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COMPARATIVE TOXICITY OF THREE ENTOMOPATHOGENS AND THEIR Mixtures ON THE EGYPTIAN COTTON LEAF WORM, *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT: The toxicity of three entomopathogens, *Bacillus thuringiensis* var. *kurstaki, Paecilomyces fumosoroseus,* nuclear polyhedrosis virus of *Spodoptera littoralis* (SLNPV) and their mixtures were tested against 2nd and 4th instar larvae of *S. littoralis* under laboratory conditions. Larvae were fed on treated castor bean leaves for 48 hours, and the LC_{50} values were determined after 4, 6, 8, 10, 12and 14 days post treatment. SLNPV was the most effective one followed by *Bacillus thuringiensis* var. *kurstaki. Paecilomyces fumosoroseus* exhibited the lowest activity. The LT_{50} values decreased with an increase in concentrations of both *Bt* var. *kurstaki* and SLNPV on both. Meanwhile, LT_{50} values of entomopathogens mixtures were shorter than those obtained from application of each pathogen individually. Mortality percentages increased with the lapse of time after treatment. All tested entomopathogens or their mixtures induced different degrees of malformation in treated larvae, pupae and moths. The larvae treated with entomopathogenic mixtunes contained lower level of total protein than the control which was more evident when SLNPV was tested. The effect of entomopathogen mixtures. This effect was more evident in treated 2nd instar.

Key Words: *B. thuringiensis* var. *kurstaki, P. fumosoroseus*, nuclear polyhedrosis virus, mixtures, *S. littoralis*.

INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) is one of the most serious insect pests attacking cotton crop, vegetables, other field and ornamental crops. Pesticides have become costly and can impact the environment and health. The biocontrol of insect pests using traditional microbial agent has been reported as a more safe and economic method (Kirschbaun, 1985), and relatively safe to cope with current insect problems, pause hazards to many kinds of non target organisms (McCopy, *et al.,* 1988). *Bacillus thuringiensis* (*Bt*) is an entomopathogenic bacterium, was isolated in 1901. It produces one or more crystalline inclusion during sporulation which is toxic to lepidopteran, some dipteran and coleopteran insects (Herrnstadt *et al.,* 1986; Donovan *et al.*, 1988 and Hoft and Whiteley, 1989). The first microorganisms found to cause diseases in insects were fungi; they cause conspicuous macroscopic growth on the surfaces of their hosts. Fungi infect many orders of insects; most common are Hemiptera, Diptera, Coleoptera, Lepidoptera and Hymenoptera (David, 1967; and Ferron, 1975). Several investigators referred to the use of the nuclear polyhedrosis virus (NPV) as a useful component in the management program of *S. littoralis* (Jones *et al.,* 1994; Tuan *et al.*, 1998 and Mabrouk *et al.*, 2001).

The present investigation was directed to study the effect of *Bacillus thuringiensis* var. *kurstaki,* the fungus, *Paecilomyces fumosoroseus* and the nuclear polyhedrosis virus of *S. littoralis* SLNPV individually or in mixtures on 2nd and 4th instar larvae of *S. littoralis*. Also, the latent effect of these treatments was investigated as well as their effect on total larval protein.

MATERIALS AND METHODS

Test Insect

A laboratory strain of the cotton leaf worm, *S. littoralis* (Boisd.) reared in the laboratory of the Plant Protection Research Institute as described by El-Defrawi *et al.,* (1964) was used. This strain was reared for ten years without any insecticidal contamination. The larval instars were fed on castor bean leaves, *Ricinus communis* L. under laboratory conditions of 26 $\pm 2^0$C and 60 ± 5%RH with a

photoperiod of 16 hrs. light and 8hrs. dark. The second and fourth instar larvae were considered in this study.

