Minufiya J. Agric. Res. Vol.40 No. 1(1): 169 - 178 (2015) "http://www.mujar.net"

EFFECT OF VERTIMEC®, A MICROBIAL INSECTICIDE ON THE PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS). A- TOXICOLOGICAL AND BIOCHEMICAL STUDIES

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(Received Sept., 16, 2014)

ABSTRACT: *The pink bollworm (PBW), Pectinophora gossypiella, is one of the most devastating and invasive insect pests causing immense damage on cotton plants across the world. Adverse effects of microbial insecticide vertimec (product of Streptomyces avermitilis - Avermectin-1.8% w/v) were evaluated against developmental stages of PBW under laboratory conditions. Mortality, LC_{50} and slope values were estimated for egg and larvae, as well as the latent effects of the vertimec LC_{50}. Bioassay tests showed that the hatchability rate of the eggs decreased in dose dependent manner and it could be noted that the vertimec effect was equal to eggs of one and four days old , where the $LC_{50(s)}$ were 127 and 230 ppm, respectively. Other developmental stage, larvae, was also affected by vertimec. The mortality rates of PBW larvae treating as 1st instar increased with the increase of the used concentration and period after treatment, where the LC_{50}-values calculated after 2 and 7 days of treatment were 0.5950 and 0.3455 ppm. In addition, the biochemical response of P. gossypiella was assessed at two larval stages (2nd & 4th instar) after treatment as newly hatched larvae with vertimec at LC_{50}. The main metabolite levels of the two larval ages (total proteins, total carbohydrates, total lipids and glucose) and some vital enzyme activities (amylase, protease, ALT, AST and AchE) were estimated.*

Key words: *Pectinophora gossypiella, pink bollworm, Streptomyces avermitilis, vertimec, biochemical response, toxicity.*

INTRODUCTION

Cotton is attacked by a number of sucking and chewing pests in Egypt. Ecologically, pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gellichidae), is considered as one of the major and important economic pests of cotton. Most of the PBW control programmers in Egypt mostly depend on the use of various synthetic insecticides. Often, the prolonged use of these chemicals has been accompanied by increased resistance. In addition, the release of these chemicals pollutes the environment and affects non-target organisms. The appearance of such problems has been accompanied by growing interest to use new safe bio-insecticide with a new mode of action specially when dealing with water.

Avermectins were originally isolated from the actinomycete *Streptomyces avermitilis*. The insecticidal activity of avermectins has been extensively reviewed (Strong & Brown, 1987). Vertimec (Avermectin B_1) is a member of Avermactins family. It has demonstrated activity against a range of insect pests, especially lepidopterous insects. It has been shown to exhibit growth regulating activity and inhibit feeding (Abo El-Ghar *et al.*, 1994). Putter *et al.* (1981) described the structure formula for vertimec and reported that, this isolate adversely affect the reproduction of some insects. Fritz *et al.* (1979) stated that vertimec blocks postsynaptic potential of neuromascular junctions. It acts on the mediation of neurotransmission by γ-aminobutyric acid (GABA) and glutamate-gated chloride channels leading to paralysis (White *et al.*, 1997).

Therefore, this study aimed to evaluate the toxicity of vertimec against the PBW, in addition to study the sub - lethal effects of the previous compound on some physiological aspects of the insect.

MATERIALS AND METHODS

Rearing of *P. gossypiella*:

Newly hatched larvae of *P. gossypiella* were obtained from a colony maintained in the Bollworms Department Laboratory, Plant Protection Research Institute, Ministry of Agriculture, Dokki, Giza for several generations at 27 ± 1°C and 75 ± 5% relative humidity (RH). Larvae were reared on a modified artificial diet as described previously by Abd El-Hafez *et al.* (1982).

Microbial formulation:

Formulation of *Streptomyces avermitilis*, vertimec [Avermectin 1.8% w/v emulsifiable concentrate; Merck Sharp & Dohme Research Laboratory (MSDRL)] was used in the present investigation. It contains at least 80% of avermectin B_{1a} (C_{48} H_{72} O_{14}, MW: 872) and not more than 20% of avermectin B_{1b} (C_{47} H_{70} O_{14}, MW: 858).

Insecticidal activity:

To assess the insecticidal activity of the tested compound, a stock solution of the tested compound was prepared by diluting the formulated compound with distilled water to obtain serial aqueous concentrations ranging from 1.25-10 ppm & 0.062-4.0 ppm, for eggs (1- & 4- days old) and newly hatched larvae, respectively.

