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**Midgut-mediated glycolipid metabolism plasticity confers adaptive tolerance to α -solanine
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Abstract: α -Solanine, potato-derived secondary metabolite, possesses toxic effects to various insects and has the potential to be developed into an insecticide. *Phthorimaea operculella* is one of the most destructive pests of potato crops worldwide. However, the effects of α -solanine on *P. operculella* larvae and the glycolipid metabolism-regulated detoxification mechanism against the toxin in this pest remain unclear. In this study, we found that α -solanine exposure at LC₅ and LC₁₀ exerted non-significant restrictive effects on larval body weight, while the relatively higher concentration LC₂₀ significantly reduced larval body weight and delayed larval developmental stages. Exposure to α -solanine at LC₂₀ suppressed larval ingestion, decreased the efficiency of conversion of ingested food (ECI) and digested food (ECD), and increased metabolic cost (MC) at 24 h. α -Solanine treatment further disturbed the expression of digestion-related genes and enzyme activities, and interfered with midgut structure. Moreover, it upregulated the expression of three *trehalase* genes and two lipid breakdown genes, corroborating the observed increase in activities of trehalase, ATP-citrate lyase, and lipase. Conversely, it downregulated the expression of five lipid biosynthesis genes and acetyl-CoA carboxylase activity, and elevated fatty acid synthase activity. Overall, our findings suggest that midgut-mediated glycolipid metabolism plays a pivotal role in orchestrating the detoxification mechanisms as counter-defense strategies larvae use to cope with α -solanine, revealing the adaptability of herbivorous insects to plant defenses and providing insights for developing effective control strategies against pests.

Keywords: Sublethal effect, Digestive enzyme, Midgut structure, Glycolipid metabolism, Adaptability

37 **Introduction**

38 Synthetic pesticides, widely used to control pests, often lead to the development of pest resistance,
39 environmental pollution, and declines in beneficial insects populations, in addition to the implications for
40 human and animal health. Integrated pest management (IPM) advocates the application of diverse control
41 techniques, reducing pests to economically acceptable levels and minimizing negative environmental
42 impacts as much as possible (Gao et al. 2025). The environmentally friendly insecticides are required to
43 control pests. Plants synthesize several types of secondary metabolites, such as alkaloids, terpenes, amines,
44 quinones, phenolics, peptides, and polyacetylenes, to cope with herbivorous insects. They are categorized
45 into four groups: phenolics, terpenoids, and nitrogen and sulfur-containing compounds. Defensive
46 functions are diverse, including feeding deterrent, repellent, and direct toxic effects, enzyme inhibitor,
47 reduced food digestibility, and inhibition of insect growth and development (Divekar et al. 2022).
48 Therefore, plant secondary metabolites function as defense compounds against herbivorous insects and
49 serve as promising alternatives for pest control.

50 Glycoalkaloids (GAs), as a class of nitrogen-containing steroidal glycosides, are prevalent in
51 Solanaceae plants, and affect the physiology of insect pests (Gao et al. 2025). Dietary *Solanum nigrum*
52 extract and solasonine exert sublethal effects on *Galleria mellonella* larvae, including disruption of larval
53 metabolism via alterations in total polyols, glucose, and trehalose levels, as well as proline concentration
54 (Spochacz et al. 2021). α -Solanine, a natural constituent of potatoes, possesses toxic effects to various
55 insects, resulting in insect mortality, developmental changes, reduced survivorship, fecundity, and fertility,
56 strong cardioinhibitory effects, altered the timing of pupal heart rhythm phases, and increased oxidative
57 stress (Nenaah 2011; Büyükgüzel et al. 2013; Adamski et al. 2014; Marciniak et al. 2019; Yan et al. 2023).
58 Notably, although potato pests can feed on potato tissues containing α -solanine and are tolerant to low
59 concentrations of α -solanine, they may be sensitive to high concentrations. The survival of *Tecia*

60 *solanivora* larvae was significantly reduced after feeding on potato tubers with a high concentration of α -
61 solanine (Karlsson et al. 2013). High concentrations of α -solanine cause the death of *Empoasca fabae*
62 adults and *Phthorimaea operculella* larvae (Sanford et al. 1996; Yan et al. 2023). It is essential to consider
63 the toxicity of α -solanine and insects' detoxification ability to cope with it, facilitating the development of
64 cost-effective ecologically friendly means to sustain agriculture. However, there are few studies on the
65 physiological consequences of α -solanine ingestion in the growth and development of insects, for which
66 feeding behavior, food conversion, digestion and absorption, excretion, and metabolism serve as key
67 indicators, especially their impacts on Lepidopteran that cause damage to agricultural crops.

68 Insect digestive enzymes break down complex food molecules into smaller molecules that insects can
69 directly absorb and use, thus regulating insect growth and development. The smaller absorbable molecules
70 (e.g., glucose and free fatty acids) in the insect gut lumen serve as the prerequisites for insect glycolipid
71 metabolism. Glucose, the universal energy source for most organisms, is derived from dietary components
72 or the breakdown of the most important carbohydrate reserves, namely trehalose and glycogen. Moreover,
73 glucose is converted to triglyceride (TG), the dominant lipid storage form, via several steps, including
74 glycolysis, pyruvate metabolism, acetyl-CoA and fatty acids synthesis (Gáliková and Klepsatel 2023).
75 Previous studies have shown that plant secondary metabolites affect biochemical indices in insects. For
76 example, *S. nigrum* extract decreases glucose and trehalose concentrations in the hemolymph of *G.*
77 *mellonella* (Spochacz et al. 2021). Tomatine and the extract from tomato leaves at higher tested
78 concentrations (10^{-5} M) decrease TG levels in the hemolymph of *Tenebrio molitor* larvae after treatment
79 for 2 h, and 10^{-5} M chaconine increases TG levels in the hemolymph and fat body (Winkiel et al. 2023).
80 Carbohydrates and lipids integrate to a complex metabolic network, essential for the maintenance of insect
81 physiological homeostasis (Pei et al. 2024; Akiki et al. 2026). However, whether α -solanine associated
82 with growth and development was regulated by glycolipid metabolism in Lepidopteran was unclear.

