Article

THE INFLUENCE OF DIETARY α-SOLANINE ON THE WAXMOTH *Galleria mellonella* L

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Plant allelochemicals are nonnutritional chemicals that interfere with the biology of herbivores. We posed the hypothesis that ingestion of a glycoalkaloid allelochemical, α-solanine, impairs biological parameters of greater wax moths Galleria mellonella. To test this idea, we reared wax moths on artificial diets with 0.015, 0.15, or 1.5 mg/100 g diet of α-solanine. Addition of α-solanine to the diet affected survival of seventh-instar larvae, pupae, and adults; and female fecundity and fertility. The diet containing the highest α-solanine concentration led to decreased survivorship, fecundity, and fertility. The diets supplemented with α-solanine led to increased malondialdehyde and protein carbonyl contents in midgut and fat body and the effect was dose-dependent. Dietary α-solanine led to increased midgut glutathione S-transferase activity and to

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decreased fat body glutathione S-transferase activitiy. We infer from these findings that α-solanine influences life history parameters and antioxidative enzyme activities in the midgut and fat body of G. mellonella. © *2013 Wiley Periodicals, Inc.*

Keywords: *Galleria mellonella*; glycoalkaloids; GST; nutrition; oxidative stress; pro-oxidant

INTRODUCTION

Plants produce allelochemicals as secondary metabolites that serve as defensive agents against herbivores. Lepidopteran insects are challenged by allelochemicals, including glycoalkaloids such as α-chaconine and α-solanine in their host plants (Weissenberg et al., 1986, 1998). *Galleria mellonella* (L.) is a pest whose larvae feed on combs, wax, and honey in beehives, leading to important financial losses in apiculture (Charriere and Imdorf, 1997). We used wax moths as a model for research into the possibility that solanine can protect plants from insect damage. Wax moth larvae have also been used as simple insect models to provide a rapid, inexpensive, and reliable evaluation of the toxicity and efficacy of new antimicrobial agents in vivo prior to the use mammalian models (Desbois and Coote, 2012). We selected wax moths because insects known to consume potatoes and other members of the Solanaceae have evolved mechanisms to avoid glycoalkaloid poisoning. The plants of the Solanaceae family produce specific steroid alkaloids such as α-chaconine, α-solanine, solamargine, solasonine, α-solamarine, β-solamarine, and tomatine for their protection from insect damage (Weissenberg et al., 1998). Most information about glycoalkaloid α-solanine toxicity comes from experiments on the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) because this beetle can effectively sequester the α-solanine found in potatoes (Krishnan et al., 2007), and most other insects and mammals are adversely affected by this glycoalkaloid (Weissenberg et al., 1998; Wang et al., 2005; Thirumalai et al., 2012). Previous studies using the Egyptian armyworm moth *Spodoptera littoralis* as a model insect confirm solanine toxcity can alter life history parameters. Dietary solanine also increases oxidative radicals and the activities of antioxidant enzymes in the digestive tract (Krishnan and Kodrík, 2006; Krishnan and Sehnal, 2006). The inhibitory effects of a series of secondary plant compounds including steroidal alkaloids and glycoalkaloids on larval growth and development of the Egyptain stemborer, *Earias insulana*, red flour betle *Tribolium castaneum*, and tobacco hornworm *Manduca sexta* were investigated (Weissenberg et al., 1986, 1998). We decided to advance this line of research by considering possible actions of α-solanine in the induction and management of oxidative stress in wax moth larvae as an alternative model system.

Insects express a suite of antioxidant and detoxification enzymes that may form a concatenated response to plant allelochemicals (Felton and Summers, 1995). The antioxidant enzymes, including glutathione S-transferases (GSTs), are described in detail elsewhere (Krishnan et al., 2007). Insect herbivores rely on GSTs for biochemical detoxification of plant allelochemicals (Vontas et al., 2001). They also act in detoxification of reactive metabolites formed by microsomal oxidation (Singh et al., 2001). The role of GSTs in metabolism of dietary glycoalkaloids, such as α-solanine has not been studied in *G. mellonella* except for their role in metabolism of a furanocoumarin compound, xanthotoxin (Büyükgüzel et al., 2010).

