INFLUENCE OF THE DESERT PLANT, LOTUS CORNICULATUS ON THE PROTEIN QUANTITY AND GLUTATHIONE-S-TRANSFERASE ACTIVITY OF SCHISTOCERCA GREGARIA

G. Elsayed, Samy M. Sayed, and Y. Naser S. Al Solaimi

ABSTRACT: The desert plant, Lotus corniculatus (Fabaceae), inhabits the Saudi Arabian Sahara Desert. In these areas the desert locust, Schistocerca gregaria (Orthoptera, Acrididae), feeds on L. corniculatus containing the plant allelochemical linamarin; a cyanogenic glucoside that is usually toxic to polyphagous insects. Under laboratory conditions this plant was fed to S. gregaria. Haemolymph proteins and allelochemicals detoxification in S. gregaria, fed on L. corniculatus were assessed. Female 5th instar nymphs were reared on L. corniculatus or on wheat seedlings, Triticum aestivum, as control. In insects, glutathione-S-transferase is one of the most important detoxifying enzymes. The activity of glutathione-S-transferase (in units/gram) in the mid-gut tissues of 5th instar nymph females fed on L. corniculatus was significantly higher than in those fed on T. aestivum. The quantity of haemolymph proteins (in mg 100 μl⁻¹) in adult locust females resulting from nymphs fed on L. corniculatus was significantly less than it was in females fed on T. aestivum. Decreasing protein synthesis in the adult females of S. gregaria might be related to detoxifying toxic compounds in L. corniculatus.

KEY WORDS: S. gregaria, Lotus corniculatus, haemolymph, proteins, glutathione-S-transferase, linamarin, detoxification

INTRODUCTION

The objective of this study was to investigate the ability of the desert locust, S. gregaria, to resist the toxicity of linamarin allelochemical in the desert plant, L. corniculatus, and the relation to the amount of protein synthesis in the haemolymph of locust adults fed as 5th instar nymphs on the desert plant L. corniculatus. The desert locust, S. gregaria, is a major insect pest in the Middle East and Northern Africa. It has a long history of devastating crops and contributing to famines in many African countries. Desert locusts have the ability to detoxify many of the toxic materials in their host plants that prevail in their preferred habitat and this allows a considerable food diversity for locusts. Ozenda (1977) mentioned that S. gregaria do not prefer L. corniculatus as food. According to Berenbaum (1986) generation of cyanide from linamarin is usually enzymatic and occurs if linamarin is exposed to linamarase. Herbivorous insects can tolerate toxic compounds because they have evolved various physiological mechanisms to avoid the harmful effects of toxins. Most herbivorous insects rely heavily on
enzymatic degradation for the neutralization of ingested allelochemicals. Poly-
substrate monooxygenases (Psmos; often called mixed function oxidases, or
MFOs) convert allelochemicals into relatively more polar compounds which are
further metabolized by secondary enzymes. Glucosinolates in Schouwia pur-
purea are metabolized by the amylorinase of S. gregaria into less toxic com-
ounds that are excreted (Mainguet et al., 2000). Vanhaelen et al. (2001), stated
that direct excretion occurs in some crucifer-feeding insects e.g. Episyrphus
balteatus which use glutathione-S-transferase as the detoxification enzyme for
the glucosinolates in Brassicaceae. Similarly, the same enzyme was found in the
ladybird Adalia bipunctata fed on aphids reared on Brassicaceae (Francis et al.,
2002). As reported by Rajurkar et al. (2003), glutathione-S-transferase provides
an important defense mechanism against plant allelochemicals and insecticides
in Helicoverpa armigera. The same phenomenon takes place in the spruce bud-
worm as indicated by Feng et al. (2001). As protein is a major nutrient required
for phytophagous insects, it is most commonly a limiting nutrient for their opti-
mal growth. Therefore, many studies have focused on the effect of diet on total
body protein content in phytophagous insects (Zanotto et al. (1997) and Telang
et al. (2004)). Insect growth and development depends, to a great extent, on the
quality and quantity of available protein in their diets (Burgess et al., 1991). The
levels of both reproductive proteins (i.e. vitellin or vitellogenin) and haemo-
lymph storage proteins (i.e. hexamerins) in grasshoppers are noticeably influ-
enced by the level of proteins in their diets (Hatle and Oppert, 2010).

