THE EFFECT OF CERTAIN BIOCONTROL AGENT ON SOME BIOLOGICAL ,BIOCHEMICAL AND HISTOLOGICAL ASPECTS OF THE COTTON LEAF WORM *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

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(Manuscript received 28 July 2010)

Abstract

The potency of three commercial bio-insecticides, Protecto namely, *Bacillus thuringiensis* var. *kurstaki*, Viruset, *Spodoptera littoralis* Nuclear Polyhydrosis Virus, SLNPV and their mixture Profect were evaluated against the cotton leaf-worm, *Spodoptera* littoralis(Boisd.) (Lepidoptera: Noctuidae). Protecto proved to be more toxic on both 2nd instar larvae than the two other tested bioagents, Viruset was more effective on 4th instar larvae than Profect. LC_{90} and LC_{50} for Viruset were $1x10^6$ and $1x10^3$ PIBs/ml, respectively, corresponding values were $5x10^8+1.6x10^7$ and $5x10^4 + 1.6x10^3$ PIBs/ml + IU/ml when Profect was tested. The tested bioagents reduced larval duration, percentages of larval pupation as well as adult emergence of *S. littoralis* fed as 2nd instar larvae on castor oil leaves treated with LC_{50} .The digestive enzymes amylase and invertase and trehalase were determined in 6th instar larvae surviving treatment of 2nd instar larvae with LC_{50} of the tested three bioagents. Amylase activity in treated larvae was found to be significantly higher in all treatments . Similarly, except in case of Viruset, invertase activity was increased. Meanwhile, the three tested compounds caused a significant decrease in trehalase activity.Midgut histological sections were carried out on 6th instar larvae treated as 4th instar larvae with LC_{50} of the three tested compounds. Profect was the most effective product in causing aberrations in the midgut layers, following by Protecto and Viruset.

Key words: Cotton leaf- worm, *Spodoptera littoralis*, *Bacillus thuringiensis* var. *kurstaki,* Nuclear Polyhydrosis Virus, digestive enzymes, midgut histopathology., insectcidal actievities.

INTRODUCTION

The entomopathogenic bacteria,*Bacillus thuringiensis* represents a good example for biological controlof insect pests. This bacterium, proved to be a highly successful for controlling some agricultural insect pests (Mohamed *et al*., 2005).

The present study was undertaken to evaluate the use of three bioagents, the bacteria (*Bacillus thuringiensis* var. *kurstaki*) "Protecto", the (*Spodoptera littoralis* Nuclear Polyhydrosis Virus, SLNPV) "Viruset" and a commercial mixture of both

"Profect", for the control of *S. littoralis* (Boisd). The study was mainly concerned with the evaluation of these compounds toxicity and their bioassay on *S. littoralis* larvae as well as their effects on larval growth and development.

A histopathological study was also conducted in the midgut of 6^{th} instar larvae surviving treatment. Furthermore, the activity of three carbohydrates enzymes was evaluated in larvae treated as 4^{th} instars.

MATERIALS AND METHODS

1. Rearing technique:

A stock culture of the cotton leaf worm, *S. littoralis* was obtained from a laboratory strain maintained at the Cotton Pest Research Department.Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, for several generations without any insecticidal pressure. The insect was reared on castor-oil leaves, *Ricinus communis,* under laboratory conditions at 25±2°C and 60 ± 5% R.H.

2. Tested compounds:-

The potency of the three tested bioagents were evaluated for their effect on *S. littoralis* larvae:- *Bacillus thuringiensis* var. *kurstaki* (Protecto) *Spodoptera littoralis* Nuclear Polyhydrosis Virus (SLNPV),(Viruset)and their binary mixture (Profect). The three mentioned microbial agents were obtained from Plant Protection Research Institute Biopesticide Unit Production.

3. Bioassay:-

The insecticidal activities of the three tested bioagents were assessed on newly ecdysed 2^{nd} instar *S. littoralis* larvae.

