

STUDIES CONCERNING SOME PROPERTIES OF THE ENZYME PROTEASE IN THE COTTON LEAF WORM, *SPODOPTERA LITTORALIS* (BOISD.)

Youssef , L. A.

Plant protection Department, Faculty of Agriculture, Ain Shams University.

ABSTRACT

The proteolytic enzyme from midgut (epithelia and peritrophic membrane) of the fifth instar larvae *Spodoptera littoralis*(Boisd.) is extracted and checked as crude preparation with casein sodium as substrate .Also, the effect of (20%) extracts of bestachia and dumb cane leaves on enzyme activity ,is studied.

The protease activity is affected by different factors;e.g incubation period, enzyme and substrate concentrations , pH number and starvation . The least activity of the enzyme occurs at 40 min incubation period.The protease is found to be alkaline as its optimum pH is about(10).Starvation shows an abrupt increase in enzyme activity only for the first two hrs., then followed by a continuous decrease in its activity.

A significant inhibition in the activities of protease,also occurs when larvae are fed on castor bean leaves treated with bestachia and dumb cane extracted in methanol and hexane.

There is a positive correlation between both enzyme and substrate concentrations and the enzyme protease activity.

Keywords: Digestive enzyme, Protease, Bestachia and Dumb cane, *Spodoptera littoralis*

INTRODUCTION

Various reviews of the literature on digestive enzymes in insects have been given by many research workers. Some have concerned themselves only with description of their sources or substrates. Others have tried to correlate the changes occurring in them with various physiological states of insects and to confirm their conclusions experimentally.

The general conclusion, reached, is that the midgut is the main site of the digestive processes in most insects. In *Spodoptera litura* proteolytic enzyme of larval stage is found to be composed of more than one protease (Ahmed *et al* 1976&1980) and the digestive proteolytic activity in *S.littoralis* is shown to be closely correlated with the protein level in the diet or with the environmental temperature (Ishaaya *et al.*, 1971)

Previous work has been done, on the biochemical interaction between the plant extracts and the activities of the digestive enzyme , Amylase in *Spodoptera littoralis*(Youssef2004).The present study is to elucidate the interaction between some physiological factors and the enzyme protease in the cotton leaf worm, *S.littoralis*.

The study includes the effect of variations in enzyme time of reaction, concentration of reactant, pH, starvation and the antifeeding bestachia and dumb cane extracts on the activity of the tested enzyme.

MATERIALS AND METHODS

The experimental insect is one day old fifth instar larvae of *S.littoralis*. They were reared under laboratory conditions ($25\pm 5^{\circ}\text{C}$ and $60\pm 5\%$ R.H.) and fed on fresh leaves of castor bean.

To prepare the enzyme, larvae were dissected and midguts only were used ; the rest of the alimentary canal was discarded.

The gut contents were washed thoroughly with 0.75% NaCl solution to remove unwanted food particles, then put in an ice cold tube and homogenized with 0.1M sodium phosphate buffer of pH 7.2 . The homogenate was also sieved to remove any remained particles. The resultant filtrate was used as protease enzyme. The proteolytic activity was estimated by the rate of hydrolysis using colorimeter spectrophotometer and was determined by using casein sodium (caseinolytic activity was measured as described by Eguchi and Kuriyama(1983)and modified by Ikeda(1988).

To study the effect of incubation period on the activity of the enzyme,a reaction mixture of equal volumes(0.2ml) of enzyme solution and 1% casein sodium was used.

The incubation periods were 0,5,10,20and 40 minutes at 30°C .The reaction was terminated by adding 0.4 ml of 20% trichloroacetic acid. After one hr., the solution was centrifuged for 5 minutes at 13000 rpm.(Ikeda 1988,Antonious1992).Absorbance of the resultant reactant was determined at 570(nm). Enzyme activity was expressed as change in optical density read at different previously mentioned (nm).

To study the effect of enzyme concentration on the caseinolytic activity was determined using various concentrations of the extracted enzyme as dilution on fixed concentration of the substrate (casein sodium);the incubation period was 40 min at 30°C .

To study pH optimum, four buffer solutions were prepared in different pH values; 0.2M of each of acetate, phosphate, glycine and potassium sodium hydroxide (KCl-NaOH) buffers. Incubation of all experiments was conducted for 40 min. under 30°C .

