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MONITORING AND MOLECULAR DIAGNOSIS OF INTERACTIVE EFFECTS OF SOME CHEMICAL AND BIOLOGICAL CONTROL AGENTS ON THE COTTON LEAFWORM

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ABSTRACT

Interactive effects of chemical and biological control agents against laboratory and field colonies of *Spodoptera littoralis* (Boisd.) were tested under laboratory conditions. The field colony was more susceptible to *Bacillus thuringiensis* than the laboratory colony, contrary with the chemical pesticides when these compounds were tested alone. Also, *B. thuringiensis* was less toxic than profenofos and more toxic than metalaxyl-M + copper oxychloride, while metalaxyl-M + copper oxychloride was more toxic than atrazine. The combinations between *B. thuringiensis* and chemical pesticides on the mortality of *S. littoralis* did not show a synergistic action between *B. thuringiensis* and both profenofos and atrazine, in contrast, to metalaxyl-M + copper oxychloride at the concentration of LC_{25} + LC_{25}. The effect of all combinations on the biochemical parameters markedly show an antagonistic action, as well as the interaction effects of combinations on the field colony did not differ than the laboratory colony. Results of RAPD-PCR and SDS-PAGE clearly differentiated between the isolates of *B. thuringiensis* as a result of treatment with the tested chemical pesticides. So, these results suggest that the tested chemical pesticides consider mutant and therefore not compatible with *B. thuringiensis*. These data may emphasize the impossibility of mixing chemical pesticides with biopesticides.

Keywords: **Monitoring, molecular diagnosis, RAPD-PCR, RAGE, interactive effects, chemical pesticides, biopesticides.**

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INTRODUCTION

Barjac and Sutherland (1990) summarized the effect of biotic and abiotic factors on the viability, toxin stability and larvicidal activity of biological control agent (*B. thuringiensis)* against many species of pests. One of the most important environmental factors affecting the larvicidal activity of these bacteria is water pollution rate (Des Rochers and Garcia, 1984; Hornby *et al.*, 1984; Nicolas *et al.*, 1987 and Berber *et al.*, 1997). Pathogenic strains of *B. thuringiensis* can lose their toxic activities in habitats polluted with organic materials, and also exhibit lower persistence (Davidson *et al.*, 1984; Lacey and Undeen, 1986 and Correa and Yousten, 1995).

So far, no studies on the effects of chemical pesticides have been carried out despite the existence of several studies on different chemical compounds on the larvicidal activity and spore viability of *B. thuringiensis*. The present investigation will undertaken to study the interactive between the entomopathogenic bacteria and certain chemical pesticides (insecticides, fungicides and herbicides) and the effects on toxicity of bacteria, using diagnostic techniques to detect the difference which occur for bacteria toxin.

MATERIALS AND METHODS

The present work was carried out at Laboratory of Pesticides Biotechnology and Molecular Toxicology, Division of Pesticides, Department of Plant Protection, Faculty of Agriculture, Zagazig University.

Tested Pesticides

1. Bio-insecticide: *Bacillus thuringiensis* Subsp. *kurstaki* (DiPel 2X 6.4% WP) supplied by May Trade Company.
2. Chemical insecticide: profenofos (Selecron® 72 % EC) supplied by Syngenta Agro Egypt.
3. Chemical fungicide: metalaxyl-M + copper oxychloride (Ridomil Gold Plus® 42.5 % WP) supplied by Syngenta Agro Egypt.
4. Chemical herbicide: atrazine (Atrazine® 80 % WP) supplied by Fluence Agrichem. China.

Tested Insect

Laboratory and field colonies of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), (Lepidoptera: Noctuidae) were used in this study.

Laboratory colony

A laboratory colony of the cotton leafworm *S. littoralis* was kindly obtained from the division of the Cotton Leafworm Department, Plant Protection Research Institute, Dokki, Giza, Egypt. The colony was maintained on a modified version of an artificial bean diet (Gelernter *et al.*, 1986) in the laboratory for more than five years away from any insecticide contamination. When larvae reached the fourth instar, they were transferred to fresh diet, and 50-60 pupae were collected from the diet containers and placed in wide glass gars until emergence. The emerged adults were provided with blotting paper or branches of tafla (*Nerium oleander*) for adult ovipostion (El-Defrawi *et al.*, 1964). Rearing was carried out under constant conditions (26 ± 2 °C and 65 ± 3 % relative humidity), with a photoperiod 14-10 h (light – dark).

Field colony

The field colony was obtained from cabbage field at Sharkia Governorate. The egg masses were collected and placed on the artificial bean diet, and the rearing procedure was carried out as described previously.

