(-38.0 [*])	51.5**
Methomyl	54	12	66	34	157.8 ± 15.6	(-38.3^*)	40.2	(-55.2^{**})	61.0**
B.T.	64	9	73	27	184.5 ± 25.4	(-27.8^*)	72.8	(-18.8)	31.3*
Npv	67	7	74	26	207.2 ± 44.5	(-21.3*)	56.1	(-37.5*)	50.7**
Untreated	5	3	8	92	255.6 ± 51.2	0.0	89.7	0.0	0.0

^{(-) =} decrease, (*) = significant, (**) = high significant

Table 3. Effect of insecticides LC₅₀ treatment on larval and pupal weight of the pink bollworm, *P. gossypiella* treated as neonate larvae.

Weight of l	arva	Weight of pupa			
Mean of larval	Change %	Mean of pupal	Change %		
weight \pm SE (g)		weight \pm SE (g)			
0.0316 ± 0.0042	(+0.6)	0.0241 ± 0.0052	(+2.6)		
0.0302 ± 0.0033	(-3.8)	0.0223 ± 0.0027	(-5.1)		
0.0214 ± 0.0063	(-31.9 [*])	0.0178 ± 0.0035	(-24.3*)		
0.0229 ± 0.0038	(-27.1 [*])	0.0193 ± 0.038	(-17.9)		
0.0236 ± 0.0052	(-24.8 [*])	0.0201 ± 0.0046	(-14.5)		
0.0221 ± 0.0074	(-29.6 [*])	0.0187 ± 0.00069	(-20.4*)		
0.0314 ± 0.0076	0.0	0.0235 ± 0.0056	0.0		
	Mean of larval weight \pm SE (g) 0.0316 ± 0.0042 0.0302 ± 0.0033 0.0214 ± 0.0063 0.0229 ± 0.0038 0.0236 ± 0.0052 0.0221 ± 0.0074	weight \pm SE (g) (+0.6) 0.0316 ± 0.0042 (+0.6) 0.0302 ± 0.0033 (-3.8) 0.0214 ± 0.0063 (-31.9*) 0.0229 ± 0.0038 (-27.1*) 0.0236 ± 0.0052 (-24.8*) 0.0221 ± 0.0074 (-29.6*)	$\begin{array}{llllllllllllllllllllllllllllllllllll$		

Table 4. Effect of insecticides LC₅₀ treatment on larval and pupal duration and adult life span of the pink bollworm, *P. gossypiella* treated as neonate larvae.

Treatment	Larval duration	n	Pupal duration	- 1 8000 JF	Adult life span			
Mean		Change	Mean duration	Change	Female		Male	
	duration± SE	%	\pm SE (days)	%				
	(days)				Mean± SE (days)	Change %	Mean± SE (days)	Change %
Spinosad	18.10 ± 0.88	(+ 3.1)	10.75 ± 1.18	(+ 6.2)	12.91 ± 1.06	(-5.9)	11.16 ± 0.82	(-2.5)
Chlorpyrifos	18.21 ± 1.06	(+3.7)	10.58 ± 1.35	(+ 4.6)	12.26 ±1.89	(- 10.6)	11.13 ± 1.37	(-2.8)
Lambda- cyhalothrin	18.42 ± 0.92	(+4.9)	11.57 ± 1.14	(+14.3)	12.86 ± 1.17	(- 6.3)	11.40 ± 1.02	(-0.4)
Methomyl	18.36 ± 1.12	(+4.6)	11.14 ± 1.26	(+10.1)	12.37 ± 1.42	(-9.8)	11.34 ± 1.85	(-1.0)
B.t.	17.84 ± 1.24	(+1.7)	10.63 ± 1.22	(+5.0)	12.74 ± 1.35	(-7.1)	11.05 ± 1.11	(-3.5)
NPV	17.72 ± 1.66	(+0.9)	10.54 ± 1.15	(+4.2)	12.66 ± 1.76	(-7.7)	11.22 ± 1.28	(-2.0)
Untreated	17.56 ± 1.43	0.0	10.12 ± 0.76	0.0	13.72 ± 1.60	0.0	11.45 ± 1.72	0.0

Biochemical Assay:

Table 5 show the effect of insecticides LC_{50} treatment on the total protein content and activity of Acetylcholinesterase (AchE) and Glutathione-S-transferase (GST) enzymes of treated larvae of $P.\ gossypiella$. The total protein of the whole-body homogenate reached to 0.0287 ± 0.0018 , 0.0318 ± 0.0026 , 0.0313 ± 0.0034 , 0.0310 ± 0.0049 , 0.0303 ± 0.0034 , 0.0327 ± 0.0016 , and 0.0308 ± 0.0051 mg protein/mg body weight of untreated and LC_{50} treated larvae with Spinosad, Chlorpyrifos, Lambda-Cyhalothrin, Methomyl, B.T., and NPV, respectively. From these data we can notice that there was insignificant increase (10.8, 9.1, 8.1, 5.6, 13.9 and 7.3%, resp.) in total protein of treated larvae than untreated. This effect was observed in specific activity of GST with NPV (1.7%), B.T. (8.6%), Methomyl (11.7%), and Lambda-Cyhalothrin (15.9%), then raised in Chlorpyrifos (23.5%) and highly increased in Spinosad (56.9%) treated larvae. The opposite effect of treatments was detected in activity of AchE enzyme which highly decreased with NPV (61.3%), Bt (58.8%), Lambda-Cyhalothrin (55.6%), Spinosad (54.4%), Chlorpyrifos (48.1%), and Methomyl (37.5%) larvae than untreated.