Eggs cards were dipped in each concentration level for 5 sec., then left to dry at room temperature. Three replicates were used for each concentration and hatchability% was recorded. The same number of replicates and eggs considered as a control (dipped in water only for 5 sec).

For larvae, seven serial aqueous concentrations ranging from 0.062 to 4.0 ppm of vertimec were tested against newly hatched larvae of PBW. One ml of each tested aqueous concentration was homogenate mixed with 50 g of fresh PBW artificial diet not containing the antimicrobial agents. After preparation of tested diets, each one was individually folded into 3 Petri dishes (9 cm in diameter). Ten healthy newly hatched larvae of PBW were gently transferred on the surface of the diet using a soft hair brush. Another group of 3 Petri dishes was prepared containing the normal diet mixed with the same volume of distilled water (used as control) and similar number of the maintained larvae was placed on their surfaces. All dishes were maintained in an incubator at 27 ± 1°C and 75 ± 5% relative humidity (RH). After two days from exposing the first instar larvae on the bioinsecticide – contaminated diet or the free one, any alive larvae of each replicate/ concentration were transferred individually into clean glass vials (2 X 7 cm) containing small piece of normal diet. Vials were plugged with absorbent cotton and incubated at the same conditions. The acute toxicity of the tested bioinsecticide was assessed after 2 days. Latent or chronic toxicity was determined by inspecting all the tubes for mortality after 7 days post treatment. Percentages of mortalities were corrected according to Abbott's formula (Abbott, 1925). The data were then subjected to probit analysis (Finney, 1971) through software Computer program to obtain the LC_{50} and slope values.

The latent effect of vertimec LC_{50} on *P. gossypiella*

Biochemical determination:

Newly hatched larvae were allowed to feed on artificial diet treated with the vertimec LC_{50} for 2 days and then transferred to feed on untreated diet. Seven days after treatment,half of the tested larvae were collected, counted, weighted and frozen (at –20°C) for biochemical analysis. The other half of larvae was left to complete their development at the same conditions. Fourteen days after treatment, the survived larvae were counted, weighted and frozen (at –20°C) for biochemical analysis. The total larval bodies (7 and 14 days old) were homogenized in distilled water using a chilled glass-teflon tissue grinder for 3 min. Homogenates were centrifuged at 3500 rpm for 10 min at 5°C and the supernatants were kept in deep-freezer till the biochemical determinations. The optical densities (OD) were read spectrophotometrically by spectronic 1201 (Milton Roy Co., USA) and centrifugation was carried out by a refrigerated centrifuge (Gs-6r, Beckman, USA).

Total proteins were determined by the method of Bradford (1976) and total lipids were estimated by the method of Knight *et al.* (1972). Total carbohydrates were extracted as described by Crompton & Brit (1967), and were determined in acid extracts by the phenol sulfuric acid reaction (Dubois *et al.*, 1956). Glucose was estimated by the method of Barham & Trinder (1972). Amylase (EC 3.2.1.1) activity was estimated by the method of Ishaaya & Swiriski (1976), while the protease activity was determined by the casein digestion method according to Brik *et al.* (1962). Alanine transaminase (ALT, EC 2.6.1.2) and Aspartate transaminase (AST, EC 2.6.1.1) activities were determined calorimetrically according to the method of Reitman & Frankel (1957). Acetylcholinesterase (AchE) (EC 3.1.1.7) activity was measured according to the method described by Simpson *et al.* (1964) using acetylcholine bromide (AchBr) as substrate.

Statistical analysis:

Toxicological data were statistically calculated through a Proban program, software computer program (Jedrychowski, 1991). The variability in response to the tested materials was determined based on LC_{50} and slope value. Analysis of variance (ANOVA) was conducted on all data using Costat computer program software. Means were compared by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

1-Insecticidal activity:

Egg stage:

Data presented in Table (1) indicated that the egg hatchability rates of the studied insect were decreased by increasing the insecticide concentrations. The highest hatchability percentages were observed with 1- and 4-old day eggs resulting 91.32 and 91.33%, respectively at 1.25 ppm, while it recorded 79.28 and 81.20%, respectively at 10 ppm. Also, it could be noted that the vertimec effect was lower in1-day than 4-old day eggs where the $LC_{50(s)}$ were 127 and 230 ppm, respectively.