Bioassay of Singly Tested Compounds

We choose the third larval instar of *S. littoralis* as a model for study monitoring and molecular diagnosis of interactive effects of chemical and bio pesticides against insects.

Third instar larvae of laboratory and field colonies of *S. littoralis* were transferred to the surface of treated artificial diet with serial concentrations of the following pesticides after air drying: *B. thuringiensis*, profenofos, metalaxyl-M + copper oxychloride and atrazine. The diet was prepared in the same way as that used for rearing, but it had no nepagin and formalin. The concentrations of the pesticides used were added to the diet thoroughly mixed and left for air drying. Ten larvae in four replicates were allowed to feed on treated diet surface for 48 h and subsequently transferred to untreated diet, control treatment was done without active material. The cups were kept under constant conditions as mentioned before.

Mortality count was taken after

8, 16, 24, 32, 40 and 48 h after treatment to recorded LT_{25} and LT_{50} values. The percentage mortality was recorded after 48 h and corrected according to (Abbott, 1925). Regression toxicity lines were established for the pesticides and the slope, LC_{25} and LC_{50} values were determined through probit analysis (Finney, 1972).

Bioassay of Mixtures

Bioassay clearance

Sublethal concentration of *B. thuringiensis* at LC_{25} was combined with LC_{25} of candidate chemical pesticides to find out the joint action of chemical and bio pesticides. The percent mortality increase/decrease over LC_{50} of chemical pesticides and *B. thuringiensis* was calculated. Also synergistic, antagonistic or additive interaction between *B. thuringiensis* and chemical pesticides were checked according to Benz equation (Benz, 1971). Also, the joint action was determined according to the equation of the co-toxicity factor given by (Mansour *et al.*, 1966). As well as mortality count was taken after 8, 16, 24, 32, 40 and 48 h after treatment to record LT_{25} and LT_{50} values.

Biochemical activities

Spectrophotometric determination of protein and certain enzymes activity using total body homogenate of the 3rd instar larvae of the field and the laboratory colonies of *S. littoralis* were carried out as follows: alkaline phosphatase (Kind and King, 1954), glutathione S-transferase (GT) (Habig *et al.* 1974) and acetylcholinesterase (Ellman *et al.* 1961) and protein content (Bradford, 1976).

The levels of enzymes induction for different treatments were subjected to analysis of variance (ANOVA) using Co-Stat software and means were separated using least significant difference (LSD) test (Gomez and Gomez, 1984).

Molecular diagnosis of *B. thuringiensis*

B. thuringiensis was isolated from both DiPel 2X formulation and the dead larvae of laboratory and field colonies of *S. littoralis* that infected with chemical and biological control agents alone and their mixtures according to Ohba and Aizawa (1978) and purified according to Smirnoff (1962).

PCR-RAPD based fingerprinting

DNA extraction from *B. thuringiensis* isolates according to Ozer *et al.* (1990). PCR-RAPD analysis was carried out using decamer oligonucleotide primers, which had minimum 60 % G+C content and lacked internal repeats (Operon Technologies, USA). Five random decamer primers were used for PCR amplifiction (Table 1). PCR was carried out according to Pattanayak *et al.* (2001).

Electrophoretic assay of protein pattern of *B. thuringiensis*

Culture conditions of *B. thuringiensis* were carried out according to Attathom *et al.* (1995). Mass separation of parasporal crystal was out according to Yunovitz *et al.* (1986). Solubilization of the crystal protein was carried out according to Faust and Bulla (1982). Protein concentration of the purified δ–endotoxin was determined spectrophotometerically according to Bradford (1976). The polyacrylamide gel electrophoresis (PAGE) was used to study the protein pattern in the reisolates of *B. thuringiensis*. SDS polyacrylamide gel was performed at room temperature in vertical apparatus as described by Laemmli (1970).

We used gelanlyzer 2010 a software for analyze gel of PCR-RAPD and SDS-PAGE.

RESULTS AND DISCUSSION

Bioassay of Singly Tested Compounds

The data presented in Table 2 represented LC_{25} and LC_{50} values for both bio and chemical pesticides individually.

These results revealed that the field colony of *S. littoralis* was more susceptible to *B. thuringiensis* than the laboratory colony at both the LC_{25} and LC_{50} levels contrary chemical pesticides.