We posed the hypothesis that the ingestion of α-solanine influences biological performance parameters and leads to oxidative stress in the midgut and fat body of *G. mellonella*. Here, we report on the outcomes of experiments designed to test our hypothesis.

MATERIALS AND METHODS

Insect Culture

Greater wax moth larvae and pupae were collected from infected hives in apicultural areas around Zonguldak, Turkey, and the newly emerged adults were used to maintain the stock culture. The insects were reared on an artificial diet (Bronskill, 1961) at 30 ± 1°C, 65 ± 5% relative humidity in constant darkness. All experiments were performed under these conditions. The methods used to prepare and dispense diets into containers, placement of larvae onto diets, and to obtain eggs and were described in previous studies (Büyükgüzel et al., 2010).

Chemicals

α-Solanine, phenylmethylsulphonyl fluoride (PMSF), dithiotreitol (DTT), dipotassium hydrogen phosphate (K_2HPO_4), potassium chloride (KCl), ethylendiaminetetraecetic acid (EDTA), butylated hydroxytoluene (BHT), thiobabituric acid (TBA), trichloroacetic acid (TCA), bovine serum albumin (BSA), Folin-Ciolcalteu reagent, guanidine hydrochloride, 2,4 dinitrophenylhydrazine (DNPH), streptomycin sulphate, glutathione (GSH), 1-chloro-2,4- dinitrobenzene (CDNB), glycerol, ethanol, sodium chloride (NaCl), phenylthiourea (PTU) were purchased from Sigma-Aldrich (St. Louis, MO). All reagents were analytical grade.

Experimental Designs

Alpha-solanine (Crystal form, 95%, $C_{45}H_{73}NO_{15}$) was directly incorporated into diets during preparation to ensure the chemical was distributed evenly in the diet, at concentrations of 0.015, 0.15, or 1.5 mg in 100 g of diets. These concentrations were used based on results of our preliminary experiments (unpublished data) within the tolerance range of *G. mellonella* and, on results of previous studies on other herbivorous insects exposed to α-solanine (Krishnan and Sehnal, 2006). These concentrations were based on actual levels that enable *G. mellonella* larvae to complete their development through to adulthood. Larvae reared on diets without α-solanine were used as controls in all treatments. Each experiment including three α-solanine concentrations and a control was repeated four times.

Survivorship

First instars were reared through adult emergence on the artificial diets amended with given concentrations of α-solanine. Survivorship of the seventh-instar larvae was recorded. Seventh instars were transferred into another jar lined with a fillter paper (to provide a dry surface) for pupation and adult emergence. Numbers of pupae and adults were recorded for each replicate. Each experiment was repeated four times and 20 larvae were used

for each replicate. Larvae that died during first 24 h and that died because of microbial contamination (especially fungal) during the assay period were excluded from the results.

Fecundity and Fertility

Neonate larvae from female adults of stock insect culture were reared to adulthood on the diets containing α-solanine concentrations. Mated females and males were obtained by placing newly emerged females with males in vials for 24 h after their emergence from the diets containing α-solanine. To determine average fecundity, females reared on α-solanine were placed in 30-ml plastic cups with screened lids. Females were kept in the cups for oviposition during 2 days. After this period, adults were removed from the cups and eggs were transferred into petri dishes using a fine brush. Fecundity was expressed as the number of eggs per female per day. Egg counts were performed in the petri dish on a black background to make the eggs more visible. Fertility, as numbers of eggs hatched per female per day were recorded. Each experiment was replicated four times with 10 females per replicate. Egg production during 2 days and larval hatch were monitored continuously from the first oviposition day until experiments were stopped.

Biochemical Assays

First instar larvae were reared through seventh-instars on an artificial diet amended with α-solanine. Thirty seventh-instar larvae (100–150 mg) were used for determining biochemical parameters with four replication. All larvae were the same chronological age.

