Impact of farnesol on the food consumption and utilization, digestive enzymes and fat body proteins of the desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae)

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The food consumption, nutritional indices and the digestive enzymes (protease, invertase, amylase, trehalase and chitinase) of *Schistocerca gregaria* were affected by treatment with farnesol. Treated insects oviposited fewer eggs. The total haemolymph and midgut protein significantly increased in farnesol-treated insects as compared with the control. Protein profiles in the fat body of treated females showed a significant ($P < 0.05$) change compared with the control females. This change in the intensity might reflect the decline in the synthesis or the increase in the utilization of this protein.

Key words: grasshoppers, locusts, terpenes, consumption indices, fecundity.

INTRODUCTION

The desert locust, *Schistocerca gregaria*, is a major pest in northern Africa and Middle East. In Egypt, *S. gregaria* is considered the most economic pest in the eastern desert, western desert, Sinai and Faiyom (Haggag 2011). Existing control methods against this pest still rely on conventional synthetic insecticides that are hazardous to the ecosystem.

Plant essential oils produce secondary metabolites, and as a consequence these components open new strategies to protect crops in modern agriculture and horticulture with integrated pest management (IPM) programmes against insect pests (Sujatha *et al*. 2010). These bioactive compounds can disrupt the immature stages and adult emergence, and act as antifeedants and toxicants to insect pests. Cardenas *et al.* (2012) mentioned that the target pests could biodegrade the bioactive metabolites to non-toxic products. Also the qualitative analysis of plant essential oils revealed the presence of several chemical groups such as alkaloids, tannins, flavonoids, lignans and terpenoids. These materials mimic the juvenile hormone and impede the moulting process (Silva *et al.* 2012).

The present study aims to clarify the effect of farnesol on the food intake and utilization, the digestive enzymes (protease, invertase, amylase, trehalase and chitinase) and the changes that may take place in the fat body proteins of *S. gregaria*.

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MATERIAL AND METHODS

Rearing insects

Adults and nymphs of the desert locust were reared for several years in insectaries in the Department of Entomology, Cairo University. The stock colony was reared in electrically heated wooden cages (60 × 60 × 60 cm). Each cage was provided with a thermostat to control temperature. The rearing conditions were 30 °C and 50–70 % RH. Insects were fed on fresh food of clover, *Trifolium alexandrinum,* from November to June and then on fresh leaves of *Sesbania sesban*. Cages were supplied with suitable ovipositional pots for egg deposition which were kept moist.

Insecticide preparation

The tested pure sesquiterpenoid, farnesol (95 %), (3, 7, 11-trimethyl-2, 6-10-dodecatrien-1-o1), was purchased from Sigma Chemical Co. A stock solution of farnesol was prepared. Five drops (≈2.5 ml) of Tween 60 were added (as emulsifier) to five drops of ethyl alcohol (95 %), and then were mixed thoroughly with 5 ml farnesol. Finally 90 ml of distilled water was added to obtain a concentration of 5 % farnesol emulsion. Six serial dilutions 2, 1, 0.5, 0.25, 0.125, and 0.0625 % were prepared by dilution of the above stock concentration with water. Application of farnesol was carried out by dipping clover leaves in the desired solution for few seconds; these leaves were air-dried and were given as food for the target insects.

Food consumption

Food consumption and utilization tests were carried out by feeding insects fresh clover leaves of a known weight dipped for 2–3 seconds in 0.25 % farnesol solution. Such a concentration was small enough for insects to continue to live and to consume food. Control insects received leaves dipped in Tween 60 and alcohol (95 %) solution. Chosen stages were newly emerged fifth instar nymphs and newly emerged adult females. Female nymphs and adults were reared separately in five cages of 25 × 25 × 25 cm, each contained five individuals. Adult females were reared without males till time of mating, when males were introduced. Parallel to this, adult males were reared in the same conditions to calculate the quantity of food consumed and faeces excreted. These quantities were subtracted from the total food eaten or faeces produced by both sexes at times of mating, so the net food consumed or faeces excreted by females could be estimated. This procedure was replicated three times (Abdel Rahman 2001).