Many authors reported that blockage of insect embryonic development occurred both when the avermectin was applied directly to the egg stage soon after oviposition or later (Mujica *et al.*, 2000 and Abo-El-Saad *et al.*, 2013). Likewise, these results make promising advantageous for PBW control programs. So, when female PBW would lay eggs on vertimec treated plants, the contact effects on eggs would inhibit embryonic development and therefore stop new infestation. On contrast, (Kathuria *et al.*, 2000) reported that the abamectin has no effect on the early stages of embryo development.

Table (1): Insecticidal activity of vertimec against1- and 4-day old eggs of the pink bollworm, *P. gossypiella*

Concentration (ppm)	% hatchability	LC_{50} (95% confidence limits)	Slope ± SE
1-day old			
1.25 2.50 5.00 10.0	91.32 88.67 84.73 79.28	127 (30-2936)	0.88 ± 0.28
4-day old			
1.25 2.50 5.00 10.0	91.33 88.93 85.64 81.20	230 (0.004 – 3921400)	0.77 ± 0.28

Larval stage:

Data (Table 2) showed that vertimec exhibited potential effects against neonate larvae. Results indicated that, the mortality rates increased with the increase of the used concentration and period after treatment (Table 2). The corrected mortality percentages after 2 days of treatment ranged from 24.0 % by using the lowest concentration (0.062 ppm) to 83.4 % by using the highest concentration (4.0 ppm). After 7 days of treatment, the corrected mortality percentages ranged between 31.73-88.74%. Also, Table (2) summarized LC_{50} and slope value of vertimec tested against the newly hatched larvae of PBW after 2 and 7 days of treatment. The LC_{50} = 0.5950 ppm (b= 1.0764) after two days of treatment. Furthermore, seven days after pesticide treatment these values decreased to 0.3455 ppm (b= 1.0615). Regarding to the cumulative mortality as deposited in Figure (1) it could be noted that at all tested concentrations, the highest percentage of mortality occurred within the first two days following treatment then the mortality continued at difference rates among larvae after they had transferred to feed on untreated diet for another 5 days. At the highest concentration (4.0 ppm), 83.40% of these larvae died after 2 days and 5.34% died within the later 5 days whereas the corrected mortality percentage reached 88.74%, while at the lowest concentration (0.062 ppm) it was 24.0 % after 2-days and it increased to reach 31.73%. So, the obtained results clearly indicate that vertimec is toxic to PBW larvae as compared to control. Vertimec has been reported to possess excellent performance as spectrum microbial insecticides both in field or laboratory (Sheeba, 2010 and El-Naggar, 2013). The toxic activity of vertimec differed according to the treated insects and the application technique.

Table (2): Insecticidal activity of vertimec after 2 and 7 days following treatment against the newly hatched larvae of pink bollworm, *P. gossypiella*.

Concentration (ppm)	% Corrected mortality	LC_{50} (95% confidence limits)	Slope ± SE
2- days after treatment			
0.062	24.00	0.5950 (0.482-0.742)	1.0764 ± 0.0900
0.125	31.73		
0.250	41.49		
0.500	52.62		
1.000	64.03		
2.000	74.59		
4.000	83.40		
7- days after treatment			
0.062	31.73	0.3455 (0.1279 – 0.7418)	1.0615 ± 0.2260
0.125	40.81		
0.250	51.34		
0.500	62.39		
1.000	72.85		
2.000	81.81		
4.000	88.74		