The potato tuber moth, *P. operculella* Zeller (Lepidoptera: Gelechiidae), damages many Solanaceae plants, especially potatoes. In this study, we investigated the sublethal effects of α -solanine intake on *P. operculella* larvae from the following aspects: the growth and development of larvae, nutritional indicators of larvae, expression of digestion-related genes and digestive enzyme activities, midgut structure, essential nutrients (including glycogen, trehalose, glucose, and triglycerides), as well as expression of key genes and enzyme activities associated with glycolipid metabolism. This study enhances our understanding of the potential insecticidal mechanisms of α -solanine and the role of glycolipid metabolism in detoxification mechanisms and the adaptability of insects to plant toxins, and provides insights into the sublethal effects of plant secondary metabolites and a theoretical basis for the development of environmentally friendly sustainable biopesticides.

Materials and methods

Insect rearing

Phthorimaea operculella used in this study was originally collected from potato fields in Yunnan Province in 2014. Subsequently, the population was established and maintained at the laboratory of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (Beijing, China) without any insecticide exposure since its collection. The larvae were reared on potato tubers under controlled conditions with a temperature at $26 \pm 1^\circ\text{C}$ and a photoperiod of 12L: 12D h. The adult moths were provided with an aqueous solution containing 10% (v/v) honey.

α -Solanine treatments and their impacts on larval growth and development

Toxicity of sublethal exposure to α -solanine on the growth and development *P. operculella* larvae was assessed using a leaf-dip method. α -Solanine (Shanghai Yuanye Bio-Technology Co., China) was dissolved in dimethyl sulfoxide (DMSO) to obtain stock solution at $200\text{ }\mu\text{g}/\mu\text{L}$ and diluted with sterile double-distilled water (ddH₂O) to prepare working solutions (LC₅, LC₁₀, and LC₂₀). Based on preliminary

106 toxicity assays, fresh potato leaves were punched to produce uniform discs of 1.5 cm diameter, which were
107 then immersed in α -solanine solutions for 30 min. The leaves were allowed to air-dry in a sterile
108 environment for subsequent experiments (Ji et al. 2025). All leaves used in this experiment were obtained
109 from uniformly grown 30-day-old potato plants.

110 The newly molted third-instar *P. operculella* larvae were starved for 6 h and reared on the these leaf
111 discs containing sublethal concentrations of α -solanine (LC₅, LC₁₀, and LC₂₀) in disposable sterile culture
112 dishes with the bottom covered by 2% agar. Throughout the experiment, the survival rate, wet weight and
113 instars of the larvae were recorded at 24 h, 48 h, 72 h, and 96 h. Each treatment comprised 150 larvae.

114 **Effect of α -solanine on feeding indices of larvae**

115 The experiment was conducted using the mentioned leaf-disc method. The newly molted third-instar
116 *P. operculella* larvae were starved for 6 h and reared on these leaf discs treated with α -solanine solutions
117 (LC₅, LC₁₀, and LC₂₀). The larvae were randomly divided into four groups with 150 larvae in each group.
118 The initial weight of the food (uniform discs of 1.5 cm diameter) in the experimental group (W), the final
119 weight of the food in the experimental group (L), undigested food and excreta (F) (or weight of larval
120 feces), and food used for growth (biomass gained) (B) were recorded. A control experiment was also
121 conducted to account for the correct water loss rate (CWL). The relative growth rate (RGR), food
122 consumption or weight of ingested food (I), relative consumption rate (RCR), approximate digestibility
123 (AD), the efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD),
124 and metabolic cost (MC) were calculated by the following formulae (Coelho et al. 2007; Yue et al. 2016).

$$125 \quad I = W - L - (aW + bL) / 2$$

$$126 \quad RGR = B / (\bar{B} \times T)$$

$$127 \quad RCR = I / B \times T$$

$$128 \quad AD = (I - F) / I$$

$$ECI = 100 \times (B / I)$$

$$ECD = 100 \times B / (I - F)$$

$$MC = 100 - ECD$$

Here, *a* is calculated as (the initial weight of the food in control group – the final weight of the food in control group) / the initial weight of the food in control group, while *b* is (the initial weight of the food in control group – the final weight of the food in control group) / the final weight of the food in control group. *T* represents the duration of feeding in days, and $\bar{B}$ represents the mean weight of larvae during the experiment.

Measurement of digestive enzyme activities

Amylase, lipase, chymotrypsin, and trypsin are the primary digestive enzymes in the insect midgut (Wu et al. 2022; Zhou et al. 2025). α -Amylase is a key enzyme for starch breakdown that hydrolyzes starch (Hámori et al. 2021). Lipase acts as an essential enzyme for lipid digestion, allowing insects to utilize dietary lipid (Holtorf et al. 2019). Chymotrypsin is a proteolytic enzyme that quickly degrades denatured proteins (Waizumi et al. 2024). Trypsin is a serine protease responsible for removing intercellular mucoproteins and glycoproteins (Akiki et al. 2026).

To reveal the sublethal effects of α -solanine (LC₂₀) on digestive functions, the samples collected at 24 h, 48 h, 72 h, and 96 h post LC₂₀ of α -solanine exposure were used to measure the activities of digestive enzymes including α -amylase, lipase, chymotrypsin, and trypsin using α -amylase activity assay kit (Solarbio, China), Lipase activity assay kit (Solarbio, China), Chymotrypsin activity assay kit (Solarbio, China), and Trypsin activity assay kit (Solarbio, China), respectively. Briefly, the weight of surviving larvae was measured and recorded. The samples were placed in a 1.5 mL centrifuge tube and homogenized in an appropriate extraction solution, and then centrifuged, with the supernatant collected to determine activities of α -amylase, lipase, chymotrypsin, and trypsin. Each experiment included three biological

152 replicates, each with three technical replicates.