Test Agents

All tested entomopathogenic agents were kindly provided by Insect Pathogen Unit at Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

***Bacillus thuringiensis* var. *kurstaki* spores**

Were produced on agar petri dishes of semi- synthetic medium according to Thiery and Francon (1997). Nutrient agar, consisted of 10g peptone, 10g NaCl, yeast 5g extract and agar 15g, these ingredierits were mixed with some distilled water and completed to one liter up to 1000ml distilled water. This medium was prepared and adjusted to pH 7.6. Petri-dishes were inoculated with *B. thuringiensis* and incubated for 72hrs at 30^0C and 50-60%RH. At the end of incubation period the spores were harvested from the surface of the culture directly by scraping with sterile solution. The resulting suspension was counted using the haemocytometer count technique (Lawrence, 1997).

The conidia of *Paecilomyces fumosoroseus*

Were produced on potato dextrose agar medium according to Smith and Onioins (1993), which consisted of 230 ml of potato-slurry,(20g glucose and 20g agar) dissolved in 770ml distilled water. The medium constituents were prepared and adjusted to pH 5.5-6.5. Petri dishes were inoculated with *P. fumosoroseus* and incubated for two weeks at 25 ± 1^0C and 50-60%RH. At the end of incubation period the conidia were harvested from the surface of culture directly by scraping with sterile solution of filtration through sterilized glass–wool. The resulting suspension was counted using haemocytometer count technique (Lawrence, 1997).

The nuclear polyhedrosis virus of *S. littoralis* (SLNPV)

Was purified from the infected larvae using Tompkins (1991) technique. Frozen larvae were weighted, thawed, crushed, left for air dryness and offered to 2^{nd} and 4^{th} instar larvae.

Bioassay

Five concentrations of each considered pathogen were prepared diluted by distilled water and 0.01% Tween-80 i.e., $2.7x10^9$,$2.7x10^8$,$2.7x10^7$,$2.7x10^6$ and $2.7x10^5$ (colony forming units/ml "c.f.u" for *B.*

thuringiensis var. *kurstaki*; spore/ml for *P. fumosoroseus* and polyhedral inclusion body (PIB/ml) for SLNPV. The leaf dipping technique was used where fresh castor bean leaves were dipped for one minute in each prepared concentration. Treated leaves were left to dry at room temperature and then offered to newly ecdysed 2nd or 4th instar larvae for 48 hours. After this time, survived larvae were transferred to other clean jars, and supplied daily with fresh clean castor bean leaves up to the termination of the larval stage. Four replicates were used each comprising 10 larvae for each prepared concentration.

A similar number of larvae was supplied with castor bean leaves immersed in distilled water with sterile solution 0.01% Tween-80 and considered as the control group. Also, three mixtures of these pathogens were prepared,(i) *Bt.* Var. kurstaki with slnpv at concentrations $2.7x10^6$ c.f.u / ml and $2.7x10^6$ PIB/ml, respectively (ii) *Bt.* var. kurstaki with *P. fumosoroseus* at concentrations $2.7x10^6$c.f.u/ml and $2.7x10^6$ spore /ml, respectively (iii) *P. fumosoroseus* with SLNPV at concentrations$2.7x10^6$ spore/ml and $2.7x10^6$ PIB /ml, respectively .Mortality counts were recorded after 4,6,8,10,12 and 14 days post-treatment and LC_{50} , LT_{50} and slope values were determined at these periods using Propan Program Soft Ware. Results were corrected according to Abbott's formula (1925).

Number of larvae pupating was calculated as a percentage. Obtained pupae were weighted and placed in wide glass jars until adult emergence which was determined as a percentage. Moreover, any malformation of larvae, pupae and adults were recorded. Data were subjected to statistical analysis by one – way ANOVA test (Gad, 2001) using SPSS software for Windows version 10. A probability of $p \leq 0.05$; $p \leq 0.01$; $p \leq 0.001$ as the level of significance was used unless stated otherwise.

