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Interactions of Host Plants and *Bacillus thuringiensis israelensis* Injection on the Performance and Midgut Protein Profile of *Schistocerca gregaria* Forskal, Adults

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ABSTRACT

This study highlights the complex relationship among the type of food, the performance of the locusts and midgut proteins as well as the impact of bacterial injection on the midgut proteins of adult. Grasshopper nymphs were supplied by different types of natural vegetation (grass, sorghum, sesban and clover) according to the seasons. The desert locust, *Schistocerca gregaria* Forskal fed on clover showed highest growth rate and largest mass and, vice versa in grass fed locusts. The insects fed on grass (low protein diet) recorded lowest midgut protein content while those fed on clover (high protein diet) recorded highest midgut protein. The change in diet produced protein profiles differences among the feeding groups of *S. gregaria* adults. Insects fed on clover had many characteristic bands in protein profile of midgut and *vice versa* in case of those fed on grass. After *Bacillus thuringiensis israelensis* (*Bti*) injection, adults fed on clover showed the least susceptibility to *Bti* and *vice versa* in grass. Analysis of midgut proteins by SDS-polyacrylamide gel electrophoresis showed appearance of new bands in the injected adults compared to normal ones. In conclusion, this study emphasizes the close relationship between the type of food and the performance of the insect. It also confirmed the impact of food and *Bti* injection on midgut proteins.

Key words: Adult performance, *Bacillus thuringiensis israelensis*, *Schistocerca gregaria,* midgut protein profile, nutrition.

INTRODUCTION

Growth and reproduction of phytophagous insects are largely dependent on the quality of their host plant (Awmack and Leather, 2002). Grasshoppers are unique among herbivorous insects in that they are largely polyphagous, feeding selectively on host plant species from a number of unrelated plant families (Chapman, 1990). Insects feeding on protein-rich plants are generally more successful than that consuming plant material containing less protein (Raubenheimer and Simpson, 1997). Negative effects on the protein economy of grasshoppers can severely reduce growth, survival and reproduction where stimulate compensatory feeding and dietary self-selection (Fagan *et al.*, 2002). In addition, food quality interacts to locust phase state through effects of crowding and locomotion. It would be profitable for desert locust to aggregate in the places where the host plants are the most nutritious. Host plant-associated ecological divergence among populations that utilize different plants is often assessed in terms of traits such as preference measures of performance in utilization of different hosts such as growth, survivorship and fecundity (Funk *et al*., 2002). The host plants with high leaf nitrogen content not only increases their fitness, but also the likelihood of gregarization and outbreaks (Van Huis *et al*., 2008).

Digestion and absorption of food materials is the main role of gut and proteins are key molecules for such function (Bezdi *et al*., 2012). Furthermore, gut of insects contains various proteins that are necessary for keeping homeostasis in normal condition (Yao *et al.,* 2009). Gut forms a barrier to entomopathogenic agents that enter through the oral route (Pauchet *et al.,* 2008). For example, *Bacillus thuringiensis (Bt)* toxins act in gut of insects and disturbed ion balances in epitheliums cells (Hakim *et al.,* 2010). Efficiency of gut in digestion and immunity system is related to express proteins in it (Pauchet *et al.,* 2008). The study of protein is a critical step in understanding physiological role of a selected organ (Liu *et al*., 2009).

Bt is a bacterium of great agronomic and scientific interest. It is an endospore-forming bacterium characterized by the presence of a protein crystal within the cytoplasm of the sporulating cell. The proteins within this crystal are toxic to insects, which explain the extensive use of *Bt* as a biological insecticide (Rudd *et al.,* 2001). Large differences in gut physiology among different insect orders might have a role in this aspect (Terra and Ferreira, 1994). The highly specific activity of *Bt* might limit its use on crops where problems with several pests occur, including non-susceptible species such as grasshoppers.

Diet and health are intimately linked and recent studies have found that caloric restriction can affect immune function. However, when given a choice between diets that differ in their macronutrient composition, pathogen–infected individuals can select a diet that improves their survival, suggesting that the nutritional composition of the diet, as well as its calorie content, can play a role in defense against disease (Cotter *et al*., 2010).

Goal of the present study was to determine the influence of experimental plants (sorghum, sesban, grass and clover) on the performance of the desert locust *Schistocerca gregaria* Forskal and its susceptibility to injected *Bt israelensis* as well as on the midgut protein content and protein profile of adult.