The effect of substrate concentrations on protease activity was accomplished by using casein sodium prepared in various concentrations , and glycine buffer of pH10 was used.The experiments were conducted after 24 hrs. from larval feeding on fresh leaves of castor bean. Incubation period was 40 min . Effect of starvation on proteolytic activity was studied by using protease enzyme from 24hrs. feeding larvae deprived of food for 2,4,8and 16hrs. Casein sodium preparation was hydrolyzed by proteolytic fluid separated from larvae starved for different periods.

It is worthwhile that in all experiments mentioned above the appropriate blanks were conducted, (some blanks without enzyme and other without substrate were also used).

To study the effect of antifeeding substances on the activity of the enzyme,bestachia and dumb cane extracts in methanol and hexane were used.

The larvae were fed on fresh leaves of castor bean dipped in 20% extracts of both antifeeding plants.

RESULTS AND DISCUSSION

(1) Effect of incubation period:The changes in the protease enzyme activity at different incubation periods are shown in table(1) and fig.(1).The obtained data show a clear positive relationship between period of reaction of enzyme and the used substrate, and the achieved enzyme activity.The enzyme activity decreases when incubation period increases and vise versa.. These data are in accordance with those reported by Ikeda (1988) on proteolytic enzyme activity in *Bombyx mori*; Antonious (1992) in *S.litura* and Youssef(1998) in *S.littoralis*.

(2) Effect of enzyme and substrate concentrations:Activity curve of the proteolytic enzyme on the substrates is shown in table (1) and fig. (2). Data also, show that using casein sodium preparation, the enzyme activity progressively increases with the increase of this substrate concentration table (1) and fig.(3). A clear positive and almost linear relationship is found between the used enzyme concentration and the achieved activity or substrate breakdown.

(3) Effect of pH:The relationships between the pH activity of protease with a discontinuous buffer systems consisting of acetate ,phosphate, glycine and potassium-NaOH buffers, after 24hrs.of feeding on castor bean leaves are shown in table(1)and fig.(4).The properties of sodium casinolytic enzyme of the midgut in *S.littoralis*, shows that the optimal activity is obtained with glycine buffer at pH10 after 24hrs. feeding. Meanwhile, weakest activity is achieved with acetate buffer at pH4.

The figured data are in great connection with previous notes of Day and Waterhouse (1953). They maintained that larvae of lepidopterous insects characteristically have weakly to strongly alkaline (8.0 to 10.0) midgut contents.Confirming this phenomenon they stated that, both activity of digestive enzyme and absorption of food are greatly influenced by hydrogen-ion concentration.

(4)Effect of starvation:When larvae were deprived of food for different periods,a change of proteolytic enzymes activity occurs ,see table (1) and fig.(5).Starvation has significant effect on enzyme activity .Long starvation period causes more inhibition of enzyme activity and vise versa.

(5)Effect of bestachia and dumb cane extracts on the proteolytic activity:

Table (2) and figs.6&7&8&9and10 indicate that the absorbency values for the resulting solutions after 24hrs. feeding show positive differences according to the examined physiological factors. (e.g.a) At40 min incubation period the absorbency value is highest (0.19) with bestachia/methanol extracts and the least(0.14)is when bestachia/ hexane is used(fig.6)

Table (1): Certain characters of protyoletic enzyme extracted from midguts 24 hrs. after feeding *S. littoralis* larvae on castor bean leaves.

Absorbency of the resultants determined as (nm) values.

Enzyme characters	Incubation time														
1- Incubation period (min)	0.0			5.0			10.0			20.0			40.0		
Absorbance value	0.02			0.05			0.11			0.14			0.23		
	Enzymes concentration														
2- Enzymes concentration	0.1			0.2			0.4			0.8			1.6		
Absorbance value	0.22			0.16			0.10			0.09			0.05		
	Substrate concentration														
3- substrate concentration	0.2			0.4			0.8			1.6			3.2		
Absorbance value	0.20			0.18			0.13			0.09			0.07		
	Effect of pH-number														
	Acetate buffer			Phosphate buffer			Glycine buffer			Potassium sod. Hydro. buffer					
4 - pH-number	4	5	6	6	7	8	8	9	10	11	12	12	13		
Absorbance value	0.02	0.03	0.04	0.07	0.1	0.16	0.19	0.24	0.27	0.31	0.39	0.65	0.18		
	Starvation period														
5- Starvation period	2			4			8			16					
Absorbance value	0.17			0.14			0.11			0.08					

Table (2): Certain protease characters extracted from midguts 24 hrs. After feeding *S. littoralis* larvae on castor bean leaves dipped in bestachia and dumbcane leaves extracted in methanol and hexane.