MATERIALS AND METHODS

Twenty newly molted 5th instar female nymphs were fed on freeze-dried
leaves of Lotus corniculatus. A similar number of nymphs of the same sex were
fed on wheat seedlings (Triticum aestivum) as control. Dry food was provided in
a glass dish with a piece of moistened cotton and a cylindrical wire was added to
help moulting. Experimental conditions were 33ºC., for 12 h (day) and 20ºC., for
12 h (night) accompanied by 50-70% RH, and 12:12 h (L: D) cycle as des cribed
by Elsayed et al. (2012). Ten-day-old 5th instar nymphs were carefully dissected
and the mid-gut tissues were extracted then washed in an insect saline solution
for the assessment of glutathione-S-transferase activity. Also, 10-day-old imma-
ture adult females were punctured at the neck membrane and haemolymph col-
lected with the aid of a Pasteur pipette for total protein estimation.

Glutathione-S-transferase (GST)

Enzyme preparation:

Collected mid-gut tissues of ten individual 5th instar female nymphs were
homogenized in cold 0.1 M phosphate buffer (pH 7.5) at a ratio of approximate-
ly 1:5 (w/v). All homogenates were centrifuged at 15000×g for 20 min at 4ºC.
Supernatants were transferred to fresh tubes and used as enzyme source.
**Glutathione-S-transferase (GST) activity assay:**

The GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) was determined according to the method of Habig et al. (1974). Briefly, 50 μL of appropriately diluted enzyme preparation was mixed with 100 μL of 9.7 mM reduced glutathione in 0.1 M phosphate buffer (pH 7.4) and 100 μL of 25 mM of CDNB. The change in absorbance was immediately recorded at 340 nm for samples. All assays were corrected for non-enzymatic conjugation using a negative control in which 50 μL of 0.1M phosphate buffer (pH 7.4) replaced the enzyme preparation. The amount of glutathione conjugate formed was calculated by applying the following formula: 

\[
\text{GST activity (U/g tissue)} = A_{340/\text{min}} \times \frac{2.812}{\text{tissue used (in grams)}}
\]

Data of enzyme activity were analyzed using the standard error equation.

**Proteins in haemolymph:**

Haemocytes of 6 individual adult females were removed by centrifugation at 20,000 rpm for 5 min at 4°C. 500 μL of reagent A (1% SDS, 1% Triton x-100, 1% Tween 20, 0.2% C12E8, 0.5 M Hcl, 0.05 M Cac12 and 0.05% Sodium azide) were added to 100 μL of supernatant and mixed; then 4 ml of reagent B ((1% CHAPS, 1% CHAPSO, 1% Octylglucoside, 0.1 M Tris, pH 8.0, 0.5 M (NH4)2 SO4 and 0.4 M Guanidine HCl) were added and mixed. Absorption was measured at 750 nm after 15 min (Peterson, 1979).

**RESULTS AND DISCUSSION**

**Glutathione-S-transferase activity**

Glutathione-s-transferase (GST), is one of the mid-gut detoxification enzymes that degrades toxic allelochemicals in plants. This enzyme, in the mid-gut tissues of female 5th instar nymphs fed on *L. corniculatus*, when measured under standard conditions of activity was significantly higher than in those fed on *T. aestivum* (p ≤ .001 probability level, t = 4.805 (Table 1)).

Table 1. Activity of GST (units/gram) in the mid-gut tissues of fifth instar nymphs of *S. gregaria* fed on *Lotus corniculatus* compared with *Triticum aestivum*.

<table>
<thead>
<tr>
<th>Host plant</th>
<th><em>L. corniculatus</em></th>
<th><em>T. aestivum</em></th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>85.62</td>
<td>52.02</td>
<td>t = 4.805 (df : 9)</td>
</tr>
<tr>
<td>SE</td>
<td>3.92</td>
<td>5.31</td>
<td></td>
</tr>
</tbody>
</table>
The histogram showing GST in mid-gut tissues of *S. gregaria* indicates that enzyme activity (in units/gram) was comparatively higher on *L. corniculatus* (85.617 ± 3.92) than after feeding nymphs on *T. aestivum* (52.02 ± 5.31), (Fig. 1).