MATERIALS AND METHODS

Insect rearing

S. gregaria was obtained from Locust and Grasshopper Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. Insects were reared and maintained as the method described by Huxham *et al.* (1989). Experimental plants were selected according to the season. In summer, insects were divided into two groups; one group was reared on sorghum (*Sorghum bicolor*), while the other was reared on sesban (*Sesbania sesban*). In winter, one group was reared on the grass (*Andropogon citratus*) and the other on Egyptian clover (*Trifolium alexandrinum*). All colonies and experiments were held at 30±2˚C, a photoperiod of 16:8 (Light: Dark) and R.H. varied between 60 and 80%.

Effect of host plant on *S. gregaria* adult performance

A bioassay of *S. gregaria* growth and development was performed on different experimental plants as follows: newly hatched nymphs were collected from the colonies and placed in cages supplied with a layer of sterilized sand. Each group was supplied daily with different diets of standard food (sorghum, sesban, grass or clover) according to the season and was observed until reaching the adult stage. Daily observation of the emerged adults was continued until death to calculate the developmental periods, adult longevity and adult body weights. Each experiment was replicated five times (20 insects/ replicate).

Bacterial pathogen

Bt israelensis (1600 IU/mg, wettable powder) was obtained from the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center, Giza, Egypt. Four groups of *S. gregaria* adults (2-4 days) were fed on the experimental plants and injected with *Bti* LD_{20} according to Barakat *et. al.* (2013), as follows, 3.1×10^4, $1.37\times10^{5,}$ 1×10^5,and 1.1×10^5cells/ml for grass, clover, sorghum and sesban, respectively. Injection was made by a 10 µl Hamilton micro-syringe fitted with a 26-gauge needle. Ten µl were injected into each insect. Control insects were injected only with equivalent volumes of sterile distilled water.

Preparation of midgut samples for the biochemical study

Adults of the desert locust (2-4 days), fed on the four experimental plants, were dissected from the ventral side and the midgut was extracted, washed rapidly in saline solution 0.9% NaCl, and intensely immersed in distilled water. The midgut was homogenized in distilled water (5 insects/5 ml) using a chilled glass Teflon tissue grinder for 3min. Homogenates were centrifuged at 3500 rpm for 10 min at 2°C in a refrigerated centrifuge. In addition, homogenates collected from un-injected, water-injected and bacteria-injected insects were referred to as normal, control and treated, respectively.

Estimation of the total midgut protein

The midgut tissue protein was estimated in normal, control and injected locust adults (at 48 h of injection) according to the method described by Bradford (1976).

Electrophoretic analysis of the midgut proteins

The midgut proteins of normal, control and 48 h *Bti* injected adults were analyzed by SDS (sodium dodecyl sulfate)-polyacrylamide gel slabs. Electrophoresis conditions and procedures followed that of Laemmli (1970). The gel was scanned with gel documentation system using a scanner (Scan tack, Sport Technology). The bands were analyzed by the 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 the software: Probit analysis program, Version 4.0. Data of the rest experiments were made using origin program, Version 8.0, expressed as mea ±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 $P \leq 0.01$.

RESULTS AND DISCUSSION

Influence of experimental plants on the performance of locust was measured by three parameters, developmental time, adult longevity and adult weight (Table 1). The insects fed on grass recorded the longest average time of development, while the ones fed on clover recorded the shortest in developmental time with significant difference. Male

Table (1): Effect of 4 different plants on *S. gregaria* performance

Experimental Plants	Developmental Time (days)	Adult longevity (days)		Adult body weight* (g)
		Male	Female	
Sorghum	47.67±1.2^{ab}	36.6±1.45^{a}	35±0.58^{a}	1.24±0.49^{a}
Sesban	46.33±0.00^{ab}	37.3±1.77^{a}	35.7±0.89^{a}	1.31±0.28^{a}
Grass	53.34±2.40^{a}	27.4±1.16^{b}	23.4±o.89^{b}	1.16±1.43^{ab}
Clover	45.23±2.52^{b}	44.7±3.10^{c}	42±1.45^{c}	1.44±0.50^{ac}

Means within a column followed by the same lower case letter are not significantly different at $P \leq 0.05$.
*: 2-4 days

Table (2): Effect of 4 different plants and LC$_{20}$ injected *Bti* on total midgut protein of *S. gregaria* adults (2-4 day old) at 48 h post-injection

