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BIOCHEMICAL AND HISTOLOGICAL EFFECTS OF CHINMIX, SPINTOR AND BIOREPEL COMPOUNDS ON LARVAE OF PINK AND SPINY BOLLWORMS. BY

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ABSTRACT

Laboratory experiments were carried out to evaluate the biochemical and histological effects of Chinmix, Spintor and Biorepel compounds on larvae of pink and spiny bollworms ($Pectinophera\ gossypiella\$ and $Earias\ insulana$). The obtained data revealed that the biochemical effects of the three compounds caused decrease in the activity of invertase, trehalase, amylase, GPT, GOT enzymes and total soluble protein in both insects. On the other hand Chinmix and Spintor compounds decreased the activity of α - and β - E enzymes in larvae of pink and spiny bollworms as compared to the control, meanwhile, the inverse is true in case of Biorepel compound. Also, results showed that Chinmix, Spintor and Biorepel compounds caused abnormalities in the mid-gut tissue of larvae of the two insect species.

INTRODUCTION

The effect of the compounds on biochemical activities of target insect must be considered as an important aspect in integrated pest management strategy to draw complete map of insecticides efficiency. The mainly biochemical responses taking in consideration especially the transaminase enzymes activity and total soluble protein content have an important role in biological and physiological activities of insects [Mead, 2000 and Khedr, 2002].

The present work aimed to evaluate the biological, biochemical and histological effects of Chinmix, Spintor and Biorepel compounds against pink and spiny bollworms (*P. gossypiella* and *E. insulana*).

MATERIALS AND METHODS

*Tested compounds:

1- Beta-cypermethrin (Chinmix; 5 % EC)

2- Spintor (Spinosad 25 % WG)

Spinosyn A: R=H, MW=731.98. Spinosyn D: R=CH₃, MW=746.00.

3- Biorepel 10 % E (a. i. Garlic juice) manufactured by JH Biotech., Inc. California, U. S. A. the recommended rate is 10 ml./L.

1-Biochemical effects of Chinmix, Spintor and Biorepel compounds on larvae of pink and spiny bollworms.

The effect of Chinmix, Spintor and Biorepel compounds on the levels of enzymes activity of pink and spiny bollworms larvae (P. gossypiella and E. insulana) was assessed under laboratory conditions using concentrations of 0.244-0.030 ppm, 0.122-0.015ppm and 200-250ppm for Chinmix, Spintor and Biorepel compounds, respectively. These concentrations were tested against newly hatched larvae of susceptible strain of pink bollworm and field strain of spiny bollworm. The larvae of both insects were fed until full grown on the treated diet (12 days-old). From both treated larvae and the control, samples (one gram larvae / sample) for biochemical assays were collected and placed in clean glass vial and kept in freezer till biochemical analysis. On the other hand, at the end of cotton season, batches of full mature larvae of pink bollworm P. gossypiella (one gram larvae/sample) were collected from treated cotton fields with Chinmix, Spintor and Biorepel compounds and untreated cotton fields (control) and placed in clean glass vial and kept in freezer till biochemical analysis. All the freezed samples were homogenized for 3 minutes in distilled water (5 ml/sample) using a teflon homogenizer surrounded with a jacket of crushed ice. The homogenates were centrifuged at 3500 r.p.m. for 10 minutes at 5 °C. The supernatants were immediately assayed to determine total soluble protein, activities of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), trehalase, amylase, invertase, alpha and beta esterases enzymes.

1-1- Determination of enzyme activities:

A- Carbohydrate hydrolyzing enzymes.

The methods used to determine the digestion of trehalose, starch and sucrose by trehalase, amylase and invertase enzymes, respectively were similar to those described by Ishaaya and Swiriski (1976).

B- Transaminase enzymes(GOT & GPT):

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme activities were determined colourimetrically according to the method of Reitman and Frankle (1957).