Effect of The Tested Entomopathogens and Their Mixtures on Total Protein Content of Infected Larvae

The 2nd and 4th instar larvae were fed on castor bean leaves dipped in $2.7x10^6$ (c.f.u/ml, spore/ml and PIB/ml, for *Bt* var. *kurstaki* , *P. fumosoroseus* and NPV, respectively) of each entomopathogen or their mixtures for 48hrs., then fed on untreated castor bean leaves until the end of 6th larval instar. Treated and

untreated larvae were frozen and used for total protein determination.

Total Protein Assay

In order to determine the total protein in the haemolymph of the entomopathogens-infected larvae, the frozen larvae were crushed and centerifugated for 10 min. at 1000 r.p.m and the supernatant was used. Total protein was determined colourimetrically using Biuret reaction (Weissman *et. al.,* 1950). The kits used for this assay were purchased from Stanbio, USA.

RESULTS AND DISCUSSION

As seen in Table 1 there were differences in the larvicidal activity of the three tested pathogens *Bt* var. *kurstaki, P. fumosoroseus* SLNPV 2nd and 4th instar larvae of *S. littoralis*. Mortality increased as time elapsed after treatment. Based on the LC_{50} values, after 8 days of treatment the virus SLNPV caused the highest toxic effect giving $0.01x10^5$ and $0.14x10^6$ PIB/ml for 2nd and 4th instar larvae respectively. This effect was followed by *Bt* var. *Kurstaki* as LC50 was $0.15x10^5$ and $5.10x10^7$ c.f.u/ml to the respective mentioned larval instar. The fungal entomopathogen, *P. fumosoroseus*, gave the weakest toxic effect against both 2nd or 4th instars and the LC_{50} and LT_{50} could not be determinated.

After 8 days of treatment, of 2nd and 4th instar larvae with the three entomopathogens insecticidal activity was higher than those obtained after 6 days of treatment. LC_{50} values decreased gradually after the first day of treatment to reach lowest level on the 14th and 8th day post treatment when 2nd or 4th instars were treated, respectively, Table 2. Salama *et al.,* (1993) reported that SLNPV had a more pathogenic effect than *B.t* on *S. littoralis* larvae. The virus SLNPV had a stronger larvicidal effect than Bt var kurstaki and P. fumosoroseus when either 2nd or 4th instars were treated as depicted by LT_{50} Table 3. The LT_{50} values for SLNPV on the 2nd instar larvae were 5.45, 6.18, 7.10, 7.55 and 8.47 days at concentrations of $2.7x10^9$, $2.7x10^8$, $2.7x10^7$, $2.7x10^6$ and $2.7x10^5$ PIB /ml, respectively. Meanwhile, these values were 8.20, 8.48, 10.30, 12.06 and 13.53 days for the respective mentioned concentrations Table 3. The LT_{50} values for *Bt* var. *kurstaki* on the 2nd and 4th instar larvae were 7.70, 8.37, 10.17, 11.95 , 13.61 and 11.66, 13.23, 13.29, 14.45, 14.45

Table1. Accumulative mortality percentages of *S. littoralis* larvae treated as 2nd or 4th instars with *B. t*, *P. fumosoroseus* and SLNPV using leaf dipping technique under laboratory conditions