Every morning, definite quantities of fresh washed clover leaves were provided to the insects, and aliquots of the same food were kept in the same conditions to calibrate the water lost from the food provided. Uneaten food was separated from the faeces and weighed. The latter weight and both the initial and final weights of the aliquots were used to calibrate the approximate food consumed by insects. Then the dry weight of the food consumed was calculated and recorded according to the following equation:

$$\text{Weight of food consumed} = (1-(A/2))(W - L(1 + B)),$$

where W is fresh weight of food provided; L is dry weight of uneaten food; A is initial weight of the aliquot; B is final weight of the aliquot (Waldbauer 1968).

The nutritional indices

1. The consumption index (CI):

   $$CI = F/(TA),$$

   where F is the dry weight of food ingested, T is duration of feeding period (in days), A is mean dry weight of the insect obtained by the following equation:

   $$A = (f - i)/2,$$

   where f and i are the final and initial dry weights of the insect (Waldbauer 1968).

2. The growth rate (GR):

   $$GR = WT/TA,$$

   where WT is dry weight gained.

3. Approximate digestibility (AD):

   $$AD = ((F - Fe)/F)\ 100,$$

   where Fe is the dry weight of faeces plus the dry weight of eggs produced. The latter was calculated by drying the whole egg-pod at 100 °C, and then its dry weight was recorded. Thereafter, the pod was heated to 250 °C for three hours, so that the sand particles become loosened. Then the burnt biomass, *i.e.* the eggs and the foamy secretion could be easily removed and sand particles were then weighed. The difference between the dry weight of the whole egg-pod and sand particles was considered as the dry weight of the biomass (Abdel Rahman 2001).

4. Efficiency of conversion of ingested food to body substance (ECI):

   $$ECI = (WT/F)\ 100.$$

5. Efficiency of conversion of digested food into body substance (ECD):

   $$ECD = (WT/(F - Fe))\ 100.$$

The experiment lasted till the death of the treated insects (45 days).

Biochemical assay

Chemical analysis of the haemolymph and midgut tissue of the fifth instar nymphs treated with 0.5 % and 0.06 % farnesol, to test the effect of a high and a low concentrations. Then after 48 hours of feeding, the activity of the enzymes was performed. Digestive enzymes were determined by using trehalose, sucrose, and soluble starch as substrates for trehalase, invertase, and α-amylase, respectively (Ishaaya *et al*. 1976). Insects were dissected in saline buffer to remove the gut tissue after 48 hours of feeding. The total proteins, main metabolites, were determined by ordinary method (Bradford 1976). The proteolytic activity was also determined by the casein digestion method (Ishaaya *et al*. 1971).

Chitinase was assayed using 3, 5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexoaminase liberated due to chitin digestion (Ishaaya & Casida, 1974).

Electrophoretic separation of the fat body protein

SDS-PAGE was carried out using a 4 % (w/v) acrylamide stacking gel and a 12 % (w/v) acrylamide separating gel (Laemmli 1970). The fat body

Table 1. Food consumption and nutritional indices of fifth instar nymphs and adult females of *Schistocerca gregaria* treated with 0.25 % farnesol.

Parameter	Fifth nymphal instars		Adult female	
	Control	Treated	Control	Treated
CI	0.067 ± 0.004	0.072 ± 0.001	0.044 ± 0.002*	0.012 ± 0.001
GR	0.0041 ± 0.0008	0.0044 ± 0.0009	0.039 ± 0.001*	0.049 ± 0.001
AD	82.54 ± 0.24	84.93 ± 1.73	51.43 ± 2.22*	28.72 ± 2.31
ECI	7.40 ± 0.49	6.69 ± 1.62	9.63 ± 1.46*	5.64 ± 1.32
ECD	8.37 ± 1.39	7.76 ± 2.27	18.73 ± 2.19*	9.64 ± 2.26
Test period (days)	10.61 ± 1.29	10.67 ± 1.57	45	45
Pods/female	–	–	2.11 ± 0.60*	1.12 ± 0.50
Eggs/pod			63.21 ± 5.58*	44.45 ± 6.73
Food consumed	4.00 ± 0.31	3.72 ± 0.25	6.78 ± 0.84*	4.71 ± 0.35
Faeces excreted	3.66 ± 0.44	2.89 ± 0.37	3.29 ± 0.23	3.36 ± 0.11
Weight gained	0.31 ± 0.02	0.30 ± 0.04	0.65 ± 0.01*	0.27 ± 0.02

