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BACILLUS THURINGIENSIS BERLINER AND SABADILLA AS BIOLOGICAL AGENTS AGAINST SPINY AND PINK BOLLWORMS

BY

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ABSTRACT

Laboratory studies were conducted to evaluate the toxicity , joint action and cytological effects of the biological agents B.T. Delfin & Dipel and Sabadilla against the spiny and pink bollworms.

The results indicated that Delfin and Dipel were more effective against the spiny bollworm than the pink bollworm. The LC_{50} values were higher on the 1st instar larvae of both insects by the film residue method when compared with those calculated from the dipping method against the 4th instar ones. Besides, Sabadilla was more effective on the spiny bollworm than the pink bollworm. It was also more effective on the 1st instar larvae by film residue method than on the 4th instar ones by dipping method.

It was noticed that the addition of Sabadilla to both pathogen agents enhanced the efficiency after 72 hrs of treatment and showed a remarkable potentiation effect in controlling these pests.

Moreover,the pathogen agents or/and Sabadilla induced several types of chromosomal aberrations,

while Delfin produced the highest percentage of abnormal male gonad cells. Sabadilla was the lowest in producing the abnormalities.

INTRODUCTION

Cotton one of the world most economic crops, is of particular importance to many countries. The spiny bollworm , Earias insulana (Boisd.) (Noctuidae) and the pink bollworm, Pectinophora gossypiella(Saund.) (Gelechiidae) are considered the most injurious cotton pests. The economic loss occurs as a result of infestation with both pests which reduce the yield, and give low fiber quality (Willcoks, 1961).

The present study was directed to explore a new function of Sabadilla,which is a medicinated type of veterinary approach to emphasize its role against harmful cotton pests and Bacillus thuringiensis was also tested. The aim of the investigation was to evaluate both biological agents as new tools from toxicity index point of view and to study their joint action against the above insect pests. The cytological effects occurring in the larval gonads of both spiny and pink bollworms were also investigated.

MATERIALS AND METHODS

A. Biological agents:

1- Delfin® (D.F.):
   - Active ingredient:
     Bacillus thuringiensis Berliner , var. Kurstaki. 53 x 10^3 SU/Kg of product.

2- Dipel® (W.P.):
   - Active ingredient:
     Bacillus thuringiensis Berliner. 16 x 10^3 IU per mg.

B. Sabadilla (greyish-white powder):

Mixture of ground Sabadilla seeds and vermiculite (SV).

Sabadilla comes from seeds of Schoenocaulon officinale (Lindl.). A Gray. Liliaceae. grown in U.S.A.,Mexico,Costa-Rica,El-Salvador. Guatimala. Hondoras. Peru and Venzuela. The toxicity of Sabadilla seed is probably associated with a complex mixture alkaloids having the group name of veratrine. which is composed of several alkaloids known as cevadine and sabadine. The alkaloids are found principally in the endosperm ,embryo and coat of the seed (Kriger,1946).

Active ingredient:

Veratrine (ceveratrum) alkaloids mixtures:

Ceveratrum alkaloids	Formula	Unclassified alkaloids	Formula
Alkamines of known Structure:		Miscellaneous alkaloids	
Veracevine (Protocevine)	$C_{27}H_{43}O_8N$	Sabine (Neosabadine)	$C_{27}H_{41}O_5N$
Cevine	$C_{27}H_{43}O_6N$	Hydroalkamine-S	$C_{27}H_{45}O_8N$
Cevagenine	$C_{27}H_{43}O_8N$	Sabadine (Sabatine)	$C_{29}H_{47}O_8N$
Esters of veracevine alkaloids.		Veragenine	$C_{31}H_{53}O_{12}N$
Cevacine	$C_{29}H_{45}O_9N$		
Cevadine	$C_{32}H_{49}O_9N$		
Vanilloylcevine	$C_{35}H_{49}O_{11}N$		
Veratridine	$C_{36}H_{51}O_{11}N$		
Other alkaloids of known structure.			
Dehyrocevagenine	$C_{27}H_{41}O_8N$		
Cevinilic acid S-lactone.	$C_{27}H_{41}O_8N$		

(C.F. Kupchan et al., 1961).