Concentrations c.f.u*/ml	Treated instar larval	Accumulative mortality %					
		Days post-treatment					
		4	6	8	10	12	14
		B`t var. *kurstaki*					
$2.7x10^9$	2nd	18.72	44.76	51.87	60.03	72.15	72.15
	4th	14.01	19.66	35.70	-	-	-
$2.7x10^8$	2nd	16.11	36.40	44.37	53.77	65.71	65.71
	4th	10.67	17.41	31.62	-	-	-
$2.7x10^7$	2nd	13.75	28.64	37.07	47.42	58.78	58.78
	4th	7.94	15.33	27.76	-	-	-
$2.7x10^6$	2nd	11.64	21.77	30.20	41.14	51.56	51.56
	4th	5.78	13.42	24.14	-	-	-
$2.7x10^5$	2nd	9.77	15.96	23.97	35.07	44.29	44.29
	4th	4.10	11.69	20.79	-	-	-
spores/ml		*P. fumosoroseus*					
$2.7x10^9$	2nd	16.60	19.92	30.94	30.94	37.21	45.70
	4th	7.57	12.37	18.29	-	-	-
$2.7x10^8$	2nd	11.77	14.76	23.98	23.98	28.71	35.77
	4th	6.71	9.78	15.29	-	-	-
$2.7x10^7$	2nd	8.03	10.58	17.98	17.98	21.26	26.71
	4th	5.93	7.61	12.67	-	-	-
$2.7x10^6$	2nd	5.27	7.33	13.02	13.02	15.08	18.99
	4th	5.22	5.83	10.38	-	-	-
$2.7x10^5$	2nd	3.32	4.91	9.10	9.10	10.23	12.82
	4th	4.58	4.40	8.41	-	-	-
**PIB/ml		SLNPV					
$2.7x10^9$	2nd	30.41	52.12	77.97	82.04	84.10	89.96
	4th	22.70	37.37	51.73	-	-	-
$2.7x10^8$	2nd	23.80	46.71	72.29	77.77	80.49	87.03
	4th	17.91	30.16	45.43	-	-	-
$2.7x10^7$	2nd	18.06	41.35	65.97	72.96	76.43	83.55
	4th	13.82	23.65	39.24	-	-	-
$2.7x10^6$	2nd	13.28	36.16	59.17	67.69	71.93	79.52
	4th	10.41	18.00	33.31	-	-	-
$2.7x10^5$	2nd	9.45	31.20	52.08	62.04	67.06	74.95
	4th	7.66	13.28	27.77	-	-	-

* Colony forming unit.
**PIB: Polyhedrosis inclusion bodies

Table 2. Accumulative LC_{50} of *S. littoralis* larvae treated as 2nd or 4th instars larvae with SLNPV and *B.t* using leaf dipping technique under laboratory conditions

Days	Treated instar larvae	LC_{50} (c.f.u*/ml)	95% Fiducial limits		Intercept	Slope
			Lower	Upper		
				B.t		
4	2nd	$1.50x10^{13}$	UND**	UND**	3.6616±0.2334	0.1016±0.0790
	4th	$9.66x10^{10}$	UND**	UND**	3.1903±0.2916	0.1647±0.0938
6	2nd	$1.10x10^{5}$	7456.2213	$1.8575x10^{9}$	3.9105±0.2025	0.2162±0.0685
	4th	$3.75x10^{14}$	UND**	UND**	3.7729±0.2239	0.0842±0.0766
8	2nd	$0.15x10^{5}$	1385.8994	$1.1940x10^{8}$	4.2115±0.1885	0.1885±0.0654
	4th	$5.10x10^{7}$	UND**	UND**	4.1380±0.1949	0.1118±0.0074
10	2nd	$0.68x10^{4}$	32.8980	$2.50341x10^{5}$	4.5479±0.1797	0.1594±0.0637
12	2nd	$0.01x10^{4}$	0.0130	156.8880	4.7774±0.1779	0.1828±0.0646
14	2nd	$0.006x10^{4}$	$1.06845x10^{9}$	16.0135	5.0303±0.1794	0.1678±0.0661
		***PIB			Virus	
4	2nd	$9.82x10^{6}$	$1.01795x10^{5}$	$1.77736x10^{19}$	3.6003±0.2287	0.2002±0.0754
	4th	$6.86x10^{8}$	$5.63426x10^{5}$	$1.0000x10^{38}$	3.4981±0.2436	0.1700±0.0801
6	2nd	$1.09x10^{5}$	526.5767	$1.92938x10^{23}$	4.4513±0.1820	0.1358±0.0639
	4th	$1.15x10^{6}$	28508.4020	$2.01288x10^{14}$	3.8015±0.2112	0.1978±0.0708
8	2nd	$0.01x10^{5}$	$1.71876x10^{6}$	23.0828	4.9745±0.1791	0.1798±0.0660
	4th	$0.14x10^{6}$	997.7681	$7.48723x10^{6}$	4.3420±0.1455	0.1583±0.0456
10	2nd	$0.02x10^{4}$	$7.80175x10^{30}$	2.8238	5.2407±0.1833	0.1256±0.0682
12	2nd	$0.001x10^{4}$	$1.0000x10^{38}$	0.8784	5.3815±0.1873	0.1393±0.0699
14	2nd	$0.0001x10^{4}$	$1.0000x10^{38}$	0.1705	5.6074±0.1990	0.1517±0.0762