Absorbency of the resultants determined as (nm) values.

Enzyme characters	Treatments					
	Control	Bestachia extract		Dumb cane extract		
		Methanol	Hexane	Methanol	Hexane	
1- Incubation period at 40 min	0.23	0.19	0.14	0.17	0.15	
2- Enzymes concentration	0.22	0.18	0.24	0.22	0.24	
3- substrate concentration	0.20	0.19	0.22	0.19	0.22	
4 - pH-number at(10)	0.75	0.56	0.48	0.39	0.37	
5- Starvation period	0.17	0.20	0.26	0.19	0.27	

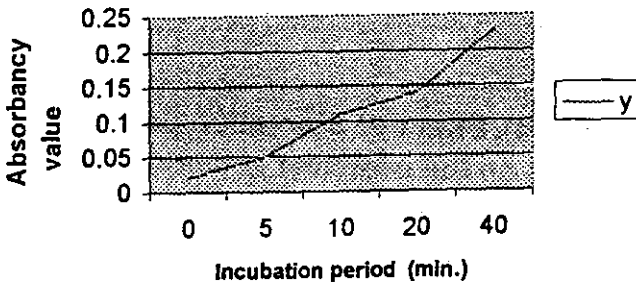


FIG (1). Effect of incubation period on the protease activity after 24 hrs. from feeding on fresh leaves of castor bean .

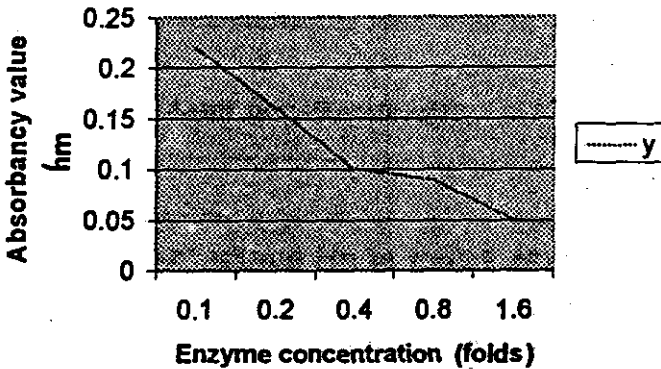


FIG (2). Effect of enzyme concentration on the protease activity after 24 hrs. from feeding on fresh leaves of castor bean .

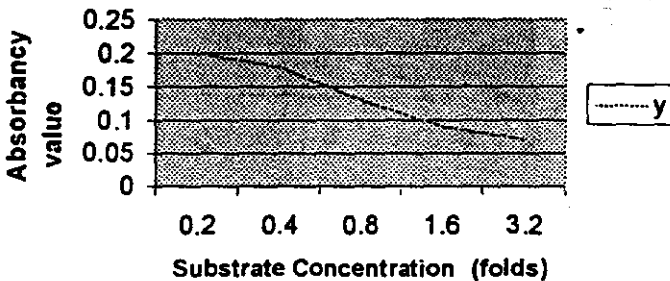


FIG (3). Effect of substrate concentration on the protease activity after 24 hrs. from feeding on fresh leaves of castor bean .

FIG (4). Effect of Starvation period (hrs.) on the protease activity after 24 hrs. from feeding on fresh leaves of castor bean .

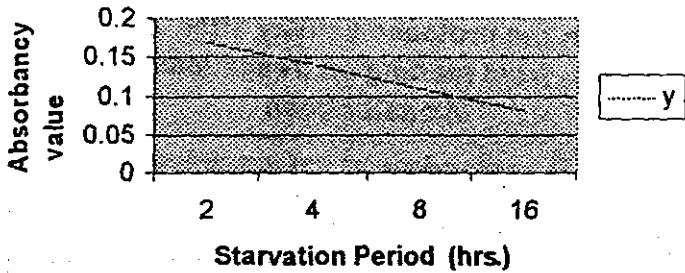


FIG (5). Effect of pH number on the protease activity after 24 hrs. from feeding on fresh leaves of castor bean .