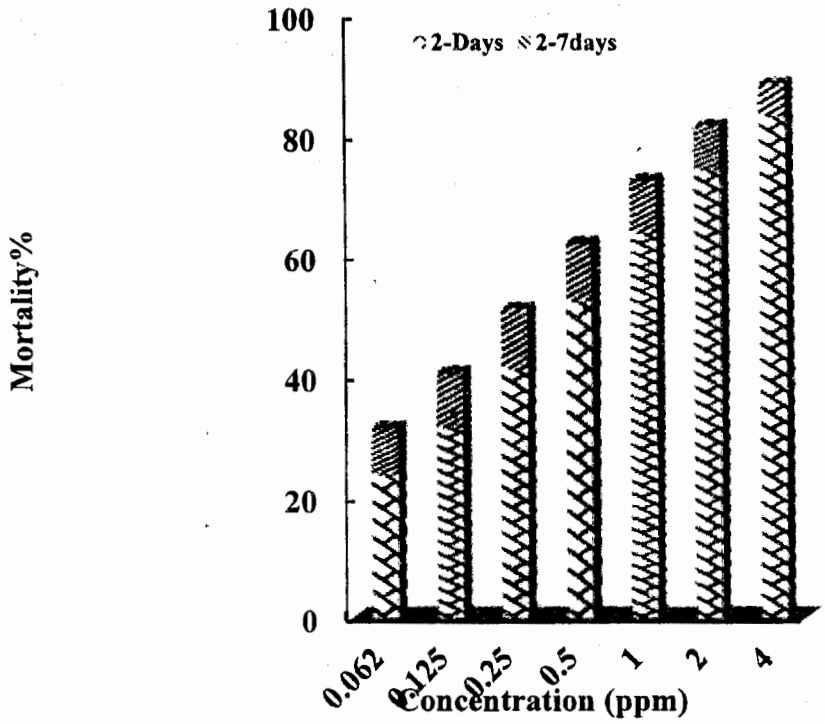

Fig (1): Cumulative mortality of *P. gossypiella* larvae after 2- and 7-days of treatment with vertimec

2- Effect of vertimec on some biochemical aspects of PBW:

2.1. Effects on main metabolites:

The main metabolites (total proteins, total carbohydrates and total lipids) are major biochemical components, which are necessary for organism to develop, grow and perform its vital activities. In the control insects, it could be noted that the total proteins and lipids contents increased with the growing of larvae. Contrarily, up growth causing decreased in the levels of carbohydrates and glucose. It could be due to the conversion of carbohydrates to lipids or protein because of that adult female insect requires a lipid to fly and protein to reproduction. Regarding to vertimec treatment, total lipids was the only parameter that increased with larval growth while the others were decreased (Table 3).

The obtained results also indicated that the content of total proteins in 2nd larval instar significantly increased when larvae were fed on diets containing LC_{50} of vertimec (44.08 mg/g BW) than of control (26.09 mg/g BW). These levels were significantly above that of control by 68.95%. These changes suggest a potentially important role for post-ingestive compensatory mechanisms in insects, such as the secretion of more proteases (Broadway & Duffey, 1986), or may be due to certain effects on the enzymes that are responsible for protein synthesis. On the contrary, when the larvae reached the full-grown, vertimec caused significant decreased in the total proteins content (41.30 mg/g BW). This value was lower that of control by 11.03%. This could be due to that the proteins are among the most important compounds of insects that bind with foreign compounds (Ahmed & Forgash, 1976).

Feeding newly hatched larvae earlier on diet treated with LC_{50} concentration of vertimec, significantly decreased the total lipids concentration in 2nd instar larvae to

53.74 mg/g BW; i.e. 12.20% below that reported in control (61.21 mg/g BW) while in the 4th instar, the means of total lipids was increased significantly than control by 96.35%. These means were 498.23 & 253.74 mg/g BW, respectively (Table 3).

In addition, results in Table (3) reported that vertimec didn't affect the mean total carbohydrates content in 2nd instar larvae. In contrary, this value in the 4th instar was above control by 12.57%. The means were 8.65 & 5.57 mg/g BW for 2nd & 4th instar of control and 8.83 & 6.27 mg/g BW for 2nd & 4th instar of vertimec, respectively.

As for glucose level, data showed that vertimec decreased significantly the glucose level in 2nd instar larvae (3.76 mg/g BW) below that of the control by 6.70%. On the other hand, glucose level in 4th instar larvae (3.43 mg/g BW) was above that of the control (3.25 mg/g BW) by 5.54% (Table 3).

2.2. Effects on the activity of some vital enzymes:

The changes in biochemical composition of treated larvae may be due to certain effects on the enzymes that are responsible for protein, lipid and carbohydrates synthesis. In this respect, our results indicated that the activity of the enzymes (except protease) decreased with the growing of control larvae. Almost the same trend was achieved in the case of vertimec treatment except for AchE which not differed with larval growing (Table 4).