C. Rearing of the spiny bollworm Earias insulana (Boisd.) and the pink bollworm Pectinophora gossypiella (Saund.) for bioassay tests:

The larvae and pupae of the spiny bollworm Earias insulana (Boisd.) were obtained from infested green cotton bolls and okra pods which had been collected from cotton and okra plantations in Beheira Governorate during the growing season of 1991. The pink bollworm Pectinophora gossypiella(Saund.) was a susceptible laboratory strain culture without being exposed to pesticides since 1977.

The two insect species larvae were reared in the laboratory on okra pods as described by Massoud (1984). The two colonies were reared at the higrothermic condition of 26 ± 1°C and 65 ± 5% R.H. The moths were sexed and transferred to jars (1 L. vol.) covered with muslin with hanged strips (black for pink bollworm and white for spiny bollworm) for mating and oviposition and supplied with a piece of cotton soaked with 10% sugar solution.

The muslin covers and strips which have singly deposited eggs were transferred to glass jars (1 L. each) covered with a polyethylene sheet. The hatched larvae were transferred on okra slices and kept under the previous conditions.

D. Treatment of bollworms larvae in the laboratory:

Progressive dilutions of each of Delfin , Dipel and Sabadilla were individually prepared in distilled water. All preparations were based on the active ingredient (W/V) and expressed as Spodoptera Units (SU) or International Units (IU) or (PPM) depending on the used compound. Each prepared dilution of Sabadilla contained

also 0.5% sugar.

Dilutions of various concentrations were tested using both methods of application, dipping and film residue, against the 1st and 4th larval instars.

The mixtures of Delfin or Dipel with Sabadilla against the 4th instar larvae of E. insulana (Boisd.) or P. gossypiella (Saund.), were prepared at the proportional rates of the calculated LC_{25} values from the dipping bioassay method. These mixtures were tested using the dipping bioassay method against the 4th larval instar of each insect species.

Bioassay Procedure:

1- Dipping method:

Okra pods were dipped for 15 seconds in the prepared dillutions of each tested Delfin, Dipel and Sabadilla. After dryness, ten 4th instar larvae of each of the spiny or pink bollworms were allowed to feed on the treated pods for 24 hrs in case of Sabadilla and 48 hrs in case of Dipel & Delfin. Then they were supplied with fresh untreated okra pods. Each concentration representing a treatment was replicated four times, for each tested insect , besides the control.

2- Film residue method:

Different concentrations of Delfin, Dipel and Sabadilla were prepared in distilled water. Two ml of each prepared concentration were distributed inside a petri-dish (15 cm in diameter) and left for complete dryness. Ten 1st instar larvae of the spiny or pink bollworms were introduced in each treated petri-dish. Each concentration was replicated four times for each insect. After one hour from initiating the test,

four okra pods were offered for larval feeding. For the control, four petri-dish replicates were used with only distilled water. This treatment was doubled at the same time to avoid the mechanical death during the inspection of mortality after 24 & 48 hrs for Sabadilla treatment or 72 & 96 hrs for Delfin or Dipel treatments.

Mortality counts were carried out throughly and recorded only after 72 & 96 hrs and 24 & 48 hrs post-treatment for the two biological pesticides and Sabadilla, respectively. The mortality data were subjected to probit analysis by the method of Litchfield and Wilcoxon (1948) after the correction by Abbott's formula (Abbott, 1925). For the direct comparison of used pesticides, the toxicity index devoted by Sun (1950) was employed. For evaluation of combinations, the values of co-toxicity coefficient were calculated according to Mansour et al. (1966).

