Egypt. Acad. J. biolog. Sci., Vol. 5 (1) 19-30 (2013) C. Physiology & Molecular Biology
Email: egyptianacademic@yahoo.com ISSN: 2090-0767
Received: 13/4/2013 www.eajbs.eg.net

Biochemical studies of *Bacillus Thuringiensis* var.*kurstaki, Serratia marcescns* and Teflubenzurone on cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptea: Noctuidae)

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ABSTRACT

Two biopesticides *Serratia marcescens* [Eubacteriales: *Enterobacteria*] (used at MC_{50}, concentration caused 50% malformation) and *Bacillus thuringiensis Var. kurstaki* (used at LC_{50}) and insect growth regulator Teflubenzuron (used at LC_{50} value) were used for treatment of 2nd instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.). Sequential combined Effect was carried out by treating 2nd instar larvae with LC_{50} value of *B. thuringiensis* or Teflubenzuron then the larvae allowed to pupate on sawdust treated with *S. marcescens* at MC_{50} .The effect of these three agents were assessed by toxicity The obtained LC_{50} values were 0.113 and 165.64 ppm, for Teflubenzuron and *B. thuringiensis Var. kurstaki*. (protecto), respectively. Moreover, the activity of Chitinase, protease, trehalase, and the main components (total lipids, proteins and carbohydrates) were significantly fluctuated during the different periods of pupal stage.

INTRODUCTION

The cotton leafworm, *S. littoralis* (Boisd.) is a highly destructive insect pest. The extensive use of insecticides to control *S. littoralis* larvae has led to several problems and hazards such as development of resistance and residual effects (Frank *et al.,* 1990). Thus, it is important to search for alternative control agents with new modes of action. Among these agents are insect growth regulators (IGR's) and microbial control agents. The bacterium *B. thuringiensis*, proved to be a highly successful weapon for fighting some agricultural pests and it offer many advantages over chemical insecticides. *B. thuringiensis* is known to be one of the most pathogenic species of bacteria, which induce larval mortality after a course of infection stages. The interest of using such agent as a microbial bioinsectcide was increased during the past decade (Dulmage and Co-operators, 1981). As well as, the bacteria of the genus Serratia are often associated with insects and have the behavior of a facultative pathogen. (Trevor *et al.*, 2004). In general, *S. marcescens* is not pathogenic to insects when present in the digestive tract in small numbers, but once it enters the hemocoel it multiplies rapidly and causes death in one to three days (Sikorowski, 1985). Furthermore, chitinase producing bacteria *S. marcescens* caused significant physiological and morphological effects on pupal and adult stages where it caused a significant increase in adult malformation % and also affected some enzymes activity El-Sheikh (2006).

The aim of this study:

Evaluate the effectiveness of the insect growth regulator Teflubenzuron as a chitin synthesis inhibitor (No moult), *B. thuringiensis* (protecto), chitinase producing bacteria *S. marcescens* and their sequential combined effect through following treatments for controlling *S. littoralis*.

MATERIALS AND METHODS

Rearing technique

The stock culture of the cotton leafworm, *S. littoralis* (Boisd.) was

obtained from a laboratory strain maintained in the Cotton Pest Research Dept, Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, for several generations without any insecticidal or microbial pressure. The insect was reared on castor-oil leaves, *Ricinus communis,* under laboratory conditions at 25 ± 2 °C and 60 ± 5 % R.H. 2nd and late 6th instars larvae were used in the current work.

Control agents

Biopesticide

Protecto: It is a wettable powder formulation, based on *B. thuringiensis Var. kurstaki*. It contains lepidopteran toxin 9.4 % produced by the Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt.

***Serratia marcescens*:** Chitinase-producing bacterial strain belongs to *Enterobactieraceae* isolated from Egyptian Soils. The isolated bacterial strain was formulated as a biocontrol agent for controlling parasitic nematodes. It was produced by Soils, Water and Environ. Res. Inst. ARC, and distributed on a commercial scale (trade name, Nemaless)

IGR

Common name: Teflubenzuron.

Trade name: No moult 15 % S.C.

This IGR was obtained from BASF Chemical Company.

Bioassay

Preliminary tests were carried out using series of concentrations (in water) for each of the bio-agent, *Serratia marcescens* (10^4, 10^5, 10^6, 10^7, 10^8. 10^9) colony forming unit/ml (cfu/ml), *B. thuringiensis* (44.187, 88.375, 176.75, 352.5, 705, 1410 ppm) and the chitin synthesis inhibitor Teflubenzuron (0.02, 0.04, 0.08, 0.16, 0.32, 0.64 ppm) were prepared using the commercial formulation. Sawdust was treated with each concentration of *S. marcescens* in glass jars and offered to late 6th instar larvae to pupate on it. The offered treated sawdust was in a wettable form, while, in case of Teflubenzuron and *B. thuringiensis*, the use of leaf-dipping technique was carried out according to Abo El-Ghar *et al.,* 1994. Castor bean leaves, *R. communis,* were dipped in each concentration then left to dry at room temperature and these were offered to the newly moulted 2nd instar larvae. Larvae were allowed to feed for 24 hrs. Then, they were provided with fresh, clean and untreated castor bean leaves until pupation. Larvae that fed on untreated castor bean leaves were used as control for Teflubenzuron and *B. thuringiensis* treatments whereas larvae kept in untreated sawdust were considered as control for *Serratia marcescens*. In all treatments, three replicates were carried out for each concentration; each replicate consisted of 20 larvae. The larval mortality and adult malformation percentages were determined. The data were then subjected to probit analysis (Finney, 1971) to obtain the LC_{50} values of both Teflubenzuron and *B. thuringiensis* as well as the concentration which causes 50% adult malformation (MC_{50}) for *S. marcescens*. The combined effect was studied by treatment of 2nd instar larvae with LC_{50} of Teflubenzuron or *B. thuringiensis* then the larvae were allowed to pupate on sawdust treated with *S. marcescens* at MC_{50}. The Toxicity index of the tested compounds was determined according to Sun (1950) as follows:

$$\text{Toxicity index} = \frac{LC_{50} \text{ of the most toxic compound}}{LC_{50} \text{ of other compounds}} \times 100$$

Biochemical determinations

Preparation of samples for biochemical analysis:

Pupal samples were collected after 2nd, 4th, 6th, 8th, 10th, 12th and 14th days of prepupation and homogenized in distilled water using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 6000 r.p.m. for 10 minutes at 5°C. The supernatant was divided into small aliquots (0.5 ml) and stored at-20°C until analysis. Three replicates were used for each biochemical determination.

Determination of Chitinase activity:

Chitinase was determined according to the method described by Ishaaya and Casida (1974).

Determination of Protease activity:

The proteolytic activity was determined by the casein digestion method described by Ishaaya *et al.* (1971).

Determination of trehalase activity:

The trehalase was determined according to the method described by Ishaaya and Swiriski (1976).

Determination of the main metabolites:

Determination of total carbohydrates

Total carbohydrates were determined as described by Singh and Sinha (1977).

Total soluble protein content:

Total soluble protein content was determined by the method of lowery *et al*. (1951)

Total lipid content:

Total lipid content was estimated according to Knight *et al*. (1972).

Statistical analysis

Data were analyzed using Proc. ANOVA in SAS (SAS Institute, 1998).

RESULTS AND DISCUSSION

Toxicological effects:

Table (1) shows the susceptibility of the 2nd instars of *S.littoralis* towards the *B. thuringiensis* and Teflubenzuron compounds. The LC_{50} of Teflubenzuron is 0.113ppm, whereas, it is 165.64ppm in case of treatment with *B. thuringiensis*. Based on LC_{50} values, it is obvious that both compounds caused considerable toxic effects against the 2nd larvae of *S. littoralis* particularly in case of Teflubenzuron which had drastical toxic effects comparing to *B. thuringiensis* toxicity. On the other hand, *S. marcescence* had very low toxic effects against larvae and high tendency to induce malformation. Thus, because of its ability to cause a high malformation percentage to *Spodoptera littoralis,* so, the concentration which causes 50 % adult malformation ($3.09x10^8$ cfu.) was used instead of LC_{50} that *S. marcescens* failed to achieve. The LC_{50} value of chitin synthesis inhibitor Teflubenzuron was similar to that obtained by Thabit (2011) who recorded LC_{50} of 0.177ppm for Teflubenzuron towards 2nd instar larvae of *S. littoralis*. Teflubenzuron in the present study had drastical toxic effect comparing to *B.thuringiensis*, this was similar to Abd El-Aziz (2007) who found that lufenuron had drastical toxic effect comparing to *B. thuringiensis* on 2nd instar larvae of *S. littoralis.* On the other hand, the concentration which causes 50% adult malformation to *S. littoralis* was similar to Tolba(2006) and EL-Sheikh *et al*.(2005) working on *Agrotis ipsilon*. Abd El-Aziz (2000) stated that the crystal toxins from most of *B. thuringiensis* serotypes are toxic to larvae of Lepidoptera up on ingestion and is quickly activated by a combination of the alkaline gut pH and proteolytic enzymes present in the mid gut of the insect.

Table 1: Toxicity of *B. thuringiensis* and Teflubenzuron against 2nd instar larvae of *Spodoptera littoralis*.

Compound	LC_{50} (ppm)	95% Fiducial Limits		Slope ± S.E.	X^2 (df)	Toxicity Index
B. thuringiensis	165.64	128.21	208.31	1.59 ± 0.12	0.523 (5)	0.068
Teflubenzuron	0.113	0.075	0.171	1.59 ± 0.13	9.94 (5)	100
Serratia marcescence concentration which causes 50% adult malformation to *Spodoptera littoralis* (Boisd).						
Compound	*MC_{50} (cfu/ml)	95% Fiducial Limits		Slope ± S.E.	X^2	Toxicity Index
Serratia marcescence	$3.09x10^8$	$7.7x10^7$	$3.81x10^9$	0.29 ± 0.055	0.966 (4)	-

* MC_{50}: Concentration caused 50% adult malformation.
**cfu: Colony forming unite.

