J. Agric. Sci. Mansoura Univ., 27(1): 631 - 637, 2002.

بسم الله الرحمن الرحيم

EFFECT OF DIFFERENT KINDS OF FOOD ON THE TRYPSINACTIVITY IN MIDGUT WALL AND MIDGUT CONTENTS OF *EUPREPOCNEMIS PLORANS* (ACRIDIDAE, ORTHOPTERA)

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ABSTRACT

Haemolymph proteins of *Euprepocnemis plorans* nymph and adult females were estimated after they feeding on different foods,and trypsin activity in midgut of nymphs. Electrophoresis of heamolymph from grasshoppers fed on lupin (*Lupinus trmis*) or horsebean (*Vicia faba*) showed much less stainable protein in comparison to those fed on clover (*Trifolium alexandrinum*). Trypsin activity in midgutt wall or in midgut were lower in nymphs fed on lupin or horsebean than those from nymphs fed on clover .

INTRODUCTION

Clover is a forage crop in Egypt. However, it usually infest with several insect pests , seriously compromising its annually yield . Among of these pests , *Euprepocnemis plorans* (Elsayed, 1998a). The rate of trypsin activity in the midgut of insects depends on the amount of food and presence of allelochemicals and its types in the food.The availability of the amount of dietary protein often influences the growth of caterpillars (McNeil and Sonthwood, 1978; Mattson, 1980; Scriber, 1984; Slansky and Scriber, 1985).

This protein-growth relationship is important because protein availability varies in space and time. Different plant species, as well as plant specimens within a species and different leaves of the same plant, may contain widely varying concentrations of protein (Suomela *et al*., 1995). The quantity of protien available for growth must depend not only on upstream processes, such as the rate of consumption of protein and the rate and location of protein breakdown within the midgut, but also on the capacity of the midgut to transport digested protein into the body (Arthur and Chamberlin, 1999) .

Good assimilation of plant protein depends on the type of allelochemicals in the host plant; the quantity of protein in haemolymph of grasshoppers was reducing on oats which containing the phenolic acid than on wheat plant (Hinks *et al.*, 1993) . Significant reduction in haemolymph protein was noted in females of *E. plorans* reared on lupin and horsebean (Elsayed, 1998a).

In this study we have tried answering the following two questions: Does midgut enzymes activity is reflecting to quantity of protein synthesis and does the plant kind and plant allelochemicals have effects on trypsin activity in the gut.

MATERIALS AND METHODS

Proteins Electrophorasis :

Ten individuals of fifth nymphal instar and ten mature adult females were collected from the stock rearing culture, and reared one batch on lupine, the second on horsebean, and the third on clover. Insects on clover several as control.

Haemolymph from ten individuals (3-5 days old fifth instar nymphs and mature adult females), per food were collected and centrifuged at 20,000 rpm for 5 min., for haemocytes precipitation. Equal amount of supernatant per food (3ای) was added to 6 ای buffer assay (pH 6.8,S.D.S. 1%), 1.2 ای sucrose 10% and 0.5 ای bromophenol blue.

In the same gel the same quantity of proteins in the nymphs or mature adults were used at different volume of haemolymph. Total protein in haemolymph of the nymphs fed on clover was 347.1 یg / ای (Elsayed,1998a). Therefore, the volume of haemolymph which contains the same quantity of protein on clover is 3.6 ای with nymphs on horsebean and 5.4 ای with nymphs on lupine. The quantity of protein in mature adults haemolymph on clover food was 229.8 یg / 3 ای (Elsayed, 1998 b), the volume of haemolymph which contains the same quantity of protein on clover is 5.1 ای and 8.1 ای on horsebean and lupine, respectively. 30 ای from the standard proteins (high molecular weight: 669,449,232 and 67 M.W) was used as indicator to calculate the molecular weight of proteins sample (Hinks *et al*., 1991).

The samples and the standard proteins were electrophorased on 12% separating gel and 5% staking gel using buffer system (Tris glycin and S.D.S. 1% pH 8.3) of Laemmli (1970). The samples were run on the gel over a 100 mA constant current. The gel was stained with methanol coomassie blue overnight and the separated bands were fixed by acetic acid 7%.

Trypsin activity:

Preparation of samples:

The midgut contents were collected in 1 ml buffer assay (Tris 100 mی, pH 8.0 cotaining 2 mی calcium chloride)and centrifuged at 3000 xg for l min. Midgut tissues were homogenised in 1 ml buffer assay after washing in insect saline and centrifuged at 1300 xg for 5 min.

Trypsin assay:

Timmins and Reynolds technique (1992) was used and modified as follows:

80 ای aliquot of the supernatant from the midgut or midgut contents were prepared as above. For the assay, 1010 ای of buffer and 250 ای of substrate were added in a cuvette to the sample. The rate of hydrolysis of the artificial substrate BAPNA (30 nM) was measured in spectrophotometer by the increase in absorption at 410 nm. Enzyme activity was expressed as nmol of BAPNase min/ml.BAPNA(N-Benzoyl-DL-Arginine-Nitroamilid).

RESULTS AND DISCUSSION

Protein electrophorasis:

Protein profiles of haemolymph of fifth instar nymphs fed on the three foods show higher concentrations of proteins (m.w.) in those fed on clover leaves than those fed on either horsebean or lupine leaves at the same volume from supernatant. Ten proteins weight were separated from the haemolymph of the nymphs which fed on clover (358, 330, 130, 64, 60, 49, 35, 33, 28 and 14 m.w) but four and six proteins weight were separated from the haemolymph of the nymphs fed on lupine (358, 330, 63 and 54 m.w.) and horsebean(358, 330, 67, 65, 62 and 54 m.w.) , respectively(Fig.1).