The LC_{25} Values were 48.6, 7.9, 912 and 1350 ppm (the laboratory colony), and 46.8, 17.9, 1118.7 and 1401.7 ppm (field colony) for *B. thuringiensis*, profenofos, metalaxyl-M + copper oxychloride and atrazine, respectively. The corresponding LC_{50} Values were 199.36, 14, 1253 and 1818.4 ppm (laboratory colony), and 120.4, 22.7, 1425 and 1849 ppm (field

Table 1. Nucleotide sequence of random primers used for RAPD analysis of *B. thuringiensis* isolates

Sr. No.	Primer name	Primer sequence
1	OPA03	AGCTCAGCCA
2	OPA08	GTCCACACGG
3	OPC06	GAACGGACTC
4	OPC20	ACTTCGCCAC
5	OPZ18	AGGGTCTGTG

Table 2. Concentration mortality responses of the third larval instar of *S. littoralis* of laboratory and field colonies to chemical pesticides and *B. thuringiensis*

Treatment	LC_{25} (ppm)		LC_{50} (ppm)		Slope	
	lab.	field	lab.	field	lab.	field
B. thuringiensis	48.6	46.8	199.36	120.4	1.1	1.65
Profenofos	7.9	17.9	14	22.7	2.7	6.5
Metalaxyl-M + copper oxychloride	912	1118.7	1253	1425	4.9	6.4
Atrazine	1350	1401.7	1818.4	1849	4	5.6

colony) for *B. thuringiensis*, profenofos, metalaxyl-M + copper oxychloride and atrazine, respectively.

Taking into consideration the relative potency at the LC_{25} and LC_{50} levels, data in Table 2 showed that *B. thuringiensis* was less toxic than profenofos and more toxic than metalaxyl-M + copper oxychloride. Also metalaxyl-M + copper oxychloride was more toxic than atrazine.

Although metalaxyl-M + copper oxychloride and atrazine caused lethal effects within treated populations, but it cannot be treated as an insecticide. LC_{50} value proved to be more hundred times higher than chemical insecticide. If one would like to obtain a significant lethal effect, using lower doses, the exposure time should be very long.

Time-mortality test were conduct using LT_{25} and LT_{50} values (Table 3). These results revealed that the field colony was faster susceptible to *B. thuringiensis* than the laboratory colony at both the LT_{25} and LT_{50} levels contrary chemical pesticides.

Bioassay of Mixtures

Bioassay clearance

Mortality percentages against the 3rd larval instar of *S. littoralis* due to the combination of *B. thuringiensis* and chemical pesticides (profenofos, metalaxyl-M + copper oxychloride and atrazine) were presented in Tables 4 and 5. It was noticed that the combination was faster in action, because of it induced mortality percentages between (30, 50 and 40 %), and (27.5, 47.5 and 37.5 %) after 48 hours for the same concentrations on laboratory and field colonies, respectively.

Lethal time of 50 % (LT_{50}) recorded (63.27, 46.15 and 68.19) hours in the laboratory colony and (70.52, 55.31 and 83.59) hours in the field colony; at the concentration (LC_{25} + LC_{25}) for all combinations, respectively. (Tables 4 and 5).

The joint action of *B. thuringiensis* and both profenofos and atrazine (i.e. observed mortality) at LC_{25} + LC_{25} was less effective than the expected mortality, indicating an antagonistic interaction, where the decrease in mortality was 29.41 and 45.45 % (laboratory colony) and 42.10 and 47.62 % (laboratory colony) less than the expected mortality at LC_{50} of both *B. thuringiensis* and profenofos, 5.88 and 20 % (laboratory colony), 21.05 and 21.05 % (laboratory colony) less than the expected mortality at LC_{50} of both *B. thuringiensis* alone and atrazine alone, respectively. In contrast to the joint action between *B. thuringiensis* + (metalaxyl-M + copper oxychloride) (i.e. observed mortality) at LC_{25} + LC_{25}, It was more effective than the expected mortality, indicating a synergistic interaction, where the increase in mortality was (17.65 and 4.76 %) and (0 and -5%) greater than the

Table 3. Time-mortality test of bio and chemical pesticides (*B. thuringiensis*, profenofos, metalaxyl-M + copper oxychloride and atrazine to the 3rd larval instar of *S. littoralis* of laboratory and field colonies

Treatment		LT_{25} (hours)		LT_{50} (hours)	
pesticides	Concentrations (ppm)	lab.	field	lab.	field
B. thuringiensis	64	41.4	32.4	74.3	63
	128	33	25.2	60.7	48.4
	192	29.5	22	50.8	39.2
	256	24.8	18.7	43	28
Profenofos	15	17	61	53.64	77.3
	20	12.3	18.8	34	60.5
	25	9.5	14	25.4	36.3
	30	6.48	7.65	16.9	22.4
Metalaxyl-M + copper oxychloride	1000	35.5	480	177	6358
	1250	12.9	15	40.4	51
	1500	7.3	12	27.4	40.5
	1750	5.5	8.6	19	29.5
Atrazine	1750	12	18.9	58	70.7
	2000	8.2	13.8	32	47.8
	2250	6.8	10.8	22.7	37
	2500	5.8	9	17.9	26