Biochemical Effects:

Effects on chitinase activity:

The obtained data in Table (2) show the change in chitinase activity in pupal stage of *S. littoralis* treated with *S.marcescence* at concentration caused 50% malformation, LC_{50} either of *B. thureinginsis* or Teflubenzuron and their sequential following treatments at which sawdust was treated with *S. marcescence* after larvae treatment either with *B. thureinginsis* (*Bt*/*serr*) or with Teflubenzuron (Teflu /*Serr*). Results confirmed that the treatments with *S. marcescens*, *Bt/Serr*, Teflubenzuron and Teflu /*Serr* caused a significant increases in the chitinase activity through the pupal stage of *S. littoralis* at all times except the last time in *Bt/Serr*, with respect, the highest induction was obvious in case of Teflu /*Serr* followed by Teflubenzuron, and *S. marcescens* in which there were gradually increases reached the maximum of 901.1 and 664.99, 785.27 and 572.15 and 324.24 and 245.58 % at 10th and 12th days of pupation, respectively, then there were decreased at 14th day to 255.79, 232.25 and 28.52%, respectively.

Table 2: Changes in chitinase activity in pupal stage of *Spodoptera littoralis* treated with *Serratia marcescence* at MC_{50}, LC_{50} of *B. thureinginsis*, Teflubenzuron and their sequential effects.

Days after treatment	Chitinase activity (µg NAGA/ min /g body weight) (*Mean ± SE).										
	TREATMENTS										
	Control	*Serratia marcescence*	% **	*B. thureinginsis*	%	Teflubenzuron	%	*B. thureinginsis* / *Serratia marcescence*	%	Teflubenzuron / *Serratia marcescence*	%
2	wx 2.778 ± 0.085	tuvw 4.285 ± 0.118	54.25	Uv 3.934 ± 0.153	41.61	no 8.639 ± 0.243	210.98	tuvw 4.344 ± 0.217	56.37	mn 9.240 ± 0.174	232.61
4	qrs 6.212 ± 0.218	no 9.093 ± 0.313	46.38	Rst 5.595 ± 0.289	-9.93	h 22.356 ± 1.013	259.88	opq 7.456 ± 0.184	20.03	h 22.510 ± 0.431	262.36
6	pqr 6.896 ± 0.183	kl 11.161 ± 0.385	61.85	Rst 5.612 ± 0.313	-18.62	e 32.102 ± 0.958	365.52	nop 8.084 ± 0.348	17.23	d 34.082 ± 1.172	394.23
8	stuv 4.610 ± 0.144	opq 7.540 ± 0.271	63.56	Vwx 3.677 ± 0.112	-20.24	f 29.142 ± 1.075	532.15	no 8.651 ± 0.216	87.66	e 31.328 ± 1.097	579.57
10	x 2.545 ± 0.059	lm 10.797 ± 0.165	324.24	Vw 3.613 ± 0.111	41.96	h 22.530 ± 0.902	785.27	kl 11.789 ± 0.386	363.22	g 25.478 ± 0.611	901.10
12	rstu 5.570 ± 0.178	i 19.249 ± 0.505	245.58	Vwx 3.983 ± 0.272	-28.49	c 37.439 ± 1.347	572.15	pqr 6.857 ± 0.144	23.11	b 42.610 ± 1.238	664.99
14	k 12.508 ± 0.367	j 16.075 ± 0.347	28.52	Nopq 7.831 ± 0.349	-37.39	b 41.558 ± 1.024	232.25	tuvw 4.331 ± 0.090	-65.37	a 44.502 ± 0.930	255.79
LSD	1.637										

Means with the same letter(s) is not significantly different.
** The reduction and induction percentage in the enzyme activity with compared to control.
NAGA : N-Acetyle glucose amine.

The individual treatment with *B. thureinginsis* revealed decreases through pupal stage except at 2nd and 10th days of pupation in which significant increases were recorded (41.61 and 41.96%, respectively). The obtained results in *S. marcescens* and Teflubenzuron treatments were in agreement with the findings obtained by Tolba (2006) who found high increases in chitinase activity during pupal stage of *A. ipsilon* treated either with *S. marcescens* or flufenoxuron. Also El-Sheikh (2006) reported that both *S. marcescens* and lufenuron cause significant increases in chitinase activity during pupal stage of *S. littoralis*. As well as similar results were reported by Lee *et al*. (1994) who found an increase in the chitinase activity of larvae of *Hyphantria cunea* treated with Diflubenzuron and Chlorofluazuron (chitin synthesis inhibitors). The increase in chitinase activity in pupae of *S. littoralis* treated with *S. marcescens* could be attributed to its ability to secrete chitinase, which facilitated penetration of bacteria within pupal skin, multiplication within tissues and killing insect by septicemia El-Sheikh (2006). In addition, the increase in chitinase activity of pupae treated with Teflubenzuron could be attributed to the secondary effect of chitin synthesis inhibitor. The primary effect involves blocking of incorporation of uridine 5'-diphospho-N-acetylglucose-amine into chitin. Chitin synthetase carried out through this polymerization step (Verloop, 1977). Moreover, the increase in chitinase activity may be a secondary effect for the reduced activity of β-ecdysone metabolizing enzymes, followed by β-ecdysone accumulation which resulted in hyper chitinase activity (Yu and Terriere, 1977). On the other hand, Abdel-Aal *et al.* (2009) found that some chitin synthesis inhibitors (CSI) increased chitinase activity of the late 6th instar larvae of *S.littoralis* and recorded that chitinase and protease are essential for digestion of old endocuticle in the moulting process. So, any changes in these enzyme activities may attribute to the interference of the (CSI) in moulting process.

