J. Agric. Sci. Mansoura Univ., 29(5): 2865 - 2872, 2004

BIOCHEMICAL INTERACTION BETWEEN THE PLANT EXTRACTS AND THE ACTIVITIES OF A DIGESTIVE ENZYME, AMYLASE IN *Spodoptera littoralis* (BOISD.)

Youssef, L. A.
Plant Protection Department, Faculty of Agriculture, Ain Shams University.

ABSTRACT

Amylase enzyme was extracted from both anterior part of the foregut and posterior part of the hindgut of the fifth instar larvae of Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.).Then it was checked using starch and mercuric chloride as substrates.

The present work shows that, activity of the enzyme depends on some important factors; incubation temperature, starvation and pH number .Amylase in the fifth instar larvae is acidic (optimum pH about 6) and the suitable incubation temperature is 35 ºC. Starvation causes an abrupt increase in the enzyme activity for the first four hours followed by a continious decrease of its activity.Feeding larvae on castor bean fresh leaves dipped in extracts of either bestachia or dumb cane leaves in methanol and hexane, results in a significant inhibition in the activities of amylase enzyme in all treatments.

Keywords: Amylase, Bestachia and Dumb cane, Spectophotometer,Egyption cotton leaf worm.

INTRODUCTION

Plants contain secondary substances which protect them from biological attacks,particularly by phytophagous insect pests,and mechanism for the action of these substances propose a reduction in availability of enzymatic digestive activity(Feeny 1976).Various reviews of the literature on digestive enzymes,their properties, sources and activities in different insects have been given by Abdel Hafez *et al.* (1983) and Teo and Woodring(1985).Moreover the interaction between these enzymes and food substance and their substrates was discussed by Ishaaya and Meisner(1973),Ishaaya and Casida(1975)and Ishaaya and Swirsik(1976).The following experiments is an attempt to investigate the biochemical interaction between some plant extracts and the activities of amylase in *S. littoralis*.

MATERIALS AND METHODS

Two days old fifth instar larvae of *S.littorals* were reared under laboratory conditions (28±2 ºC and 60±5%R.H.).Some were fed on fresh castor bean leaves dipped in extracts of either bestachia or dumb cane with methanol and hexane as solvents,(20%).Fresh leaves of castor bean dipped in the organic solvents only were used as control.

The anterior and posterior parts only of the foregut and hindgut respectively,were separated in different Petri dishes at room temperature.The contents of each segment were thoroughly rinsed out with 0.1ml (Ringer's

solutions)and gut tissues were separately put in test tubes and placed in an ice bath,after adding 0.3ml (Ringer's solutions) to each tube for homogenization . The tissue extracts were then centrifuged at 10000 rpm for 2 min, and the supernatants were stored in the freezer. The reaction mixture used in the present experiments was prepared as described by Teo and Woodring (1985).It consists of 0.3ml enzyme extract, 0.7ml buffer solution (acetate or phosphate buffers)and 1ml (1%soluble starch solution),0.2ml mercuric chloride and incubation was done at 37 ◦C for 20-60 min.The absorbance was read at 530 nm against a reagent blank, using the spectophotometer.

To study the effect of temperature on the activity of amylase three different incubation temperatures of20,35,50◦C,were used. Starvation effect on amylase activity was studied on the fifth instar larvae starved for 2, 4,8,16hrs. The reaction mixture was incubated at 35◦C for 1 hr.as control.

To study the optimum pH two buffer solutions were prepared in different pH values, 0.7 M of each acetate buffer(4 -6) and phosphate buffer(6-8).Incubation period was 25 min at 35◦C.

RESULTS AND DISCUSSION

(1)Effect of incubation temperature on amylase activity:

The changes in the amylase enzyme activity at different incubation temperature in *S. littoralis* are shown in table(1) and fig.(1).The obtained data show an obvious positive relationship between incubation temperature and the achieved enzyme activity.

Table 1: Certain characters of amylase enzyme extracted from fore guts 48 hrs after feeding *S. littoralis* larvae on castor bean leaves. Absorbency of the resultants determined as (nm) values.

