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Effect of diet on the haemolymph protein profile of the desert locust, *Schistocerca gregaria* Forskal (Orthoptera: Acrididae) and susceptibility of adult to *Bacillus thuringiensis israelensis*

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ABSTRACT

In this study the effect of four types of plants diets (grass, sorghum, sesban and clover) on the changes in haemolymph protein and haemolymph protein profile of the adult desert locust *Schistocerca gregaria* (Forskal) and the susceptibility of locusts to *Bacillus thuringiensis* (*Bt*) have been investigated. The highest protein content was found in the haemolymph of locusts fed on clover and the lowest was found in insects fed on grass. In *Bt*-injected locusts, there were significant decreased in the total haemolymph proteins at all the experimental diets compared with control insects except those fed on sorghum which showed insignificant differences with controls. The susceptibility of the locusts to *Bt* was the lowest, when fed on clover, while it was the highest in locusts fed on grass and an intermediate response was shown in locusts fed on sorghum or sesban. Analysis of proteins by SDS-polyacrylamide gel electrophoresis showed disappearance of some protein bands and appearance of other new bands in the injected adults compared with controls. Some of these new proteins may affect adult immunity.

Key words: *Schistocerca gregaria, Bacillus thuringiensis*, plant diets, grass, clover, sorghum, sesban, haemolymph proteins and electrophoresis.

INTRODUCTION

The Red Sea coastal plains of Africa and the Arabian Peninsula are important breeding area for locusts and grasshoppers. This area has been implicated as a source or transit area for locust swarms that threaten agriculture. The desert locust, *Schistocerca gregaria* represents a very destructive herbivore. Locusts of only a part of a moderate swarm have devouring an amount of food plants suffice for 2500 persons (El-Ebiarie, 2011). Insects in their adult stage require carbohydrates, proteins and lipids to perform biological activities necessary for survival and reproduction (Chapman, 2012).

The quality of dietary protein is an important determent of key-history traits in phytophagous insects (Lee, 2007). Protein has been shown to affect important individual-level fitness-associated traits such as body size, growth rate, and fecundity; and at higher levels of organization has been linked to population dynamics, life histories, and even biological diversification (Fagan *et al.*, 2002).

Insect haemolymph is influenced by several factors, among them are age, diet, temperature and disease. The haemolymph plays a very important role in transport and storage of nutrients and is crucial for the recognition and defense against microorganisms (Bogaerts *et al.*, 2009).

Haemolymph usually contains, in addition to a large amount of water, protein which is the main nitrogenous constituent of all living materials. A considerable attention has been paid to the characterization of haemolymph proteins and their role in defense reactions. Pathogenic infection produces drastic changes in the haemolymph protein content of the infected host (Barakat and Meshrif, 2007).

Due to the successful use of *Bacillus thuringiensis* (*Bt*) formulations in the Egyptian fields against Lepidoptera and Diptera, it was necessary to evaluate the use of this pathogen against the most destructive orthopteran pest, *Schistocerca gregaria*. The maintenance of the immune system can be costly, and a lack of dietary protein can increase the susceptibility of organisms to disease (Alaux *et al*., 2010). Therefore this work aimed to investigate the relationship between the nutritional value of four different host plants and protein quantity and quality of insect haemolymph and impact of this relation in susceptibility of the desert locust to *Bt.*

MATERIALS AND METHODS

Insects rearing:

The desert locust, *Schistocerca gregaria* (Forskal) was obtained from the Locust and Grasshopper Research Department, Plant Protection Research Institute, Agricultural Research Center, Cairo, Egypt. Methods used for rearing and maintaining locusts were those reported by Huxham *et al.* (1989). The locusts were held at 30 ± 2˚C, a photoperiod of 16:8 (Light: Dark) and the relative humidity varied between 60 and 80%.

Plant diets and analysis of nutritional values:

Locusts were divided into four groups; in summer two groups reared on sorghum (*Sorghum bicolor*) and sesban (*Sesbania sesban*), while the other two groups were reared on grass (*Andropogon citratus*) and clover (*Trifolium alexandrinum*) in winter.