Effects on protease activity:

Results in Table (3) show that *S. marcescence* caused significant increases in protease activity at all most pupation times recording the maximum increase (25.29% at 12th day) moreover, treatment with *B. thureinginsis* gave higher increases (maximum of 96.47 % at the last time) through the pupal stage, while recorded -58.51 %,-71.39% and -66.72% at 6th, 8th and 12th day of pupation time. However, *Bt/Serr* treatment exhibited higher significant effects in protease activity at 2nd and 4th days -.of pupation time (-32.00 % and 81.31%, respectively) than that of treatment with *B. thureinginsis* (27.47% and 35.21%, respectively). While, there were less effect at other times, as compared with control. Treatment with Teflubenzuron revealed significant decreases (maximum of -70.06 % at 8th day) as compared to control. Moreover, the treatment with Teflu /*Serr* caused significant increases from 6th to 14th days of pupation time. Treatment with Teflu /*Serr* had more effect in protease activity at last three times (60.33 %, 37.43% and 59.62%, respectively) than either *S. marcescence* (22.10%, 25.29% and19.71%, respectively) or Teflubenzuron (25.72%,-28.71% and -14.58%, respectively) treatments, as compared to control .

The increase in protease activity was similar to the results obtained by El-Sheikh (2006) who observed significant increases in protease activity after 3, 8 (logarithmic phase) and 13 (stationary phase) days of *S. littoralis* pupation in sawdust treated with *S. marcescens*. On the other hand, lufenuron significantly decreased protease activity at the 3rd, 8th and 13th days of pupation. Also, Abdel-Aal *et al*. (2009) recorded that Teflubenzuron decreased the porotease activity of the late 6th instar larvae of *S.littoralis*.

Table 3: Changes in protease activity in pupal stage of *Spodoptera littoralis* treated with *Serratia marcescence* at MC_{50}, LC_{50} of *B. thureinginsis*, Teflubenzuron and their sequential effects.

Time in day	Protease activity (ug protein/min./ g body weight.) (*Mean ± SE).										
	TREATMENTS										
	Control	*Serratia marcescence*	% **	*B. thureinginsis*	%	Teflubenzuron	%	*B. thureinginsis* / *Serratia marcescence*	%	Teflubenzuron / *Serratia marcescence*	%
2	st 250.00 ± 9.877	pqr 277.33 ± 6.574	10.93	mno 318.67±13.299	27.47	pqrs 268.00 ± 4.168	7.20	wx 170.00 ± 6.437	-32.00	stu 238.67 ± 6.367	-4.53
4	jkl 367.33 ±15.044	hi 403.33 ±14.127	9.80	f 496.67 ±13.312	35.21	pq 279.33 ± 8.120	-23.96	b 666.00± 18.605	81.31	rstu 242.67 ± 7.064	-33.94
6	klm 348.67 ±10.410	ijk 373.33 ± 8.120	7.07	xy 144.67 ± 2.909	-58.51	nop 299.33 ± 9.967	-14.15	tu 230.67 ± 6.367	-33.84	hij 387.33 ± 9.273	11.09
8	g 452.00 ±14.017	e 536.00 ±21.411	18.58	y 129.33 ± 4.672	-71.39	xy 135.33 ± 4.672	-70.06	hij 390.67 ±16.766	-13.57	c 593.33 ±13.792	31.27
10	w 184.00 ± 6.437	tu 224.67 ± 7.064	22.10	qrst 247.33 ± 8.120	34.42	t 231.33 ± 7.544	25.72	v 207.33 ± 3.532	12.68	op 295.00 ± 3.610	60.33
12	fg 466.67 ±12.930	cd 584.67 ±20.691	25.29	wxy 155.33 ± 3.532	-66.72	lmn 332.67 ±12.143	-28.71	c 592.00 ± 8.728	26.86	b 641.33 ±22.840	37.43
14	h 416.00 ±15.639	f 498.00 ±14.486	19.71	a 817.33 ±28.136	96.47	jkl 355.33 ±10.426	-14.58	de 555.33 ±16.033	33.49	b 664.00 ±23.208	59.62
LSD	35.752										

*Means with the same letter(s) is not significantly different.