*c.f.u: colony forming unit

** UND: undetectable values.

***PIB: Polyhedrosis inclusion bodies

Table 3. LT_{50} values of *S. littoralis* treated as 2nd or 4th instar larvae with *B.t* and SLNPV using leaf dipping technique under laboratory conditions

Concentrations c.f.u*/ml	Treated instar larvae	LT_{50} (days)	95% Fiducial limits		Intercept	Slope
			Lower	Upper		
	2nd	7.70	6.5275	8.8963	2.6212±0.4461	2.6831±0.4761
$2.7x10^9$	4th	11.66	8.161	1327.382	2.4468±0.8462	2.3934±1.0708
	2nd	8.37	7.2967	9.5645	2.2268±0.4630	3.0061±0.4904
$2.7x10^8$	4th	13.23	8.8416	5817.875	2.1555±0.9138	2.5357±1.1470
	2nd	10.17	8.8512	12.1920	2.1996±0.4773	2.7802±0.4989
$2.7x10^7$	4th	13.29	9.0389	810.4899	1.7094±0.09614	3.0529±1.1978
	2nd	11.95	10.2310	15.3629	2.1188±0.4469	2.6746±0.5147
$2.7x10^6$	4th	14.45	9.4870	4433.7414	1.5549±1.0698	2.9702±1.3231
	2nd	13.61	11.390	18.955	2.0401±0.5173	2.6108±0.5322
$2.7x10^5$	4th	14.45	9.4870	4433.7414	1.5549±1.0698	2.9702±1.3231
PIB**/ml				Virus		
	2nd	5.45	4.4659	6.2505	2.5242±0.4570	3.3603±0.5085
$2.7x10^9$	4th	8.20	6.5232	27.2198	2.7171±0.7716	2.4990±0.9870
	2nd	6.18	5.2212	6.9961	2.3338±0.4560	3.3710±0.4994
$2.7x10^8$	4th	8.48	6.9244	12.8917	2.3119±0.5732	2.8957±0.7204
	2nd	7.10	6.1791	7.9618	2.0564±0.4641	3.4581±0.5001
$2.7x10^7$	4th	10.30	7.7003	83.9346	2.3609±0.8325	2.6059±1.0541
	2nd	7.55	6.6427	8.4555	1.9225±0.4709	3.5042±0.5036
$2.7x10^6$	4th	12.06	8.5551	214.7427	1.9897±0.9162	2.7840±0.5237
	2nd	8.47	7.5229	9.5377	1.8006±0.4824	3.4467±0.5092
$2.7x10^5$	4th	13.53	9.1417	976.5839	1.7482±0.9974	2.8748±1.2408

*c.f.u: colony forming unit

**PIB: Polyhedrosis inclusion bodies

days at the concentrations of $2.7x10^9$, $2.7x10^8$, $2.7x10^7$, $2.7x10^6$ and $2.7x10^5$ c.f.u/ml, respectively Table 3.