Enzyme characters	Incubation temperature °C					
1- Incubation temperature	20	35	50			
Absorbance value	0.46	0.53	0.60			
	Starvation period (h)					
2- Starvation period (hrs)	2	4	8	16 hrs.		
Absorbance value	0.42	0.38	0.22	0.12		
	pH number					
	Acetate buffer (4-6)			Phosphate buffer (6-8)		
3- pH number	4	5	6	6	7	8
Absorbance value	0.29	0.60	0.58	0.59	0.34	0.37

a change of

(2)Effect of starvation:

When larvae are deprived of food for different periods,a change of amylase enzyme activity occurr as shown in table (1) and fig.(2).The longest period of starvation the more significant inhibition is in amylase enzyme activity.

(3) Effect of pH:

The relationships between pH and the activity of *S. littorals* amylase enzyme with either acetate buffer, or phosphate buffer after 48hrs. feeding on castor bean leaves are shown in table(1)and fig.(3).The properties of mercuric chloride of amylase enzyme of the foregut of *S.littoralis*,show that the optimal activity is obtained with phosphate buffer at pH6. Meanwhile,the least activity occur using acetate buffer at pH4.

(4) Effect of bestachia and dumb cane extracts on amylase activity:

Table (2) indicates that the optimal incubation temperature after 48hrs.feeding is at 35C.The absorbancy values are 0.20,0.34,0.18,0.34 and 0.53 for bestachia extracts in (methanol&hexane),dumb cane extracts in (methanol&hexane) and control,respectively. The absorbancy values of bestachia and dumb cane in hexane are equal,(0.34)while in methanol the absorbancy values of bestachia and dumb cane extracts is less(0.20&0.18),respectively.Thus solvents play important part in the absorbancy values of substrates.The higher polarity the lesser absorption value,see,Youssef(1998).

Table 2: Certain characters of amylase enzyme extracted from fore guts 48 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.

Treatmes / Enzyme characters	Treatments				
	Control	Bestachia extract		Dumb cane extract	
		Methanol	Hexane	Methanol	Hexane
1- Incubation temp. at 35°C	0.53	0.20	0.34	0.18	0.34
2- Starvation period (4 hrs.)	0.38	0.15	0.20	0.15	0.18
3- pH number at (6)	0.58	0.21	0.49	0.27	0.42

Moreover the absorbancy value of bestachia extracts in methanol is higher than that of dumb cane extract in the same solvent.It can be said that dumb cane extract has higher toxic activity.Insects digestive enzymatic activity could be used as aparameter for determining antifeeding activity or phagostimulation in phytophagous insects(Ishaaya&Meisner,1973)and (Ishaaya&Casida,1975).Goldstein and Swain(1965) noted that the mechanism of action of these rejected plant extracts cause inhibition in the enzymatic digestive activity.

Concerning lepidopterous insects, (Ishaaya&Swirski,1976)explained that the reduction in food consumption caused inhibition of enzyme activity.

(5) Effect of bestachia and dumb cane extracts on the amylase activity when larvae were starved for 4hrs.:

Data in table (2)show that in four hrs. starved larvae, the absobancncy values are (0.15,0.20) (0.15,0.18)and 0.38 for extractions of bestachia and dumb cane in (methanol & hexane)and control. respectively.Amylase activity is inhibited in both bestachia and dumb cane, extracted in methanol, at the same degree (fig.5). However, using hexane, inhibition of amylase enzyme differs; bestachia extract has more effect than dumb cane. Thus there is a corrolation between the solvent used, food and activity of enzyme. In this case the polarity of the solvent plays a part in the enzyme activity.

(6) Effect of bestachia and dumb cane extracts on the amylase activity when using phosphate buffer pH=6:

Previous experiments in the present work show that activity of amylase is at higher degree when phosphate buffer pH6 is used, after 48 hrs. feeding, see table (1).The effect of bestachia and dumb cane extacts on amylase activity at the same buffer pH is shown in table (2). The absorbancy values are 0.21, 0.49, 0.27, 0.42 and 0.58 for bestachia and dumb cane extracts in (methanol & hexane) and control, respectively (fig.6). The results indicate that the activity of the enzyme is inhibited. However, there are different degrees of inhibition in amylase activity; hexane cause less inhibition than methanol in either bestachia or dumb cane extracts.Moreover dumb cane extract in methanol has less effect on amylase activity than that of bestachia extract.Vise versa effect occurred when hexane is used.