Control mortality was zero % throughout the period of experiment

Table 4. Expected and observed percentage of mortality of the 3rd larval instar of the laboratory colony of *S. littoralis* treated with *B. thuringiensis*, combined with profenofos, metalaxyl-M + copper oxychloride and atrazine

Treatment		Cumulative mean mortality% at the indicated days after treatment											
		1 day			2 day								
										% Increases over			
B. thuringiensis	Chemical pesticides	8 h	16 h	24 h	32h	40 h	48 h	Expected	Co-toxicity factor	*Bt* at LC_{50}	C P at LC_{50}	LT_{50} (hours)	Toxic action
LC_{25}		0	0	7.5	12.5	17.5	20					84.45	
LC_{50}		0	12.5	22.5	32.5	37.5	42.5					52.94	
	$LC_{25}P$	0	10	15	20	25	30					81.84	
	$LC_{50}P$	10	20	25	30	40	55					52.82	
	$LC_{25}M$	0	10	17.5	20	22.5	25					100.61	
	$LC_{50}M$	17.5	30	37.5	47.5	50	52.5					40.67	
	$LC_{25}A$	10	15	17.5	22.5	25	27.5					246.87	
	$LC_{50}A$	22.5	30	45	47.5	50	50					40.63	
LC_{25}	$LC_{25}P$	0	0	5	10	20	30	44	-31.82	-29.41	-45.45	63.27	antagonistic
LC_{25}	$LC_{25}M$	20	30	37.5	45	47.5	50	40	25	17.65	-4.76	46.15	synergistic
LC_{25}	$LC_{25}A$	10	20	30	35	37.5	40	42	-4.76	-5.88	-20	68.19	antagonistic

Control mortality was zero % throughout the period of experiment.

p= profenofos, M= metalaxyl-M + copper oxychloride, A= atrazine and CP= chemical pesticides

expected mortality of the 3rd larval instar of laboratory and field colonies of *S. littoralis*, respectively at LC_{50} of both *B. thuringiensis* alone and metalaxyl-M + copper oxychloride, respectively.

Data in Tables 4 and 5 showed that interaction effects in the field colony did not differ than the laboratory colony.

Biochemical activities

The results indicated that the field colony had higher total protein, and enzymes activities compared with the laboratory colony (Table 6). These results

Table 5. Expected and observed percentage of mortality of the 3rd larval instar of the field colony of *S. littoralis* treated with *B. thuringiensis*, combined with profenofos, metalaxyl-M + copper oxychloride and atrazine

Treatment		Cumulative mean mortality% at the indicated days after treatment											
		1 day			2 day								
										% Increases over			
B. thuringiensis	Chemical pesticides	8 h	16 h	24 h	32h	40 h	48 h	Expected	Co-toxicity factor	*Bt* at LC$_{50}$	C P at LC$_{50}$	LT$_{50}$ (hours)	Toxic action
LC$_{25}$		0	0	5	10	20	22.5					73.38	
LC$_{50}$		0	15	22.5	30	40	47.5					49.87	
	LC25P	7.5	15	20	22.5	22.5	27.5					198.05	
	LC50P	12.5	27.5	35	40	45	52.5					45.67	
	LC25M	10	15	17.5	20	22.5	22.5					611.42	
	LC50M	17.5	25	35	42.5	47.5	50					47.39	
	LC25A	0	12.5	15	20	22.5	25					105.88	
	LC50A	15	27.5	40	45	47.5	47.5					45.46	
LC$_{25}$	LC25P	0	2.5	10	20	25	27.5	43.81	-37.23	-42.10	-47.62	70.52	antagonistic
LC$_{25}$	LC25M	17.5	25	30	40	45	47.5	39.3	20.87	0	-5	55.31	synergistic
LC$_{25}$	LC25A	5	17.5	20	25	30	37.5	41.86	-10.42	-21.05	-21.05	83.59	antagonistic

Control mortality was zero % throughout the period of experiment.

P= profenofos, M=metalaxyl-M + copper oxychloride, A= atrazine and CP= chemical pesticides

agree with the previous study of Hendawy (1997). All combinations were antagonist, because the effect of mixed pesticides was less than the sum effect of *B. thuringiensis* and chemical pesticides respectively, where very highly significant difference between treatments on all parameters.