Increase of the GST activity in mid-gut in nymphs fed on *L. corniculatus* is probably attributed to the existence of linamarin. It seems that the enzyme degrades linamarin and its metabolites to less toxic compounds or, possibly, converts these compounds to products that can be easily excreted. Detoxification process requires high energy that affects protein synthesis in insect haemolymph resulting in lower weight gain. This confirms several previous studies: Elsayed et al. (2012) recorded the highest activity in GST in the tropical grasshopper, *Poeecilocerus bufonius* after feeding on *Calotropis procera* which contain cardenolids. Also, GST and general esterases play an important role in the detoxifying of numerous substances including allelochemicals from plants and chemical pesticides (Ramzi et al., 2009). GST pattern in *Myzus persicae* was discussed in terms of insect adaptation to the presence of plant secondary substances such as Brassicaceae glucosinolates and isothiocyanates (Francis et al., 2005). GST in the fat body and mid-gut of *Oxya chinensis* played a significant role in detoxifying xenobiotics including plant allelochemicals (Hai-hua et al., 2008). GST activity in *Spodoptera*

![Fig. 1. Quantity of proteins in haemolymph of adult females of *Schistocerca gregaria* fed on *Lotus corniculatus* compared with *Triticum aestivum*.](image-url)
litura larvae reached a maximum when larvae were fed on tobacco leaves followed by Chinese cabbage and cowpea while minimum activities were observed in larvae fed on sweet potato leaves (Ming et al., 2010). GST and glutathione peroxidases are essential components of the cellular detoxification system that defend cells against reactive oxygen compounds (Qian et al., 2009). According to Falk and Gershenzon (2007) myrosinase eliminates the formation of toxic glucosinolate hydrolysis products in the mid-gut of S. gregaria feeding on Schouwvia. Mixed function oxidase is thought to play a critical role in insect tolerance to noxious compounds (Wu et al., 2007 and Rodriguez et al., 2011).

**Proteins in haemolymph**

Haemolymph proteins of immature adult females of S. gregaria fed on L. corniculatus were significantly lower than those for adults fed on T. aestivum (p ≤ .0001, t = 10.23 (Table 2). The histogram of haemolymph proteins (Fig. 1) indicates that the mean quantity of proteins in the haemolymph of adult females was higher on T. aestivum than it was on L. corniculatus (1.80 ± 0.357).

![Figure 2](https://example.com/figure2.png)

**Host plant**

Fig. 2. Glutathione-S-transferase activity in midgut tissue of fifth instar nymphs of S. gregaria fed on L. corniculatus leaves compared with T. aestivum.
Table 2. Quantity of protein in the haemolymph of *Schistocerca gregaria* adult fed on *Lotus corniculatus* compared with *Triticum aestivum* (Mean ± S.E.).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th><em>L. corniculatus</em> mg proteins 100μl⁻¹ ± S.E.</th>
<th>N</th>
<th><em>T. aestivum</em> mg proteins 100μl⁻¹ ± S.E.</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. corniculatus</em></td>
<td>6</td>
<td>1.80 ± 0.357</td>
<td>6</td>
<td>4.97 ± 0.413</td>
<td>t:10.23 df: 5 P≤ .0001</td>
</tr>
</tbody>
</table>

Decrease of the quantities of haemolymph proteins of adult females fed on *L. corniculatus* (Fig. 2) suggests that protein synthesis was obviously affected by the toxic compounds in *L. corniculatus*. It is anticipated that linamarin in *L. corniculatus* probably inhibits protein digestion in the mid-gut of adult females. This seems to agree with the previously mentioned fact that protein synthesis in *S. gregaria* is influenced by glucosinolates in *S. purpurea* (Elsayed et al., 1996). Haemolymph proteins of the nymphs of *Euprepocnemis plorans* fed on horse beans and lupine were adversely affected by hydrogen cyanide and quinolizidine, respectively (Elsayed, 1998). Three desert plants, *Calotropis procera*, *Pulicaria crispa* and *Zygophyllum simplex* inhibited protein synthesis in *Anacridium aegyptium* nymphs (Elsayed, 2004). The levels of reproductive protein (*i.e.* vitellin or vitellogenin) and haemolymph storage proteins (*i.e.* hexamerins) in grasshoppers were affected by protein level in their diets (Hatle and Oppert, 2010). The current investigation sheds light on the possibility there is a positive relationship between the detoxification of harmful compounds and the amount of protein produced in insects’ bodies.

**LITERATURE CITED**