A series of dilutions were prepared from 1 gm of the product obtained as wettable powder, $6.4x10^6$, $3.2x10^5$, $3.2x10^4$, $3.2x10^3$, $3.2x10^2$ and 3.2x10 IU/ml of Protecto. (IU= International Unit)., $1x10^7$,$1x10^6$,$1x10^5$,$1x10^4$,$1x10^3$, $1x10^2$ and 1x10 PIBs/ml. (PIBs= Polyhedral Inclusion Bodies)of Viruset and $5x10^8+1.6x10^7$, $5x10^6+1.6x10^5$, $5x10^5+1.6x10^4$, $5x10^4+1.6x10^3$, $5x10^3+1.6x10^2$, $5x10^2+1.6x10$ and 5x10 +1.6 PIBs/ml+ IU/ml of Profect.

Treatment of larvae was conducted by the leaf dipping technique, Mortality was recorded daily and accumulative larval mortality was determined at the end of the larval stage. The mortality percentages were corrected by Abbott's formula (Abbott, 1925). Results were illustrated graphically as log/probit regression lines, and toxicity LC_{90} and LC_{50} values as well as the slope were obtaind according to Finney, (1971).

4. Biological studies:

Anewly ecdysed 2nd instars larvae were offered castor oil leaves treated with the determined LC_{50} of each of the tested compounds for 24 hr., and then reared on untreated leaves. The number of treated larvae was 20 larvae placed in sets of five and each experiment was replicated 3 times. The following biological aspects were determined Larval duration, percentage pupation, pupal weight, pupal stage duration, adult emergence and life span of adult moths.

5. Biochemical studies:-

The activity of three carbohydrate enzymes :- invertase, amylase and trehalase was determined in 6th instars larvae surviving treatment of 2nd instars with LC_{50} of each the three tested compounds, as described by Ishaaya and Swirski, (1970) and Ishaaya *et al.,* (1971).

6. Histopathological studies

The effect of each of the three tested compounds at LC_{50} values was studied on the cellular structure of the mid gut of 6th instar larvae surviving treatment of 2nd instars larvae with the LC_{50} values. Larvae were dissected in Ringer solution and their mid guts rapidly removed and placed in aqueous Bouin's solution for 24 hrs. for fixation. Specimens were washed in water, dehydrated in a series of ethyl alcohols and cleared in xylene; paraffin wax embedding procedure was then followed. Cross sections were cut at 6µ thickness, using a microtome, and mounted on glass slides. Sections were stained with Heamatoxylin and counterstained in alcoholic aqueous Eosin for microscopic examination. Similarly, sections of the mid gut of non-treated larvae were also prepared for comparison.

3.7. Statistical analysis:-

Statistical analysis (ANOVA) of the obtained data was performed by using COSTAT program, which run under WIN. Also the difference between means was conducted by using Duncan's multiple range tests in this program.

RESULTS

1. Insecticidal activities:-

The efficiency of the, three bioagents were evaluated on 2nd instars larvae of *S littoralis* (Boisd.).The results indicated that Protecto (*Bacillus thuringiensis* var. *kurstaki*) gave the highest larvicidal activity as compared to the other two bioagents. The LC_{90} and LC_{50} for 2nd instar larvae were $6.4x10^6$ and $3.2x10^2$ IU/ ml. respectively (Table1). The slope values were 0.37 and 0.31 for 2nd instars larvae, respectively, which proved the homogeneity of the tested larvae .

The bioagent Viruset (*Spodoptera littoralis* Nuclear Polyhydrosis Virus, SLNPV) exhibited an intermediate toxic effect to treated larvae, while the bioagent Profect which is a mixture of *Bt* and the SLNPV proved to be the least toxic to both treated larval instars as depicted by the calculated LC values.Also the determined LT_{50},s of the tested bioagents recorded 12.02, 12.47 and 14.12 days to the treatments by Protecto, Viruset and Profect, respectively (Table 1) .

Table 1. Susceptibility of *Spodoptera littoralis* 2nd instar larvae to three bioinsecticides.