These plants were analyzed for protein, fat and carbohydrate content according to the methods described by Bradford (1976), Knight *et al.,* (1972) and Crompton and Birt (1967), respectively. All biochemical measurements were conducted in the micro-analysis unit at Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

All experiments outlined below were carried out with 10 adults (both sexes) after the second generation and all being within 2 - 4 days after ecdysis at the same rearing condition.

Susceptibility of locusts to the bacterial pathogen:

The bacterium, *Bacillus thuringiensis* formulated as wettable powder (AGERIN, 3200 IU/mg) was produced by The Agricultural Genetic Engineering Research Institute, Ministry of Agriculture, Egypt. *Bt* was grown aerobically at 28 ± 2˚C in nutrient broth tubes for 48 hr according to Barakat and Meshrif (2007). The grown bacteria were harvested by suspending in sterile distilled water and centrifuged at 6000 rpm for 30 min. The sediment bacteria were washed several times with a sterile saline solution and centrifuged again at the same rate till the saline solution becomes completely clear.

Ten µl of the bacterial concentration was injected into each locust in the last coxal corium (treated). Control insects were injected only with equivalent volumes of sterile distilled water. Another normal group of insects was not injected.

To determine the effect of food on the susceptibility level of *S. gregaria* adults to *Bt*. groups of insects, each containing 10 individuals, were injected as previously described with four doses: $2.2x10^4$, $2.2 x10^5$, $2.2 x10^6$ and $2.2 x10^7$ cells/ ml for insects fed on sorghum and sesban (summer season) and $2.72x10^4$, $2.72 x10^5$, $2.72 x10^6$ and $2.72 x10^7$ cells/ml for insects fed on grass and clover (Winter season). Final mortality percentages were scored at 48 hr post-injection. Collection and processing of haemolymph from normal, control and treated insects were done according to Hoffmann (1980).

Estimation of the total haemolymph protein:

A stock suspension of a dose of *Bt* that produces 20% mortality was injected into the haemocoel of the locusts for investigating the influence of pathogenic infection on the total haemolymph protein of the desert locust. The total protein content of the haemolymph was estimated in normal, control and injected locust adults according to the method described by Bradford (1976).

Electrophoretic analysis of the haemolymph proteins:

Ten µl of plasma (normal, control and immune) injected with LD_{20} of *Bt* was diluted with three volumes of treatment buffer (0.125 M tris-HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol), and then denatured by heating at 95 ºC for one min in water bath and chilled on ice until use. Then, the denatured plasma was analyzed by SDS-polyacrylamide gel slabs using P9DS apparatus from Owl separation systems. Electrophoresis conditions and procedures were as described by Laemmli (1970). Mixture of proteins marker (Sigma): 212, 120, 97, 66, 45, 31, 20, 14 and 7 kDa, was dissolved in bidistilled water and diluted in the treatment buffer and treated in the same manner as the samples to be used as a reference for the molecular weight (MW) of the apparent separated protein fractions. Electrophoresis was carried out at 40 volts for 24 hr at room temperature. After electrophoresis, the gels were removed. The resolving gel was fixed for an hour in fixing solution and then, was stained for 2 hr in Coomassie blue R-250. It was then socked in destain solution for 4 hr then, further destained for 24 hr with excess of destaining solution or until bands were visible.

Scanning and analysis of the bands:

The gel was scanned with gel documentation system by using a scanner (Scan tack, Sport Technology) and then, the bands were analyzed by using software: Gel-Pro Analyzer, version 3.1 for windows 95/NT, from Media Cybernetics 1993-1997. U.S.A.

Data analysis:

Statistical analysis of data was made by using software: probit analysis program, Version 4.0. Data of the rest experiments were made using origin program, Version 8.0, expressed as mean ± standard error (SE). Levels of significance for differences of means were determined using Student's *t*-test for paired samples and Anova one way. The level of significance for each experiment was set at $P \leq 0.05$ or $P \geq 0.01$.