153 **Analysis of midgut ultrastructure**

154 Hematoxylin and eosin (HE) staining. The collected midgut tissues were immediately fixed in
155 histological fixative with 4% paraformaldehyde (Servicebio, China) for 24 h, rinsed with distilled water
156 for 10 min, dehydrated in a graded ethanol series (60–90% gradient concentrations for 30 min; 95% and
157 100% gradient concentrations for 20 min), and cleared by xylene for 30 min. Subsequently, the midgut
158 tissues were embedded in paraffin, sectioned (4 μ m), stained with HE, and examined by optical microscopy.

159 Scanning electron microscopy (SEM). The collected midgut tissues were immediately fixed in a
160 fixative solution with 2.5% glutaraldehyde as the effective element (Servicebio, China) at room
161 temperature for 2 h for electron scanning. After fixation, the samples were washed with 0.1 M phosphate-
162 buffered saline (PBS) (Solarbio, China) three times for 15 min each, placed in 1% OsO₄ solution for 2 h
163 at room temperature, and then consistently washed with 0.1 M PBS three times for 15 min each. The
164 samples dehydrated with alcohol (30–50–70–80–90–95–100% gradient concentrations for 15 min at a time)
165 and isoamyl acetate (for 15 min), and dried in the critical point dryer. Finally, the samples were sputter-
166 coated with gold using an ion-sputtering apparatus (Hitachi, Japan) and subsequently visualized via an
167 electron scanning microscope (Hitachi, Japan).

168 Transmission electron microscopy (TEM). The collected midgut tissues were immediately placed in
169 fixative solution with 2.5% glutaraldehyde as the effective element (Servicebio, China) at room
170 temperature for 24 h, post-fixed in 1% OsO₄ solution for 2 h at room temperature, and then stained with
171 4.8% uranyl acetate. The midgut tissues were dehydrated in a graded ethanol series (50–70–80–90–95–
172 100% gradient concentrations) and acetone (100%), inserted into embedding plates, cut into ultra-thin
173 sections, and stained with magnesium-uranyl acetate and lead citrate. Finally, the samples observed by
174 transmission electron microscopy (Hitachi, Japan).

175 **Determination of nutrient contents**

176 Larvae were collected at 24 h, 48 h, 72 h, and 96 h after LC₂₀ of α -solanine treatment for examining
177 the contents of glucose, trehalose, glycogen, and TG using Glucose content assay kit (Solarbio, China),
178 Trehalose content assay kit (Solarbio, China), Glycogen content assay kit (Solarbio, China), and TG assay
179 kit (Nanjing Jiancheng, China), respectively. Samples were weighed in 1.5 mL centrifuge tubes and
180 homogenized in an appropriate extraction solution following the instructions by the respective kit
181 manufacturers. The homogenate was centrifuged to obtain the supernatant for content determination. The
182 OD value was tested with a microplate reader (BioTek, USA). Each treatment comprised three biological
183 replicates, each with three technical replicates.

184 **Measurement of enzyme activities in glycolipid metabolic processes**

185 The key enzyme activities in glycolipid metabolic processes were also detected using the Trehalase
186 activity assay kit (Solarbio, China), ATP citrate lyase activity assay kit (Grace, China), Acetyl-CoA
187 carboxylase activity assay kit (MLBio, China), and Fatty acid synthases activity assay kit (Grace, China).
188 According to the manufacturer's instructions, larvae treated with LC₂₀ of α -solanine were collected,
189 weighed, and subsequently homogenized in a 1.5 mL centrifuge tube after treatment with LC₂₀ of α -
190 solanine for 24 h, 48 h, 72 h, and 96 h. All experiments were performed by three biological replicates, each
191 with six larvae.

192 **RT-qPCR analysis**

193 The expression of key genes in glycolipid metabolic processes was assessed by RT-qPCR. After LC₂₀
194 of α -solanine treatment, larvae were collected at 24 h, 48 h, 72 h, and 96 h. Total RNA was extracted from
195 each sample using an animal tissue total RNA extraction kit (TIANGEN, China). The first-strand cDNA
196 template for RT-qPCR was synthesized by reverse transcription with 1 μ g of total RNA. RT-qPCR was
197 performed on the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific Inc., USA)

with PerfectStart[®] Green qPCR SuperMix (TransGen, China) utilizing a three-step PCR with cycling parameters as follows: 1 cycle of 94 °C for 30 s, followed by 40 cycles of 94 °C for 5 s, 60 °C for 34 s, and 72 °C for 10 s. The internal reference genes, *P. operculella* elongation factor 1 α (*PoEF1 α*) and ribosomal protein L13 (*PoRPL13*), were used to normalize the expression of genes related to digestion, sugar metabolism, and lipid metabolism (Shen et al. 2022). The results were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak et al. 2001).

The sequences of α -amylase 2/4 (*Poa-Amy2/4*), lipase3 (*PoLIP3*), chymotrypsin-1 (*PoChy1*), trypsin CFT1 (*PoTryp CFT1*), trypsin C (*PoTryp C*), glycogen synthase (*PoGS*), glycogen phosphorylase 1/2 (*PoGP1/2*), glycogen debranching enzyme (*PoGDE*), trehalose 6-phosphate synthase (*PoTPS*), soluble trehalase 1 (*PoTreS1*), membrane-bound trehalase (*PoTreM*), soluble trehalase 2 (*PoTreS2*), ATP-citrate lyase (*PoACLY*), acetyl-CoA carboxylase (*PoACC*), fatty acid synthase 1/2/3/4 (*PoFAS1/2/3/4*), mammalian adipocyte triglyceride lipase homologue *Brummer* (*PoBmm*), and hormone-sensitive lipase (*PoHSL*) were obtained from the genome and transcriptome data of *P. operculella*. Gene-specific primers were detailed in Supplementary Table S1. All groups were performed in three biological replicates.