Table (3). Effect of LC_{50} concentration of vertimec on certain total body contents (mg/g BW) of 2nd and 4th instar larvae of *P. gossypiella*

Treatment	Proteins		Lipids		Carbohydrates		Glucose	
	Mean ± SD	Change %*	Mean ± SD	Change %	Mean ± SD	Change %	Mean ± SD	Change %
2nd stage larvae								
Control	26.09^{Bb} ± 2.03	—	61.21^{Ab} ± 4.02	—	8.65^{Aa} ± 0.11	—	4.030^{Aa} ± 0.007	—
Vertimec	44.08^{Aa} ± 2.38	+68.95	53.74^{Ab} ± 3.84	-12.20	8.83^{Aa} ± 0.98	+2.08	3.76^{Ba} ± 0.002	-6.70
LSD (treatment)	5.02		8.91		1.59		0.01	
4th stage larvae								
Control	46.80^{Aa} ± 1.00	—	253.74^{Ba} ± 2.98	—	5.57^{Bb} ± 0.02	—	3.25^{Bb} ± 0.003	—
Vertimec	41.30^{Ba} ± 0.72	-11.03	498.23^{Aa} ± 2.29	+96.35	6.27^{Ab} ± 0.31	+12.57	3.43^{Ab} ± 0.02	+5.54
LSD (treatment)	1.97		6.27		0.50		0.04	
LSD Control	3.63		8.24		0.17		0.01	
Vertimec	3.84		7.16		1.66		0.03	

Means within columns with the same letter (s) are not significantly different at 5% level of probability.
- Capital letter treatment
- Small letter ages
* Change % = treatment – control / control x 100

Table (4). Effect of LC_{50} concentration of vertimec on certain enzyme activities* of 2nd and 4th instar larvae of *P. gossypiella*.

Treatment	Amylase		Protease		ALT		AST		AchE	
	Mean ± SD	change %**	Mean ± SD	change %	Mean ± SD	change %	Mean ± SD	change %	Mean ± SD	change %
2nd instar larvae										
Control	0.678^{Aa} ± 0.031	—	0.467^{Aa} ± 0.040	—	0.192^{Ba} ± 0.003	—	0.130^{Ba} ± 0.010	—	0.097^{Ba} ± 0.11	—
Vertimec	0.639^{Aa} ± 0.090	-5.75	0.598^{Aa} ± 0.074	+28.05	0.524^{Aa} ± 0.014	+172.92	0.246^{Aa} ± 0.015	+89.2	0.400^{Aa} ± 0.044	+312.37
LSD	0.152		0.135		0.023		0.029		0.071	
4th instar larvae										
Control	0.229^{Bb} ± 0.037	—	0.422^{Aa} ± 0.051	—	0.130^{Ab} ± 0.017	—	0.080^{Ab} ± 0.003	—	0.075^{Bb} ± 0.006	—
Vertimec	0.463^{Ab} ± 0.035	+102.18	0.370^{Ab} ± 0.051	-12.32	0.162^{Ab} ± 0.017	+23.08	0.062^{Bb} ± 0.001	-22.50	0.355^{Aa} ± 0.019	+373.33
	0.081		0.116		0.038		0.005		0.031	
LSD Control	0.08		0.10		0.03		0.02		0.02	
Vertimec	0.15		0.14		0.04		0.02		0.08	

Means within columns with same letter (s) are not significantly different from each other at 5% level of probability.

- Capital letter treatment
- Small letter ages

* Enzyme activities are given as enzyme units as follows: Amylase unit; mg glucose/g BW, Protease unit; OD/min/g BW, ALT unit; mg puryvate/min/g BW, AST unit; mg puryvate/min/g BW, AchE unit; mg AchBr/min/g BW.

** Change % = treatment – control / control x 100

In case of 2nd instar, data in Table (4) indicated that vertimec did not affect amylase activity. The means of amylase activity were 0.678 & 0.639 µg glucose/g BW for control & vertimec, respectively. The activity of amylase in untreated 4th instar larvae decreased to 0.229 µg glucose/g BW. Treatment with vertimec (0.463 µg glucose/g BW) caused significant increase in amylase activity than control by 102.18%. Vertimec did not cause significant variances in the protease activity than control in both ages. The means were 0.467 & 0.422 OD/min/g BW for control and 0.598 & 0.370 OD/min/g BW for vertimec, respectively.

The obtained results in Table (4) show that vertimec treatment caused a significant increase in the activity of ALT in 2nd instar larvae of PBW when treated as newly hatched larvae with vertimec LC_{50}. The mean was 0.524 mg pyruvate/min/g BW and it exceeded that of control (0.192 mg pyruvate/min/g BW) by 172.92%. Meanwhile, when larvae became older, data showed that there is no significant difference between control (0.130 mg pyruvate/min/g BW) and vertimec treatment (0.162 mg pyruvate/min/g BW).