Finally, it could be said from the present results that there is a positive correlation and interaction between feeding, food consumption, solvent polarity, buffer pH, antifeeding substances and the activity of amylase enzyme in *S. littoralis*.

This is in agreement with the findings of other worker. For example, Ishaaya and Casida (1975) in *Tribolium confusum*, Ishaaya & Swirski (1976) in black scale scale *S.oleae*, Teo and Woodring (1985) in house cricket, *Acheta domesticus* and clockalingam *et al* (1989) in *Spodoptera litura*.

FIG. (1) Effect of incubation temperature on the Amylase activity after 48 hrs. from feeding on fresh leaves of Castor bean

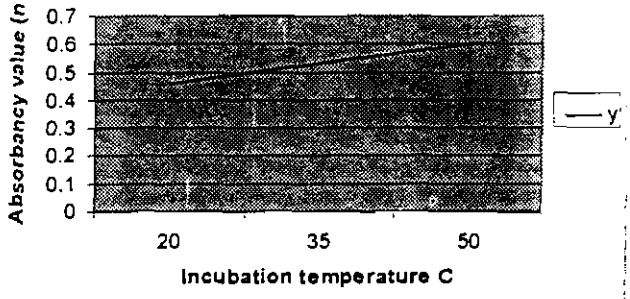


FIG. (2) Effect of Starvation Period (hrs.) on the Amylase Activity after 48 hrs. from feeding on fresh leaves of Castor bean.

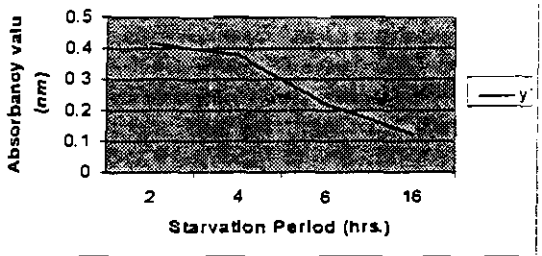


FIG.(3) Effect of pH value on the Amylase activity after 48 hrs. from feeding on fresh leaves of castor bean.

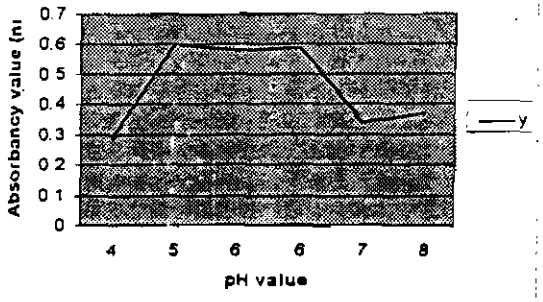
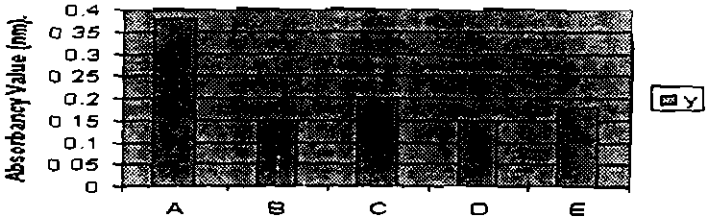


FIG .(4) Effect of bestachia and dumbcana extracts on the Amylase enzyme Activitie of S.littoralis larvae after 48 hrs. from feeding.

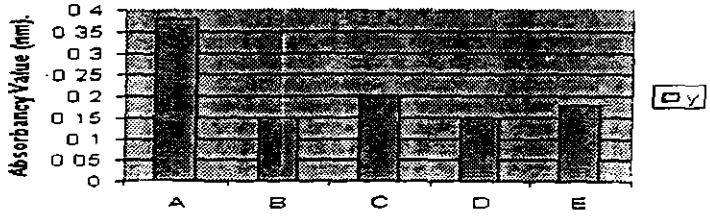
Treatment { A (control) , B (best.methanol) , C (best.hexane) , D (dumb.(methanol) , E (dumb.hexane) }.

FIG .(5) Effect of bestachia and dumbcana extracts on the Amylase enzyme Activitie after 48 hrs. from feeding when the larvae of S.littpralis were starved for 4hrs.



Treatment (A (control) , B (best.methanol) , C (best.hexane) , D (dumb.(methanol) , E (dumb.hexane) }.