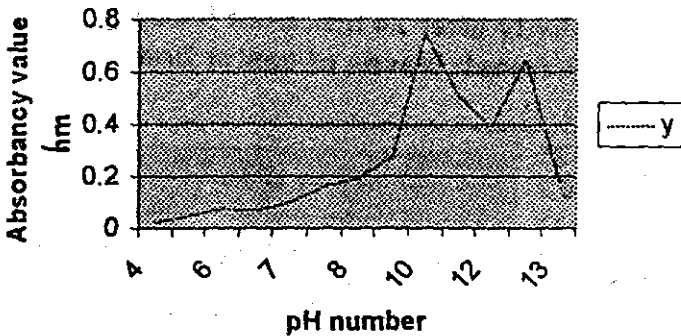


FIG (6). Effect of bestachia and dumbcane extracts on the protease enzyme activities of *S. littoralis* larvae after 24 hrs. from feeding.

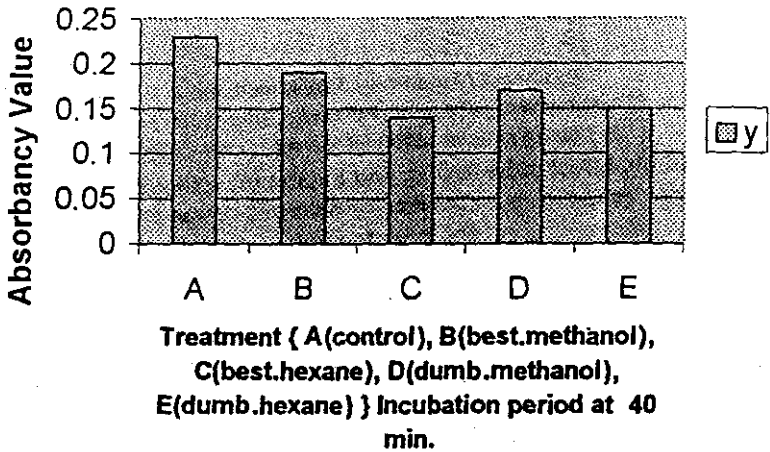
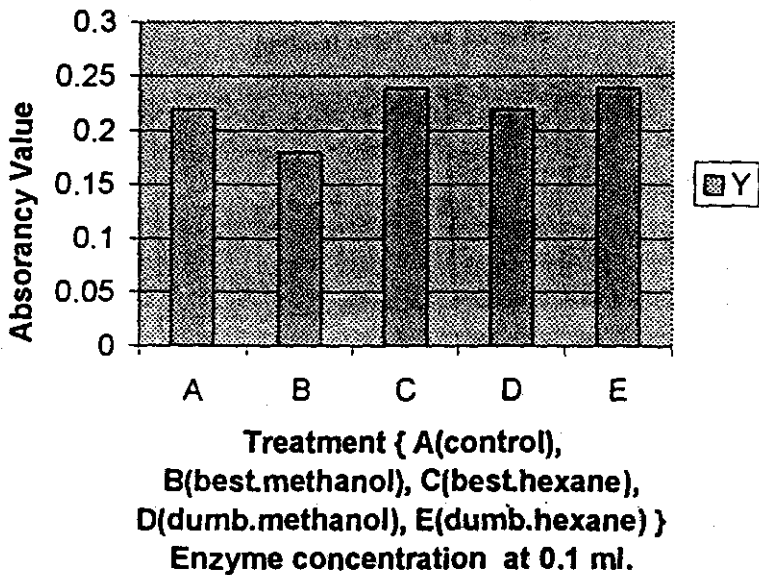


FIG (7).Effect of bestachia and dumbcane extracts on the protease enzyme activities of *S. littoralis* larvae after 24 hrs. from feeding.



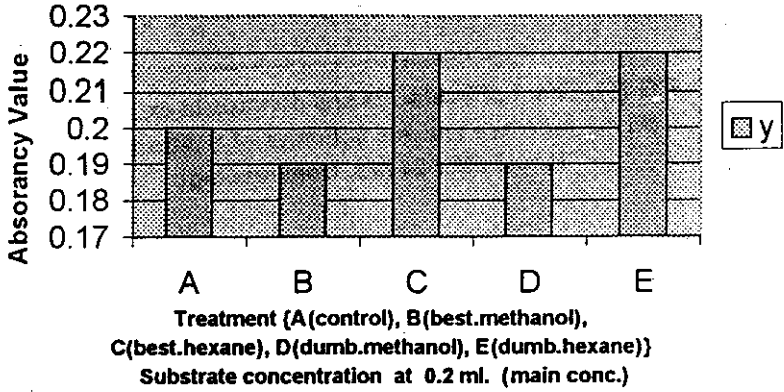


FIG (8). Effect of bestachia and dumbcane extracts on the protease enzyme activities of *Sittoralis* larvae after 24 hrs. from feeding