E. Cytological studies:

For studying the cytological effects, the dillutions of separately tested biological pesticides and Sabadilla were prepared in distilled water at the rate of their calculated LC_{50} values after 96 and 48 hrs, respectively. The okra pods were dipped for 15 seconds in each of the prepared dillutions. After dryness of the okra pods, two replicates of 25 4th instar larvae of each of the spiny or pink bollworms were fed on the treated okra pods for 48 hrs and 24 hrs for Delfin & Dipel and Sabadilla, respectively. After 72 and 48 hrs post-treatment, the healthy alive larvae treated with Delfin & Dipel and Sabadilla were collected for the cytological studies. These studies were carried as follows:

1-Preparations from the gonads of the full-grown male larvae (4th instar).

2-According to North et al. (1981) and Shalabi et al. (1983), squash technique on the testes by using 2% aceto-orcein stain solution was

used to study the meiotic chromosomes of Earias insulana and Pectinophora gossypiella.

RESULTS AND DISCUSSION

A. Toxicity index of Sabadilla and B.T. (Delfin and Dipel) on the spiny and pink bollworms:

1. Biological agents:

a) Against 4th larval instar:

Delfin and Dipel as two selective microbial pesticide formulations of Bacillus thuringiensis (B.T.)were studied against the 4th instar larvae of Earias insulana (Boisd.) and Pectinophora gossypiella(Saund.) by using the dipping method.

In general, for the evaluated two formulations of Bacillus thuringiensis, the data in Table (1) show that Delfin achieved superior toxic effect than Dipel on the tested larvae of both insects. The LC_{50} values of Delfin were 38 x 10^3 SU against the spiny bollworm and 42 x 10^3 & 39 x 10^3 SU against the pink bollworm after 72 & 96 hrs of treatment,successively. The LC_{50} values of Dipel were 51 x 10^3 & 45 x 10^3 and 64 x 10^3 & 58 x 10^3 IU on the spiny and pink bollworm larvae, after 72 & 96 hrs,respectively.

Moreover,it was noticed that Delfin and Dipel were more effective against the spiny bollworm than the pink bollworm. However, the toxicity index of Delfin and Dipel were 100 and 77.77 against the spiny bollworm, while they were 89.74 and 60.34 against the pink bollworm after 96 hours (Table 1).

Table 1. Toxicity index of Bacillus thuringiensis against 4th instar larvae of A. arabiensis and C. quinquefasciatus (dipping method).

Biological compound	Insects	Function of Ld-P line										Confidence limits of Ld_{99}				Confidence limits of slope				Toxicity index	
		Ld_{84}		Ld_{50}		Ld_{25}		Ld_{16}		Slope		Upper		Lower		Upper		Lower			
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Delfin (DU)	A. arabiensis	82 x 10^3	89 x 10^3	38 x 10^3	35 x 10^3	23 x 10^3	19.5x10^3	18 x 10^3	15 x 10^3	0.13	0.34	64000	61475	32759	29536	2.64	2.94	1.69	1.81	100	100
	C. quinquefasciatus	89 x 10^3	79 x 10^3	40 x 10^3	39 x 10^3	24.5x10^3	24.9x10^3	21.5x10^3	19.5x10^3	1.93	0.81	48700	49310	34807	33563	2.45	2.61	1.52	1.95	80.48	89.76
Dipel (DU)	A. arabiensis	230 x 10^3	130 x 10^3	51 x 10^3	45 x 10^3	27 x 10^3	24 x 10^3	20 x 10^3	18 x 10^3	0.96	0.47	81300	64000	49500	39500	3.49	3.20	1.85	1.98	74.91	77.77
	C. quinquefasciatus	260 x 10^3	175 x 10^3	64 x 10^3	50 x 10^3	35 x 10^3	34 x 10^3	24 x 10^3	26 x 10^3	0.68	0.10	96000	67970	53333	40760	3.79	3.74	1.85	1.75	59.36	60.34

a = 72 hrs.

b = 96 hrs.

Table (2). Toxicity index of Bacillus thuringiensis against 3rd instar larvae of A. arabiensis and C. quinquefasciatus (Pile rondeu method).