At the same amount of protein loaded onto gel, we have observed three bands were recorded in the panel of nymphs which fed on clover (66, 34 and 6 m.w.) compared with the above bands on the same food. Also four and three bands more than above with nymphs fed on horsebean (40, 35, 30 and 14m.w.) and lupine (65, 30 and 16 m.w.).

Electrophorasis on 12% separating gel shows reduction in haemolymph proteins band of mature adult females fed on either lupine or horsebean than those fed on clover. At the same volume of haemolymph, eleven molecular weights protein were recorded in the haemolymph of females fed on clover (330, 220, 67, 65, 63, 61, 60, 47, 37 and 28 m.w.) while, seven bands with females fed on lupine (330, 67, 66, 63, 61, 60 and 47 m.w.), and nine bands with females fed on horsbean (330, 220, 67, 65, 64, 63, 61, 60, and 47 m.w.).

At the same amount of protein, one additional band (15 m.w.) was separated from haemolymph of females fed on clover and two bands with females fed on lupine than above bands but no additional bands were recorded with females fed on horsebean (Fig. 2).

Trypsin activity :

Trypsin is one of the midgut enzymes that hydrolyses the peptide bonds, was tested in the nymphs of *E. plorans* fed on colver and compared with lupine and horsebean. Trypisn activity in midgut wall and midgut contents was measured under the standard conditions of enzyme activity.

Midgut wall trypsin of nymphs fed on clover (Fig. 3) had higher activity (1.3 nmol/min/ml) than those fed on horsebean or lupine leaves (1.07 and 0.82 nmol/min/ml, respectively). The same was observed in Fig. (4), the activity of trypsin in the midgut contents of the nymphs fed on clover was 1.15 nmol/min/ml but there was a reduction in the trypsin activity in the nymphs fed on horsebean or lupine (0.98 and 0.75 nmol/min/ml, respectively).

Reduction in the number of bands of separated proteins in the haemolymph of grasshopper fed on lupine and horsebean as compared with those fed on clover may be due to the adaptation of grasshopper on clover. Elsayed (1998 a) found that the grasshopper, *E. plorans* grow normally on clover but the presence of allelochemicals such as, quinolizidine alkaloid and non-protein amino acids in lupine and horsebean plants, respectively are responsible for antixenosis and antibiosis. The amount of haemolymph proteins was significantly lower in fifth instar nymph and mature adult females reared on lupine and horsebean than in females reared on clover (Elsayed, 1998 a & b).

Fig1,2

Fig3,4

The reduction of total proteins and protein bands in heaemolymph of *Schistocerca gregaria* fed on *Schowia purpurea* than those fed on wheat seedlings may be attributed to the glucosinolates in the leaves of *S. purpurea*

(Elsayed, 1994). Total protein in whole gut homogenates was significantly higher in kochia fed grasshopper in the second instar and significantly lower in those fed on oat in the fifth instar (Hinks and Erlandson, 1995). In Lepidoptera, concentrations of arylphorin (a major haemolymph storage protein) have been related to feeding and nutrient availability (Kamost *et al*., 1990). Six proteins with different molecular weights were separated from oocytes of *Melanoplus sanguinipes* fed on oats (Hinks *et al.*, 1993).

Many of plants contain proteinase inhibitors active against animal and insect enzymes (Hilder *et al.*, 1987). In this study, the trypsin activity in midgut wall or in midgut contents was of higher activity in grasshopper fed on clover than those fed on lupine or horsebean, which may be due to the presence of allelochemicals (quinolizidine alkaloid and non-protein amino acids) in these plants, which have had an effects on the enzymes system.Oak tannins inhibits the digestive enzymes in a herbivores gut (Feeny, 1969). Azadirachtin inhibits the production of trypsin in midgut of *Manduca sexta* (Timmins and Reynold, 1992); also Elsayed (1994) suggested that, the glucosinolates in *S. purpurea* may inhibit trypsin activity in gut of *S. gregaria*.

The results suggest that, proteins in the tissues of midgut wall and in midgut contents were markedly lowered by feeding on lupine or horsebean, this may be a reflection of trypsin reduction activity.The data of trypsin activity revealed that, feeding on lupine or horsebean may caused an effect on the enzyme system by the available allelochemicals and consequently on metabolism.This effect will decrease the female fecundity , which will be reflected on reduction of this pest population.

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تأثير أنواع الغذاء المختلفة على نشاط إنزيم التربسين فى جدار ومحتويات القناة الهضمية الوسطية لنطاط البرسيم ***Euprepocnemis plorans***

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تم تقدير البروتين فى هيموليمف حوريات العمر الخامس والطور الكامل للإناث فى نطاط البرسيم بعد التغذية على عوائل مختلفة وهى الترمس والفول و البرسيم وذلك لدراسة العلاقة بين نوع الغذاء ونشاط إنزيم التربسين فى القناة الهضمية الوسطية. أظهرت نتائج تحليل البروتين فى هيموليمف الحوريات بعد التغذية على كل من الترمس والفول نقص فى البروتين مقارنة بالتغذية على البرسيم. وكان نشاط إنزيم التربسين فى جدار القناة الهضمية الوسطية منخفضا فى الحوريات المتغذية على الترمس والفول عنه فى حالة تغذيتها على البرسيم.