Statistical analysis

The toxicity experiment data were adjusted for control mortality using Abbott's formula prior to analysis (Abbott 1925). The normality of data distribution was examined using the ShapiroWilk test for normality. Equality of variance between groups was tested using the Levene test for homogeneity of variance. The differences in wet weight, nutritional indices, activities of digestive enzymes, as well as sugar-lipid metabolic processes-related enzymes, nutrient contents, and relative gene expression between the treatments and the control were assessed using One-way analysis of variance (ANOVA) or Student's *t*-test. These statistical analyses were conducted using SAS 8.1 (SAS Institute, Cary, NC, USA) and GraphPad Prism 8.0.2 (GraphPad Software, San Diego, California, USA).

221 **Results**

222 **Sublethal effects of α -solanine on the growth and development of *P. operculella* larvae**

223 The newly molted third-instar *P. operculella* larvae were subjected to α -solanine (Fig. 1A). Exposure
224 to LC₅ and LC₁₀ concentrations increased larval wet weight at 24 h by 6.29% and 8.94% respectively but
225 had no effect at 48 h, 72 h, and 96 h compared with the control. LC₂₀ treatment significantly decreased
226 larval wet weight at 24 h, 48 h, 72 h, and 96 h by 8.70%, 19.21%, 23.28%, and 28.34%, respectively (Fig.
227 1B). In addition, LC₅ treatment with α -solanine slightly accelerated the development from 3rd to 4th instar
228 larvae of *P. operculella* at 48 h and 72 h, but delayed at 96 h. Exposure to LC₁₀ and LC₂₀ concentrations
229 also delayed larval development at 48 h, 72 h, and 96 h (Fig. 1C).

230 **Sublethal effects of α -solanine on the nutritional indicators of *P. operculella* larvae**

231 Exposure to LC₅ and LC₁₀ of α -solanine increased food consumption at 24 h and did no affect food
232 consumption at 48 h, 72 h, and 96 h. The LC₂₀ inhibited food consumption at 48 h, 72 h, and 96 h (Fig.
233 2A). The larval feces was not significantly different at 24 h and 48 h and decreased at 96 h post α -solanine
234 treatment (LC₅, LC₁₀, and LC₂₀), and exhibited a remarkable downregulation at 72 h post the LC₂₀
235 treatment (Fig. 2B). Treatment with LC₅, LC₁₀, and LC₂₀ of α -solanine failed to induce changes in AD at
236 24 h, 48 h, 72 h, and 96 h (Fig. 2C). Following 24 h, 48 h, 72 h, and 96 h of treatment with LC₅, LC₁₀, and
237 LC₂₀ of α -solanine, RCR per larva had no significant differences (Fig. 2D). Additionally, treatments with
238 LC₅, LC₁₀, and LC₂₀ of α -solanine had no significant effect on RGR , ECI, ECD, and MC per larva at 48
239 h, 72 h, and 96 h (Fig. 2E–H). In contrast, distinct impacts were observed at 24 h: LC₂₀ treatment decreased
240 ECI and ECD as well as increased MC (Fig. 2F–H).

241 **Sublethal effects of α -solanine on gene expression and activity of digestive enzymes**

242 Given that LC₂₀ showed significant effects compared with LC₅ and LC₁₀ (Fig. 1,2,S1), subsequent
243 experiments were conducted using LC₂₀ treatment. The effects of α -solanine on digestion function of larval

244 midgut were investigated (Fig. 3A). The α -amylase activity significantly increased at 24 h, 48 h, and 72 h
245 in LC₂₀ treatment (Fig. 3B). Compared with the control, the lipase activity increased at 24 h and 48 h
246 (although the differences at 48 h did not reach significance), and decreased at 72 h and 96 h (Fig. 3C).
247 Furthermore, the activity of chymotrypsin was markedly upregulated at 24 h and 48 h, and downregulated
248 at 72 h and 96 h (Fig. 3D). In comparison, trypsin activity was greatly downregulated at 24 h and 48 h,
249 and upregulated at 72 h and 96 h (Fig. 3E).

250 RT-qPCR analysis showed that, compared with the control, *Poa-Amy2* expression was significantly
251 downregulated at 24 h, 48 h, 72 h, and 96 h (Fig. 3F). The expression of *Poa-Amy4* was upregulated at 24
252 h, 48 h, 72 h, and 96 h in the LC₂₀ treatment (Fig. 3G). The expression of *PoLIP3* exhibited a remarkable
253 downregulation at 72 h and 96 h post-treatment with LC₂₀ of α -solanine (Fig. 3H). The expression of
254 *PoChyl* was increased at 72 h and decreased at 96 h post LC₂₀ of α -solanine exposure (Fig. 3I). And the
255 expression of *PoTryp CFT1* and *PoTryp C* was reduced at 48 h and 96 h, and increased at 72 h after LC₂₀
256 treatment (Fig. 3J,K).

257 Sublethal effects of α -solanine on intestinal structure of larvae

258 To further investigate the α -solanine-induced alterations in the absorptive capacity of the larval
259 midgut, midgut ultrastructure was observed. HE staining revealed that clear differences between the
260 control and treatment group. In the control group, the midgut cells were arranged neatly, and the surface
261 of epithelial cells was covered by long microvilli (Mi). However, intestinal Mi were shortened at 24 h, 48
262 h, 72 h, and 96 h of α -solanine exposure (Fig. S2). Similar findings were found using SEM and TEM,
263 where LC₂₀ of α -solanine induced the shortening of Mi. SEM also showed that Mi were well developed
264 and continuously, densely, and abundantly distributed on the surface of larval midgut epithelial cells, while
265 Mi structure was abnormal and heterogeneous in *P. operculella* larvae that had ingested potato leaf discs
266 treated with LC₂₀ of α -solanine (Fig. 4A). Importantly, the accumulation of intracellular lipid droplets in

267 midgut exhibited high abundance in the control group as we have observed, but the accumulation
268 intracellular lipid droplets maintained lower abundance in larvae treated with LC₂₀ of α -solanine for 24 h,
269 48 h, and 72 h (Fig. 4B).