Larvae were chilled on ice for 5 min and surface sterilized in 95% ethanol for collection of the midgut and fat body. Midguts and fat body were separately collected into a chilled Eppendorf tube charged with cold homogenization buffer [w/v 1.15% KCl, 25 mM K_2HPO_4, 5 mM ethylen-diaminetetraacetic acid (EDTA), 2 mM phenylmethylsulphonyl fluoride (PMSF), 2 mM dithiotreitol (DTT), pH 7.4] and stored at −80°C. A few crystals of phenylthiourea (PTU) were added to each sample to prevent melanization. Dissection and extraction of midguts and fat body are described in earlier studies (Büyükgüzel and Kalender, 2007; Hyrsl et al., 2007). Protein concentrations were determined according to Lowry et al. (1951) by using bovine serum albumin (BSA) as a quantitative standard. A dual beam spectrophotometer (Shimadzu 1700, UV/VIS Spectrophotometer, Kyoto, Japan) was used for all absorbance measurements.

Determination of Malondialdehyde and Protein Carbonyl Contents, and GST Activity

Malondialdehyde (MDA) content was determined after incubation at 95°C with TBA (1% w/v). Absorbances were measured at 532 nm and MDA content expressed as nmol/mg protein by using 1.56×10^5 M^{-1} cm^{-1} for extinction coefficient (Jain and Levine, 1995).

Protein carbonyl (PCO) was assayed according to Levin et al. (1990) with some modifications (Krishnan and Kodrík, 2006). Carbonyl content was quantified after reacting carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) leading to the formation of a stable 2,4-dinitrophenylhydrazone. Absorbances were measured at 370 nm. Results are expressed as nmol/mg protein using 22×10^{-3} M^{-1} cm^{-1} as the extinction coefficient. Protein concentrations in guanidine solutions were measured at 280 nm and quantified

Table 1. Effects of α-Solanine on Survivorship of* G. mellonella.** ***Each Value Was Calculated from Four Replicate Experiments (mean ± SE, n = 20)

α-solanine (mg/100 g of diet)	*Survival to seventh instar (%) (mean ± S.E.)*[*]	*Survival to pupal stage (%) (mean ± S.E.)*[*]	*Survival to adult stage (%) (mean ± S.E.)*[*]
0.000[§]	88.88 ± 7.8a	83.33 ± 7.8a	77.77 ± 6.6a
0.015	77.77 ± 5.7a	61.11 ± 4.5b	38.88 ± 4.7b
0.15	72.22 ± 6.6a	66.66 ± 5.3b	55.54 ± 5.4b
1.5	44.44 ± 4.8b	33.33 ± 4.2c	16.66 ± 3.8c

[*]Values followed by the same letter are not significantly different from each other ($P > 0.05$; LSD test).
[§]Control (without α-solanine).

with a bovine serum albumin standard curve. Protein carbonyls values were corrected for interfering substances by subtracting protein measured without DNPH at 370 nm.

GST (EC 2.5.1.18) activity was assayed by measuring the formation of the GSH and 1-chloro-2,4-dinitrobenzene (CDNB) conjugate (Habig et al., 1974). The increase in absorbance was recorded at 340 nm for 3 min. The specific activity of GST was expressed as nmol GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient 9.6 mM^{-1} cm^{-1}. All assays were corrected for nonenzymatic reactions using corresponding substrate in phosphate buffer (50 mM, pH 7.0).

Statistical Analysis

Data on the egg production, fertility, and midgut and fat body MDA, protein carbonyl content and GST activity were analyzed by one-way analysis of variance (ANOVA). The least significant difference (LSD) test (SPSS, 1997) was used to determine significant differences between means, significance set at $P < 0.05$. Data on survivorship were compared by a chi-squared test (Snedecor and Cochran, 1989). When the F and χ^2 estimate exceeded the probability of 0.05, the differences were considered significant. Regression analysis also was performed to determine the relationship between α-solanine concentrations and life table parameters, and between α-solanine concentrations and oxidative stress biomarkers (SPSS, 1997).