** The reduction and induction percentage in the enzyme activity with compared to control.

Effect on trehalase activity:

The obtained data in Table (4) show the change in trehalase activity in pupal stage of *S. littoralis* treated with *S. marcescence* at concentration causes 50% malformation ,LC_{50} of *B. thureinginsis*, Teflubenzuron and their sequential following treatments at which sawdust was treated with *S. marcescence* after larvae treatment either with *B. thureinginsis Bt*/*serr* or with Teflubenzuron (Teflu /*Serr*). The results revealed potentiality of *S. marcescens* and *B. thureinginsis* to cause significant decreases in trehalase activity in pupal stage of *Spodoptera littoralis* by - 66.32-43.83 and -23.67-18.76 at 8^{th} and 10^{th} day of pupation time, respectively, in comparison with control. On the other hand, significant increases in trehalase activity were found during intervals from 4^{th}, 6^{th}, 8^{th} and 10^{th} days by 26.96, 30.07, 38.88 and 52.74% for Teflubenzuron and by 26.84, 30.57, 56.50 and 57.41% for *Bt/Serr*, respectively. Treatment with *S. marcescence*, Teflubenzuron and Teflu /*Serr* exhibited significant decreases at 12^{th} and 14^{th} days of pupation time by (-37.37, -53.16, and -66.68%) and (-35.72, -22.13 and -80.27), respectively, as compared with control. The reduction in trehalase activity of pupae treated with *S. marcescens* was similar to that published by Tolba (2006) who revealed potentiality of *S. marcescens* to cause significant decrease in trehalase activity during pupal stage of *A. ipsilon*. In addition, the effect of Teflubenzuron on trehalase activity in the present study may be in harmony with Tolba (2006) who recorded significant increases in trehalase activity at the most times during pupal stage of *A. ipsilon* treated with Flufenoxuron. It is known that in insects, trehalase degrades trehalose to glucose for internal energy supply and generation of glucose needed for chitin build-up (during moulting), so the inhibition of trehalase observed in the present work might affect chitin build-up. The mode of action of CSIs revealed that these compounds affect the integument composition of insect, especially that of chitin (Ishaaya and Casida, 1974). The reduced level of chitin synthesis in the cuticle formation is due to the inhibition of biochemical processes leading to chitin build-up (Post and Vincent, 1973). Trehalase has the important function for liberating glucose for energy, and is activated during moulting to generate glucose for chitin build up (Meisner *et al.*, 1978). In addition, trehalase played a significant role in the supply of energy to

the insect and the activity of trehalase might serve as an indicator of energy reserves resulting from availability of carbohydrate nutrients (Wyatt 1967). During moulting cycles, the trehalose-trehalase system is activated to generate glucose needed, probably, for chitin build-up in the newly synthesized cuticle (Candy and Kilby, 1962).

Table 4: Changes in trehalase activity in pupal stage of *Spodoptera littoralis* treated with *Serratia marcescence* at MC_{50}, LC_{50} of *B. thureinginsis*, Teflubenzuron and their sequential effects.

Time in day	Trehalase activity (µg glucose/min /g body weight) * Mean ± S.E.										
	TREATMENTS										
	Control	*Serratia marcescence*	% **	*B. thureinginsis*	%	Teflubenzuron	%	*B. thureinginsis* / *Serratia marcescence*	%	Teflubenzuron / *Serratia marcescence*	%
2	ijk 1447.57 ± 41.26	op 1019.68 ± 32.84	-29.56	klm 1375.45 ± 28.27	-4.98	n 1203.15 ± 30.78	-16.89	fg 1642.01 ± 25.12	13.43	de 1780.63 ± 39.17	23.01
4	d 1842.88 ± 44.53	ef 1709.25 ± 34.21	-7.25	pq 963.25 ± 15.12	-47.73	a 2339.76 ± 41.86	26.96	a 2337.47 ± 38.33	26.84	klm 1387.60 ± 34.30	-24.70
6	hi 1523.03 ± 41.80	hij 1519.55 ± 45.24	-0.23	jk 1442.55 ± 34.10	-5.28	c 1981.04 ± 23.24	30.07	c 1988.64 ± 34.61	30.57	h 1538.26 ± 25.45	1.00
8	m 1336.42 ± 17.67	vw 450.17 ± 12.56	-66.32	op 1020.03 ± 17.54	-23.67	d 1856.06 ± 32.35	38.88	b 2091.45 ± 36.42	56.50	lm 1350.61 ± 25.92	1.06
10	op 1028.27 ± 33.95	u 577.59 ± 16.72	-43.83	rs 835.36 ± 21.58	-18.76	gh 1570.53 ± 35.95	52.74	g 1618.61 ± 31.52	57.41	s 787.35 ± 12.53	-23.43
12	qr 913.03 ± 17.83	u 571.85 ± 14.59	-37.37	uv 516.76 ± 11.23	-43.40	w 427.62 ± 15.65	-53.16	vw 444.12 ± 12.70	-51.36	x 304.25 ± 8.71	-66.68
14	o 1095.28 ± 36.91	t 704.09 ± 17.66	-35.72	kl 1429.53 ± 15.88	30.52	rs 852.91 ± 19.68	-22.13	n 1251.29 ± 26.91	14.24	y 216.12 ± 7.43	-80.27
LSD	80.167										

*Means with the same letter(s) is not significantly different.
** The reduction and induction percentage in the enzyme activity with compared to control.