270 **Sublethal effects of α -solanine on biochemical indices**

271 A scheme of catabolism and anabolism of energy reserves in *P. operculella* was presented, illustrating
272 that glucose and trehalose can be converted into either glycogen or TG, and glycogen can be reversibly
273 converted to glucose (Fig. 5A). The relative contents of glycogen, trehalose, glucose, and TG were
274 significantly reduced at 24 h, 48 h, 72 h, and 96 h in *P. operculella* treated with LC₂₀ of α -solanine (Fig.
275 5B–E).

276 **Effects of α -solanine on the expression of sugar metabolism-related genes and key enzyme activities**

277 To further investigate the effect of LC₂₀ treatment on larval sugar metabolism, the expression profiles
278 of eight genes in the sugar metabolism and trehalase activity were evaluated. The schematic diagram of
279 sugar metabolism in *P. operculella* was successfully drawn (Fig. 6A). The expression of *PoGS* increased
280 at 24 h and 48 h, and decreased at 72 h and 96 h after LC₂₀ treatment with α -solanine compared with the
281 control (Fig. 6B). The expression of *PoGPI*, *PoGP2*, and *PoGDE* was significantly reduced at 24 h and
282 72 h and remarkably enhanced at 48 h after LC₂₀ exposure (Fig. 6C–E). The expression of *PoTPS* was
283 increased at 48 h and decreased at 72 h in the LC₂₀ treatment (Fig. 6F). Exposure to LC₂₀ concentration
284 significantly downregulated *PoTreS1* and *PoTreM* expression at 24 h, downregulated *PoTreS1* expression
285 and upregulated *PoTreM* expression at 48 h, upregulated *PoTreS2* and *PoTreM* expression at 72 h, and
286 upregulated *PoTreS1* and *PoTreS2* expression at 96 h (Fig. 6G–I). Furthermore, trehalase activity exhibited
287 a decrease at 24 h, and an increase at 48 h, 72 h, and 96 h after LC₂₀ treatment with α -solanine (Fig. 6J).

288 **Effects of α -solanine on the expression of lipid metabolism-related genes and key enzyme activities**

289 The lipid metabolism model examined in this study is also shown (Fig. 7A). The expression of

PoACLY was decreased at 24 h, 48 h, and 72 h post-treatment with LC₂₀ of α -solanine, although the differences did not reach significance at 72 h (Fig. 7B). The expression of five lipogenesis genes—*PoACC*, *PoFAS1*, *PoFAS2*, *PoFAS3*, and *PoFAS4*—was downregulated at 24 h, 48 h, 72 h, and 96 h in LC₂₀ treatment (only *PoACC* expression showed no significant difference at 96 h), except *PoFAS3* expression at 96 h (Fig. 7C–G). The expression of two lipolysis genes, *PoBmm* and *PoHSL*, was increased at 24 h, 48 h, and 72 h following LC₂₀ treatment, with the exception that *PoBmm* expression decreased at 48 h. And the expression of these two genes was downregulated after 96 h of exposure to LC₂₀ of α -solanine, while no significant difference was observed in the expression of *PoBmm* (Fig. 7H,I).

The key enzyme activities in lipid metabolism were assessed. The activity of ATP-citrate lyase was remarkably elevated at 24 h, 48 h, and 72 h, but depressed at 96 h post-treatment with LC₂₀ of α -solanine (Fig. 7J). The activity of acetyl-CoA carboxylase was prominently downregulated at 48 h, 72 h, and 96 h, but not at 24 h in the LC₂₀ treatment (Fig. 7K). Meanwhile, increased fatty acid synthase activity was observed at 24 h, 48 h, 72 h, and 96 h in the LC₂₀ treatment (Fig. 7L).

Discussion

Plants and herbivorous insects have complex and close interrelationships. During the long-term interaction process, plants have evolved multiple defense mechanisms against herbivorous insects, among which the synthesis and accumulation of abundant secondary metabolites are important components. Herbivorous insects have also evolved corresponding counter-defense strategies, such as detoxification against the host's secondary metabolites. This study evaluated sublethal effects of α -solanine on biological and nutritional indicators, the functions of digestion and absorption, as well as glycolipid metabolism, providing a theoretical basis for investigating the adaptation of herbivorous insects to their hosts and facilitating the exploration of new green pest control methods targeting host adaptability.

The sublethal effects of pesticides are an important part of their toxicity, and the sublethal effects of

313 plant secondary metabolites that can serve as biopesticides also function as a critical component. Previous
314 studies have shown that sublethal concentrations of biopesticides inhibit the growth and development of
315 many insects. Tannic acid from cotton plants at a concentration as low as 5% significantly reduced the
316 relative growth rate of *Malacosoma disstria* (Karowe 1989). A sublethal dosage of 2-tridecanone retards
317 the development of *Helicoverpa armigera* larvae (Zhang et al. 2016). Tannic acid and gossypol
318 significantly reduced the intrinsic rate of increase, the finite rate of increase, and the net reproductive rate
319 in *Aphis gossypii* (Ma et al. 2019). β -caryophyllene interferes with the moulting process of *S. litura*,
320 resulting in a significant prolongation in the pupal period in treated larvae (Mahajan et al. 2022). After
321 exposure to the LC₁₅ concentration of 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one from
322 maize, some surviving individuals failed to develop into adults (Chen et al. 2025). Our results revealed
323 that exposure to sublethal doses of α -solanine did not consistently cause larval weight loss and
324 developmental slowdown (Fig. 1), indicating that insects can resist plant-toxin stress and exhibit adaptive
325 feeding. Moreover, given that the *P. operculella* larvae are boring pests, the potential interference of
326 multiple factors (e.g., brush touching and weighing) on their developmental duration during the experiment
327 cannot be completely ruled out, as the present study found that the developmental duration of larvae in the
328 control group exhibited an extension phenomenon compared with those that were only provided with fresh
329 leaf discs replacement.