RESULTS

The Effects of α-Solanine on Survivorship of G. mellonella

The influence of α-solanine on survivorship is presented in Table 1. Compared to controls, the diets containing the low concentrations of α-solanine did not influence the survivorship ($\chi^2 = 3.2$, $df = 1$, $P = 0.073$). The highest dietary α-solanine concentration (1.5 mg) decreased seventh-instar survivorship ($\chi^2 = 32$ $df = 1$, $P < 0.0001$) by 50%. Regression analysis also showed a significant negative relationship between α-solanine concentrations and larval survival ($R^2 = 0.91$, $P = 0.046$). Low dietary concentrations of α-solanine led to decreased survival of pupae ($\chi^2 = 7.91$ $df = 1$, $P = 0.0049$ for 0.015 mg; $\chi^2 = 6.14$ $df = 1$, $P < 0.013$ for 0.15 mg; $\chi^2 = 37.02$, $df = 1$, $P < 0.0001$) and adults ($\chi^2 = 22.4$ $df = 1$, $P < 0.0001$ for 0.015 mg; $\chi^2 = 8.0$ $df = 1$, $P = 0.0047$ for 0.15 mg; $\chi^2 = 53.94$, $df = 1$, $P < 0.0001$). The highest α-solanine treatment (1.5 mg) decreased survivorship in pupal ($\chi^2 = 37.02$, $df = 1$, $P < 0.0001$) and adult stages (from 78 to 17%; $\chi^2 = 53.94$, $df = 1$, $P < 0.0001$).

Table 2. α-Solanine Influences Fecundity and Fertility of* G. mellonella. *Fecundity Is Presented as Numbers of Eggs Laid/Day/Female and Fertility Is Presented as Percent Hatchability of Deposited Eggs. Each Value Was Calculated from Four Replicate Experiments (mean ± SE, n = 10)

α-solanine (mg/100 g of diet)	*Fecundity (eggs/day/ female) (mean ± S.E.)*[*]	*Fertility hatchability of eggs (mean ± S.E.)*[*]
0.00[§]	95.08 ± 8.9a	89.16 ± 7.5a
0.015	51.40 ± 6.4b	41.65 ± 6.4b
0.15	62.70 ± 5.3b	51.02 ± 6.3b
1.5	32.00 ± 3.5c	16.87 ± 3.3c
F	29.63	59.10
Df	3	3
P	<0.05	<0.05

[*]Values followed by the same letter are not significantly different from each other ($P > 0.05$; LSD test).
[§]Control (without α-solanine).

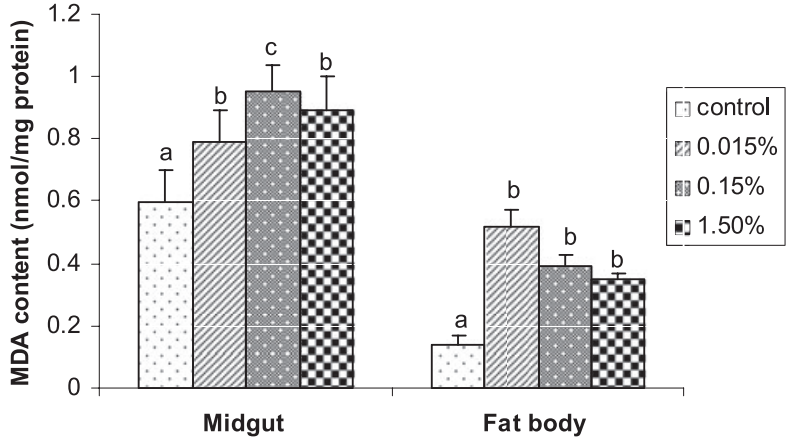

Figure 1. The effects of dietary α-solanine on midgut and fat body MDA content of *Galleria mellonella* larvae. Each histogram bar represents the mean of four replicates (± S.E., $n = 30$) in each of treatment groups. Vertical bars represent standard deviation. Means followed by the same letter are not significantly different ($P > 0.05$; LSD test).

The Effect of α-Solanine on Fecundity and Fertility of G. mellonella

The influence of α-solanine on fecundity and fertility is presented in Table 2. Relative to the controls, adults from larvae reared on diets with α-solanine decreased egg production ($F = 29.63$, $df = 3$, $P < 0.05$) and fertility ($F = 59.10$, $df = 3$, $P < 0.05$). The effects were registered in a dose-dependent manner.