RESULTS

Nutritional value of plants:

The mean nutrient composition of the investigated four plants (per 100 g fresh weight basis) on which the insects fed is presented in Figure (1). Results showed that the protein content was higher in clover (13.08 +1.28 g) followed by sesban (7.55+ 0.92 g), sorghum (6.2 +0.54 g), and grass (1.82 +0.31g). For carbohydrate content, sorghum had the highest carbohydrate content of 69.5 +0.38 g and grass had the least carbohydrate content of 25.31 +1.09 g. The fat content was highest in sesban (2.69 ± 1.37g) and least in grass (0.49 ± 0.84 g). The present results revealed that protein: carbohydrates (P: C) ratio in clover was the highest (1:3.3) followed by sesbsn (1: 3.7) then sorghum (1:11) and the lowest ratio was in grass (1:13.9).

Susceptibility of locusts to the bacterial pathogen:

Table 1 (A &, B) showed the susceptibility tests of *S. gregaria* adults fed on grass, sorghum, sesban and clover to the injection with *Bt*. The percent mortality in insects fed on clover was lower than the insects fed on sesban then those fed on sorghum and those fed on grass. The LD_{50} in locusts fed on the clover recorded the highest value (1.04×10^6 cells/ml), followed by seban fed locusts (8.4×10^5 cells/ml) then sorghum fed locusts (5.8×10^5 cells/ml) and finally grass fed locusts (5.2×10^5 cells/ml), The estimated LD_{20} for adults fed on sorghum, sesban, grass and clover were 3.1×10^4, 1×10^5, 1.1×10^5 and 1.37×10^5 cells/ml, respectively. These doses were used as sublethal dose to investigate the subsequent tests.

Effect of diet and *Bt* on the total haemolymph protein:

The influence of the different diets on the haemolymph protein of adult *S. gregaria* was studied (Fig. 2). It was noticed that the total haemolymph protein content varied significantly in the normal insects fed on the four diets where the insects fed on grass recorded the lowest haemolymph protein content (24.62 ± 1.46 mg/ml), while those fed on clover recorded the highest haemolymph protein content (62.1 0 ± 1.05 mg/ml). The susceptibility of the injected locusts with *Bt* (LD_{20}) on the total haemolymph protein was shown also in Figure (2). There was significant decrease ($P\leq0.05$) in the total haemolymph proteins at all the experimental diets compared with control, except those fed on sorghum which showed insignificant differences with control.

Electrophoretic analysis of the haemolymph proteins:

Normal haemolymph of *S. gregaria* adults fed on four experimental plants (Fig. 3) was totally fractionated into 26 protein bands, with MW ranging from 14.619 to 177.29 kDa (1 polymorphic, 4 monomorphic and 21 unique). Insects fed on sesban and clover, were found to have the highest number (11 bands), those fed on grass were characterized by having (10 bands), while those fed on sorghum was found to have the lowest number (8 bands). Insects fed on grass had 5 characteristic bands of MW 118.419, 51.755, 42.648,30.552 and 26.019 kDa, while in insects fed on sorghum there were 3 characteristic bands of MW 48.255, 37.536 and 27.791 kDa. In locusts fed on sesban, 6 characteristic bands of MW 111.685, 48.855, 40.591, 31.967, 27.224 and 24.259 kDa were detected. Concerning those fed on clover, haemolymph showed a great difference in protein bands with 7 characteristic bands of MW 73.144, 54.826, 42.124, 32.365, 24.764, 18.184 and 14.619 kDa.

The effect of *Bt* on haemolymph protein of *S. gregaria* adults fed on grass was shown in Figure (4). Control (10 bands) was characterized by the bands of molecular weights 75.75, 50.775, 43.775, 31.248, 26.106 and 3.597KDa, while the treated (12 bands) was characterized by the bands of molecular weights 82.875, 50.095, 40.738, 28.179 and 24.295 KDa.

The effect of *Bt* on hemolymph protein of *S. gregaria* adults fed on sorghum was presented in Figure (5). Control (10 bands) was characterized by seven bands of molecular weights 117.906, 77.312, 52.997, 42.441, 30.55, 26.151 and 3.495 KDa, while the treated (12 bands) was characterized by five bands of molecular weights 112.283, 50.47, 41.879, 28.708 and 24.574 KDa.