*Significant difference ($P \leq 0.05$).

proteins of both normal and farnesol (0.25 %) treated adult females were dissolved in sample buffer; and for the reducing conditions, samples were denatured with 2 % SDS containing 5 % β-mercaptoethanol and boiled for 3 min. Treated samples were centrifuged at 10000 *g* for 5 min before being loaded onto the gels. Samples were subjected to electrophoresis at 20 mA and the gel was stained with Coomassie Brilliant Blue R-250 (Meyer & Lamberts1965). Molecular weight standards used (30–200 kDa) from Bio-Rad (La Jolla, CA) were carbonic anhydrase (30), hen egg white ovalbumin (45), bovine serum albumin (66), rabbit muscle phosphorylase b (97), β-galactosidase from *E. coli* (116), and myosin from porcine heart (200).

Statistical analysis

Statistical analysis was done using SPSS©.

RESULTS AND DISCUSSION

Food consumption and nutritional indices

Indices of food consumed by *S. gregaria* fifth instar nymphs and adult females are summarized in Table 1. There was no significant difference ($P \geq 0.05$) in these indices for the nymphal stage. This might be attributed to the short lifespan of this stage (Table 1). The consumption index for the adult control females was greater than that of the treated ones. This indicates that control females consumed more food than the treated females by 3.7-fold. Perhaps farnesol retarded digestion, hence treated individuals were unable to consume enough food (De Geyter *et al*. 2007).

The growth rate was higher in treated females by 1.25-fold compared with the control individuals ($P \leq 0.05$). The approximate digestibility was 1.8-fold higher in control females than treated ones ($P \leq 0.05$). These could be interpreted as the control insects consumed more food than the treated ones (Table 1) (Abdel Rahman 2001).

The efficiencies of conversion of ingested and digested food to body substance were significantly higher in control females than in treated groups ($P \leq 0.05$). This might be explained by the toxicity of farnesol to the digestive cells, and so treated insects were unable to digest food properly (Meyer & Lamberts 1965).

Survived female adults had reduced size as compared to the control. A growth regulator such as juvenile hormone could be assumed to be the cause of the long nymphal periods due the high non-specific esterase induction. Also, a pronounced prolongation was reported in *Agrotis ipsilon* larval duration with a reduction in weight when fed on leaves sprayed with farnesol (Awad 2001). In addition to the antifeedant activity of farnesol, a stress on the enzyme expression system to synthesize new and higher amounts of detoxification enzymes which may be a possible reason for the arrested growth (Shekari *et al*. 2008).

Egg-pod production in treated females was lower than the control ones by about 47 % ($P \leq 0.05$). Also, eggs per pod decreased by about 30 % ($P \leq 0.05$). Perhaps treated females were unable to utilize the digested food (Abdel Rahman

Table 2. Total protein and digestive enzymes in the midgut and haemolymph of theadult females of *Schistocerca gregaria* treated with 0.06 and 0.5 % farnesol.