C- Non-specific esterases:

Alpha esterases (α -E) and beta esterases (β -E) were determined according to the method of Van Aspern (1962)

1-2- Determination of total soluble protein:

Colourimetric determination of total soluble protein in total supernatant of the homogenated larvae of pink and/or spiny bollworms was carried out as described by (Gornall et al., 1949)

2- Histological effects of Chinmix, Spintor and Biorepel compounds on larvae of pink and spiny bollworms:

To detect the effect of Chinmix, Spintor and Biorepel compounds on mid gut tissues of *E. insulana* and *P. gossypiella* larvae, the newly hatched larvae of susceptible strain of pink bollworm and field strain of spiny bollworm were fed on the artificial diets treated with 0.244 ppm. Chinmix, 0.122 ppm Spintor and 2000 ppm Biorepel. After 12 days from treatment, the alive larvae which show sluggishness were taken for starting the histological process. Sections in the larval mid-gut were done similar to a manner which has been described previously by Gad (1951).

RESULTS AND DISCUSSION

- 1-Biochemical effects of Chinmix, Spintor and Biorepel compounds on larvae of pink and spiny bollworms:
- 1-1 Under laboratory condition:

Carbohydrate hydrolyzing enzymes:

Data in Table (1) showed that Chinmix, Spintor and Biorepel compounds decreased the activity of invertase, trehalase and amylase enzymes in the larvae of pink and spiny bollworms (P. gossypiella and E. insulana) as compared to the control. In addition, in case of pink bollworm the highest decrease in invertase and trehalase activity recorded with Chinmix compound, while the highest decrease in amylase activity recorded with Spintor compound. The lowest decrease in invertase, trehalase and amylase activity was recorded with Biorepel compound. In state of spiny bollworm (E. insulana) the highest decrease in invertase, trehalase and amylase activity recorded with Spintor compound, while the lowest one was for Biorepel.

Transaminase enzymes (GPT & GOT)

The present data in Table (2) revealed that the tested compounds decreased the activity of GPT and GOT enzymes in larvae of pink and spiny bollworms as compared to the control, in addition in case of pink bollworm the highest decrease in GPT and GOT activity recorded with Chinmix compound, while in case of Spiny bollworm the highest decrease in GOT and GPT activity recorded with Spintor compound. On the other hand lower GOt and GPT activity was noticed in Biorepel treated larvae of pink and spiny bollworm.

Alpha and beta esterase ($\alpha - E \& \beta - E$):

Chinmix and Spintor compounds decreased the activity of α - and β - E enzymes in larvae of pink and spiny bollworms (*P. gossypiella & E. insulana*) as compared to the control, meanwhile, the inverse is true in case of Biorepel compound; Table (3).

Table (1): Changes in activities of carbohydrate hydrolyzing enzymes (µg glucose/ minute/ ml) in larvae of pink and spiny bollworms treated with, Chinmix, Spintor and Biorepel compounds as % to control under laboratory conditions.

	Insects Pink bollworm Spiny bollworm								
			A HIS BOHWOLD			Spiny bouworm			
Com- pounds	Concentration (ppm)	Invertage*	Trebalas*	Amyline	Invertuse	Treheles*	Amylane*		
	0.244	-22.03	-39.58	-41.03	-20.31	-23.53	-27.50		
.벌	0,122	-16.95	-20.83	-28.21	-14.06	-19.61	-25.00		
Chinmix	0.061	-10.17	-10.42	-15.38	-9.40	-11.76	-15.50		
ט	0.030	-6.78	-14.58	-10.26	-6.25	-11.76	-7.50		
!	Mean	-13.98	-20.35	-23.72	-12.50	-16.37	-18.88		
	0.122	-20.34	-35.42	-35.90	-25.00	-29.41	-32.50		
ı,	0.061	-13.56	-25.00	-25.64	-17.19	-23.53	-22.50		
Spintor	0,030	-10.17	-12.50	-17.95	-15.63	-19.61	-20.00		
S	0.015	-6.78	-10.42	-17.95	-9.38	-11.76	-12.50		
	Mean	-12.71	-20.84	-24.36	-16.80	-21.08	-21.88		
	2000	-11.86	-16.67	-17.95	-15.63	-17.65	-15.00		
Biorepel	1000	-8.47	-10.42	-17.95	-12.50	-11.76	-17.50		
	500	-8.47	-10.42	-7.69	-6.25	-5.88	-10.00		
	250	-5.08	-4.17	-10.26	-3.13	-9.80	-5.00		
	Mean	-8.47	-10.42	-13.46	-9.37	-11.27	-11.88		