Compound	Unit	LC_{90}	LC_{50}	Slope	LT_{50}	Slope
Protecto	IU/ ml	$6.4x10^6$	$3.2x10^2$	0.37	12.02	2.49
Viruset	PIBs/ml	$1x10^7$	$1x10^2$	0.19	12.47	5.24
Profect	PIBs/ml +IU/ml	$5x10^8$+ $1.6x10^7$	$5x10^3$+ $1.6x10^2$	0.33	14.12	9.53

2. Biological effects of the three tested bioagents on *S. littoralis* (Boisd.) treated as 2nd instars larvae:

2.1. Effects on the development of *S. littoralis*:-

The result in Table 2 indicated that there was no effect on the larval duration as the result of treatment the *S. littoralis* 2nd instars by LC_{50} of the three tested bioagents recorded 14.5±0.4 ,15±0.4 ,15±0.3 days for Protecto ,Viruset and profect respectively compared to 15±0.4 days in control . However, treatment with Protecto insignificantly slightly shortened the larval duration than the control by 0.5 day (Table 2).

The percentage of larvae treated as 2nd instars with LC_{50} of the three bioagents metamorphosing to pupae was markedly reduced to approximately half the value of their control (Table 2). Pupation percentage of 47.5 , 50 and 52.2%, recorded when Protecto ,Viruset and Profect were tested respectively.

It is noteworthy, that the weight of the apparently normal appearing pupae soon after pupation developing from treated larvae was comparable to the control and no differences were found recorded a mean of 0.35mg.

Under conditions of the present work, in untreated insects the pupal stage lasted 13.6 days, this period was shortened to 11.3 days ; i.e. less by 2.3 days ,when LC_{50} of Protecto or Viruset was used and to 12.6 days with the application of Profect i.e. less by 1 day. These differences were found to be statistically significant (Table 2).

Percentage of adult ecolosion was affected as result of treatment of 2nd instar larvae with LC_{50} of the three bioagents, it was less than the control by 37.4%, 40% and 42%

when LC_{50} of Protecto, Viruset and Profect were tested, respectively (Table 2) . Life span of moths was an average of 12.67, 13.67 and 16 days to the respective mentioned bioagents, as compared to 12.33 days in untreated insects.

Table 2. Percentages of pupation, mean pupal duration and adult emergence % of *S. littoralis* fed as 2nd instar larvae on castor oil leaves treated with LC_{50} of Protecto, Viruset and Profect.

Compound	Mean larval duration (days ± S.E.)	Pupation %	Pupal stage duration (days ±S.E.)	% Adult emergence
Protecto	14.5±0.4 [a]	47.5 [c]	11.3±0.3 [c]	79 [b]
Viruset	15±0.4 [a]	50 [bc]	11.3±0.6 [c]	80 [b]
Profect	15±0.3 [a]	52.2 [b]	12.6±0.1 [b]	80.9 [b]
Control	15±0.2 [a]	100 [a]	13.6±0.3 [a]	100 [a]
F values	4.836 *	293.594 ***	21.34 ***	60.3636 ***
L.S.D.	1.25	4.20605	0.67	4.3028

Means with the same letter are not significantly different ($p<0.05$).

3. Biochemical studies:

The effects of LC_{50} of Protecto, Viruset and Profect on the activities of three carbohydrate digestive enzymes in *S. littoralis* 6th instar larvae treated as 2nd instars larvae was estimated (Table 3). In untreated larvae amylase activities were found to be 205.4 µg glucose/min /gm. Following treatment with LC_{50} of Protecto, Viruset or Profect, the activity of amylase was significantly increased to 330.5, 209.8 and 285 µg glucose/min /gm., respectively.

Invertase activity was 478.2 µg glucose/min /gm. in untreated larvae this level was relatively unaffected in treated larvae with LC_{50} of Viruset i.e. 474.2 µg glucose/min /gm. Meanwhile, when Protecto or Profect were used at LC_{50} values, invertase activity was significantly decreased to 405, and 432 µg glucose/min /gm., respectively.