Biological compound	Insects	Function of Ld-P line										Confidence limits of Ld_{99}				Confidence limits of slop				Toxicity index	
		Ld_{84}		Ld_{50}		Ld_{25}		Ld_{16}		Slope		Upper		Lower		Upper		Lower			
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Delfin (DU)	A. arabiensis	100 x 10^3	97 x 10^3	46 x 10^3	39 x 10^3	29.5x10^3	23 x 10^3	19.0x10^3	17.7x10^3	0.84	0.82	51000	46820	37700	33051	2.91	2.79	1.75	1.96	100	100
	C. quinquefasciatus	92 x 10^3	82 x 10^3	47 x 10^3	37.5x10^3	30 x 10^3	26.5x10^3	24 x 10^3	19.3x10^3	1.96	0.86	54500	46847	40517	33305	2.91	2.72	1.93	1.96	93.62	94.73
Dipel (DU)	A. arabiensis	155 x 10^3	150x 10^3	70 x 10^3	56 x 10^3	41 x 10^3	28 x 10^3	32 x 10^3	29 x 10^3	0.90	0.90	68000	64500	59322	45736	2.79	3.90	1.76	1.85	82.85	69.64
	C. quinquefasciatus	170 x 10^3	160x 10^3	80 x 10^3	76 x 10^3	47 x 10^3	44 x 10^3	37 x 10^3	34 x 10^3	0.14	0.17	98000	80540	68644	64936	2.60	2.71	1.71	1.74	59.00	51.32

b) Against 1st larval instar:

Similar trends of mortality were observed when the film residue method was used. In addition, it was noticed that the LC_{50} values of Delfin and Dipel were higher when compared with those calculated from the dipping method (Table 2). For Delfin, the LC_{50} values were 44 x 10^3 and 39 x 10^3 SU against the spiny bollworm and 47 x 10^3 and 39.5 x 10^3 against the pink bollworm after 72 & 96 hrs, respectively. While for Dipel, these LC_{50} values were 70 x 10^3 and 56 x 10^3 IU and 80 x 10^3 and 76 x 10^3 IU on the spiny and pink bollworm larvae after 72 & 96 hrs of treatment, respectively.

2. Sabadilla:

a) Against 4th larval instar:

The toxicity of Sabadilla was evaluated against the 4th instar larvae of both spiny and pink bollworms. The data were recorded 24 & 48 hrs after treatment. The data in Table (3) indicate that the Sabadilla induced a moderate toxicity against both insects. The calculated LC_{50} values were 3500 & 2600 ppm and 5200 & 3350 ppm against the spiny and pink bollworms after 24 & 48 hrs of treatment, respectively.

b) Against 1st larval instar:

As shown in Table (4), similar results were recorded , using the film residue method. In general, it was noticed that the LC_{50} values of Sabadilla were highly decreased when compared with those obtained from the dipping method. The calculated LC_{50} values of Sabadilla were 1300 & 800 ppm and 3000 & 2400 ppm against the spiny and pink bollworms after 24 & 48 hrs successively.

Table (3). Toxicity index of Bacillus against 4th instar larvae of A. [illegible] and [illegible] (dipping method).

Other compound	Insects	Function of Ld-P line										Confidence limits of LC_{50}				Confidence limits of slope				Toxicity index	
		LC_{84}		LC_{50}		LC_{25}		LC_{16}		Slope		Upper		Lower		Upper		Lower			
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Bacillus (ppm)	A. [illegible]	16000	9800	3500	2400	1300	1100	800	760	0.47	3.64	4970	3640	2459	1857	6.93	5.09	2.80	2.60	100.00	100.00
	[illegible]	96000	14500	9200	3350	1800	1300	970	880	5.33	6.98	7000	4690	3467	2193	9.99	6.17	7.96	7.86	67.30	77.61

Table (4). Toxicity index of Bacillus against 1st larvae of A. [illegible] and [illegible] (Film residue method).