On the other hand, the effect of *Bt* on hemolymph protein of *S. gregaria* adults fed on sesban was shown in Figure (6). Control (10 bands) was characterized by the band of molecular weights 115.171, 52.238, 43.21, 30.514 and 3.396 KDa, while the treated (11 bands) was characterized by three bands of molecular weights 111.585, 49.036, 40.561, 27.752 and 24.455 KDa.

In *S. gregaria* adults fed on clover and injected with *Bt* (Fig. 7), Control (11 bands) was characterized by three bands of molecular weights 163.087, 98.327 and 3.643 KDa, while the treated (10 bands) was characterized two bands of molecular weights 155.053 and 92.797 KDa.

DISCUSSION

The physiological processes of insects are influenced by different biotic factors such as the quality as well as the quantity of food (Musa and Ren, 2005). In the present investigation, haemolymph protein content was assessed as an indication of insect condition. The results showed that the haemolymph protein contents in *S. gregaria* adults fed on clover was the highest followed by sesban then sorghum and finally grass. This variation was directly proportional to the ratio of protein to carbohydrate content of the plants. Protein to carbohydrate (P: C) ratio in clover was the highest (1:3.3) followed by sesbsn then sorghum and the lowest ratio was in grass (1:3.7). The same results were found by Telang *et al.* (2002) who showed that larvae of the moth *Heliothis virescens* reared on foods with a low protein to carbohydrate ratio gained less mass, grew more slowly, and contained significantly less storage protein reserves than individuals fed balanced or high protein diets. Bouayad *et al.* (2008) studied the influence of four commodities (wheat flour, dates, sorghum and barley) on protein content of *Plodia interpunctella* moth and found that protein content was lower for larvae fed on dates than for those fed on other commodities. The biochemical composition of different commodities showed that dates are a poor source of protein compared to the other commodities.

The present study clearly demonstrated that the locusts, injected with *Bt* (LD_{20}), fed on grass were the most susceptible group to *Bt* and vice versa in locusts fed on clover. This may be due to the powerful defense mechanisms of the locustst fed on high protein. Studies on the autumnal moth, *Epirrita autumnata* (Borkhausen) have demonstrated that intra-specific variation in host plant quality can alter the insect immune defense where eating a diet with low quality protein has been associated with high mortality, reduced growth, delayed development, and low reproductive output in insect herbivores (Lee, 2007). Interactions between insect herbivores and their pathogens can be modulated by host plants. Inter- and intraspecific differences in plant chemistry and structure can alter the susceptibility of insects to infection and the production and environmental persistence of pathogens (Cory and Hoover, 2006).

Locusts fed on four diets were injected with *Bt* (LD_{20}). After 48 hr, there was a significant decrease in the total haemolymph protein at all the experimental diets compared with control, except those fed on sorghum which showed insignificant differences with control. This may be attributed to the action of bacteria. Barakat and Meshrif (2007) found variation of total haemolymph proteins of the desert locust *Schistocerca gregaria* (Forskal) with changing the rearing diet and the injection of *Bacillus thuringiensis kurstaki* Berliner (*Bt*). Blanco *et al.* (2009) demonstrated that susceptibility of tobacco budworm *Heliothis virescens* to the Cry1Ac toxin from *Bacillus thuringiensisin* can be affected by the type of diet that this protein is incorporated into. In studies with natural plant material, Diamond and Kingsolver (2010) compared the effect of high and low quality food plants on the immune response of *Manduca sexta* and found that the better quality host plant improved body condition and encapsulation response of larvae following an immune challenge. This was also demonstrated by Bauce *et al*. (2002) who showed that the impact of an increase in *Bt* concentration on larval mortality was strongly dependent upon food quality where increase of *Bt* concentrations resulted in moderate mortality of spruce budworm, *Choristoneura fumiferana* (Clem.) fed on high protein diet, greater larval mortality on medium protein diet. Natural diets fed locusts were found to be more resistant to *Bt* than artificial diet fed locusts. These results was also found by Shikano *et al.* (2010) who found that cabbage loopers were more resistant to viral infection when reared on the better food plant, broccoli, than on cucumber. Encapsulation, phenoloxidase (PO) activity, and lysozyme activity are all enzyme-based immune responses to foreign invasion or wounding that may be affected by protein deficiency (Siva-Jothy *et al*., 2005). Caterpillars of *Spodoptera* moth fed protein-rich diets had enhanced immunity to a viral pathogen relative to those fed on carbohydrate-rich diets (Lee *et al*., 2006). Dietary protein limited PO activity with direct consequences to antibacterial activity, and *Spodoptera* caterpillars increased their protein intake in response to infection while ingesting similar levels of carbohydrates (Povey *et al*., 2009).