330 Assessment of nutritional parameters has been identified as a strategy for evaluating the post-
331 ingestive effects, antifeedant activity, and/or toxicity of secondary metabolites (Wheeler and Isman 2001).
332 Evaluation of nutritional parameters could yield a more comprehensive understanding of the detrimental
333 effect of α -solanine on larval growth and developmental processes. Our results revealed that sublethal
334 doses of α -solanine inhibited the feeding of larvae (Fig. 2A), suggesting that α -solanine had antifeeding
335 effect. Interestingly, the low-concentration α -solanine treatment (LC₅ and LC₁₀) promoted larval feeding

at 24 h (Fig. 2A), which may be related to the fact that insects' adaptive radiation to host plants enables them to feed on low doses plant toxins. Given that its properties such as analgesic, anti-inflammatory, antitumor, cardiovascular, and antioxidant effects, or activity that prevents muscle wasting in mammals, it is hypothesized that the increased food consumption is required for potential beneficial functions of α -solanine. We also noticed that α -solanine (LC₂₀) reduced key feeding indicators including ECI and ECD at 24 h (Fig. 2F,G), suggesting that the effect was not solely an antifeedant effect but also a toxicity mediated physiological inhibition. Additionally, α -solanine (LC₂₀) increased MC at 24 h in *P. operculella* larvae (Fig. 2H), which is consistent with the phenomenon where *Annona coriacea* lectin (ACLEC) increased MC of *Anagasta kuehniella* larvae (Coelho et al. 2007). Differences observed in metabolic cost estimation may be explained by factors such as study type and dietary quality (Méndez-Ortíz et al. 2019). Our results cannot rule out differences of dietary quality, even if relatively uniform leaves were chosen. The findings demonstrated that α -solanine exhibits toxicity to *P. operculella* larvae, while this pest has developed adaptability.

Some studies indicate that plant secondary metabolites can inhibit insect digestive enzymes. Dietary stress from plant secondary compounds reduces the activities of amylase, lipase, and chymotrypsin, and lowers the expression of *Chy2* gene (Huang et al. 2017). Treatment with the trans-anethole decreases the activities of α -amylase, lipase, chymotrypsin-like, and trypsin-like enzymes (Pour et al. 2022). Meliaceae secondary metabolites may interfere with enzyme secretion and nutrient absorption (Zhou et al. 2025; Senthil-Nathan 2013). When *S. litura* larvae are treated with TSN, the activities of α -amylase, lipase, and trypsin decreased, and the expression of some key genes involved in carbohydrate metabolism (α -Amy 2 and α -Amy 4), lipid metabolism (*LIP3*), and protein metabolism (*Chy1*, *Tryp CFT1*, and *Tryp C*) was downregulated (Zhou et al. 2025). In our study, LC₂₀ of α -solanine disrupted the expression of six digestive enzyme genes and the activities of α -amylase, lipase, chymotrypsin, and trypsin, and LC₂₀ of α -solanine

359 did not consistently suppress or enhance enzyme activity and gene expression (Fig. 3), suggesting that
360 insect digestive enzymes might be involved in the toxicity mechanism of α -solanine. A factor that cannot
361 be overlooked is that gene expression and activity of digestive enzymes display inconsistent variation (Fig.
362 3), which could be a regulatory adjustment strategy of insects in response to the adverse effects of α -
363 solanine on their digestion. Additionally, since both chymotrypsin and trypsin are involved in digestion
364 and regulate protein metabolism—and considering the findings of this study on the sublethal regulatory
365 effects of α -solanine on these two enzymes' activities (Fig. 3D,E)—it is conceivable that they may serve
366 as a complementary or redundant system. This could be an essential adaptive response allowing insects to
367 counteract toxins and maintain proper development.

368 The insect midgut, the main organ of detoxification, plays a very important role in food digestion and
369 nutrient absorption (Hakim et al. 2010). Midgut damage and apoptosis cause digestive enzyme disorders,
370 affecting the digestion and absorption of nutrient metabolites (Zhao et al. 2019). Mi, formed by the apical
371 membrane of midgut columnar cells (CCs), serve as the primary site for many physiological, biochemical,
372 and biological interactions between insects and the environment. They not only are accountable for the
373 synthesis and secretion of digestive enzymes but also contribute to nutrient uptake (Caccia et al. 2019).
374 The initial target of TSN is situated on the Mi of the midgut epithelium cells, and Mi were messed and
375 swelled as well as degradation or myelination after treatment with TSN, leading to the regurgitation,
376 paralysis and death of *Mythimna separate* larvae (Li et al. 2020). Azukisapogenol triterpenoid saponins
377 from *Oxytropis hirta* Bunge result in the destruction and irregular arrangement of Mi, decrease α -amylase
378 activities, and increase lipase and trypsin activities to affect the digestion system of *Acyrtosiphon pisum*
379 (Ye et al. 2023). In addition, azadirachtin destroyed the normal midgut cell structure and the intestinal wall
380 of *Bactrocera dorsalis* larvae (Zhao et al. 2019). Similar with these results, we found that α -solanine
381 exerted toxicological effects to midgut cells, including disordered arrangement, abnormal morphology,

382 and decreased length of Mi (Fig. 4,S2), further demonstrating that α -solanine governed the digestive and
383 absorptive functions in the larval midgut. Interestingly, the integrity of midgut cells was impaired at 72 h
384 of α -solanine treatment, and microorganisms invaded the intracellular environment (Fig. 4B), suggesting
385 that insect immunity is activated and the role of gut microbiota need be further investigated.