Other compound	Insects	Function of Ld-P line										Confidence limits of LC_{50}				Confidence limits of slope				Toxicity index	
		LC_{84}		LC_{50}		LC_{25}		LC_{16}		Slope		Upper		Lower		Upper		Lower			
		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Bacillus (ppm)	A. [illegible]	7000	4500	1300	800	450	270	260	155	5.17	5.39	1768	1200	956	533	8.96	9.16	3.39	3.17	100.00	100.00
	[illegible]	15000	12500	3000	2400	1000	800	620	450	5.00	5.27	4350	3600	2069	1600	8.50	9.96	2.94	3.06	43.33	33.33

Thus, it was obvious from the results shown in Tables (1-4) that the B.T. formulations were more toxic to the 4th instar larvae of the spiny and pink bollworms when the dipping method was used. Sabadilla was also more effective on the 1st instar larvae of the spiny and pink bollworm when the film residue method was applied. Moreover , it was clear that the 1st or/and 4th instar larvae of the spiny bollworm were more susceptible to B.T. formulations or Sabadilla than those of the pink bollworm. The efficiency of B.T. formulations depends on their mode of action as stomach poisons through feeding, which agrees with the results reported by Saad et al. (1985).

It is an interesting to state that Bacillus thuringiensis as typified by subspecies,kurstaki ,produces toxins in a parasporal body during sporulation. The parasporal body consists of a single bipyramidal crystal, with or without a small cuboidal inclusion. The bipyramidal crystal is composed of one or more proteins with a molecular size of 135 kda, while the cuboidal inclusion typically consists of a single protein of 65 kda,actually a protoxin, which is solubilized from the bipyramidal crystal and then cleaved by midgut proteases to produce an active 65 kda toxin. The cuboidal inclusion protein, 65 kda in size, is active upon solubilization, and is toxic without further processing (Georghiou, 1990).

In case of Sabadilla, the apparent symptoms on treated 4th instar larvae show that its toxicity is due to the effect on C.N.S. as a contact poison which highly agrees with the statements of Catteral (1980) who reported that veratridine (Sabadilla alkaloids) has a broad spectrum of pharmacological activity causing muscle contraction, repetitive firing of nerves and irregular heart rhythms. Veratridine causes depolarization of nerves due to increased sodium

permeability. Thus, the depolarization of excitable cells by veratridine is caused by two effects, namely block of inactivation and shift of activation to more negative potentials.

However, plants of the veratrum group have been used for medicinal purpose for hundreds of years, and as insecticides. The insecticidal action of dried S. officinale, characteristics of cevadine and veratridine, is found only in the seeds (C.F. Kupchan et al.,1961).

B. Joint action of B.T. with Sabadilla against the 4th instar larvae:

The results in Table (5) show the actual average percent mortalities of the 4th instar larvae of both E. insulana and P. gossypiella at different intervals after treatment with the mixtures of Sabadilla/Delfin or/and Dipel. Apparently, the calculated values of co-toxicity factor of these mixtures, primarily indicated the additive or antogonistic effect after 24 and 48 hrs from larval treatment. After 72 hrs, all the evaluated mixtures were potent. From these results, it could be realized that the addition of Sabadilla to both pathogen agents enhanced the efficiency and showed a remarkable potentiation effect in controlling both pests. These findings could be attributed to the mode of action, as stomach or contact pesticide and toxicity to the central nervous system.Morecver, the presence of sugar in the prepared Sabadilla dilutions (as 0.5%) which acts as an additive feeding stimulant for the insect-larvae. Similarly, El-Husseini et al.(1980) and Mesbah (1985) concluded that a sugar concentration not exceeding 0.5% (W/V) increased the pathogenic effect of Entrobackterin-3 and Dipel in all treated larval instars of Earias insulana.

C. Cytological effect of B.T. and Sabadilla against the 4th larval instars:

Cytological studies were made on preparations from male gonads of healthy mature larvae of both treated and untreated insects. Observations indicated that the male of these insects had a chromosome complex of 31 pairs (Fig. 1-A,B) in the metaphase stage which was more frequent than the anaphase or telophase stage (Fig. 2). Similar results were obtained in Spodoptera littoralis by Shalabi et al.(1983)and El-Feel et al.(1990). The results presented in Table (6) also show that the percentage of abnormal cells for all treatments was higher than in the untreated control.