Data in Tables 4 and 5 indicate that the LT_{50} until larval mortality in treated 2nd and 4th instar larvae was shorter in the mixtures of the entomopathogens SLNPV with *Bt* var. *Kurstaki* than those obtained with the use of each pathogen separately. LT_{50} for this mixture on the 2nd and 4th instar larvae was 5.07and 6.25days, respectively which was lower than LT_{50} when either virus or bacteria were tested individually. Similarly,LT_{50} for *Bt* var. *kurstaki* +*P. fumosoroseus* on the 2nd and 4th instar larvae was 5.82 and 8.20 days respectively, and NPV with *P. fumosoroseus* caused a 7.72 and 11.92 days at the same concentration . Slope values for the mixtures of entomopathogens were higher than those obtained when either of SLNPV, *Bt* var. *kurstaki* and *P. fumosoroseus* used separately, which reflect the highest mortality and lower LT_{50s}. El-Nagar *et al.,* (1985) mentioned that when nuclear polyhedrosis virus was administered orally to *S. littoralis* larvae, there was a general increase in the concentration of DNA and RNA in treated larvae, as compared with untreated ones. Mabrouk (2001) mentioned that incubation period until morality among the treated 2nd instar larvae was shorter in NPV mixed with *Bt* than when each pathogen was used separately.

All three entomopathogens induced some pupal or moth malformations, Also, larval-pupal or pupal-adult intermediates were observed as a result of 2nd and 4th instar larval treatment. These effects were more apparent when SLNPV was tested, followed by *Bt* var. *kurstaki* and least by *P. fumosoroseus* Table 6. The mixture of SLNPV +*Bt* var. *kurstaki* represented the highest effects and the least one was the mixing SLNPV + *P. fumosoroseus* as shown in Table 6. The number of larval entering the pupal stage as well as moth emergence was affected; this effect was more evident when SLNPV was used for either treated instar. This was followed *Bt* var. *kurstaki* and least by *P. fumosoroseus*.

Generally, mixture of pathogens caused a lower percentages of pupation and moth ecolosion, especially the mixture of SLNPV with *Bt* var. *kurstaki* Table 6.

Table 4. Accumulative mortality percentages of 2nd and 4th instars larvae of *S. littoralis* treated with three entomopathogens mixtures

Concentrations* of mixture	Treated instar larvae	Cumulative mortality % indicated days after treatment			
		4	6	8	10
B. t + NPV	2nd	34.82	61.02	77.49	86.95
	4th	32.26	48.09	59.66	
B. t + *P. fumosoroseus*	2nd	31.21	51.61	66.16	76.08
	4th	21.82	36.75	48.96	
P. fumosoroseus + NPV	2nd	20.57	37.63	51.76	62.65
	4th	16.67	27.16	36.20	

* : each entomopathogen was used at concentration $2.7x10^6$

Table 5. Toxicity regression line parameters of 2nd and 4th instar larvae of *S. littoralis* treated with pathogen mixtures using leaf dipping technique

Concentrations * of mixture	Treated instar larvae	LT_{50} (days)	95% Fiducial limits		Intercept	Slope
			Lower	Upper		
B. t + NPV	2nd	5.07	4.1138	5.7837	2.3193 ±0.6044	3.8046 ± 0.7434
	4th	6.29	4.796	10.8645	3.1299 ±0.7361	2.3416 ± 0.9512
B. t + *P. fumosoroseus*	2nd	5.82	4.6722	6..7965	2.6962 ±0.5861	3.0126 ± 0.7068
	4th	8.20	6.5232	27.2198	2.7171 ±0.7716	2.499 ± 0.987
P. fumosoroseus + NPV	2nd	7.72	6.5646	9.8444	2.4475 ±0.6063	2.8751 ± 0.7178
	4th	11.92	7.9942	10.00	2.8041 ±0.8115	2.0405 ± 1.0330

* : each entomopathogen was used at concentration $2.7x10^6$

Table 6 . Latent effect of three entomopathogens and their mixtures treated on 2nd and 4th instar larvae of *S. littoralis* using leaf dipping technique