Effects on the main components:

Effects on total lipids content:

The obtained data in Table (5) revealed significant sharp decreases in total lipid content of untreated pupae at 12th and 14th days of pupation. *S. marcescens*, Teflubenzuron and Teflu /*Serr* caused significant decreases at all times of pupation, except significant increase of 24.24% at 8th day for Teflubenzuron. With respect, significant sharp decreases in total lipid content in pupae treated with *S. marcescens* were found at early time of pupation in which decreased by about -63.25%, -71.17 % and -59.13% after 2nd, 4th and 6th days of pupation time, respectively. *B. thureinginsis* exhibited significant decreases from 4th to 10th days of pupation, whereas, significant increases at last two times were found. As well as, *Bt/Serr* caused significant decreases from 2nd to 10th days, while, significant increase at last time was recorded. Regarding to all treatments, it is worth to mention that all treatments contained *S. marcescens* had higher decreasing effect than the others, as compared to control. The reduction in total lipid content of pupal stage after treatment with Teflubenzuron is similar to the data obtained by El-sheikh, (2002) and Abdel-Aal (2003) after using Flufenoxuron against *Agrotis ipsilon* and *S. littoralis* larvae, respectively. Also Tolba (2006) reported that Flufenoxuron reduces the total lipid content in *Agrotis ipsilon* pupal stage and that the sharp reduction in total lipid content at early time of pupation resulted in pupae treated with *Serratia marcescens* may be due to bacterial growth in the insect body and consuming lipids for energy requirement. Similar reduction in total lipid content of pupae treated with *B. thuringiensis* was recorded by Abd El-Aziz (2000) who found a sharp decrease in lipid content of *S. littoralis* larvae treated with *B. thuringiensis* var. *kurstaki*. Similarly lipid content in tobacco cutworm *Spodoptera litura* (fab.) larvae was studied by Tripathi and Singh (2002)

who reported that the infection resulted in significant reduction in total lipid content of infected larvae suggesting that the reason for the lower fat content could be due to extended larval period of the treated insects and blocked food ingestion so, the fat reserves might have been utilized for the maintenance during larval period. Also, Abdel-Aal (2006) found that *B. thuringiensis* var. *kurstaki* caused a significant reduction in the lipid content of *S. littoralis* larvae. Bennett and Shotwell (1972) suggested that the infected larvae might produce enzymes that utilize lipids in effort to remove the invading organisms. They also, assumed another possible reason for the decreasing in haemolymph lipid as infection progressed or pathogens may break down haemolymph lipids to simpler moieties that can be utilized as a carbon source for growth and sporulation.

Table 5: Changes in total lipids in pupal stage of *Spodoptera littoralis* treated with *Serratia marcescence* at MC_{50}, LC_{50} of *B. thureinginsis*, Teflubenzuron and their sequential effects.

Time in day	Total lipid content (*Mean ± SE)										
	TREATMENTS										
	Control	*Serratia marcescence*	% **	*B. thureinginsis*	%	Teflubenzuron	%	*B. thureinginsis* / *Serratia marcescence*	%	Teflubenzuron / *Serratia marcescence*	%
2	b 13.445 ± 0.318	lm 4.941 ± 0.134	-63.25	b 13.668 ± 0.355	1.66	d 10.651 ± 0.327	-20.78	fg 8.953 ± 0.208	-33.41	d 11.061 ± 0.316	-17.73
4	a 14.896 ± 0.364	no 4.294 ± 0.108	-71.17	h 8.213 ± 0.175	-44.86	e 9.585 ± 0.197	-35.65	e 9.487 ± 0.253	-36.31	h 8.432 ± 0.268	-43.39
6	c 11.832 ± 0.292	m 4.836 ± 0.102	-59.13	d 10.570 ± 0.280	-10.67	h 8.364 ± 0.227	-29.31	h 8.349 ± 0.270	-29.44	mno 4.450 ± 0.090	-62.39
8	j 6.963 ± 0.153	o 4.022 ± 0.089	-42.24	kl 5.422 ± 0.094	-22.13	gh 8.651 ± 0.180	24.24	k 5.880 ± 0.139	-15.55	p 3.435 ± 0.083	-50.67
10	ef 9.243 ± 0.199	j 6.722 ± 0.174	-27.27	i 7.638 ± 0.144	-17.36	lm 4.954 ± 0.099	-46.40	mn 4.551 ± 0.097	-50.76	p 3.494 ± 0.083	-62.20
12	q 2.358 ± 0.094	r 1.702 ± 0.048	-27.82	p 3.035 ± 0.076	28.71	r 1.753 ± 0.044	-25.66	q 2.352 ± 0.056	-0.25	st 1.184 ± 0.061	-49.79
14	r 1.821 ± 0.078	rs 1.331 ± 0.067	-26.91	p 3.053 ± 0.045	67.66	st 1.176 ± 0.037	-35.42	q 2.469 ± 0.045	35.58	t 0.807 ± 0.030	-55.68
LSD	0.5146										

*Means with the same letter(s) is not significantly different.
** The reduction and induction percentage in the enzyme activity with compared to control.