Delfin produced the highest percentage of abnormal cells of 20.0 and 23.0% in P. gossypiella and E. insulana, respectively. Dipel produced percentage of 20.90 and 17.33% for both pests, while Sabadilla produced the lowest percentage of abnormal cells of 15.29 and 15.90% in the pink and spiny bollworms, respectively.

The preparations revealed that the most frequent aberration was irregular distribution of the chromosomes which may be attributed to the disturbance of the spindle apparatus(El-Feel et al.,1990). The preparations also showed that Delfin induced high percentage of irregularity in E. insulana (Fig. 3), while Sabadilla showed equal percentages in both pests.

The second frequent aberration was the lagging chromosomes (Fig.4) which may be attributed to the delayed terminilization or due to the stickiness of chromosomes (Kaur and Grovers, 1985). As to the effect of Delfin , Dipel and Sabadilla produced descendingly percentages of 9.41, 6.36 and 5.88 of lagging chromosomes, respectively, in P. gossypiella. The same trend

Table 5. Toxicity factor for mixtures of biological agents and subadilla against the 4th instar larvae of S.inqulans and P.gossypiella.

Mixtures				Tested insects	Average percent mortality of the two insects											
					After 24 hrs.			After 48 hrs.			After 72 hrs.			After 96 hrs.		
1	2	1	2		Obser-ved	Co-toxicity	Type	Obser-ved	Co-toxicity	Type	Obser-ved	Co-toxicity	Type	Obser-ved	Co-toxicity	Type
Subadilla	Delfin	25	25	S.inqulans	47.5	- 5	(00)	60.0	20	(00)	75.0	50	(+)	87.5	75	(+)
				P.gossypiella	45.0	-10	(00)	52.5	5	(00)	70.0	40	(+)	82.5	65	(+)
	Dipel	25	25	S.inqulans	40.0	-20	(-)	55.0	10	(00)	72.5	45	(+)	80.0	60	(+)
				P.gossypiella	35.0	-30	(-)	45.0	-10	(00)	65.0	30	(+)	75.0	50	(+)

(+) - Potentiation effect.

(00) - Addition effect.

(-) - Antagonism effect.

Table (6). The percentages of the different types of abnormalities in the abnormal meiotic cells of treated larvae of S.inqulans and P.gossypiella.

Treatment	Insect	No. of divided cells	No. of abnormal cells	Abnormal cell %	Chromosomal abnormalities %			
					Stickness	Irregular distribution	Lagging	Sticky bridge
Delfino	S.inqulans	200	46	23.00	6.00	10.00	8.00	1.00
	P.gossypiella	253	51	20.00	4.71	5.88	9.41	0.00
Dipel	S.inqulans	225	39	17.33	5.33	4.00	0.00	0.00
	P.gossypiella	220	46	20.90	4.54	9.09	6.36	0.91
Subadilla	S.inqulans	264	42	15.90	3.41	7.95	4.55	0.00
	P.gossypiella	255	39	15.29	2.35	7.06	5.88	0.00
Untreated	S.inqulans	300	20	6.67	1.00	3.00	2.67	0.00
	P.gossypiella	300	12	3.00	1.00	0.00	2.00	0.00

was observed in E. insulana, while the untreated larvae had percentages of 2 and 2.67 for P. gossypiella and E. insulana, respectively.Jain and Sarbhoy (1987) reported that the chromosomes could not reach the poles and remained scattered in the cytoplasm as a result of chemical treatments.

The third chromosomal aberration was the sticky chromosomes (Fig. 5) which were generally regarded as physiological and unspecific disturbances and may be attributed to the action on the chromosomal proteins (Devadas et al.,1987). The data also showed that Dipel was more obvious in producing sticky chromosomes in E. insulana than the others, while both Dipel and Delfin induced equal percentage of sticky chromosomes in P. gossypiella, which were higher than those induced by Sabadilla. The preparations showed that there were few cells which had sticky bridges in the anaphase stage (Fig. 6).