Variable	Tissue	Concentration (%)		
		0	0.06	0.5
Total protein	M	$0.54 \pm 6.74 \times 10^{-3}$ [a]	$0.59 \pm 9.87 \times 10^{-3}$ [a]	$0.35 \pm 6.9 \times 10^{-3}$ [b]
	H	$0.63 \pm 4.98 \times 10^{-3}$ [a]	$4.23 \pm 0.16 \times 10^{-3}$ [b]	$2.49 \pm 9.2 \times 10^{-3}$ [c]
Protease	M	160.00 ± 12.662 [a]	330.00 ± 15.503 [b]	443.33 ± 16.272 [c]
	H	114.67 ± 8.686 [a]	152.40 ± 0.635 [b]	105.00 ± 9.539[c]
Chitinase	M	18.210 ± 0.5685 [a]	43.255 ± 0.5729 [b]	47.93 ± 0.8011 [c]
	H	14.796 ± 0.547 [a]	34.332 ± 0.691 [b]	122.894 ± 0.854 [c]
Invertase	M	158.800 ± 1.5454 [a]	208.809 ± 1.5784 [b]	103.332 ± 0.9615 [c]
	H	688.90 ± 1.3042 [a]	726.13 ± 0.9114 [b]	669.85 ± 1.7450 [c]
Amylase	M	182.5013 ± 0.555 [a]	181.5283 ± 1.207 [a]	93.5986 ± 0.798 [b]
	H	501.1073 ± 1.987 [a]	737.907 ± 2.453 [b]	493.722 ± 1.079 [c]
Trehalase	M	467.215 ± 1.146 [a]	721.390 ± 2.336 [b]	530.840 ± 1.520 [c]
	H	192.540 ± 1.201 [a]	196.778 ± 0.859 [b]	99.695 ± 0.901 [c]

Figures followed by different letters in the same row for the midgut (M) (µg) or haemolymph (H) (µl) are significantly different ($P \leq 0.05$).

2001). Also Ghazawy *et al*. (2007) mentioned that azadirachtin reduced egg production in *Heteracris littoralis*. Such chemicals might retard ovarian development, causing an increase in the receptor tyrosine kinase present on the adjacent follicle cells Lim & Lee (1982). This leads to completely suppressed oocyte development (Polivanova & Triseleva 1989).

Biochemical assay

Table 2 shows that the total haemolymph protein increased significantlyin farnesol-treated insects at lower and higher concentrations to 6.76- and 3.98-fold as that of the control insects, respectively ($P \leq 0.05$). A significant decrease was observed in the total midgut protein of the treated insects at 0.5 % farnesol by 1.5-fold than the control ($P \leq 0.05$). The protease activity showed a significant increase in the midgut tissue of insects treated with the lower and higher concentrations to 2.06- and 2.77-fold than that of the control, respectively ($P \leq 0.05$). Only a significant increase was observed in the haemolymph of treated nymphs at the lower concentrations to 1.3-fold that of the control ($P \leq 0.05$).

Table 2 shows that invertase, amylase and trehalase activities of the midgut tissue of treated individuals at higher concentration decreased significantly to nearly 0.65-, 0.51- and 0.52-fold as compared with control individuals, respectively ($P \leq 0.05$). However, invertase and trehalase activities showed a significant increase at lower concentration of farnesol as compared to the control ($P \leq 0.05$).

The invertase, amylase and trehalase activities in the haemolymph of treated insects at lower concentration increased significantly to nearly 1.05-, 1.5- and 1.5-fold as that of the control, respectively ($P \leq 0.05$). The amylase and trehalase activities in the haemolymph of treated insects at the higher concentration decreased significantly to 1.01- and 1.93-fold as that of the control, respectively ($P \leq 0.05$). While the invertase activity showed a significant decrease to 0.97-fold as that of the control when the nymphs feed on 0.5 % farnesol-treated clover. It was clear that the midgut digestive enzyme synthesis in the treated nymphs were highly increased with the low farnesol concentration compared to the control. This depends mostly on the secretion rate of the enzymes. The results agree with other authors (Terra & Ferreira 1994). This is particularly true on the treatment with farnesol. In *S. gregaria* females, amylase was more sensitive to farnesol and gut amylase activity significantly decreased. Thus, the mastication process is important in digestion. On the other hand, trehalase is an important enzyme in which insects degrade trehalose to glucose for internal energy supply, thus the activity of trehalose might serve as an indicator of energy reserves resulting from availability of carbohydrate nutrients. There seemed to be a complex interaction which included

lowered food consumption and the general increase in the total protein content in the midgut tissue or haemolymph of the *S. gregaria* nymphs fed on farnesol-treated clover. This may be attributed to the toxic properties of farnesol and new immune-proteins appeared. The midgut cells also activate some enzymes leading to activation of the toxin, farnesol, to destroy the midgut epithelial cells (Terra & Ferreira 1994).