Table 5. Effect of insecticides LC₅₀ treatment on the total protein content and activity of Glutathione-S-transferase and Acetylcholinesterase of the pink bollworm *P*.

gossypiella larvae.

	gossypicite iai v						
Treatment	nent Total protein content		GST		AchE		
	Mean ± S.E. (mg/mg b.w)	Change %	Mean activity ± S.E. (nmole/	Change %	Mean activity ± S.E. (nmole/ min/mg protein)	Change %	
~ · ·	0.0210 0.000	(: 10.0)	min/mg protein)	/ = C 0**\	0.704 . 0.404 . 405	***	
Spinosad	0.0318 ± 0.0026	(+10.8)	49.76 ± 3.211	$(+56.9^{**})$	$0.731 \pm 0.191 \times 10^{-5}$	(-54.4**)	
Chlorpyrifos	0.0313 ± 0.0034	(+9.1)	39.16 ± 2.271	$(+23.5^*)$	$0.032 \pm 0.260 \times 10^{-5}$	(-48.1**)	
Lambda- cyhalothrin	0.0310 ± 0.0049	(+ 8.1)	36.74 ± 5.303	(+ 15.9)	$0.710 \pm 0.062 \times 10^{-5}$	(-55.6**)	
Methomyl	0.0303 ± 0.0034	(+ 5.6)	34.42 ± 7.104	(+11.7)	$1.004 \pm 0.492 \times 10^{-5}$	(-37.5 [*])	
B.t.	0.0327 ± 0.0016	(+ 13.9)	34.42 ± 4.331	(+ 8.6)	$0.661 \pm 0.058 \times 10^{-5}$	(-58.8**)	
NPV	0.0308 ± 0.0051	(+7.3)	32.25 ± 1.318	(+1.7)	$0.623 \pm 0.057 \times 10^{-5}$	(-61.3**)	
Untreated	0.0287 ± 0.0018	0.0	31.71 ± 3.064	0.0	$1.602 \pm 0.510 \times 10^{-5}$	0.0	

DISCUSSION

The aforementioned results proved the high insecticidal activity of chemical insecticides than bioinsecticides, and Spinosad had the highly toxic effect on neonate larvae of the pink bollworm. Other findings of Radwan (2002) mentioned that toxicity of Spinosad was high than Dipel-2X (Bacillus thuringiensis kurstaki) and Agerin (Bacillus thuringiensis aegypti) to larvae of the Spiny bollworm, Earias insulana. Rashed et al., (2012) reported that Abamectin and Spinosad were more effective on third larval instar of the pink bollworm than Esfenvalerate and Dipel-2X. Emamectin benzoate and Spinosad were highly potent insecticides than Chlorpyrifos, Azadirachtin, and profenofos against the newly hatched larvae of P. gossypiella (Massoud et al., 2011). Mahmoud et al., (2009) reported that the second and fourth larval instars of the cotton leafworm, Spodoptera littoralis, were sensitive to the toxic effect of three different isolates of Egyptian baculovirus (NPV). The second larval instar was highly susceptible than 4th one. NPVcairo isolate was more effective then NPV_{giza} and NPV_{alex} isolates. NPV's decreased the moth longevity, fecundity, egg hatchability, and altered the sex ratio. Our data show that LC₅₀ treatments of the tested insecticides produced several changes in biological aspects of the pink bollworm

treated as neonate larvae. The high larval and total mortalities with low adult emergency were resulted from NPV and Bt treatments of the neonate larvae. The reduction in weight of larvae and pupae was presented in all treatments except Spinosad. A slight prolongation in duration of larvae and pupae with shortened in adult life span were resulted from all treatments. A high reduction in female fecundity and hatchability of their eggs with high sterility percentages were resulted from all treatments. Other studies reported that some biological parameters of the pink bollworm P. gossypiella were affected when treated as newly hatched larvae with LC_{50s} of B. thuringiensis and Beauveria bassiana. There was prolongation in pupal duration, decreasing in egg hatchability, larval duration, male adult longevity, mating ability and frequency, fecundity of females, egg hatchability, and increasing in female longevity and sterility in treated insects than control (Reda et al., 2008). Treatment of neonate larvae of the pink bollworm with LC₅₀ of Abamectin, Spinosad, B. thuringiensis kurstaki, and Esfenvalerate caused high larval and pupal mortalities, prolongation of larval and pupal durations, and decreasing in pupal weight, adult emergence and fecundity, and egg hatchability compared with control (Hassan, 2014). In the majority of studies that have examined the effect of low level Bt exposure, target insects have exhibited decreased weight gain (Kullk et al., 2011; Nathan et al., 2005) and increased development time (Arshad and Suhail 2011; Ramalho et al., 2011). Small larvae of Trichoplusia ni were susceptible to the effect of B. thuringiensis and they exhibited reductions in growth and frass production (Janmaat et al., 2014).