Meanwhile, trehalase activity was highly significantly decreased from 313.5 in untreated larvae to 303.2, 196.9 and 262 µg glucose/min /gm. in larvae treated with Protecto, Viruset and Profect, respectively.

Table 3. Activity of amylase, invertase and trehalase in 6th instar larvae of *S. littoralis* treated with LC_{50} of Protecto, Viruset and Profect as 2nd instar larvae.

Treatments	Mean carbohydrases activity µg glucose/min /gm. ±S. E.		
	Amylase	Invertase	Trehalase
Protecto	330.5 ±0.2 [a]	405.0 ± 1.2 [c]	303.2 ± 0.1 [b]
Viruset	209.8 ±0.2 [d]	474.2 ± 0.1 [a]	196.9 ± 0.5 [e]
Profect	285 ±0.6 [c]	432 ±0.3 [b]	262 ±0.2 [c]
Control	205.4 ±0.2 [e]	478.2 ±0.1 [a]	313.5 ±0.3 [a]
F values	1760.026***	4558.533***	7798.393***
L.S.D	4.175	4.41	1.883

Means with the same letter are not significantly different ($p<0.05$).

4. Histopathological studies:-

The histological structure of midgut in larvae of Lepidoptera is well documented **(Chapman, 1988).** As seen in Fig.1 the mid gut is lined with an epithelial layer, which rests on a basement membrane, and is composed of a single layer of three types of cells. (i) A majority of columnar cells containing a large coarse nucleus which occupies a middle position within the cell and bears a striated or brush-like border (microvilli). (ii) Goblet cells; which are somewhat calyx-shaped and are seen between the columnar cells; each of these cells has a large ampulla opening by a narrow neck through a small aperture on the inner surface. (iii) Regenerative cells are small in size and rest on the basement membrane between the bases of the other cells, and are round or elongated and contains a large nucleus surrounded by a small amount of strongly basophilic cytoplasm.

Within the midgut lumen, there is a thin peritrophic membrane, that surrounds the food mass. A musculosa surrounds the epithelial layer, composed of an inner circular layer and an outer layer of longitudinal muscle.

The histological structure of 6th instar larvae surviving their treatment with LC_{50} of the three bioagents as 2nd instar larvae appears somewhere impaired. Induced cellular changes were relatively similar when either of the bioagents were tested. However, they were more enhanced where Profect was tested. Generally, the changes in the histological structure of midgut could be summarized as follows:-

i. The lumen of the gut appears somewhere collapsed and globular bodies and cytoplasmic fragments were observed pinching off from the tip of some of the epithelial cells vicinal to the deteriorated peritrophic membrane. This observation was most apparent when Protecto was tested. (Fig.2).

ii. The muscularis layers lose their compact appearance, and the loss of many circular muscles was evident. The peritrophic membrane appears somewhat deteriorated, and the striated border together with the regenerative cells was obliterated when larvae were treatment with Viruset (Fig.3).

iii. Vacuolization in the midgut epithelium and disruption of both the peritrophic membrane and the striated boarder were evident. Some of the degenerated columnar cells were fused with the disrupted peritrophic membrane. This observation was more evident when larvae were treatment with LC_{50} of Profect (Fig.4).

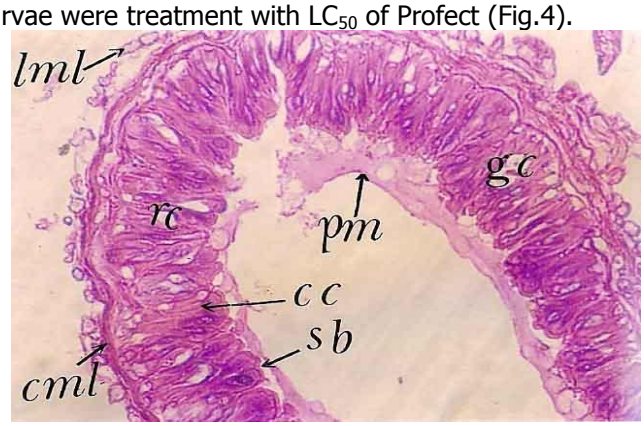

Fig. 1.T. S. in the mid gut of untreated 5 days old 6th instar *S. littoralis* larvae (X 160).