As for AST activity, treatment with vertimec significantly elevated in the activity of AST (0.246 mg puryvate/min/g BW) than control (130.24 mg puryvate/min/g BW) by 89.23%. When larvae became older (full-grown) the activity of AST decreased in case of control (0.08 mg puryvate/min/g BW) and the tested compound (0.062 mg puryvate/min/g BW). It could be noted that vertimec caused significance decrease in the level of AST below that of control by 22.5%.

Rizk (1998) found that vertimec compound affected on the enzymes weakly in total homogenates of *P. gossypiella* full-grown larvae (-0.26 and –3%).

Generally, according to the results of this study, and the study done in sequence by Ahmed (2014) , vertimec proves to be an effective bio-insecticide against developmental stages of PBW and can be a possible candidate to be applied on cotton plants by the Ministry of Agriculture after successful field experiments.

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تأثير المبيد الحيوي فيرتيمك على دودة اللوز القرنفلية *Pectinophora gossypiella*
أ- دراسات سمية وبيوكيميائية

دينا عبد الظاهر أحمد

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي- جيزة

الملخص العربى

تعد دودة اللوز القرنفلية واحدة من الآفات الحشرية الأكثر تدميرا لنباتات القطن حول العالم. يهدف هذا البحث لدراسة التأثيرات السمية والسلبية للمبيد الحيوي فرتيمك ضد الأطوار غير الكاملة لدودة اللوز القرنفلية تحت الظروف المعملية، حيث تم تقدير التركيز السام النصفي (LC_{50}) للفيرتيمك ضد طوري البيض واليرقات بالإضافة إلي التأثيرات المتأخرة لهذا التركيز.

أفادت النتائج بزيادة معدل الخفض في فقس البيض مع زيادة التركيز المستخدم من الفيرتيمك ولوحظ أيضا تساوي التأثير علي كلا عمري البيض تحت الدراسة. أما بالنسبة للعمر اليرقي الأول فقد ارتبطت معدلات الموت أيضا بزيادة التركيز المستخدم والفترة بعد المعاملة حيث كانت قيم التركيز السام النصفي (LC_{50}) المسجلة بعد يومين وسبع أيام علي التوالي هي ٠,٥٩٥، ٠,٣٤٥٥ جزء في المليون.

أيضا تم تقييم الاستجابة البيوكيميائية ليرقات العمر الثاني والرابع لدودة اللوز القرنفلية بعد معاملة الفقس الحديث بالتركيز السام النصفي للفيرتيمك،حيث تم تقدير نواتج الأيض الرئيسة (البروتينات الكلية، الكربوهيدرات الكلية، الليبيدات الكلية والجلوكوز)، بالإضافة لنشاط بعض الإنزيمات الحيوية (الاميليز، البروتييز، ALT, AST و الأسيتيل كولين استيريز).

وقد أشارت النتائج إلي ارتفاع المحتوي الكلي للبروتينات في يرقات العمر الثاني نتيجة المعاملة بالفيرتيمك وذلك مقارنة بالكنترول، في حين انخفض محتوي الحشرة من الليبيدات نتيجة المعاملة.وعلي العكس من ذلك فعند وصول اليرقات للعمر الرابع انخفض محتوى البروتينات وارتفع محتوى الليبيدات في اليرقات المعاملة مقارنة بالكنترول. هذا ولم تؤثر المعاملة بالفرتيمك علي محتوى العمر اليرقي الثاني من الكربوهيدرات الكلية في حين ارتفع هذا المنسوب في يرقات العمر الرابع مقارنة بالكنترول. وعند دراسة تأثير المعاملة بالفيرتيمك على محتوى الجلوكوز فقد لوحظ تغييرات طفيفة سواء بالزيادة أو النقصان في محتوى الجلوكوز في كلا العمرين اليرقيين مقارنة بالكنترول.

أما بالنسبة للأنزيمات محل الدراسة فقد لوحظ انخفاض نشاطها (فيما عدا البروتييز) وذلك مع نمو اليرقات وبلوغها العمر الرابع وذلك بالنسبة لليرقات غير المعاملة، كما أدت المعاملة بالفرتيمك للوصول إلي نفس النتيجة فيما عدا في حالة الأسيتيل كولين استيريز حيث لم يحدث أي تغير في محتواه مع نمو اليرقات.