SDS-Polyacrylamide gel electrophoresis (PAGE) has been extensively used as an excellent tool for the separation of proteins, lipoproteins and glycoproteins from both plant and animal sources (Zacharius *et al.*, 1969). Locusts fed on grass had 5 characteristic bands, while those fed on sorghum had 3 characteristic bands. In locusts fed on sesban, 6 characteristic bands were determined. Concerning those fed on clover; haemolymph showed a great difference in protein bands with 7 characteristic bands. The quality of plant protein is primarily determined by its amino acid compositions, which is highly variable between plant species and between tissues within a plant (Felton 1996). Huerta *et al.* (2007) reported similar findings with the larvae of *Gonipterus scutellatus* (Coleoptera: Curculionidae) fed on three different eucalyptus species which showed three different protein profiles. They suggested that the change in diet could have produced the differences observed in proteins bands among larvae. Habibi *et al.* (2001) also found that the past dietary history of the phytophagous hemipterans influenced their salivary protein composition. For example, in the saliva of *Empoasca fabae* (Harris) fed on broad bean, four extra bands became visible compared with treatment simple diet. The present results demonstrated disappearance and appearance of protein bands after *Bt* injection. This may be attributed to their involvement in the immune reactions. Food quality can also affect the number or quality of the components that make up the immune system (Szymas & Jedruszuk, 2003). Plants that are higher in nitrogen and lower in carbon are considered to be of high quality (Strengbom *et al.,* 2008). Grasses cover over 40 percent of the Earths landscape, which provide a large, but nutritionally poor resource for herbivores (Tscharntke & Greiler, 1995). While clover *Trifolium alexandrinum* not only has a high quality protein but also has a variety of biologically active molecules such as vitamins, minerals and other nutrients (Burris and Roberts, 1993). Lee *et al.* (2008) observed that *Spodoptera littoralis* larvae fed on the low quality (Zein) protein diet had a lower haemolymph protein pool than those fed on the high-quality (casein) protein diet. They revealed that the lower protein quality resulted in fewer resources being available for provisioning the substrates (tyrosine) or enzymes (phenoloxidase, PO) required for melanin synthesis.

In conclusion, the insects fed on grass recorded the lowest haemolymph protein content while those fed on clover recorded the highest haemolymph protein. The change in diet produced differences observed in the protein profiles among groups of *S. gregaria* adults. Adults fed on clover (high protein diet) showed the least susceptibility to *Bt* , while those fed on grass (low protein quality) showed the least susceptibility. Host plant may alter the vulnerability of insects to *Bt*.

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تأثير الغذاء على المحتوى الكلي والفصل الكهربي لبروتين الهيموليمف للجراد الصحراوي *شيستوسيركا جريجاريا* (فورسكال) وحساسية الحشرة للبكتريا *(باسيلس ثيرونجنسيز)*

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1- قسم علم الحشرات- كلية العلوم- جامعة عين شمس
2- قسم علم الحشرات- كلية العلوم- جامعة بنها