Experimental plants	Total protein• (mg/ml) (mean ± SE)		
	Normal adults	Control adults	Treated adults
Sorghum	16.03 ± 0.57^{b}	16.78 ± 1.72	11.91 ± 1.2**
Sesban	18.20 ± 0.65^{a}	18.85 ± 1.31	15.10 ±1.15*
Grass	14.30 ± 1.08^{b}	13.65 ± 2.11	9.22 ± 1.90**
Clover	22.14 ± 2.64^{a}	21.37 ± 2.70	17.39± 1.91*

Vertically means bearing different letters are significantly different at $P \leq 0.05$.
* Significance at $P \leq 0.05$. ** High Significance at $P \leq 0.01$.

and female longevity of insects fed on clover were the highest, while those fed on grass recorded the lowest one and varied significantly. Highest weight was recorded for insects fed on clover, followed by those fed on sesban, and then on sorghum, while the lowest weight occurred in insects fed on grass. This observation showed that the type of food had clear impact on locust performance. Protein and amino acid requirements of locusts and grasshoppers are similar to those of other manipulate, phytophagous insects. Negative effects on the protein economy in grasshoppers can severely reduce growth, survival, reproduction and stimulate compensatory feeding and dietary self-selection (Hinks *et al*., 1993). Barakat *et al.* (2013) found that the protein content was higher in clover, followed by sesban, sorghum and grass. Plants that are higher in nitrogen and lower in carbon are considered high quality (Strengbom *et al.*, 2008). Protein is more limiting to the growing nymphs. At the nutrient levels, locusts regulated their intake of food with respect to protein where they consume more of lower protein diets than of higher protein diets. Decrease of weight gained in the male and female is due to an effect on protein synthesis (El-Sayed and Al-Otaibi, 2006). This finding was confirmed by Van Huis *et al.* (2008) who found that locusts reared on high-nitrogen leaves were larger, developed faster, had higher survival, reproduced more and earlier, and showed greater synchronization than those fed on low-nitrogen leaves. Also, Hahn (2005) determined that 5th nymphal instar of *Schistocerca americana* fed intermediate on high nutrient content diets as nymphs were significantly larger than individuals fed low nutrient content diets. Low growth rate of *S. gregaria* on grass could be attributed to low conversion rate of the digested food. In agreement with Bin *et al.* (2011) who found that with increased protein content, the development period of the beet armyworm *Spodoptera exigua* (Hübner) decreased. In addition, pupal weight increased, pupal period decreased and adult longevity tended to be longer.

The influence of food type and injection of grasshoppers with *Bti* LD$_{20}$ on the midgut protein was presented in table (2). Normal adults fed on clover recorded highest protein content, while those fed on grass recorded lowest midgut protein content. A significant change at ($P \leq 0.05$) was observed between insects fed on clover and those fed on sorghum and grass.

In *Bti*-injected locust, there were significant decreases at ($P \leq 0.05$) in the total midgut protein recorded for insects fed on sesban and clover, respectively, compared to those of control insects fed on the same plants. High significance decreases at ($P \leq 0.01$) in the total midgut protein derived from insects fed on sorghum and grass compared to control insects fed on the same plants, respectively. It was noticed that the total protein content of bacterial treated locusts, fed on clover, was also the highest, while the lowest value was observed between those fed on grass and the intermediate values were recorded to adult insects fed on sorghum and sesaban.

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, the midgut protein contents were assessed as an indication of insect condition. The variation of insects protein content was directly proportional to differentiation in protein content of the eaten food. Jones and Raubenheimer (2001) mentioned that the nymphs of the German cockroach, *Blattella germanica* (L.) given the high protein food contain more nitrogen in their body content. Telang *et al.* (2002) also 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. Furthermore, 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 (poor source of protein) than for those fed on other commodities (rich source of protein).