Generally,the pathogenic microbial pesticides, Delfin and Dipel, were more effective than Sabadilla in producing cytological aberrations in such treated pests. This may be due to inhibiting considerably synthesis of protein and nucleic acids and disturbing the ratios of protein, RNA and DNA (Emara et al.,1991).

In conclusion, the B.T. formulations of Delfin and Dipel were more effective on the 4th instar larvae of the spiny and pink bollworms by the dipping method than on the 1st instar larvae by the film residue method. Sabadilla was effective on the two insect species larvae,especially the first instar ones, as a contact poison. The combinations of Sabadilla/Delfin or / and Dipel produced a potentiation effect which means that Sabadilla is a strongly potential factor to B.T. which will be useful in controlling the cotton bollworms either the newly-hatched larvae or the mature ones. In addition, these mixtures will

Fig. (1). Metaphase I in (A) P. gossypiella and (B) E. insulana showing 31 pairs of chromosomes (bivalents). Magnification Power (M.P) : 4×10^3 x

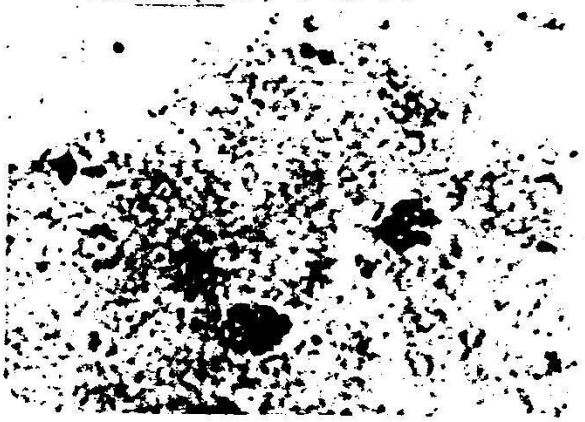

Fig. (2). Normal telophase I stage of P. gossypiella, M.P: 4×10^3 x.

Fig. (3). Metaphase I showing irregular distribution of biva- lents in E.insulana, MP: $4x10^3$x.

Fig. (4). Metaphase I in P. gossypiella showing 2 lagging bivalent. MP. :$4x10^3$ x.

Fig. (5). Metaphase I in E. insulana showing stickiness bivalents, M.P. : 4×10^3 x.

Fig. (6). Early anaphase in E. insulana showing sticky bridge, M.P.; 4×10^3 x.

reduce the amount of used pathogen pesticides with a highly positive control and at the same time without any environmental pollution.

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REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Catteral, W.S. (1980). Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes , Ann. Rev. Pharmacol. Toxicol., 20: 15-43.

Devadas, N.; A. Sodanandam; R. Kishan Rao and K. Subhas (1987).Induced somatic aberrations and chlorophyll mutation in capsicum by insecticides. Cytologia, 52: 235-241.

El-Feel, E.A.; H.A.H. Omar; A.E.M. El-Sorady and Y.H. Issa (1990). Toxicological , enzymological and cytological effects of some oil-insecticide mixtures against Spodoptera littoralis (Boisd.). Com. Sci. & Dev. Res., 30: 31-49.

El-Husseini, M.M. and A.I. Afify (1980). Increasing the efficacy of Bacillus thuringiensis. Berliner against the spiny bollworm, Earias insulana (Boisd.) by adding a feeding stimulant. Proc. 1st Conf. Pl. Prot. Res. Ins., 11: 397-403.

Emara, S.A. ; G.M. Moawad and A.I. Gadallah (1991). Effect of three formulations of Bacillus thuringiensis on the American bollworm,Heliothis armigera HBN. 4th Arab Gongr. of Pl. Prot. Cairo 1-5 December.

Georghiou, G.P. (1990). Resistance potential to biopesticides and consideration of countermeasures. J. of Pesticides and Alternatives: 409-420.