Chitinase showed significant increase in the midgut (by 2.4- and 2.6-fold) and haemolymph, (by 2.3- and 8.3-fold) for the lower and higher concentrations, respectively. Chitin is the major component of the cuticle in insects and other body parts. Chitinase is the major enzyme which is responsible for cuticle degradation into a high molecular weight polymer of NAGA, N-acetyl-glucoseamine (Koga *et al*. 1983). Epidermal cells from the gut, salivary glands and fat bodies are usually responsible for chitinase production (Kramer & Koga 1986). However, during the moulting process, many enzymes work together in harmony and the trehalase is activated to produce glucose leading to chitin build up.

Effect of farnesol on protein profile of fat body

The proteins extracted from the female fat body were resolved into a number of bands ranging in molecular weight from 400 kDa to 30 kDa in 10 % SDS-PAGE.

The protein profiles of fat body in control and treated females are presented in Fig. 1. The females treated with a dose of 0.25 % started to moult and their fat body showed three polypeptides at 400 and 150 kDa, while the control ones did not show these three polypeptides (Fig. 1). High intensities of protein in treated samples may be due to failure in the sequestration of these proteins by fat body. At 200 kDa there were expressions of protein intensities in the control females than in treated ones. There is no change in the intensity of protein profiles in the control and treated females at 66 kDa. The protein profile of fat body of control females had two polypeptides at 116 kDa region, but the treated females had only one polypeptide protein band at 97 kDa. Furthermore, a protein band (molecular weight 45 kDa) appeared reduced in quantity in treated samples compared to control. Proteins of 45 kDa were expressed more in control than in treated ones. The intensity of protein band at 30 kDa of treated females is higher

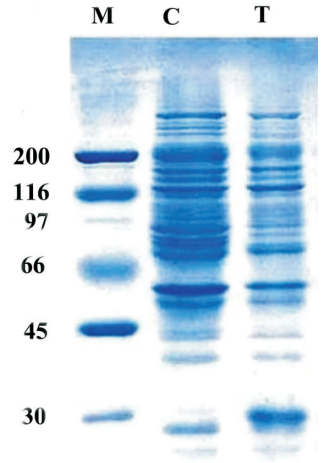

Fig. 1. Profile of fat body protein bands of *Schistocerca gregaria* adult females treated with farnesol. M = Marker, C = control, T = treated.

than that of control. It seems that there is a complex interaction between the decrease in food utilization, inhibition in enzymatic activity and the disappearance of normal proteins. The disappearance of the protein bands may be attributed to the presence of toxins which could increase protease activity (Table 2), leading to protein hydrolysis. Such proteins are presumed to be associated with enzymatic activities related to the cuticle (sclerotization, melanin formation, and catecholamine metabolism). The decrease in the intensity might be taken as evidence for a decline in synthesis or an increase in the utilization of this protein.

Compounds that have a juvenile hormone analogue activity affect protein bands in many insects. Such a result was also reported in *S. gregaria* treated with dieldrin and fenitothion (Kulkarni & Mehrotra 1975). Also, when *S. gregaria* was injected with azadirachtin, the fat body protein was not as efficient as in the control locusts (Paranagama *et al*. 1993). These authors concluded that the prevention of stimulatory action of neurohormones on protein synthesis (Paranagama *et al*. 1993). Changes in the chemical composition of the egg proteins of *S. gregaria* treated with azadirachtin was also reported (Ghazawy *et al*. 2010).

Several authors working on different insects obtained the same results (*e.g*. Salokhe *et al*. 2006). Also, protein bands decreased considerably in treated *Tribolium castaneum* larvae with flufenoxuron as compared to the control (Salokhe *et al*. 2006).

CONCLUSION

Farnesol could be used as a safe insecticide against locusts, since it decreases food consumption, changes the digestive enzymes activities and affects positively protein synthesis in the fat body.

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