Effects on total soluble protein content:

From results recorded in Table (6) it could be stated significant decreases of-12.45% and -15.05% in total soluble protein content after 8th and 10th days of pupation in sawdust treated with *Serratia marcescens*, respectively. In addition, Teflubenzuron caused significant decreases by about -12.76 and -7.29 in total soluble protein content at 2nd and 4th days of pupation. Whereas, Teflu /*Serr* treatment caused significant decreases in total soluble protein content at all times of pupation (except 14th day of pupation) comparing to control. Results also revealed significant decreases in total soluble protein content in pupal stage of *S. littoralis* treated with *B. thuringiensis* at 6th and 8th days (-22.37% and -24.27%, respectively). On contrary, there was a significant increase (6.06%) at the first time of pupation, as compared with control. Once more, *Bt/Serr* caused significant decreases in total soluble protein content in pupal stage of *S. littoralis* at all times of pupation with exception at 2nd and 14th days of pupation comparing to control. Thus, it could be stated that both *Bt*/*serr* and Teflu /*Serr* had more significant effect on the reduction in total soluble protein content than treatments with *B. thuringiensis*, Teflubenzuron and *S. marcescens*. Similar results were obtained by Abdel-Aal (2006) who found that *B. thuringiensis* var. *kurstaki* caused a significant reduction in the protein

content of *S.littoralis* larvae. Kamel *et al.* (2010) observed significant reduction in total protein content of *S. littoralis* larvae after treatment with *B. thuringiensis* var. *kurstaki*. This could be due to the break down of protein into amino acids which help to supply energy for the insect. El-Shershaby *et al.* (2008) indicated that, *B. thuringiensis* var. *kurstaki* resulted in a great reduction in protein content of *S. littoralis* larvae and these toxins of *B. thuringiensis* are responsible for the inhibition of protein synthesis by forming a protein complex. Also Tolba (2006) reported that treatment with either Flufenoxuron or *S. marcescens* reduces the protein content of *Agrotis ipsilon* pupal stage. The same reduction in total soluble protein content due to Teflubenzuron treatment was found by (El-sheikh, 2002 and Abdel-Aal 2003) after using Flufenoxuron against *Agrotis ipsilon* and *S. littoralis* larvae, respectively. In addition, Abdel-Aal (2006) reported that treatment with either chlorfluazuron or *B. thureinginsis* var. *kurstaki* decreased total soluble protein content of *S. littoralis* larvae. The decrease in the protein content of the pupae in the present work in all treatments might be due to the protein was binding with foreign compounds as the compounds used.

Table 6: Changes of total protein in pupal stage of *Spodoptera littoralis* treated with *Serratia marcescence* at MC_{50}, LC_{50} of *B. thureinginsis*, Teflubenzuron and their sequential effects.

Time in day	Total protein (mg /g body weight) (*Mean ± SE).										
	TREATMENTS										
	Control	*Serratia marcescence*	% **	*B. thureinginsis*	%	Teflubenzuron	%	*B. thureinginsis* / *Serratia marcescence*	%	Teflubenzuron / *Serratia marcescence*	%
2	ef 31.816 ± 0.834	fg 29.955 ± 0.922	-5.85	cde 33.745 ± 0.696	6.06	hi 27.756 ± 0.897	-12.76	fg 30.026 ± 0.713	-5.63	ijk 26.537 ± 0.736	-16.59
4	b 36.445 ± 1.067	bcd 34.985 ± 1.329	-4.01	bc 35.706 ± 1.056	-2.03	cde 33.787 ± 1.077	-7.29	cde 33.881 ± 0.889	-7.04	ef 31.774 ± 1.146	-12.82
6	a 41.788 ± 1.270	a 39.724 ± 0.943	-4.94	e 32.439 ± 1.078	-22.37	a 40.098 ± 1.052	-4.04	bcd 35.278 ± 0.772	-15.58	b 36.828 ± 0.875	-11.87
8	de 33.284 ± 0.889	gh 29.140 ± 0.774	-12.45	jklm 25.206 ± 0.273	-24.27	e 32.414 ± 0.614	-2.61	ijkl 25.599 ± 0.718	-23.09	jklm 25.144 ± 0.744	-24.46
10	ijk 26.048 ± 0.869	n 22.129 ± 0.643	-15.05	ij 26.736 ± 0.750	2.64	klm 24.447 ± 0.543	-6.15	Lmn 23.807 ± 0.661	-8.60	mn 23.010 ± 0.668	-11.66
12	o 18.424 ± 0.618	opq 16.345 ± 0.543	-11.28	op 17.413 ± 0.435	-5.49	op 17.200 ± 0.535	-6.64	pqr 16.137 ± 0.433	-12.41	pqr 15.661 ± 0.482	-15.00
14	qrs 14.892 ± 0.390	rs 14.019 ± 0.526	-5.86	qrs 14.900 ± 0.346	0.05	qrs 14.628 ± 0.395	-1.77	s 13.260 ± 0.398	-10.96	s 12.790 ± 0.398	-14.11
LSD	2.198										

*Means with the same letter(s) is not significantly different.