The Effects of α-Solanine on MDA and PCO Contents and GST Activities

The diet with α-solanine at 0.15 mg led to maximized midgut MDA content relative to the highest and lowest α-solanine doses. Midgut ($F = 9.699$, $df = 3$, $P = 0.022$) and fat body ($F = 13.041$, $df = 3$, $P = 0.006$) MDA contents were increased by α-solanine treatments (Fig. 1). Similar results were obtained with 0.015 mg α-solanine treatment in fat body MDA content. The content of PCO in fat body ($F = 4.628$, $df = 3$, $P = 0.023$) was increased in larvae reared on high dietary concentrations of α-solanine and each solanine

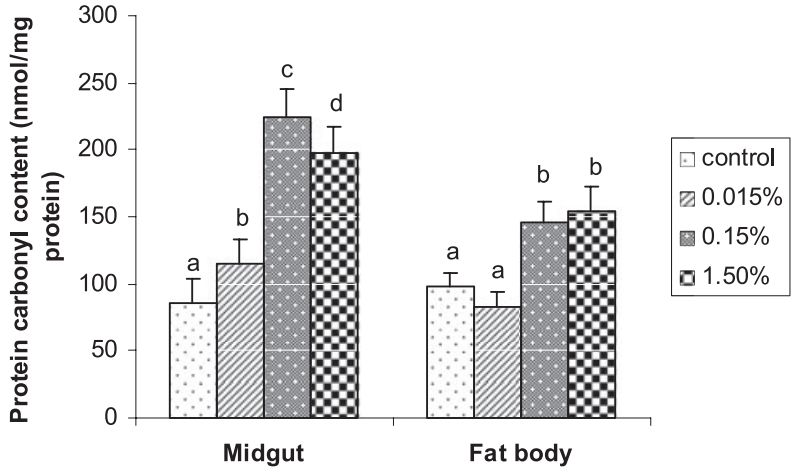

Figure 2. The effect of dietary α-solanine on midgut and fat body protein carbonyl content of *Galleria mellonella* larvae. Each histogram bar represents the mean of four replicates (± S.E., $n = 30$) in each of treatment groups. Vertical bars represent standard deviation. Means followed by the same letter are not significantly different ($P > 0.05$; LSD test).

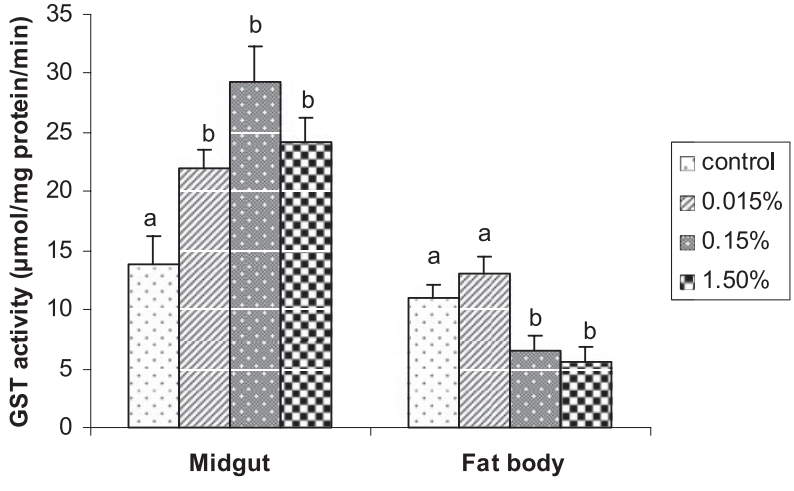

Figure 3. The effect of dietary α-solanine on midgut and fat body GST activity of *Galleria mellonella* larvae. Each histogram bar represents the mean of four replicates (± S.E., $n = 30$) in each of treatment groups. Vertical bars represent standard deviation. Means followed by the same letter are not significantly different ($P > 0.05$; LSD test).

concentrations led to increased midgut PCO content ($F = 8.56$, $df = 3$, $P = 0.003$) (Fig. 2). Midgut MDA and PCO levels were enhanced in a dose-dependent manner in larvae reared on 0.015, 0.15, and 1.5 mg of α-solanine. Relative to controls, each α-solanine concentration increased midgut GST activities ($F = 7.060$, $df = 3$, $P = 0.005$). However, the larvae reared on diets with high α-solanine concentrations yielded decreased fat body GST activity by about 50% in comparison to control ($F = 6.021$, $df = 3$, $P = 0.010$; Fig. 3).