After *Bti* injection, there were significant decreases in the total midgut protein at all the experimental diets compared to control. Perhaps, the locusts were not able to feed at normal rate when injected with *Bti* and there could be a change in digestion and absorption of nutrient (Anandakumar and Michael, 2011). It was noticed that the total protein content of treated locust with *Bti*, fed on clover, was also the highest, but the lowest appeared in those fed on grass, while the intermediate values were recorded in locusts fed on sorghum and sesban. Barakat and Meshrif (2007) determined variation of total haemolymph proteins of the desert locust, *S. gregaria* with changing the rearing diet and injection of *Bt kurstaki* (*Btk*). They noticed that locusts fed on natural diets were more resistant to *Btk* than artificial diet fed locusts. Further, *Spodoptera* moth caterpillars that fed on protein-rich diets had enhanced immunity to pathogen relative to those fed on carbohydrate-rich diets (Lee *et al.*, 2006). Protein limitations constrain the synthesis of the antimicrobial peptides (AMP), and therefore expression of the genes encoding them and then increased protein availability in the diet would allow a greater investment in AMP production (Fellous and Lazzaro, 2010). This confirmed the finding of Bauce *et al*. (2002) who showed that the impact of increased *Bt* concentration on larval mortality was strongly dependent upon food quality. They found that increased *Bt* concentrations resulted in moderate mortality of spruce budworm, *Choristoneura fumiferana* (Clem.) fed on high protein diet and greater larval mortality occurred with medium protein diet but not on low diet. On this last diet, mortality was mainly due to food unsuitability than to *Bti*. Blanco *et al.* (2009) demonstrated that susceptibility of tobacco budworm, *Heliothis virescens* to the Cry1Ac toxin from *Bt* can be affected by the type of diet that this protein is incorporated into. Shikano *et al.* (2010) revealed that the high mortality observed in cabbage loopers larvae reared on cucumber with poor nutritional quality result in less investment in immune factors.

Results of midgut protein of *S. gregaria*, fed on experimental plants (sorghum, sesban, grass and clover), were present in plate (1). The protein bands of the midgut varied in number and density. The total number of polypeptide bands was 22 bands, with the molecular weight (MW) ranging from 174.02 to 2.55 kDa and to induce the protein component, the mobility rate (Rm) varied from 0.021 to 0.987. Variability analysis showed two polymorphic, four monomorphic and 16 were unique bands. In insects fed on clover, midgut protein had the highest number of bands (13 bands), while those fed on grass had the lowest number of (6 bands).

The protein pattern in LD_{20} *Bti* injected adults fed on sorghum at 48h post-injection; results were present in plate (2). The midgut protein was separated into 15 bands with MW ranged from 180.53 to 2.55 kDa and Rm values from 0.012 to 0.987. Variability analysis resulted four monomorphic, three polymorphic bands, while eight bands were unique. Number of bands in treated insects (12 bands) exceeded that of the control (7 bands) by five bands. The control was characterized by bands with MW of 106.78, 67.68 and 28, 77 kDa. In treated locust, it was characterized by bands with MW of 180.53, 100.55, 48.38, 43.46, 22.76, 17.38, 9.33 and 5.29 kDa.

Concerning, the effect of injected *Bti* on midgut protein profile of locust fed on sesban was appeared in plate (3), the protein profile was found separated into 16 bands with MW ranged from 142.31 to 2.55 kDa and Rm from 0.067 to 0.987. Variability analysis showed four monomorphic, six polymorphic bands and six unique bands. Number of bands in treated locust (10 bands) and that of control (10 bands) were equal. Control was characterized by bands with MW of 142.31, 115.44, 73.68, 44.98, 11.70 and 7.74 kDa. Treated locust was characterized by bands with MW of 117.63, 68.48, 34.34, 16.45, 11.55 and 7.23 kDa appeared in the bacterial injected adults.

Plate (4) showed the effect of *Bti* injection on midgut protein bands of locust fed on grass, the bands differed in number and density. Total number was 10 bands with MW ranged from 118.50 to 2.55kDa and with R_m values from 0.108 to 0.987. Variability analysis showed five monomorphic, one polymorphic band and four unique bands. Number of bands in treated locust (9) exceeded that of control (6) by three bands. Control was characterized by band with MW of 108.16 kDa and R_m values of 0.129. Treated locust was characterized by bands with MW of 118.50, 64.99, 44.15and 34.18kDa.

The midgut protein profile of *Bti* injected locust fed on clover was in (plate 5), showed total number

Normal adults: un-injected, Control: water-injected, treated adults: bacteria-injected.

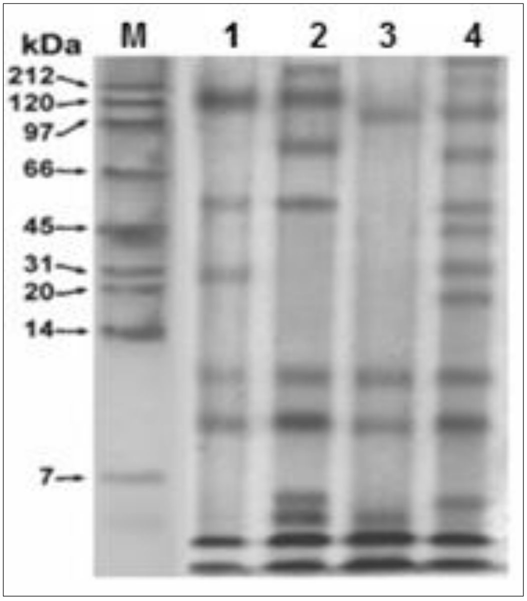

Plate (1): Changes in the midgut protein banding patterns of *S. gregaria* adults (2-4 days old) fed on 4 different plants.