Molecular diagnosis of *B. thuringiensis*

PCR-RAPD based fingerprinting

In all primers (A3, A8, C6, C20 and Z18) the results proved that, ten bands appeared only in the second lane, these bands were considered as specific bands for

Table 6. Total protein content, AchE, ALP, and GT activity in the 3^{rd} larval instar of the field colony of *S. littoralis* treated with bio and chemical pesticides alone and its mixtures at LC_{25}

Treatments	Total protein µg/mg b.w.		AchE nmmol/min/mg protein		GT nmmol/min/mg protein		ALP IU/min/mg protein	
	Lab. colony	Field colony	Lab. colony	Field colony	Lab. colony	Field colony	Lab. colony	Field colony
Control	54.00 ± 3.56	73.60 ± 5.6	44.29 ± 1.64	62.70 ± 2.17	295.63 ± 1.72	402.30 ± 3.8	609.00 ± 1.53	787.13 ± 2.92
***B. thuringiensis* (*Bt*)**	73.20 ± 8.17	89.07 ± 4.8	38.08 ± 1.63	55.40 ± 1.02	268.53 ± 7.86	345.67 ± 2.33	565.17 ± 3.24	721.00 ± 11.39
Atrazine (A)	45.60 ± 4.54	64.80 ± 3.8	23.98 ± 0.38	40.79 ± 1.03	428.87 ± 6.12	506.67 ± 6.98	366.67 ± 5.04	556.33 ± 12.76
Metalaxyl-M + copper oxychloride (M)	24.00 ± 4.16	33.20 ± 2.8	30.42 ± 2.7	48.83 ± 1.79	491.23 ± 7.24	664.83 ± 20.05	265.67 ± 5.17	444.57 ± 15.3
Profenofos (p)	37.20 ± 1.83	60.80 ± 2.62	12.88 ± 0.91	25.44 ± 0.79	450.93 ± 6.71	524.70 ± 15.16	317.23 ± 9.48	501.43 ± 4.38
***Bt* + A**	33.60 ± 1.83	54.00 ± 6.93	16.56 ± 0.69	29.85 ± 0.53	322.78 ± 4.14	429.53 ± 8.67	499.67 ± 7.62	679.33 ± 2.[illegible]7
***Bt* + M**	18.00 ± 2.08	25.20 ± 3.67	20.64 ± 1.35	36.72 ± 0.95	398.87 ± 12.73	493.70 ± 7.51	410.67 ± 8.87	608.80 ± 19.24
***Bt* + P**	29.60 ± 3.82	40.40 ± 2.88	9.61 ± 0.78	20.35 ± 1.11	378.03 ± 7.83	441.23 ± 2.64	451.50 ± 10.26	641.20 ± 7.31