cc: columnar cell.

cml: circular muscle layer**.**

gc: goblet cell.

lml: longitudinal muscle layer.

pm : peritrophic membrane**.**

rc: regenerative cell**.**

Sb: striated border**.**

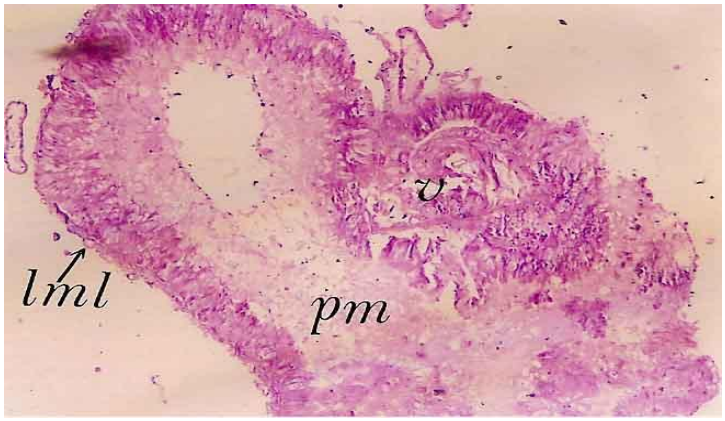

Fig . 2. T. S. in the mid gut of 5 days old 6th instar *S. littoralis* larvae treated with LC_{50} of Protecto as 4th instars (X 400).

lml: longitudinal muscle layer.

pm : peritrophic membrane**.**

V: vacuole.

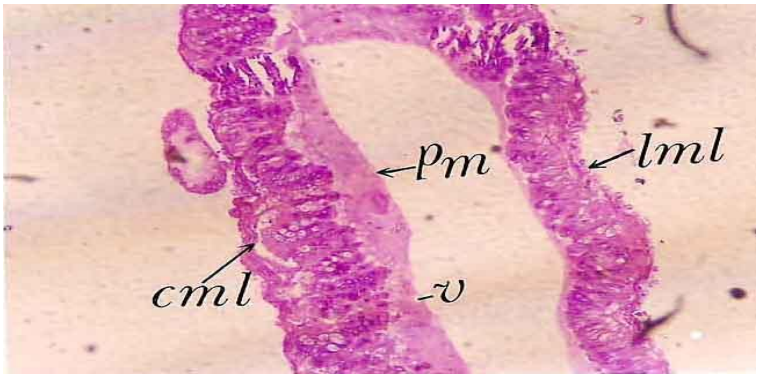

Fig . 3. T. S. in the mid gut of 5 days old 6th instar *S. littoralis* larvae treated with LC_{50} Viruset as 4th instars (X 400).

cml: circular muscle layer**.**

lml: longitudinal muscle layer.

pm : peritrophic membrane.

V: vacuole.

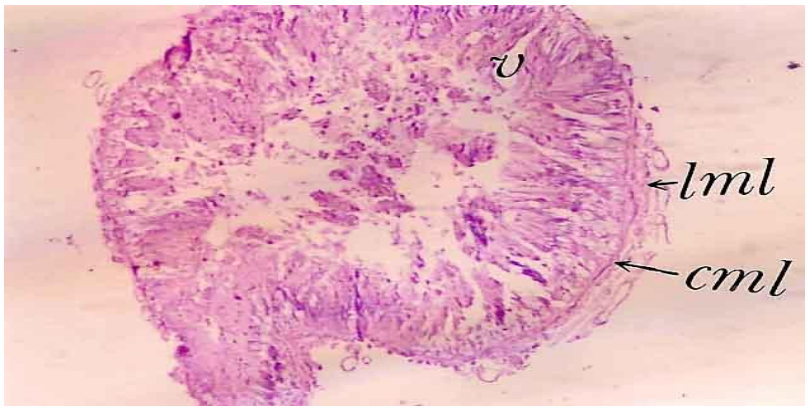

Fig . 4. T. S. in the mid gut of 5 days old 6th instar *S. littoralis* larvae treated with LC_{50} of Profect as 4th instars (X 400).

cml: circular muscle layer**.**

lml: longitudinal muscle layer.