تختلف النباتات عن بعضها البعض في العناصر الأساسية من البروتينات والكربوهيدرات ؛ ويعتبر البروتين من أهم العوامل التي تؤثر على الحشرات من الناحية الفسيولوجية والبيولوجية والبيئية. وفى هذه الدراسة تم مقارنة تأثير أربع أنواع من النباتات وهم البرسيم والسيسبان والذرة والحشيشة على حشرة الجراد الصحراوي *شيستوسيركا جريجاريا* (فورسكال) من حيث المحتوى الكلي والفصل الكهربي للبروتينات وحساسية هذه الحشرة للبكتريا *(باسيلس ثيرونجنسيز)* من خلال الحقن. وتبين من النتائج أن اختلاف محتوى البروتين في النباتات كان له علاقة طردية على المحتوى الكلي لبروتينات الدم في الجراد الصحراوي؛ حيث سجلت الحشرات المتغذية على البرسيم أعلى نسبة في المحتوى البروتيني يليه المتغذية على السيسبان ثم الذرة فالحشيشة. أيضا سجل الفصل الكهربي للبروتينات اختلافا واضحا بين الأربع مجموعات من الجراد.و قد انعكس هذا التأثير عند إصابة الحشرة بالبكتريا بشكل عكسي حيث سجلت الحشرات المتغذية على البرسيم أقل نسبة إصابة بينما الحشرات المتغذية على الحشيشة سجلت أعلى نسبة إصابة. هذا يدل على أن مناعة الحشرة قد تزداد كلما زاد المحتوى البروتيني للحشرة.

Table (1-A): Susceptibility of *S. gregaria* adult, reared on sorghum and sesban (summer season) to *Bt* at 48 h post injection.

Dose (cells / ml)	Locusts fed on sorghum		Locusts fed on sesban	
	Observed mortality (%)	Expected mortality(%)	Observed mortality (%)	Expected mortality(%)
$2.2X10^4$	10	9.52	10	6.75
$2.2X10^5$	30	35.39	20	28.76
$2.2X10^6$	80	71.24	70	64.61
$2.2X10^7$	90	93.25	90	90.48
Control	-	-	-	-
Chi^2calculated	0.67		0.67	
Chi^2 tabulated	6.0		6.0	
Slope	0.94 +/- 0.25		0.94+/-0.25	
LD_{50}	5.8×10^5 cells/ml		8.4×10^5 cells/ml	
LD_{20}	1×10^5 cells/ml		1.1×10^5 cells/ml	

Table (1-B): Susceptibility of S. *gregaria* adult, reared on grass and clover (Winter season) to *Bt* at 48 h post injection.

Dose (cells/ml)	Locusts feed on grass		Locusts feed on clover	
	Observed mortality (%)	Expected mortality (%)	Observed mortality (%)	Expected mortality (%)
$2.72X10^4$	20	15.73	10	6.75
$2.72X10^5$	30	41.52	20	28.76
$2.72X10^6$	80	71.81	70	64.61
$2.72X10^7$	90	91.43	90	90.48
Control	-	-	-	-
Chi^2calculated	1.04		0.67	
Chi^2 tabulated	6.0		6.0	
Slope	0.7914 +/- 0.2259		0.9352 +/- 0.2467	
LD_{50}	5.2×10^5		1.04×10^6	
LD_{20}	3.1×10^4		1.37×10^5	

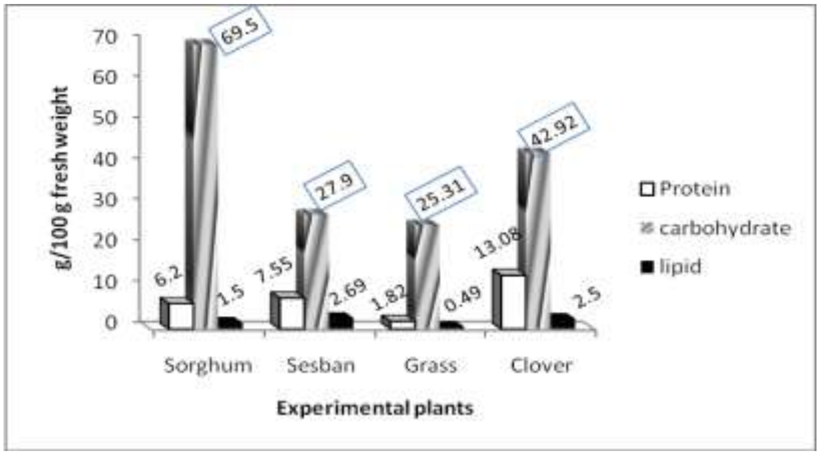

Fig. 1: Nutritional value of four experimental plants (per 100 g fresh weight basis).