Jain, A.K. and R.K. Sarbhoy (1987). Cytological studies on the effect of some chlorinated pesticides. I. Effect on somatic chromosomes of lenus and pisum. Cytologia ,52: 47-53.

Kaur, P. and I.S. Grover (1985). Cytological effects of some organophosphorus pesticides. II. Meiotic effects. Cytologia, 50: 199-211.

Krieger, C.H. (1946). Sabadilla , Agricultural Chemicals, 1: 19-21, 32.

Kupchan, S.M. ; J.H. Zimmerman and A. Afonso (1961). The alkaloids and taxonomy of veratrum and related genera. J. of Pharm. & Allied Biol. Sc. (LLOYDIA) 24: 1-26.

Lichfield, J.T.Jr. and Wilcoxon (1948).A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Terap., 69: 99-113.

Mansour, N.A.; M.E. El-Dafrawi; A. Tappozada and M.I. Zeid (1966). Toxicological studies on the Egyptian cotton leafworm Prodenia littura. VI. Potentiation and antagonism of organophosphorus and carbamate insecticides. J. Econ. Ent., 56 (2): 307-311.

Massoud, M.A. (1984). Toxicological studies on spiny bollworm Earias insulana. M. Sc. Thesis , Fac. of Cotton Sciences, Helwan Univ.

Mesbah, H.A. (1985). Evaluation of the potency of the biological compound Dipel against the spiny bollworm Earias insulana (Boisd.). J. Agric. Sci., Camb.,105: 589.

North, D.T. ; R.A. Leopold and Chidress (1981). Meiotic and mitotic chromosomes of cotton boll-weevil (Coleoptera : Curculionidae). Can. J. Genet. Cytol., 23: 443-447.

Saad, A.S.A.; M.A. Elewa;O.A. Zaghloul;H.A. Awad and M.A. Massoud (1985). Toxicological and histopatholoical studies on spiny bollworm Earias insulana. Med. Fac. Landbouww.Rijksuniv.,Gent.,50/2b:735-750.

Shalabi, H.G.; N. Shaker; E. Badr; F. Taman and A. El-Sebae (1983). Studies on meiotic and mitotic chromosomes of cotton leafworm Spodoptera littoralis. Proc. Conf. Univ. Haz. Agrochem., II: 932-940.

Sun, Y.P.(1950).Toxicity index and proved method of comparison the relative toxicity of insecticides.J. Econ. Entomol.,40(3):45-53.

Willcoks, F.C. (1961). The insects and related pests of Egypt. Vol. I. The insects and related pests injurious to the cotton plant.Part I. The pink bollworm. Sultanic Agricultural Society. 339 pp. Cairo.

الملخص العربي

أجريت دراسات معملية لتقييم درجة السمية والفعل المشترك والتأثيرات السيتولوجية لكل من المبيدات الحيوية (ديلفين ، تايبل) والسابانيلا ضد دودة اللوز الشوكية والقرنفلية .

أوضحت النتائج ان كل من الديلفين والدايبل كانا اكثر فعالية ضد دودة اللوز الشوكية عن القرنفلية وان قيم الـ LC50 ازدادت مع العمر الاول لكلا الحشرتين عند المعاملة بطريقة متبقى المبيد بالمقارنة بطريقة الغمر مع العمر الرابع .

كما أظهر السابانيلا فعالية اكبر على دودة اللوز الشوكية عن القرنفلية ، ولكن هذه الفعالية ظهرت بوضوح على العمر الاول بطريقة متبقى المبيد اكثر منها على العمر الرابع بطريقة الغمر.

لوحظ ايضا ان اضافة السابانيلا الى كل من المبيدين البكتيريين اعطى تأثير تضوية ملحوظ عند مكافحة هذه الحشرات. علاوة على ان المبيدات البكتيرية والسابانيلا اظهرت شذوذات كروموسومية حيث أعطى الديلفين اعلى نسبة من خلايا البراعم التناسلية الذكرية الغير طبيعية بينما كان السابانيلا اقلهم في اظهار هذه ، الخلايا الغير طبيعية .