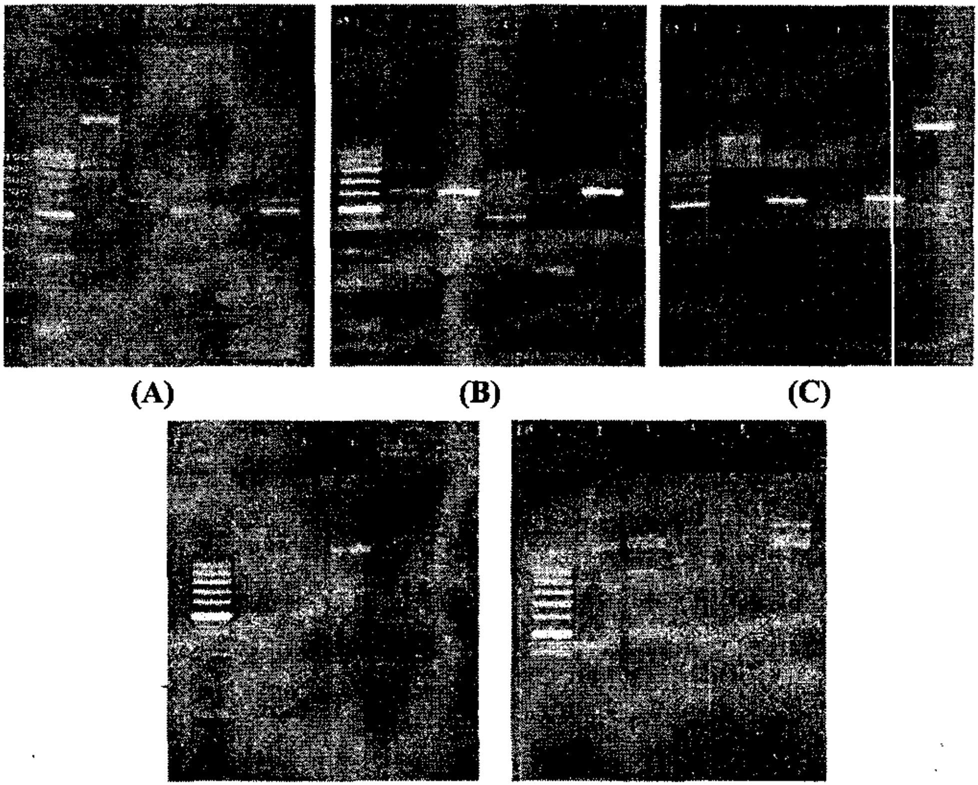
-Values are the mean ± SD

the original strain of *B. thurinigiensis kurstaki*. Twelve bands appeared only in the fourth lane, these bands were considered as a response to effect of atrazine on *B. thurinigiensis*. Seven bands appeared only in the fifth lane, these bands were considered as a result to effect of metalaxyl-M + copper oxychloride on *B. thurinigiensis*. Six bands appeared only in the sixth lane, these bands were considered as a response to effect of profenofos on *B. thurinigiensis*. Three bands appeared only in the fourth and fifth lanes, these bands were considered as a response to effect of both atrazine and metalaxyl-M + copper oxychloride. There was two bands appeared only in the fourth and fifth lanes, these bands were considered as a response to effect of both atrazine and profenofos on *B. thuringiensis*. There was one band appeared only in the fifth and sixth lanes, this band was considered as a response to effect of both metalaxyl-M + copper oxychloride and profenofos on *B. thuringiensis*. Ten bands appeared only in the fourth, fifth and sixth lanes, these bands were considered as a response to effect of atrazine, metalaxyl-M + copper oxychloride and profenofos on *B. thuringiensis*. One band appeared in all lanes, this band was considered as specific bands for the original strain of *B. thuringiensis kurstaki* and resistance to effect of atrazine, metalaxyl-M + copper oxychloride and profenofos (Figure 1).

Our study indicates that RAPD provides a high degree of discrimination between *B. thuringiensis* reisolates. On analysis of the dendrogram (Figure 2) it was observed that the *B. thuringiensis* reisolates clustered according to its treatment with chemical pesticides. It was observed that all the primers used for RAPD analysis showed amplification and generated RAPD fingerprint for *B. thuringiensis* isolates (Figure 1). An average of 15 fragments was produced per primer. The primer OPZ18 was found most discriminatory as it produced the highest number of 20 fragments. Cluster analysis of the dendrogram indicated that *B. thuringiensis kurstaki* reisolated from the dead larvae of *S. littoralis* that treated with DiPel 2X (lane 3) was the closest to reisolate that reisolated from the commercial product of DiPel 2X formulation (lane 2) followed with lane 6 and lanes (4 and 5). While the reisolate that reisolated from the dead larvae that treated with mixture of *B. thuringiensis* + (metalaxyl-M + copper oxychloride) (lane 5) and DiPel 2X was the closest to reisolate that reisolated from the dead larvae that treated with mixture of atrazine + DiPel 2X (lane 4).

Electrophoretic assay of protein pattern of *B. thuringiensis*

Electrophoresis patterns for reisolates of *B. thuringiensis* are illustrated in Figure 3. The reisolates that reisolated from thelaboratory colony (lanes 3, 4, 5 and 6), the results showed that, a total of 9 bands numbers (1, 2, 3, 5, 6, 7, 8, 9 and 10) with motilities



(A) (B) (C)

(D) (E)

Figure 1. Representative RAPD profiles showing polymorphism among *B. thuringiensis* isolates, electrophoresed on 1% agarose gel and stained with ethidium bromide. The amplification of DNA was carried out using (A) for primer OPA3, (B) for primer OPA8, (C) for primer OPC6, (D) for primer OPC20 and (E) for primer OPZ18. Lane 1 molecular weight marker (100 bp) ladder. Lane 2 *B. thuringiensis* was isolated from the commercial product DiPel 2X, Lane 3 *B. thuringiensis* was isolated from the dead larvae of the laboratory colony of *S. littoralis* that infected with *B. thuringiensis* strain was obtained from the commercial product DiPel 2X, Lanes 4, 5 and 6 *B. thuringiensis* was isolated from the dead larvae of the laboratory colony of *S. littoralis* that infected with mixtures of *B. thuringiensis* + atrazine, *B. thuringiensis* + (metalaxyl-M + copper oxychloride) and *B. thuringiensis* + profenofos, respectively