** The reduction and induction percentage in the enzyme activity with compared to control.

Effects on Total Carbohydrate contents:

Table (7) shows that all treatments cause significant decreases in total carbohydrate content at all times except treatment with Teflubenzuron in which there were significant increases at 2nd and 10th days . The highest decrements were observed in case of treatments with *S. marcescence*, *Bt/Serr* and Teflu /*Serr.* The decrements for *S. marcescence* were gradually pronounced with time reaching drastical decreases at 10th and 12th days of pupation time. These decrements were of -27.60, -32.00,-40.14, -55.28, -80.93, -81.57 and – 79.1 % at 2nd, 4th, 6th, 8th, 10th, 12th and 14th. Whereas, they were -46.40, -46.84, -58.27, -74.95, -82.11, -66.25 and -51.36 % for *Bt/Serr* at the same times, respectively, which were significantly higher than those of *B. thureinginsis*. On the other hand, the decrements were -33.33, -31.48, -50.45,-60.31,-42.47,-50.26 and -52.74 % in case of Teflu /*Serr* at all times, respectively, which were significantly higher than those of treatment with Teflubenzuron, as compared to control . Thus, it could be stated that all treatments contained *Serratia marcescence* induced more

reducing effect on total carbohydrate content of *S. littoralis* pupae than those of Teflubenzuron or *B. thuringiensis*. *S. marcescens* caused a high decrease in total carbohydrates because of their requirements to glucose as energy source for propagation and growth so bacteria utilize carbohydrates as carbon source for energy and built a new cell, this may decrease the available carbohydrates in treated insect especially glucose which plays an important role in energy supply, adult maturation and builds up a new chitin (Tolba, 2006; El-sheikh *et al*. 2005 and El-sheikh 2006) that may explain the high malformation% obtained in the present study.

Table 7: Changes of total carbohydrates concentration in pupal stage of *Spodoptera littoralis* treated with *Serratia marcescence* at MC_{50}, LC_{50} of *B. thureinginsis*, Teflubenzuron and their sequential effects.

Time in day	Total carbohydrates content (ug glucose/g body weight) (*Mean ± SE)										
	TREATMENTS										
	Control	*Serratia marcescence*	% **	*B. thureinginsis*	%	Teflubenzuron	%	*B. thureinginsis* / *Serratia marcescence*	%	Teflubenzuron / *Serratia marcescence*	%
2	c 36.634 ± 0.905	e 26.522 ± 0.862	-27.60	ghi 22.898 ± 0.843	-37.50	a 45.590 ± 1.271	24.45	kl 19.635 ± 0.669	-46.40	fg 24.424 ± 0.703	-33.33
4	d 30.169 ± 0.596	jk 20.514 ± 0.522	-32.00	d 31.289 ± 1.423	3.71	efg 24.615 ± 0.776	-18.41	mn 16.037 ± 0.562	-46.84	jk 20.673 ± 0.577	-31.48
6	c 36.138 ± 0.700	ij 21.633 ± 0.623	-40.14	d 30.178 ± 0.876	-16.49	hij 22.300 ± 0.689	-38.29	no 15.080 ± 0.480	-58.27	lm 17.907 ± 0.583	-50.45
8	a 45.708 ± 0.983	jk 20.440 ± 0.607	-55.28	ijk 21.445 ± 1.220	-53.08	ef 26.183 ± 0.710	-42.72	pqr 11.451 ± 0.381	-74.95	l 18.141 ± 0.504	-60.31
10	c 37.191 ± 0.759	t 7.092 ± 0.224	-80.93	fg 24.350 ± 0.927	-34.53	b 42.113 ± 1.069	13.23	t 6.653 ± 0.278	-82.11	ijk 21.395 ± 0.489	-42.47
12	d 30.886 ± 0.627	tu 5.693 ± 0.198	-81.57	gh 24.077 ± 0.783	-22.05	gh 24.182 ± 0.942	-21.71	qrs 10.423 ± 0.329	-66.25	n 15.364 ± 0.243	-50.26
14	kl 19.612 ± 0.378	u 4.098 ± 0.098	-79.10	pq 11.669 ± 0.434	-40.50	op 13.257 ±0.520	-32.40	rs 9.539 ± 0.374	-51.36	s 9.268 ± 0.166	-52.74
LSD	1.9851										

*Means with the same letter(s) is not significantly different.
** The reduction and induction percentage in the enzyme activity with compared to control.

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ARABIC SUMMARY

التأثيرات البيوكيميائية لبكتيريا باسلليس ثيورينجينسيز و سيراشيا مرسيسنس، ومنظم النمو الحشرى تيفلوبينزورون على دودة ورق القطن *سبودوبتيرا ليتوراليس* (بويذد.).

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اشتمل البحث على دراسة بعض التأثيرات البيوكيميائيه الناتجه عن استخدام التركيز المتسبب فى تشوه 50% من الفراشات لبكتيريا سيراشيا مرسيسنس المعامل بها فى التربه والتركيز القاتل للنصف لكلا من المستحضر التجارى بروتيكتو (بكتيريا باسلليس ثيورينجينسيز) ومنظم النمو الحشرى تيفلوبينزورون ضد العمر الثانى لدودة ورق القطن وايضا تاثيراتهم المشتركة من خلال معاملة العمر الثانى بالتركيز القاتل للنصف لكلا من الباسلليس ثيورينجينسيز والتيفلوبينزورون ثم تعريض اليرقات عند التعذير لتربه معامله ببكتيريا سيراشيا مرسيسنس عند التركيز المتسبب فى تشوه 50% من الفراشات.. أيضا تسببت المعاملات فى تغيرات معنويه فى انزيمات الكيتينيز،البروتييزوالتريهاليزوالمحتوى الكلى لكلا من البروتين والكربوهيدرات والليبيدات أثناء الفترات المختلفة للطور العذرى .