386 Insects predominantly store energy in the forms of glycogen and triacylglycerols. Reduced food
387 intake triggers a state of starvation in insects, compelling them to mobilize stored energy reserves to sustain
388 growth and development (Zhang et al. 2019). During this physiological process, glycogen and trehalose
389 are converted into glucose in insects, accompanied by the consumption of energy reserves indispensable
390 for growth and detoxification metabolism (Al et al. 2019; Sudhakar et al. 2020). Decreased fat synthesis
391 rate increased lipid mobilization, and a rapid decline in TG levels were also observed (Hafeez et al. 2019).
392 Consistently, given reduced food intake and decreased the contents of glycogen, trehalose, glucose, and
393 TG after LC₂₀ treatment (Fig. 2A and 5B,C,E), a state of larval starvation forcing them to utilize stored
394 energy, including glycogen, trehalose, and TG. Alternatively, exposure to LC₂₀ of α -solanine significantly
395 increased the expression of sugar metabolism-related genes and elevated trehalase activity (Fig. 6),
396 suggesting that α -solanine disrupts sugar metabolism and promotes glucose production to support insect
397 growth and development. Given that glucose-induced TG synthesis exists in insects with the participation
398 of ACLY, and that a sublethal dose of α -solanine inhibits the expression of *PoACLY* and stimulates ACLY
399 activity in *P. operculella* larvae (Fig. 7), α -solanine modulates the metabolic conversion of glucose into
400 triglycerides, which may account for the observed reduction in glucose levels. Interestingly, the inverse
401 relationship between downregulated expression of *PoACLY* gene and upregulated enzyme activity of
402 ACLY (Fig. 7B, J) indicated that insects remodel metabolism to avoid over-transformation of glucose into
403 triglycerides under α -solanine treatment. Our study demonstrated that *P. operculella* larvae treated with
404 LC₂₀ of α -solanine exhibited the downregulated expression of key genes and decreased activity of acetyl

CoA carboxylase associated with lipid synthesis in the preliminary stage post α -solanine treatment (Fig. 7B–G,K), accompanied by upregulated expression of key genes and increased activity of lipase (Bmm and HSL are two key lipases for lipolysis) linked to lipid degradation (Fig. 3 and 7H, I), indicating that LC₂₀ of α -solanine could inhibit adipogenesis and promote lipolysis. As we have observed, although lipolytic gene expression was upregulated, lipase activity was reduced at 72 h (Fig. 3 and 7H, I), suggesting that the capacity of a sublethal dose of α -solanine to stimulate lipolysis is diminished. We further found that *Fas3* gene expression was significantly upregulated at 96 h, coupled with decreased activity of acetyl CoA carboxylase (Fig. 7F,K), an event which might be associated with the weakened ability to suppress adipogenesis. Notably, the activity of fatty acid synthases showed a sustained elevation—potentially an adaptive response of insects to avoid excessive fat loss, enabling them to sustain growth and development and cope with insecticide toxicity. Collectively, these results revealed that α -solanine disrupted digestion and absorption, thereby modulating sugar metabolism and lipid synthesis and degradation, ultimately governing insect growth and development (Fig. 8). Further studies will be needed to understand the toxicity of α -solanine to insects and glycolipid metabolism-regulated detoxification mechanisms as counter-defense strategies larvae use to cope with α -solanine.

Conclusions

Our study revealed sublethal effects of potato-derived α -solanine on *P. operculella* larvae, including wet weight, developmental instar, and various nutritional indicators. The results indicated that sublethal α -solanine exposure did not always suppressed larval growth and development: a high concentration (LC₂₀) result in reduced larval wet weight and slowed development, whereas low concentrations (LC₅ and LC₁₀) had no effects on larval wet weight. Sublethal α -solanine (LC₂₀) possessed antifeedant property, inhibited various nutritional indicators, disrupted the expression of digestive enzyme genes and enzyme activities, damaged the structure of the midgut, and interfered with sugar and lipid metabolism. These findings are

428 crucial for developing environmentally friendly botanical insecticides, providing a basis for further
429 investigations on the toxicity of plant secondary metabolites to herbivorous insects and on the adaptability
430 of these insects to host-plant secondary metabolites.

431 **Author contributions**

432 Y.G.: Supervision, Writing – review & editing, Project administration, Funding acquisition. Y.J.:
433 Supervision, Data curation and analysis, Methodology, Writing - original draft. M.H.: Investigation. J.Y.:
434 Formal analysis. Subba Reddy Palli: Formal analysis, Writing - review and editing.

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438 Agricultural Sciences (CAAS-2060302).