V: vacuole.

DISCUSSION

In the present investigation, the potency of three entomopathological bioagents, the bacteria (*Bacillus thuringiensis* var. *kurstaki*) "Protecto", the (*Spodoptera littoralis* Nuclear Polyhydrosis Virus, SLNPV) "Viruset" as well as their mixture "Profect" was assessed.

These bioagents are widely used for the control of many lepidopteran insects (Lacey *et al.,* 2001).In the present work, Protecto exhibited a lower LC values than the other two bioagents, following by Viruset then Profect. Gamil (2004) recorded high rate of mortality in 3rd instar *S. littoralis* larvae treated with Protecto than in SLNPV treated larvae. The median lethal concentration LC_{50} of the three tested bioagents did not kill larvae rapidly. Therefore mortality rate was low during the first and second days following treatment but their toxic effects became most apparent at the termination of the larval stage. LT_{50} was similar when either Protecto or Viruset was used, it was slightly extended when Profect was tested.

Treated 2nd instars larvae of *S. littoralis* were susceptible to the bacteria, virus and their mixture, however, younger instar was more susceptible. It is well documented that older instar were usually more tolerant to the toxic effect of many compounds, (Hanafy *et al*., 2005), these authors showed the considerable variations in susceptibility between bioagents against different instar larvae.

Treatment with the tested bioagents prolonged the duration of the subsequent larval instar of treated larvae, as well as the pupal stage, also, pupation and adult moth emergence rates were reduced. Mohamed, (2006) reported the delay of ecdysis in larvae treated with NPV. Gamil, (2004) and Mohamed, (2006), also found that the development time of larvae and pupae were extended as well as adult emergence as a result of treatment with bacterial or viral agents. Hegazy and Antonious, (1987) reported the antifeedant effect of thuricide and SAN 415 both containing *B. thuringiensis* on *S. littoralis* this effect was than reflected in reductions of larvae and pupae weights. In the results of the present work, the weight of formed pupae (from treated larvae) was not impaired.

In the present work, a general disturbance in three carbohydrates enzymes was detected in larvae treated as 2^{nd} instars with LC_{50} of any of the three tested bioagents .

On the other hand, treatment with the tested compounds caused disruption in the midgut tissue which therefore, must have caused disturbance of its digestive carbohydrase enzymes. These suggestion are supported by similar observations by Ishaaya *et al.,* (1971) who mentioned that generally reduction in larval digestive enzymes could be an inhibitory effect of tested compounds as well as a result of its binding to inactive (zymogens) or active digestive enzymes.

The histological structure of the midgut in *S. littoralis* infected by the bioagents was affected. The mode of action of *Bt* is well documented, various proteins produced by the bacteria, known as □- endotoxin form crystals inside the bacteria. In midgut of susceptibly insects, this endotoxin become activated and is dissolved in the insect's midgut liberating the protoxins Gill *et al.*, (1992). These protoxins bind to the midgut cells creating spores in the cell membrane and leading to equilibration of ions (Parenti *et al*., 1993). Furthermore, Van Rie *et al*., (1989) showed that the activated portions toxins disrupt the osmotic balance of these cells causing them to lyse. According to Sampson and Gooday, (1998), *B.thuringiensis* endogenous enzyme weakens the peritrophic membrane which allows more readily access of the bacterial toxins to the gut epithelia. Salama *et al*., (1993) showed that the initial destruction by *Bt* toxin facilitates the penetration and entry of virus. This observation might therefore explain the fact that the midgut epithelia were more impaired when Profect was used.