^{* =} activities of enzymes as % to control

Total soluble protein:

The tested compounds decreased total soluble protein in larvae of pink and spiny bollworms (*P. gossypiella & E. insulana*) as compared to the control. Also, in case of pink bollworm the highest decrease in total soluble protein recorded with Biorepel compound while in case of spiny bollworm the highest decrease in total soluble protein recorded with Chinmix compound. The least dcrease in total soluble protein was noticed in Spintor treated larvae of pink and spiny bollworms (*Pectinophera gossypiella* and *Earias insulana*) tabulated in Table (3).

1-2- Under field condition:

Carbohydrate hydrolyzing enzymes:

Chinmix and Spintor compounds caused increase in the activity of invertase and amylase enzymes and decrease in the activity of trehalase in larvae of pink bollworm (P. gossypiella). On the other hand, Biorepel caused a decline in the activity of invertase, trehalase and amylase enzymes; Table (4).

Table (2): Changes in activities of transaminase enzymes (GOT and GPT, µg/l) in pink and spiny bollworms treated with Chinmix, Spintor and Biorepel compounds as % to control under laboratory conditions.

Insects		Pink bo	oliworm	Spiny bollworm	
Com- pounds	Parameter Concen- tration (ppm)	GPT.	GOT.	GPT.	GOT.
	0.244	-29.17	-45.95	-20.00	-33,33
Chinmix	0.122	-22.50	-37.84	-16.00	-33.33
Ē.	0.061	-16.67	-27.03	-12.00	-22.22
Ŗ	0.030	-14.17	-18.92	-8.00	-11.11
	Mean	-20.63	-32.44	-14.00	-25.00
	0,122	-25.00	-32.43	-24.00	-44.44
5	0.061	-20.83	-32.43	-16.00	-33.33_
Ĭ.	0.030	-14.17	-18.92	-16.00	-22.22
Spintor	0.015	-10.83	-10.81	-1200	-22.22
"	Mean	-17.71	-23.65	-17.00	-30.55
Biorepel	2000	-10.83	-18.92	-12.00	-22.22
	1000	-10.83	-10.81	-8.00	-22.22
	5 00	-8.33	-10.81	-8.00	-11.11
	250	-5.83	00.00	-4.00	-11.11
	Mean	-8.96	-10.14	-8.00	-16.67

Table (3): Changes in activities of total soluble protein (gr/100ml) alpha-esterase and beta-esterase (gr/100ml) in pink and spiny bollworms treated with Chinmix, Spintor and Biorepel compounds as % to control under laboratory conditions.