M : marker protein.
Lane 1: protein bands of insect fed on sorghum.
Lane 2: protein bands of insect fed on sesban.
Lane 3: protein bands of insect fed on grass.
Lane 4: protein bands of insect fed on clover**.**

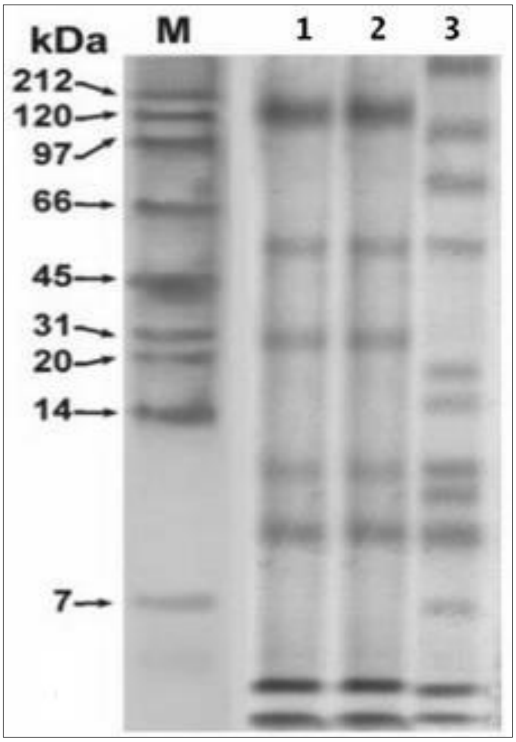

Plate (2): Changes in the midgut protein banding patterns of *S. gregaria* adults (2- 4 days old) injected with LC_{20} of Bti, fed on sorghum at 48 hr post-injection.

M : marker protein.
Lane 1: normal
Lane 2: control
Lane 3: treated

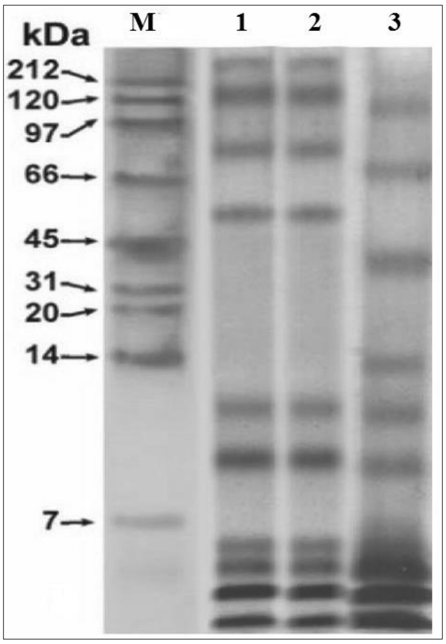

Plate (3): Changes in the midgut protein profile of *S. gregaria* adults (2-4 days old) injected with LC_{20} of Bti fed on sesban after 48 hr post-injection.