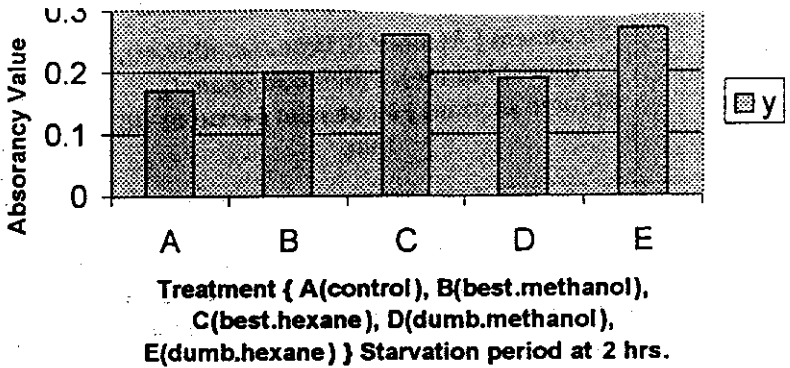


FIG (9). Effect of bestachia and dumbcane extracts on the protease enzyme activities of *S. littoralis* larvae after 24 hrs. from feeding.

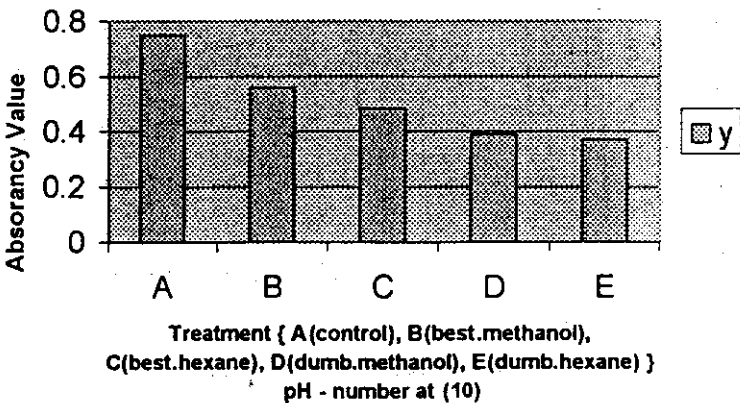


FIG (10). Effect of bestachia and dumbcane extracts on the protease enzyme activities of *S. littoralis* larvae after 24 hrs. from feeding.

- b) Using enzyme concentration at 0.1ml folds results in equal absorbency values with both bestachia &dumb cane/hexane(0.24) while the least value (0.18) is when bestachia/methanol is used.(fig.7)
- c) Substrate concentration at 0.2ml also, causes equal absorbency values(0.22)with bestachia &dumb cane/hexane and equal absorbency values(0.19) with bestachia and dumb cane/methanol extracts(fig.8)
- d) After starvation period of 2hrs. the absorbency values is the highest (0.27)with dumb/hexane extracts while it is lowest(0.19)with dumb cane/methanol extracts.(fig.9)
- e) pH number of 10 causes the least absorbency values(0.37) with dumb/hexane and the highest absorbency values(0.56) with bestachia/methanol(fig.10).

The above results show a positive interaction and correlation between the different physiological factors and the activity of protease enzyme in *S.littoralis*. This is in agreement with Chockalingam *et al* (1989) who explained that the growth inhibition in *S.litura* by the extracts of *C.roseus* appears to be a complex interaction which includes lowered food consumption and depressed activities of the digestive enzymes. Goldstein and Swain (1965) noted that the mechanism of action of these rejected plant extracts is an inhibition in the enzymatic digestive activity, while Feeny (1970) explained the mechanism as a reduction in availability of dietary protein.

ACKNOWLEDGEMENT

The author wishes to express his deepest thanks and profound gratitude to Prof. Dr. Nabila M. Azmy, Plant Protection Department, Faculty of Agriculture, Ain Shams University for her reviewing the manuscript and preparing it in the final form.