	Insects	Pink bollworm			Spiny bollworm		
Com- pounds	Parameter Concentration (ppm)	Total solubie protein	Alpha coterases	Beta' esterases	Total soluble protein	Alpha* esterases	Beta esterases
	0.244	-38,30	-20,00	-29.03	-35.56	-20.69	-30.30
ij	0.122	-31.21	-14.55	-25.81	-28.15	-17,24	-24.24
Chinmix	0.061	-13.48	-14,55	-16.13	-21.48	-13.79	-15.15
ರ	0.030	-4.26	-9.09	-12.90	-14.07	-8.62	-15.15
	Mean	-21.81	-14.55	-20.97	-24.82	-15.09	-21.21
	0.122	-24.82	-14.55	-19.35	-42.96	-17.24	-24.24
i i	0.061	-10.64	-14.55	-19.35	-28,15	-15.52	-21.21
Spintor	0.030	-17.73	-10,91	-12.90	-14.07	-13.79	-15.15
Sp	0.015	-8.51	-5.45	-9.68	-6.67	-8.62	-12.12
Ì	Mean	-15.43	-11.37	-15.32	-22.96	-13,79	-18.13
	2000	-35.46	+5.45	+9.68	-34.07	+5.17	+9.09
Biorepel	1000	-48.94	+3.64	+9.68	-23.70	+6.90	+9.09
	500	-26.95	0.00	+6.45	-28.15	+3.45	+3.03
	250	-41.13	+3.64	0.00	-9.63	+1.72	+3.03
	Mean	-38.13	+3.18	+6.45	-23.89	+3.88	+6.06

^{* =} activities of enzymes as % to control

Table (4): Changes in activities of carbohydrate hydrolyzing enzymes (g-glucose/minute/ml) and total soluble protein (gr/100 ml) in larvae of pink boliworm in cotton fields treated with Chinmix, Spintor and Biorepel compounds as % to control.

Compounds	Parameter Concentration (ppm)	Invertase	Trehalase	Amylase	Total soluble protein
Chinmix	500 ml/feddan	+7.69	-26.09	+25.00	+74.36
Spintor	50 g/ feddan	+13.46	-15.22	+41.67	+123.08
Biorepel	10 ml/ liter	-23.08	-34.78	-16.67	+48.72

^{* =} activities of enzymes as % to control

Total soluble protin:

Chinmix, Spintor and Biorepel compounds showed on increase in the total soluble protein in the larvae of pink bollworm (*P. gossypiella*) as compared to control; Table (4).

Transaminase enzymes (GPT & GOT):

Chinmix, Spintor and Biorepel compounds caused increase in the activity of GPT&GOT enzymes in larvae of pink bollworm as compared to control; Table (5).

Alpha and beta esterase ($\alpha - E & \beta - E$):

Chinmix, Spintor and Biorepel compounds caused increase in the activity of α -E & β -E enzymes in larvae of pink bollworm (*P. gossypiella*) as compared to control; Table (5).

Table (5): Changes in activities of transaminase enzymes (GOT and GPT, μg/l) alpha and beta-esterase in larvae of pink bollworm in cotton fields, treated with Chinmix, Spintor and Biorepel compounds as % to control.

Compounds	Para- meter Concen- ration (ppm)	GPT '	сот.	Alpha * esterases	Beta esterases
Chinmix	500 ml/ feddan	+87.5	+50.0	+26.92	+21.88
Spintor	50 g/ feddan	+125.0	+75.0	+19.23	+15.63
Biorepel	10 ml/ liter	+50.0	+25.0	+11.54	+6.25

^{* =} activities of enzymes as % to control

This Results are similar with the finding of Abd El-Hafez et al. (1985) who found that the colorimetric evaluation indicated absence of both level esterase's phosphates (acid and alkaline) and aminotransferases (GOT and GPT) enzymes from the adult moths of pink bollworm treated with synthetic pyrethroids and organophosphorous insecticides. Synthetic pyrethroids had less or no effect on