DISCUSSION

The data reported in this study support our hypothesis that dietary α-solanine influences biological and biochemical parameters of wax moth larvae. As expected with a glycoalkaloid, α-solanine increased MDA and PCO contents and impaired GST activity. Most work on the oxidative effects of glycoalkaloids has focused on detoxification within midgut tissues of insects reared on diets amended with potato or on potato plants. The degradation of ingested potato diet containing α-solanine and other glycoalkaloids is often associated with the formation of toxic metabolites leading to production of ROSs (singlet oxygen, superoxide anion, hydroxyl radical, and hydrogen peroxide) that damage midgut tissues of some lepidopteran insects (Krishnan and Kodrik, 2006; Krishnan et al., 2007). GST and other anti-oxidant systems operate to reduce local ROS concentrations.

Increased concentration of ROS can produce oxidative damage to lipids, proteins, and nucleic acids (Rikans and Hornbrook, 1997). Although all classes of macromolecules are susceptible to radical attack, polyunsaturated fatty acids are especially sensitive to oxidation owing to their double bond structures. A radical attack on lipids leads to the formation of lipid-hydroperoxides and finally aldehydes (Cheeseman, 1993). Oxidatized proteins are generally dysfunctional, losing catalytic or structural integrity. Protein carbonyls tend to accumulate on the side chains of proteins as a result of oxidative stress. We observed substantial increases in MDA and PCO in fat body and midgut tissue of *G. mellonella* fed on α-solanine compared to controls. Our results are relevant to research on Egyptian armyworms *S. littoralis*. The armyworms also responded to α-solanine in their diet with increased superoxide content concomitant with increased total peroxide in foregut contents and protein carbonyl levels in foregut and midgut tissue (Krishnan and Kodrik, 2006).

The induction of GST in larval wax moth midgut tissue is likely due to the differences between the midgut lumen (topologically outside the animal) and the fat body (topogically inside); that is, toxic compounds within the body have potential to exert more overall damage to the body than compounds still outside the body. Hyršl et al. (2007) reported that an inorganic insecticide altered GST activities in correlation to elevated MDA content in wax moth larval and pupal hemolymph and fat body. We interpret their finding to show a strong correlation between GST activities and MDA content. The adverse effects of α-solanine on the wax moth larvae may be attributed to its activated metabolites, rather than the parent compound per se. The idea that activated α-solanine metabolites lead to oxidative stress and alterations of GST activity was suggested for the toxicity of some potato leaves in *S. littoralis* larvae (Krishnan and Kodrik, 2006).

Our results show a reduction in the survivorship, fecundity, and egg-hatchability of *G. mellonella* reared on α-solanine. An increase in midgut and fat body MDA and protein carbonyl levels along with decreased egg production and hatching suggests to us that α-solanine-induced oxidative stress affected the fecundity of *G. mellonella*. This is supported by Melisa et al. (2005), who reported that increased oxidative stress was associated with reduced fecundity in *Drosophila melanogaster* (Meigen). Previous studies demonstrate that sublethal oxidative effects of a dietary supplement on survivorship and development depends on its interaction with dietary nutrients and thus alters the feeding rate of larvae (Büyükgüzel and İçen, 2004; Büyükgüzel and Kalender, 2007, 2009). Perhaps α-solanine similarly degrades diet quality. The diets with high α-solanine concentrations may have led to impaired feeding rates in larvae. We speculate that the increased α-solanine toxicity at high concentrations is due to nutritional deficiency, leading to reduced survivorship and fecundity in adults. This is in line with results of Adamski et al. (2009) who showed that plant-extracted glycoalkaloids decreased hatching success of *Spodoptera exigua* eggs,

which was correlated with chemical concentrations. Hussein et al. (2006) also reported that potato plants decreased life-table parameters including growth and reproduction of the lepidopteran pest moth, *S. littoralis*. We infer that GST reduces α-solanine-induced oxidative stress in *G. mellonella* as suggested for some other insect groups (Leszczynski et al., 1994).

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