M : marker protein.
Lane 1: normal
Lane 2: control
Lane 3: treated

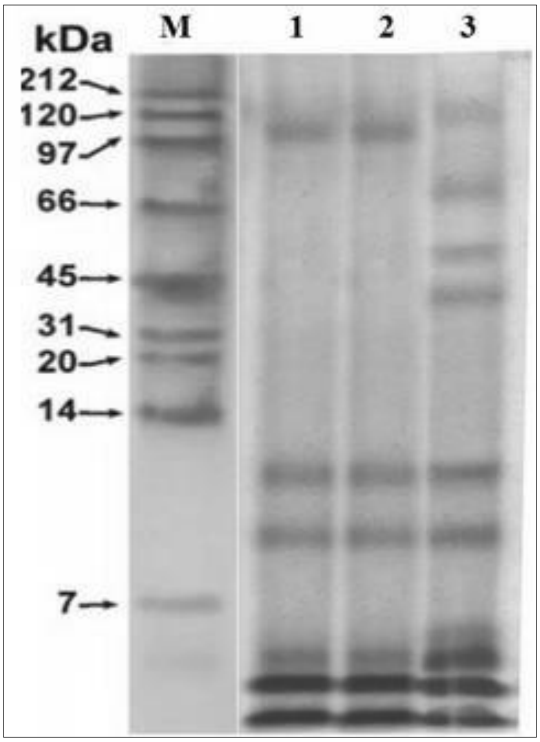

Plate (4): Changes in the midgut protein profile of *S. gregaria* adults (2-4 days old) injected with LC_{20} of Bti, fed on grass at 48 hr post-injection.

Lane 1: normal
Lane 2: control
Lane 3: treated

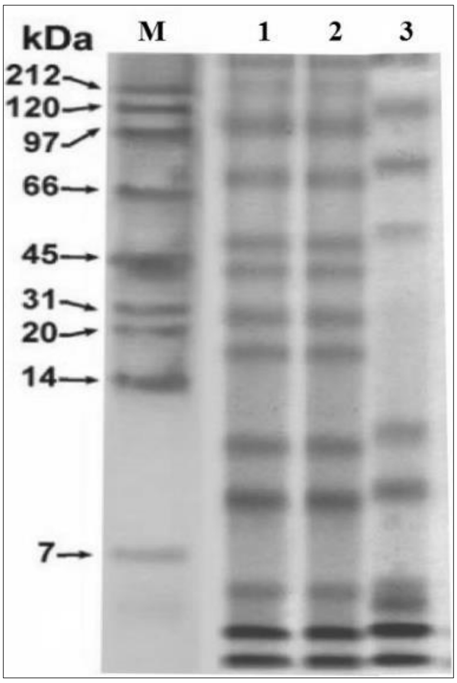

Plate (5): Changes in the midgut protein banding profile of *S. gregaria* adults (2-4 days old) injected with LC$_{20}$ of Bti, fed on clover at 48 hr post-injection.

M : marker protein.
Lane 1: normal
Lane 2: control
Lane 3: treated

of bands (20 bands) and had MW ranged from 180.38 to 2.55kDa with R$_m$ values from 0.012 to 0.987. Two of them were monomorphic, 11 polymorphic bands and seven unique bands. Number in treated locust bands (9) lowered that of control (13 bands) by four bands. Control locust was characterized by bands with MW of 174.02, 147.61, 113.32, 72.34, 47.50, 38.24, 29.53, 21.41, 11.70, 7.74 and 4.22 kDa. Treated locust was characterized by bands with MW of180.38, 104.96, 72.76, 45.79, 11.31, 7.22 and 3.67 kDa.

This study showed clearly that the type of food had an influence in the midgut protein profile. Habibi *et al.* (2001) found that the saliva of *Emposca fabae* fed on broad bean had four extra bands that became visible compared with treatment simple diet. Huerta *et al.* (2007) reported that larvae of *Gonipterus scutellatus* (Coleoptera: Curculionidae), fed on three different eucalyptus species, showed three different protein profiles and suggested that the change in diet could have produced the differences observed in protein bands among larvae. Qualitative analysis of silkworm proteins by El-Akkad *et al.* (2008) showed obvious variations in the number and position of bands when fed different mulberry varieties. Appearance of particular bands, when larvae are fed on certain variety that disappears with another variety, may be explained by varietal differences in crude protein content. Lee *et al.* (2008) observed that *S. littoralis* larvae fed on the low-quality protein diet had a lower hemolymph protein pool than those fed on the high-quality protein diet and suggested two possible explanations for this observation, the first is that larvae fed on the low-protein diet had reduced intake rates, *i.e*. nutrient acquisition and the second is that they had reduced nitrogen utilization efficiency post-ingestion.

Obtained results indicated that disappearance and appearance of protein bands in midgut protein profile after *Bti* injection 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 and Jedruszuk, 2003). In the 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 the encapsulation response of larvae following an immune challenge. Overall, better food quality is frequently associated with increased immune responses, but not always to increased disease resistance as different immunity factors that are likely to target different pathogens and parasitoids (Strand, 2008).

In conclusion, obtained results confirm the previous field observations that the type of food has obvious effect on the performance of locusts, as well as the immune system and its ability to overcome diseases. Such an understanding of the mechanisms of response towards dietary changes would improve the control of *S. gregaria* by using *Bt* and other pathogens.

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