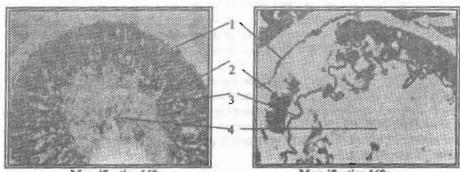
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esterases enzyme activities comparing with organophosphorous compounds. Avvangar and Rao (1990) found that Azadirachtin significantly decreased total haemocyte count, protein and trehalase in S. litura, Raslan et al., (1994) indicated that the activity of α -and- β esterase's in pink bollworm larvae was variably affected according to the insecticide used. All the tested compounds (synthetic pyrethroids, op compounds and carbamates) reduced \(\alpha\) -esterase's activity, whereas the treated larvae gave high level of 6- esterase's comparison with the untreated ones. Also all tested insecticides reduced the GOT activity except Esfenvalerate exhibited slightly increase in GOT activity. In contrast, GPT activity was increased in all insecticides treated larvae. Also, all treatments increased the amylase and invertase activity. Concerning trehalase activity cis-cyfluthrin showed high reduction in trehalase activity, but there was no great difference in trehalase activity between Esfenyalerate, Cypermethrin treated larvae and untreated once. Both K Cyhalothrin and Fenpropathrin revealed high increase in trehalase activity. Tawfik (1998) recorded reduction in GOT and GPT activity of pink and spiny bollworms after treatment with NeemAzal. Mead (2000) recorded an increase in the activities of transaminase enzymes (GOT & GPT), carbohydrate hydrolyzing enzymes (trehalase, invertase and amylase) and total soluble protein of apterous adults of A. craccivora after treatment with the aqueous extracts of garlic bulbs. Khedr (2002) reported decrease in the activity of GOT and GPT enzymes and increasing of trehalase, invertase and amylase activities as well as in the total soluble protein of S. littoralis (2nd & 4th instars larvae) after treatment with Biorepel. While Chinmix compound caused destruction of epithelial cells, peritrophic and basment membranes in mid-gut larvae of pink and spiny bollworms. On the other hand, Biorepel compound caused separation of epithelial cells from basement membranes and thickness of epithelial cells in the mid-gut larvae of pink bollworm and caused deformed of epithelial cells and degeneration in some points in mid-gut larvae of spiny bollworm.

2- Histological effects of Chinmix, Spintor and Biorepel compounds on larvae of pink and spiny bollworms:

The cross section of pink and spiny bollworms(P. gossypiella & E. insulana) mid-gut larvae treated with Chinmix, Spintor and Biorepel compounds showed certain abnormalities in the internal components of the mid-gut compared with the control; Plats (1:8) Spintor compound caused destruction of the epithelial cells in some points and separation of epithelial cells from basement membranes in the mid-gut larvae of pink and spiny bollworms (P. gossypiella & E. insulana). While Chinmix compound caused destruction of epithelial cells, peritrophic and basment membranes in mid-gut of larvae of the two insects. On the other hand, Biorepel compound caused separation of epithelial cells from basement membranes in some points and thickness of epithelial cells in the mid-gut larvae of pink bollworm and caused deformed of epithelial cells and degeneration in some points in mid-gut larvae of spiny bollworm (P. gossypiella and E. insulana). In respect with previous results it is clear that both Chinmix and Spintor are neurotoxic compounds and their toxic effect associated with hyperactivity. Therefore hyperactivity reflected in the decrease of the digestion of carbohydrates and protein constituent of target insects, while the Biorepel compound effect associated whith bio-compounds.

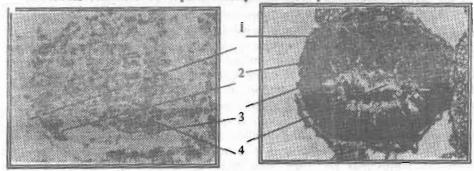
These results partially agree with the findings of Appassy et al. (1984). who found that Cypermethrin caused cytapathological changes in the mid-gut epithelium of pink bollworm such as elongation, vacuolization and detachment of the peritrophic membrane, chromatin clumping, dissolution of cell boundaries and detachment of basement membranes. Saad et al. (1985) studied the effect of Fenvalerate on spiny bollworm larvae in the 4th instars. They reported that damage caused by the treatment was found to be characterized by elongation and vacuolization of epithelial cells detachment of membranes and lysis. Tawfik (1998) reported that histological studies indicated that Vertimec and Neemazal compounds caused distraction for the mid-gut epithelium of the pink and spiny bollworms and affected on the uniform of the epithelial cells. Zidan et al., (1998) indicated that Bacillus thuringiensis (MVP II) caused morphological changes to mid gut epithelium of treated larvae of pink and spiny bollworms. Mohamed (2002) found that plant seed oil extracts, i.e., sunflowers, soybean, castor and cotton caused abnormalities in the tissue of mid gut larvae of P. gossypiella,



Magnification 160x Plate (1): Cross section of mid - gut of P. Plate (2): Cross section of mid - gut of P. gossypilla feeding on diet untreated (control) at newly hatched larvae.