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تأثير بعض المركبات الحيوية على بعض النواحى البيولوجية والبيوكيميائية والهستوباثولوجية على دودة ورق القطن سبودبترا ليتورايس

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وقد استهدفت الدراسة الحالية ما يلى :

1 – تقييم فاعلية المركبات محل الدراسة ضد يرقات العمر الثانى لدودة ورق القطن :

اختبرت فاعلية 3 مركبات حيوية وهى بروتكتو ، فيروست، وبروفكت والتى أظهرت الآتى:

كانت قيمة التركيز النصف مميت للمركبات بروتكتو ، فيروست ، وبروفكت المتحصل عليه عند معاملة يرقات العمر الثانى هى عند PIB s/ml+IU/ml ^{2}10X1.6 + ^{3}10X5, PIB s/ml ^{2}10X1, IU/ml ^{2}10X3.2 على التوالى. تم حساب الوقت اللازم لإماتة 50% بالتركيز النصف مميت للبروتكتو ، فيروست ، وبروفكت هى 12.02 ، 12.47 ، 14.12 يوم على التوالى . كما تم حساب التركيزات الميتة اــ 90 % من يرقات العمر الثانى لجميع المركبات محل الدراسة.

2 – تأثير المركبات المختبرة على بعض القياسات البيولوجية لدودة ورق القطن :

أظهرت النتائج عدم وجود فروق معنوية فى طول العمر اليرقى ليرقات العمر الثانى المعاملة بالبروتكتو ، فيروست وبروفكت ، حيث كان متوسط العمر اليرقى هو 14.5 ، 15 ، 15 يوم ، على التوالى . أدت المعاملة بالمركبات محل الدراسة إلى التأثير السلبى على نسبة التعذر فى اليرقات المعاملة فى العمر اليرقى الثانى حيث كانت نسبة التعذر 52.5 ، 50 ، 47.5% ليرقات العمر الثانى المعاملة البروتكتو ، الفيروست والبروفكت على التوالى . تسببت المعاملة فى خفض فترة العمر العذرى بيوم واحد ليرقات العمر الثانى المعاملة البروفكت عن اليرقات غير المعاملة وسجلت متوسط 12.6 يوم . وعند معاملة يرقات العمر الثانى بالبروتكتو والفيروست انخفضت فترة العمر العذرى بـــ 2.3 يوم عن الكنترول بمتوسط 11.3 يوم لكلا المعاملتين.

3 – التأثيرات الكيميائية الحيوية :

تسببت المعاملة بالمركبات المختبرة عند تقدير نشاط إنزيمات الهضم المستخلصة من طحن يرقات العمر السادس الناتجة من معاملة يرقات العمر الرابع بالمركبات محل الدراسة فى :

زيادة معنوية فى نشاط إنزيم الأميليز عند المعاملة بكل من البروتكتو والبروفكت ، بينما لم يسجل الفيروست فروق معنوية مقارنة باليرقات الغير معاملة . من جهة أخرى سجلت زيادة معنوية لنشاط إنزيم الإنفرتيز فى كل من البروتكتو والبروفكت وعدم وجود فروق معنوية للفيروست . وقد سجلت زيادة معنوية لنشاط إنزيم التريهاليز لجميع المركبات محل الدراسة باستثناء البروتكتو حيث كانت التغيرات غير معنوية مقارنة باليرقات الغير معاملة.

4 – التأثيرات الهيستوباثولوجية المتأخرة:

أوضحت هذه الدراسة التغيرات الهيستولوجية الناتجة من المعاملة فى طبقات المعى المتوسط ليرقات العمر السادس الناتجة من معاملة يرقات العمر الرابع حديث الانسلاخ بالتركيز القاتل لـــ 50% من اليرقات المعاملة بالمركبات محل الدراسة ، وقد بينت هذه الدراسة تأثير جميع المركبات المستخدمة على طبقات المعى الأوسط مقارنة بمثيلتها فى اليرقات الغير معاملة ، وقد كان أكثر المركبات تأثيراً على المعى الأوسط هو مركب البروفكت يليه البروتكتو ثم الفيروست.