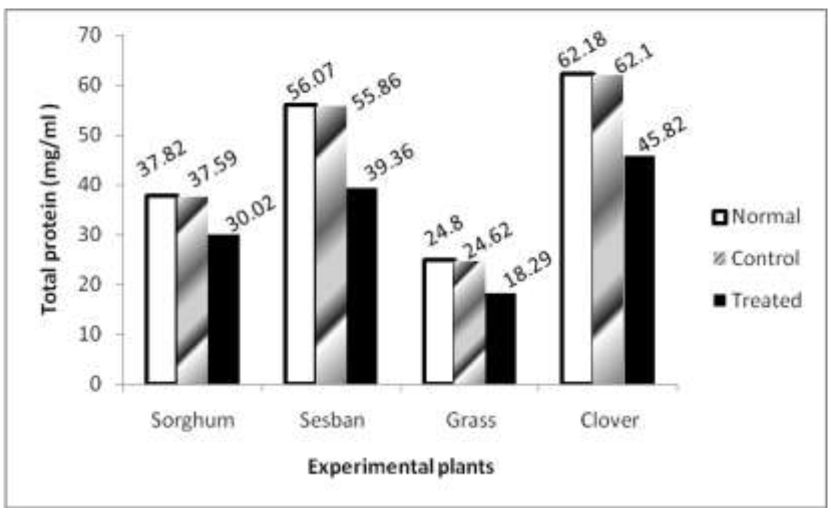

Fig 2: The total protein content (mg/ml) of *S. gregaria* adult haemolymph fed on four experimental plants after 48 hr post-injection with *Bt*.

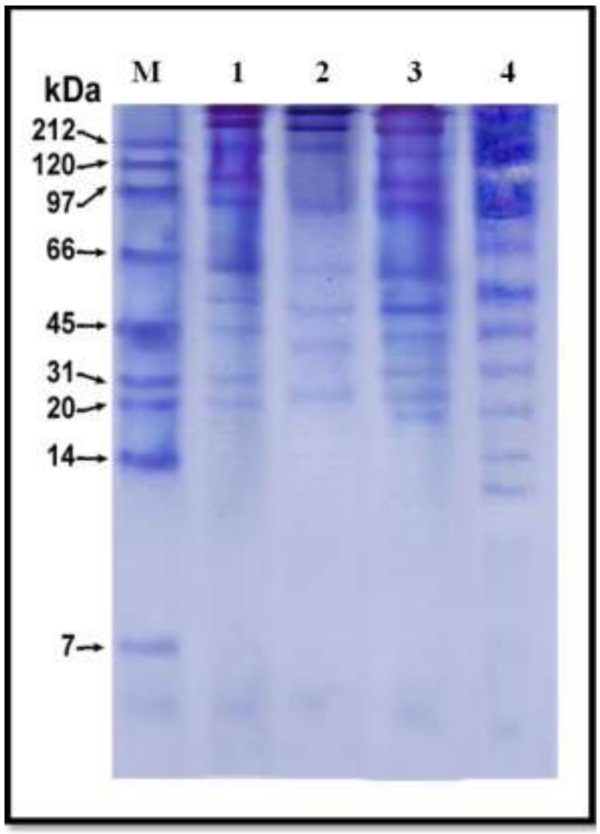

Fig 3: Haemolymph protein banding patterns of *S. gregaria* fed on four experimental plants.

M : marker protein.

Lane 1: protein bands of insect fed on grass.

Lane 2: protein bands of insect fed on sorghum.

Lane 3: protein bands of insect fed on sesban.

Lane 4: protein bands of insect fed on clover.