Studied data showed that treatment of the pink bollworm larvae with LC₅₀ of all tested insecticides elevated the activity of Glutathione-S-transferase enzyme and total protein contents in the whole-body homogenate of treated larvae untreated ones. A high inhibition of Acetylcholinesterase enzyme was presented in all treated larvae in comparing with untreated. Also, Rashed *et al.*, (2012) reported that Abamectin, Spinosad, Dipel-2X, and Esfenvalerate treatments at LC₅₀ concentration produced increase the proteins and activity of protease and Glutathione-S-transferase enzymes in gut tissues of the pink bollworm larvae. Proteins are among most important compound of insects that bind with foreign compounds, the increase in treated insect proteins may reflect the increase in the activity of various enzymes related to insecticides (Zidan *et al.*, 2012). Insects GST have been induced and is becoming recognized for their importance in the metabolic detoxification of insecticides (Yu, 1996). AchE activity of pests was reduced by >50% in insecticides treatment, it was associated with mortality and knockdown (Edwards and Fisher, 1991).

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ARABIC SUMMERY

تقييم الأثر الأكيد للمبيدات على يرقات ديدان اللوز القرنفلية، بكتينوفورا جوسبييلا (سويندرز)

إيمان محمد مصطفى رضوان ومحمد علي الملا ومحمد عبد الحي فودة ورجب عبد الله سليمان مصباح الله عليمان مصباح الله عمل المركزي للمبيدات مركز البحوث الزراعية وزارة الزراعة - جيزة - مصر الحيوان والحشرات -جامعة الأزهر -مدينة نصر - القاهرة - مصر

التجارب الخاصة بدراسة الآثار السمية والحيوية لثلاثة مبيدات حشرية كيميائية (كلوربيريفوس، ولمبادا- سيهالوثرين، والميثوميل) ومبيد كيموحيوي (سبينوساد)، ومبيدين حيويين (باسيلس ثيرونجينسس وبيكلوفيروس) على يرقات دودة اللوز القرنفلية، بكتينوفورا جوسيبيلا (ساوند) أجريت تحت الظروف المعملية. حيث سجلت أُعلى أثر لمبيدات الآفات المختبرة موتا للبرقات بعد أربعه أيام من التغذية على بيئة معاملة. وكان سبينوساد هو الأعلى سمية ($LC_{50} = 1.4$) جزء من المليون لليرقات حديثة الفقس يليه كلوربيريفوس ($LC_{50} = 1.4$ $(LC_{50} = 0.99)$ جزء من المليون يُليها لمبادا-سيهالوثرين $(LC_{50} = 0.99)$ جزء من المليون ثم الميثوميل (C_{50} عن معاملة البرقات حديثة الفقس بالتركيزات النصفية المميتة لكل المبيدات المختبرة نقص في أوزان البرقات والعذاري الناتجة وخصوصا مع معاملة مبيد لمبادا سيهالوثرين (٣١.٩ و٣٤.٢ %على التوالي) بالمقارنة بالحشرات غير المعاملة. كما ظهر هذا النقص في خصوبة وعمر إناث الفراشات وانعكس ذلك على ارتفاع نسب العقم لدى الحشرات المعاملة بمبيدي كلوربيريفوس (٦٦.٧ %) وميثوميل (٦١ %) وأيضا أثرت المعاملة بالتركيز النصفي المميت لكل المبيد المختبرة في نقص المحتوى الكلي للبروتين ونشاط إنزيم جلوتاثيون إس ترانسفيراز بطريقة غير معنوية في أجسام اليرقات المعاملة مقارنة باليرقات مرتفع (٩٦.٩ %) في نشاط الإنزيم مع معاملة مبيدي كلوربيريفوس وسبينوساد. كما سجل انخفاضا معنويا جدا في نشاط إنزيم أسيتيل كولين استير از بأنسجة البرقات المعاملة بالفيروس (٣٠.١٣ %) والبكتريا (٨٨.٥ %) ولمبادا سيهالوثرين (٦.٥٠ %) وسبينوساد (٤٤٥ %) كلوربيريفوس (٤٨٧ %) ثم ميثوميل (٣٧٠ %) بالمقارنة باليرقات غير المعاملة.