FIG .(5) Effect of bestachia and dumbcana extracts on the Amylase enzyme Activitie after 48 hrs. from feeding when the larvae of S.littpralis were starved for 4hrs.



Treatment (A (control) , B (best.methanol) , C (best.hexane) , D (dumb.(methanol) , E (dumb.hexane) }.

ACKNOWLEDGEMENT

The authors wish 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

Abdel-Hafez, F.A.; A. R. Nassar and F.E. Abbasai (1983). The changes in the digestive enzymes trehalase, amylase and invertase during the development of *Spodotera littoralis* (Boisd.) Annals. Of Agric. Science, p. 71-77.

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.

El-Saidy, M.F. and D. Degheele (1990). Effect of diflubenzuron on growth, development and digestive enzymes in *Spodoptera littoralis*(Boisd.). Med Van-de Fac. Landbouww. Rijks. Gent, 55(26): 583-592.

Feeny, P.P. (1976). Biochemical interaction between plants and insects. Recent advances in phytochemistry, 10: 1-36.

Goldstein, J.L. and T. Swain (1965). The inhibition of enzymes by tannins. Phytochemistry, 4: 185-192.

Ishaaya, I. and J. Meisner (1973). Physiological effects of sugar on varius digestive enzymes of *Spodoptera littoralis* larvae. J. Comp. Physiol., 86: 117-124.

Ishaaya, I. and E. Casida (1975). Phenyltin compounds inhibit digestive enzymes of *Tribolium confusum* larvae. Pest. Biochem. Physiol., 5: 350-358.

Ishaaya, I. and E. Swirski (1976). Trehalose, invertase and amylase activities in the black scale, *Saissetia oleae* and their relation to host adaptability. J. insect physiol., 22: 1025-1029.

Teo, L.H. and J.P. Woodring (1985). Digestive enzymes in the House Cricket *Acheta domesticus* with special reference to amylase comp. Biochem. Physiol. Vol. 82A, (4):871-877.

Thomas, K.K. and J.L. Nation (1984). Protease, amylase and lipases activities in the midgut and hindgut of the cricket, *Gryllus rubens* and mole crickets, *Scapteriscus* acletus, Comp. Biochem. Physiol., 79: 297-304.

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.

التأثيرات البيوكيميائية بين بعض المستخلصات النباتية ونشاط انزيم الاميليز فـــى دودة ورق القطن

لطفى عبد الحميد يوسف
قسم وقاية النبات - كلية الزراعة - جامعة عين شمس - شبرا الخيمة - مصر

إستخلص انزيم الاميليز من الجزء الأمامى (للمعى الأمامى) والجزء الخلفى (للمعى الخلفـــى) من القناة الهضمية ليرقات العمر الخامس لدودة ورق القطن واختبر باســـتخدام (النشـــا وكلوريـــد الزئبقيك) كمواد تفاعل . واتضح من النتائج ان نشاط انزيم الاميليز يتأثر بعدة عوامل وهى :

١- درجة الحرارة التى يتم عندها تحضين مواد التفاعل الانزيمية - ووجد أن أعلى نشاط للانزيـــم يكون عند التحضين على ٣٥°م.

٢- أعلى نشاط للانزيم يكون عند درجة حموضة = ٦ .

٣-عند تجويع الحشرات تزداد درجة نشاط الانزيم خلال الأربع ساعات الأولى ، يحـــدث بعدهـــا انخفاض فى معدل النشاط الانزيمى .

٤-بدراسة تأثير بعض المستخلصات النباتية على نشاط انزيم الاميليز وجد ان مســـتخلص أوراق نباتى البستاشيا والدفينجيا بكتافى الميثانول والهكسان (٢٠%) وجد تثبيط معنـــوى فـــى معـــدل النشاط الانزيمى عند تغذية اليرقات على هذه المستخلصات فى كل المعاملات.

وأخيراً يمكن القول أن درجة حرارة تحضين الانزيم ودرجة الحموضة والتغذيـــة والتجويـــع والمستخلصات النباتية والمذيبات كلها عوامل تؤدى إلى تنشيط أو تثبيط فى نشاط انزيم الاميليز فى يرقات العمر الخامس لدودة ورق القطن.