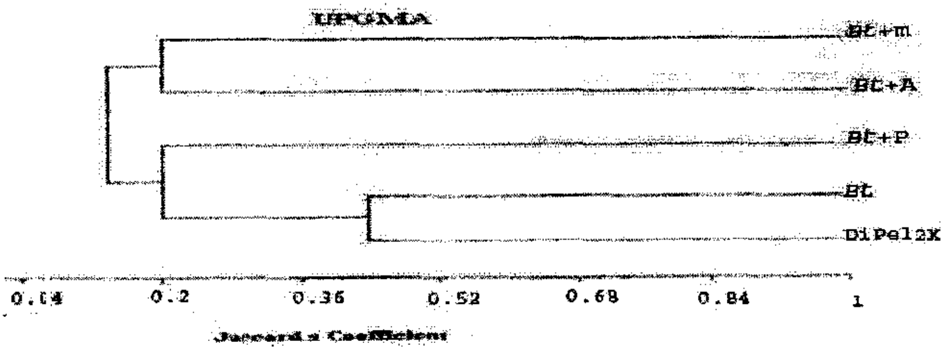


Figure 2. Dendrogram based on the RAPD profiles of the 5 *B. thuringiensis* reisolates generated by primer OPA3 using the Dice Coefficient and UPGMA cluster analysis

(0.261, 0.306, 0.365, 0.514, 0.541, 0.608, 0.644, 0.716 and 0.788) and MW (99, 81, 65, 45, 44, 41, 40, 38 and 37) respectively, appeared in all lanes, these bands were considered as specific bands for the original strain of *B. thurinigiensis kurstaki* and resistance to effect of atrazine, metalaxyl-M + copper oxychloride and profenofos. As well as there was one band number (11) with mobility (0.869) and MW (36) which appeared only in the fourth, fifth and sixth lanes, this band was considered as a response to effect of both atrazine, metalaxyl-M + copper oxychloride and profenofos on *B. thurinigiensis*. Also one band number (4) with mobility (0.446) and MW (52) which absent only in the fifth lane, this band was considered as a result to effect of metalaxyl-M + copper oxychloride on *B. thurinigiensis*.

Considering the reisolates that isolated from the field colony (lanes 7, 8, 9, and 10), a total of 9 bands numbers (1 and 9) with mobilities (0.261 and 0.716) and MW (99 and 38) respectively, appeared in all lanes. These bands were considered as specific bands for the original strain of *B. thurinigiensis kurstaki* and resistance to effect of atrazine, metalaxyl-M + copper oxychloride and profenofos. As well as there was also three bands number (3, 7 and 11) with motilities (0.365, 0.608 and 0.869) and MW (65, 41 and 36) respectively, which appeared only in the eighth, ninth and tenth lanes, these bands were

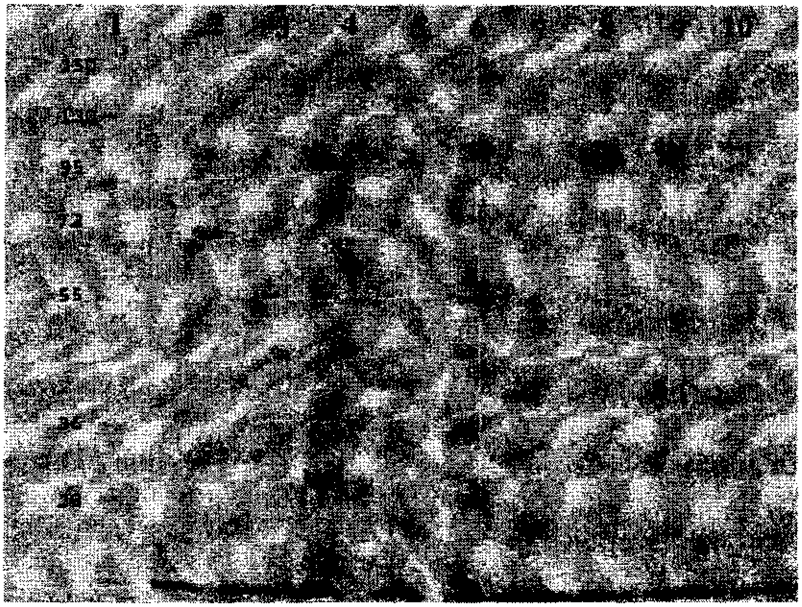


Figure 3. SDS-PAGE of reisolates of *B. thuringiensis* show polymorphism among *B. thuringiensis* isolates. Lane 1 molecular weight marker (ladder). Lane 2 *B. thuringiensis* was isolated from the commercial product DiPel 2X, Lanes 3, 4, 5 and 6 *B. thuringiensis* was isolated from the dead larvae of the laboratory colony of *S. littoralis* that infected with *B. thuringiensis* was obtained from the commercial product DiPel 2X, mixture of *B. thuringiensis* + atrazine, mixture of *B. thuringiensis* + (metalaxyl-M + copper oxychloride) and mixture of *B. thuringiensis* + profenofos, *respectively.* Lanes 7, 8, 9 and 10 *B. thuringiensis* was isolated from the dead larvae of the field colony of *S. littoralis* that infected with *B. thuringiensis* was obtained from the commercial product DiPel 2X, mixture of *B. thuringiensis* + atrazine, mixture of *B. thuringiensis* + (metalaxyl-M + copper oxychloride) and mixture of *B. thuringiensis* + profenofos, *respectively*