Concentrations	% Pupation		Pupal weight (mg)		% Adult emergence		% Malformed adult	
			c.f.u*/ml					
	2nd	4th	2nd	4th	2nd	4th	2nd	4th
$2.7x10^9$	10	60	$226.88^{a} \pm 0.01513^{***}$	268.24 ± 0.00334	50	54.1	50	45.9
$2.7x10^8$	12.5	70	$265.75^{b} \pm 0.00286^{**}$	274.00 ± 0.00398	60	60.7	40	39.3
$2.7x10^7$	15	70	$272.50^{b} \pm 0.00119^{*}$	279.72 ± 0.00709	66.6	64.2	33.4	35.8
$2.7x10^6$	25	72.5	$275.50^{b} \pm 0.00132^{*}$	276.00 ± 0.01354	70	72.4	30	27.6
$2.7x10^5$	32.5	75	$280.00^{bc} \pm 0.00108$	292.75 ± 0.00363	84.6	80	15.4	20
Control	95	97.5	$297.25^{c} \pm 0.00259$	308.50 ± 0.02564	95	97.5	5	2.5
			Spore/ml					
$2.7x10^9$	57.5	70	252.75 ± 0.00536	264.25 ± 0.00143	73.9	89.2	26.1	10.8
$2.7x10^8$	62.5	72.5	267.00 ± 0.00372	267.5 ± 0.00075	76	93.1	24	6.9
$2.7x10^7$	67.5	77.5	270.25 ± 0.00562	280.00 ± 0.00451	77.7	93.5	22.3	6.5
$2.7x10^6$	70	82.5	277.50 ± 0.00602	290.75 ± 0.01199	82.1	96.9	17.9	3.1
$2.7x10^5$	70	85	282.50 ± 0.00441	294.50 ± 0.00375	89.2	97	10.8	3
Control	95	100	279.30 ± 0.01650	295.50 ± 0.02356	95	100	5	0
			PIB**/ml					
$2.7x10^9$	10	47.5	$143.25^{a} \pm 0.01006^{***}$	$174.50^{a} \pm 0.00532^{***}$	50	31.5	50	68.5
$2.7x10^8$	12.5	52.5	$167.25^{b} \pm 0.00511^{***}$	$180.25^{a} \pm 0.00511^{***}$	60	38	40	62
$2.7x10^7$	17.5	55.5	$177.25^{b} \pm 0.00405^{***}$	$200.25^{ab} \pm 0.00915^{***}$	71.4	40.9	28.6	58.1
$2.7x10^6$	20	67.5	$184.75^{bc} \pm 0.00565^{***}$	$199.75^{ab} \pm 0.00534^{***}$	75	48.1	25	51.9
$2.7x10^5$	25	72.5	$198.50^{c} \pm 0.00612^{***}$	$219.75^{b} \pm 0.00352^{***}$	80	51.7	20	48.3
Control	95	97.5	$292.50^{d} \pm 0.00259$	$308.75^{c} \pm 0.01869$	95	97.3	5	2.5
B.t + NPV	7.5	22.5	$116.75^{a} \pm 0.00921^{***}$	$150.75^{a} \pm 0.00375^{***}$	33.33	55.5	66.67	44.45
B.t + *P. fumosoroseus*	20	35	$130.00^{a} \pm 0.00091^{***}$	$168.00^{b} \pm 0.00255^{***}$	50	65	50	35.72
NPV + *P. fumosoroseus*	27.5	47.5	$145.00^{a} \pm 0.00082^{***}$	$179.25^{c} \pm 0.00338^{***}$	63.63	68.42	36.37	31.58
Control	97.5	97.5	$352.25^{b} \pm 0.01636$	$363.50^{d} \pm 0.00343$	97.5	97.5	2.5	2.5

Values represent means ± SEM (n = 4). Significance level: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ compared with control.