Magnification 160x gossypilla feeding on diet - treated with 0.122 ppm Spintor at newly hatched larvae

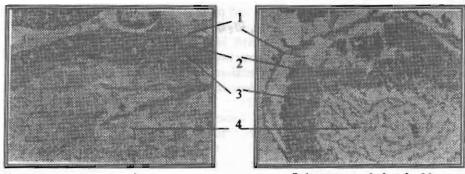
1- Basement membrane 2- Epithelium layer 3- Proitrophic membrane 4- Lumen



Magnification 160x Plate (3): Cross section of mid - gut of P.gossypilla feeding on diet - treated with 0.244 ppm Chinmix at newly hatched larvae

Magnification 160x Plate (4): Cross section of mid - gut of P.gossypilla feeding on diet - treated with 2000 ppm Biorepel at newly hatched larvae.

1- Basement membrane 2- Epithelium layer 3- Proitrophic membrane 4- Lumen

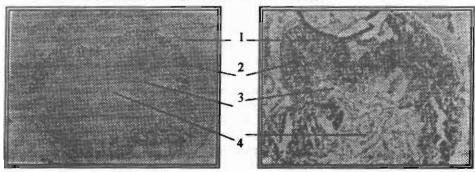


Spintor at newly hatched larvae.

Magnification 160x Plate (5): Cross section of mid - gut of E. insulan feeding on diet - untreated (control) at newly hatched larvae.

Magnification 160x Plate (6): Cross section of mid - gut of E. insulana feeding on diet - treated with

1- Basement membrane 2- Epithelium layer 3- Proitrophic membrane 4- Lumen



Magnification 160x Plate (7): Cross section of mid - gut of E. insulana feeding on diet - treated with 0.244 ppm Chinmix at newly hatched

Magnification 100x Pinte (8); Cross section of mid - gut of E. insulana feeding on diet - treated with 2000 ppm Blorepel at newly hatched

1- Basement membrane 2- Epithelium layer 3- Proitrophic membrane 4- Lumen

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التأثيرات البيوكيمياتية والهستولوجية لمركبات الشنمكس والسبينتور والبيوريل على يرقات ديدان اللوز الشوكية والقرنفلية

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** معهد بحوث وقاية النبات - مركز البحوث الزراعية - دكي - جيزة.

أجرت هذه التجارب لتقييم التأثيرات البيوكيماوية والهمتولوجية لكمل مسن مركب Chinmix ومركب Spintor ومركب Biorepel على يرقات ديدان اللوز الشوكية والقرنفلية. أوضعت النتائج المتحصل عليها في هذه الدراسة أن التأثيرات البيوكيماويه للمركبات الثلاثة المذكورة سببت انخفاضا في نشاط إنزيمات الـ invertase و trehalase و GOT و GPT و GOT كما انخفضت نسبة البروتين الكليي القابل للنوبان أيضا في الحشرات تحت الدراسة. من ناحية أخرى سبب مركب Chinmix ومركب Spintor انخفاضا واضعا في نشاط إنزيمات α- E and β- E في يرقات ديسدان اللوز الشوكية والقرنفلية مقارنة بالكنترول. بينما أعطى مركب Biorepel نتائج مغايرة لذلك. كما أوضحت النتائج المتحصل عليها أيضا أن المركبات الثلاثة تحت الدر اسة أحدثت تغيرات غير طبيعية (تشوهات) في أنسجة الأمعاء الوسطى ليرقات كل من دودة اللوز الشوكية والقرنفلية.