considered as a response to effect of both atrazine, metalaxyl-M + copper oxychloride and profenofos on *B. thurinigiensis*. Also three bands number (5, 6 and 10) with motilities (0.514, 0.541 and 0.788) and MW (45, 44 and 37) which absent only in the eighth lane, this band was considered as a result to effect of metalaxyl-M + copper oxychloride on *B. thurinigiensis*. also there was one band number (8) with mobility (0.644) and MW (40), which appeared only in the eighth and tenth lanes, this band was considered as a response to effect of both atrazine and profenofos on *B. thurinigiensis*.

Our results of RAPD PCR and SDS PAGE clearly differentiated *B. thuringiensis* isolates based on its treatment with chemical pesticides. These results suggest that the insecticide (profenofos), the fungicide, (Ridomil Glod Plus) and Herbicide (atrazine) are mutant and not compatible with *B. thuringiensis*.

A recent study related to the effects of chemical compounds on spore viability, larvicidal activity and toxin stability of *B. sphaericus* 2362 strain reported that the reason for the loss of larvicidal activity is the chemical degradation of toxin proteins by the generation of free radicals and pH differences (Berber, 1998).

It was reported that mosquito pathogenic colonies of *B. thuringiensis* could lose their toxic activity in habitats polluted with organic and chemical materials, and also exhibit lower persistence, whereas the insecticidal activity of *B. sphaericus* was prolonged in this kind of habitat (Silapanuntakul *et al*., 1983; Davidson *et al*., 1984 and Correa and Yousten, 1995). Nevertheless, there was no significant difference between spore germination and larvicidal activity in either biological control agent treated with pesticides. However, the insecticidal activity of *B. thuringiensis* var. *israelensis* and *B. sphaericus* 2362 spores was quite tolerant to inactivation by the applied pesticides (Berber, 2004).

There is still no general mechanism to describe how the accelerated degradation of pesticides occurs. Some scientists speculate that, as with microbial resistance to antibiotics and heavy metals, the genes for pesticide breakdown may be carried on plasmids that can be treated freely among various microbes to speed adaptation to the pesticides (Chapalamadugu and Chaudhry, 1991). It would be better to use genetically modified colonies that contain genes resistant to pesticides in habitats polluted with chemicals. Berber (2004) indicated that chemical pesticides prevented the effect of bioinsecticides, thus causing unreliability in biological control methods and resulting in the loss of millions of dollars spent on biological control.

Finally, we not recommend mixing chemical pesticides with *B. thuringiensis*.

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الرصد والتشخيص الجزيئى للتأثيرات التفاعلية لبعض مواد مكافحة الآفات الكيميائية والحيوية على دودة ورق القطن

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تم اختبار التأثيرات التفاعلية بين المبيدات الكيميائية والحيوية على السلالة المعملية والحقلية لدودة ورق القطن تحت الظروف المعملية. عند استخدام المبيدات منفردة وجد أن السلالة الحقلية أكثر حساسية ببكتريا الباسيلس ثورينجنسيس عن السلالة المعملية على عكس المبيدات الكيميائية، كذلك وجد أن بكتريا الباسيلس ثورينجنسيس كانت أقل سمية عن البروفينوفوس وأكثر سمية عن الميتالاكسيل-إم + أوكسى كلوريد النحاس، بينما الميتالاكسيل-إم + أوكسى كلوريد النحاس كان أكثر سمية عن الأترازين. لم تظهر نتائج الخلط تأثير تنشيطى على نسب الموت لدودة ورق القطن بين الباسيلس ثورينجنسيس مع كل من البروفينوفوس والأترازين على عكس الميتالاكسيل-إم + أوكسى كلوريد النحاس عند تركيز (LC_{25} + LC_{25}) لكل منهم. أيضاً، وجد أن نتائج الخلط كانت تثبيطية على المقاييس البيوكيميائية، كذلك التأثيرات المتداخلة للمخاليط لم تختلف بين السلالتين المعملية والحقلية. نتائج RAPD-PCR و SDS-PAGE أوضحت إختلافات بين عزلات الباسيلس ثورينجنسيس نتيجة معاملتها بالمبيدات الكيميائية وبالتالى تقترح هذه النتائج أن المبيدات الكيميائية قد تكون مطفرة للباسيلس ثورينجنسيس. وتفيد هذه المعلومات بعدم إمكانية خلط المبيدات الكيميائية مع المبيدات الحيوية.