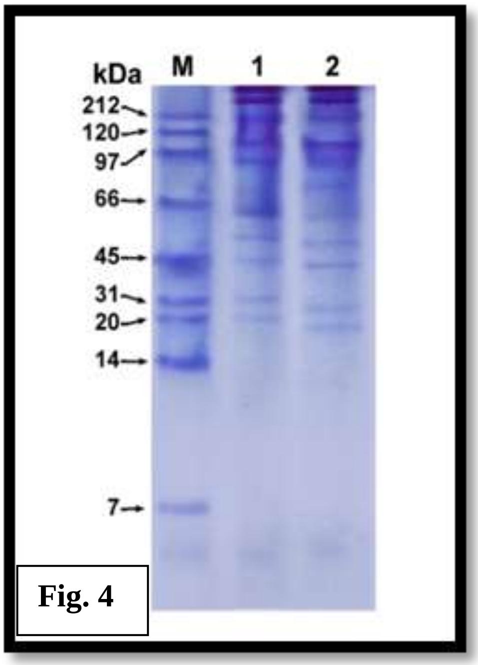

Fig. 4

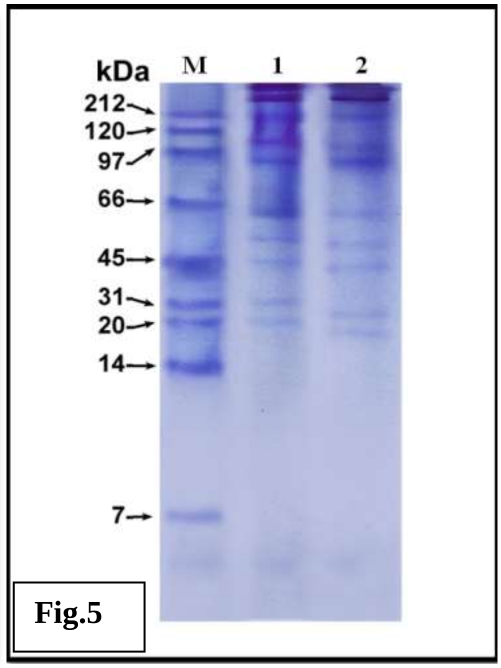

Fig.5

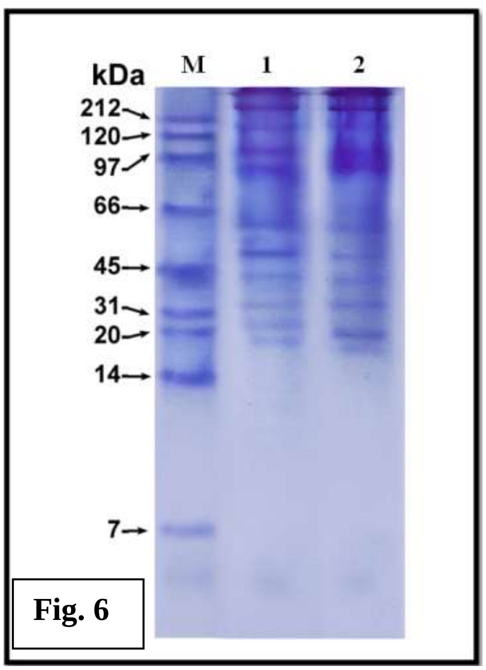

Fig. 6

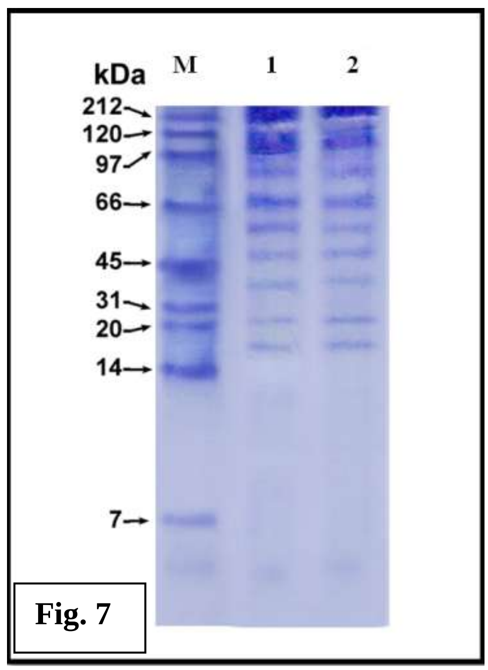

Fig. 7

Figures 4-7: Haemolymph protein banding patterns of *S. gregaria* after 48 hr *Bt* post-injection from feeding on grass (Fig. 4), sorghum (Fig. 5), sesban (Fig. 6) and clover (Fig. 7). {M: marker protein, Lane 1: control , Lane 2: treated}