*c.f.u: colony forming unit **PIB: Polyhedrosis inclusion bodies

Table 7. Total protein content in 6th instar larvae of *S. littoralis* treated as 2nd or 4th instars with three entomopathogens or their mixtures

Larval instar	Entomopathogene	Total protein(g/dl)
2nd	*Bacillus thuringiensis* var.*kurstaki*	$6.240^{I} \pm 0.125$
	Paecilomyces fumosoroseus	$4.172^{e} \pm 0.752$
	Nuclear polyhedrosis virus	$6.911^{j} \pm 0.208$
	B.t + NPV	$1.998^{bc} \pm 0.276$
	B.t + *P. fumosoroseus*	$1.524^{ab} \pm 0.105$
	NPV + *P.. fumosoroseus*	$1.373^{a} \pm 0.041$
4th	*Bacillus thuringiensis* var.*kurstaki*	$5.663^{g} \pm 0.152$
	Paecilomyces fumosoroseus	$5.151^{f} \pm 0.081$
	Nuclear polyhedrosis virus	$5.880^{h} \pm 0.099$
	B.t + NPV	$2.490^{c} \pm 0.068$
	B.t + *P. fumosoroseus*	$2.490^{c} \pm 0.068$
	NPV + *P. fumosoroseus*	$3.617^{d} \pm 0.109$
	control	$16.373^{k} \pm 0.372$

Similar results were reported in different insect pests treated with entomopathogens such as (El- Tantawy, 1973; Moftah *et al.* , 1990; Hou and Chou, 1993; Farrag , 2000; Sondos *et al.*, 2000).

Spectrophotometer determinations of total protein are shown in Table 7. Data reveal that treatment with the entomopathogen mixtures caused the highest decrease in total protein content in both treated larval instars than when using each pathogen alone. It is not worthy that protein level was more detected when 2nd larval instar than the larvae treated at the 4th instar. The effect of the fungus, *P. fumosoroseus* in decreasing protein content was more noticeable in treated 2nd instar than the other two pathogens. While the effect of each pathogen alone in protein level reduction in 4th treated instar larvae was more evident.

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السمية المقارنة لثلاث من الممرضات الحشرية ومخاليطها على دودة ورق القطن

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أجريت دراسة معملية بمعهد بحوث وقاية النبات- الدقى- جيزة-مصر وذلك بهدف دراسة تأثير البكتريا *Bacillus thuringiensis* والفطر *Paecilomyces fumosoroseus* والفيروس nuclear polyhedrosis virus والخلط بين الممرضات الثلاثة على نسبة الموت والتأثير المتأخر والبروتين عند معاملة العمر اليرقى الثانى والرابع لدودة ورق القطن المغذى على التركيزات ٢,٧x ١٠^{٩} و٢,٧ x ١٠^{٨} و٢,٧x ١٠^{٧} و٢,٧ x ١٠^{٦} و٢,٧ x ١٠^{٥} لمدة ٤٨ ساعة وتم حساب نسب الموت على فترات مختلفة من المعاملة وأظهرت النتائج أن نسبة الموت تزداد بزيادة انقضاء الوقت بعد المعاملة.كذلك وجد ان الفيروس أكثر الممرضات تأثيرا على نسبة الموت ثم البكتريا ثم الفطر حيث كانت قيم LT_{50} للفيروس عند التركيزات المذكورة أقل من البكتريا والفطر.

وعند خلط الممرضات الثلاثة بالتركيز 2.6x10^{6} (البكتريا + الفيروس و البكتريا+ الفطر والفيروس+الفطر) قلت قيمة T_{50} بالمقارنة بكل مسبب مرضى بمفرده. كذلك أحدثت المركبات المختبرة تشوهات فى العذارى والحشرات الكاملة الناتجة من اليرقات المعاملة (عمر ثاني وعمر رابع) ومخاليطها وكان أكثرها فعالية مركب الفيروس NPVوأقلها الفطر *P. fumosoroseus* بينما فى حالة المخاليط كان مخلوط *B.t* + NPV أكثرها فعالية وأقلها مخلوطNPV+*P. fumosoroseus*. أكد هذه النتائج تقدير البروتين الكلى وكذا استخدام التفريد الكهربى لنمط البروتين الذى تم تكسيره باستخدام SDS لاختلاف عدد الشرائط البروتينية وتركيزها قل عدد bands فى المعاملة عن الكنترول.