REFERENCES

- Ahmed, Z.; M. Saleemuddin and M. Siddiqi (1976). Alkaline protease in the larvae of the armyworm, *Spodotera litura* Insects Biochem. 6:501-505.
- Ahmed, Z.; M. Saleemuddin and M. Siddiqi (1980). Purification and characterization of three alkaline protease from the gut of larva of armyworm, *Spodotera litura*. insects Biochem. 10:667-673.
- Antonious, A.G. (1992). some properties of the digestive fluid protease in the tobacco cutworm, *Spodotera litura* (F.) Annals Agric. Sci., Ain Shams Univ., Cairo, 37(2):621-628.
- Chockalingam, S.; M.S.N. Sundari and S. Thenmozhi (1989). Impact of the extract of *Catharanthus roseus* on feeding and enzymatic digestive activities of *Spodoptera litura*. J. Environ. Biol., 10 (3): 303-307.
- Day, M.F. and D.F. Waterhouse (1953). The mechanism of digestion Insect physiology. (ed. Kenneth D. Roeder). John Wiley & Sons, N.Y., PP311-349.

- Eguchi, M. and K. Kuriyama (1983). Relation-ship between alkaline protease from the midgut lumen and epithelial of the silkworm, partial purification and comparison of properties of both protease 6B3. *Comp. Biochem. physiol.* 76B:29-34.
- Feeny, P.P. (1970). Sesonal changes in oakra leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology*, 51:565-581.
- Goldstein, J.L. and T. Swain (1965). The inhibition of enzymes by tannins. *Phytochemistry*, 4: 185-192.
- Ikeda, M. (1988). Purification and characterization of proteolytic enzymes from the gut of the silkworm, *Bombyx mori* L.M.Sc. thesis, Nagoya Univ., Nagoya, Japan. p.120.
- Ishaaya, I., I. Moore and D. Joseph (1971). Protease and amylase activity in larvae of the Egyptian cotton leafworm, *Spodoptera littoralis*. *J. insects physiol.* 17:945-953.
- Youssef, L.A. (1998). Physiological disorders of the cotton leafworm *Spodoptera littoralis* (Boisd.) fed on certain plants and synthetic media. Ph.D. Thesis, Fac. Agric., Ain Shams Univ.
- Youssef, L.A. (2004). Biochemical interaction between the plant extracts and the activities of the a digestive enzyme, amylase in *Spodoptera littoralis* (in press).

دراسات متعلقة ببعض صفات انزيم البروتينيز في دودة ورق القطن
لطفى عبد الحميد يوسف
قسم وقاية النبات - كلية الزراعة - جامعة عين شمس - شبرا الخيمة - مصر

- استخلصت العصارة الهاضمة المحتوية على الانزيمات المحللة للبروتينات من المعى الاوسط (النسيج الطلائى والغشاء الحول غذائى) من يرقات العمر الخامس لدودة ورق القطن وتم تحضير مستخلص انزيم البروتينيز. وقد استخدمت مادة الكازين كمادة تفاعل لانزيمى كما تم اختبار تأثير تغذية يرقات العمر الخامس على مستخلص لورق نباتى البستاشيا والدفينبخيا بكتا فى الميثانول والهكسان (٢٠%) على معدل نشاط انزيم البروتينيز
- وجد ان النشاط الانزيمى يتاثر بعوامل مختلفة
- ١- فترة التحضين بين العصارة الانزيمية ومواد التفاعل .
 - ٢- تركيز العصارة الانزيمية المستخدمة .
 - ٣- تركيز مادة التفاعل التى يعمل عليها الانزيم .
 - ٤- با استخدام اكثر من منظم يعطى اكثر من درجة حموضة ثبت ان العصارة الانزيمية للمعى الوسطى فى هذا الطور لهذه الحشرة قلوى حيث يعمل على درجة حموضة = ١٠
 - ٥- عند تجويع الحشرة لفترات مختلفة وجد ان العصارة المحتوية على المستخلص الانزيمى يزداد نشاطها حتى مرور ساعتين من تجويع الحشرات ثم حدث نقص تدريجى فى نشاط العصارة الانزيمية .
 - ٦- عند تغذية اليرقات على لورق الخروج بعد معاملةها بمستخلص لورق البستاشيا والدفينبخيا بكتا فى الميثانول والهكسان (٢٠%) وجد ان هناك خفض معنى فى معدل نشاط الانزيم .