439 **Data availability**

440 The data that support the findings of this study will be made available on request.

441 **Declarations**

442 **Conflict of interest**

443 All of the authors have read and approved the manuscript.

444 **References**

- 445 Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18 (2):
446 265–267. <https://doi.org/10.1093/jee/18.2.265a>.
- 447 Adamski Z, Marciniak P, Ziemnicki K, Büyükgüzel E, Erdem M, Büyükgüzel K, Ventrella E, Falabella P,
448 Cristallo M (2014) Potato leaf extract and its component, α -solanine, exert similar impacts on
449 development and oxidative stress in *Galleria mellonella* L. Arch Insect Biochem Physiol 87(1): 26–
450 39. <https://doi.org/10.1002/arch.21177>.
- 451 Al Baki MA, Lee DW, Jung JK, Kim Y (2019) Insulin-like peptides of the legume pod borer, *Maruca*
452 *vitrata*, and their mediation effects on hemolymph trehalose level, larval development, and adult
453 reproduction. Arch Insect Biochem Physiol 100(2): e21524. <https://doi.org/10.1002/arch.21524>.
- 454 Akiki P, Delamotte P, Montagne J (2026) Lipid metabolism in relation to carbohydrate metabolism. Adv
455 Exp Med Biol 1494: 239–259. https://doi.org/10.1007/978-3-032-04842-4_821.
- 456 Büyükgüzel E, Büyükgüzel K, Snela M, Erdem M, Radtke K, Ziemnicki K, Adamski Z (2013) Effect of
457 boric acid on antioxidant enzyme activity, lipid peroxidation, and ultrastructure of midgut and fat
458 body of *Galleria mellonella*. Cell Biol Toxicol 29(2): 117–129. [https://doi.org/10.1007/s10565-013-](https://doi.org/10.1007/s10565-013-9240-7)
459 9240-7.
- 460 Caccia S, Casartelli M, Tettamanti G (2019) The amazing complexity of insect midgut cells: types,
461 peculiarities, and functions. Cell Tissue Res 377(3): 505–525. [https://doi.org/10.1007/s00441-019-](https://doi.org/10.1007/s00441-019-03076-w)
462 03076-w.
- 463 Chen Y, Chi H, Zeng W, Liu C, Zheng X, Wang P, Fu H, Yang F (2025) Adaptive glutathione S-transferase
464 genes induced by DIMBOA as potential RNAi targets against *Ostrinia furnacalis*. Pestic Biochem
465 Physiol 214: 106557. <https://doi.org/10.1016/j.pestbp.2025.106557>.
- 466 Coelho MB, Marangoni S, Macedo MLR (2007) Insecticidal action of *Annona coriacea* lectin against the

flour moth *Anagasta kuehniella* and the rice moth *Corcyra cephalonica* (Lepidoptera: Pyralidae).

Comp Biochem Physiol C 146: 406–414. <https://doi.org/10.1016/j.cbpc.2007.05.001>.

Divekar PA, Narayana S, Divekar BA, Kumar R, Gadratagi BG, Ray A, Singh AK, Rani V, Singh V, Singh AK, Kumar A, Singh RP, Meena RS, Behera TK (2022) Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. Int J Mol Sci 23(5): 2690. <https://doi.org/10.3390/ijms23052690>.

Gáliková M, Klepsatel P (2023) Endocrine control of glycogen and triacylglycerol breakdown in the fly model. Semin Cell Dev Biol 138: 104–116. <https://doi.org/10.1016/j.semcdb.2022.03.034>.

Gao Y, Alyokhin A, Prager SM, Reitz S, Huseeth A (2025) Complexities in the Implementation and Maintenance of Integrated Pest Management in Potato. Annu Rev Entomol 70(1): 45–63. <https://doi.org/10.1146/annurev-ento-120523-023156>.

Hafeez M, Liu S, Jan S, Gulzar A, Fernández-Grandon GM, Qasim M, Khan KA, Ali B, Kedir SJ, Fahad M, Wang M (2019) Enhanced effects of dietary tannic acid with chlorantraniliprole on life table parameters and nutritional physiology of *Spodoptera exigua* (Hübner). Pestic Biochem Physiol 155: 108–118. <https://doi.org/10.1016/j.pestbp.2019.01.012>.

Hakim RS, Baldwin K, Smagghe G (2010) Regulation of midgut growth, development, and metamorphosis. Annu Rev Entomol 55: 593–608. <https://doi.org/10.1146/annurev-ento-112408-085450>.

Hámori C, Remenyik J, Kandra L, Gyémánt G (2021) Colorado potato beetle alpha-amylase: purification, action pattern and subsite mapping for exploration of active centre. Int J Biol Macromol 168: 350–355. <https://doi.org/10.1016/j.ijbiomac.2020.12.071>.

Holtorf M, Lenaerts C, Cullen D, Vanden Broeck J (2019) Extracellular nutrient digestion and absorption in the insect gut. Cell Tissue Res 377(3): 397–414. <https://doi.org/10.1007/s00441-019-03031-9>.

490 Huang X, Ma J, Qin X, Tu X, Cao G, Wang G, Nong X, Zhang Z (2017) Biology, physiology and gene
 491 expression of grasshopper *Oedaleus asiaticus* exposed to diet stress from plant secondary compounds.
 492 Sci Rep 7(1): 8655. <https://doi.org/10.1038/s41598-017-09277-z>.

493 Ji Y, Yan J, Palli SR, Gao Y (2025) Identification of carboxylesterase genes and function of three inducible
 494 α -esterase genes involved in insecticidal activity of α -solanine in *Phthorimaea operculella*. J Agric
 495 Food Chem 73(31): 19764–19777. <https://doi.org/10.1021/acs.jafc.5c05732>.

496 Karlsson MF, Birgersson G, Witzgall P, Lekfeldt JD, Nimal Punyasiri PA, Bengtsson M (2013) Guatemalan
 497 potato moth *Tecia solanivora* distinguish odour profiles from qualitatively different potatoes *Solanum*
 498 *tuberosum* L. Phytochemistry 85: 72–81. <https://doi.org/10.1016/j.phytochem.2012.09.015>.

499 Karowe DN (1989) Differential effect of tannic acid on two tree-feeding Lepidoptera: implications for
 500 theories of plant anti-herbivore chemistry. Oecologia 80(4): 507–512.
 501 <https://doi.org/10.1007/BF00380074>.

502 Li H, Zhang J, Ma T, Li C, Ma Z, Zhang X (2020) Acting target of toosendanin locates in the midgut
 503 epithelium cells of *Mythimna separate* Walker larvae (Lepidoptera: Noctuidae). Ecotoxicol Environ
 504 Saf 201: 110828. <https://doi.org/10.1016/j.ecoenv.2020.110828>.

505 Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative
 506 PCR and the $2^{-\Delta\Delta C(T)}$ method. Methods 25: 402–408.
 507 <https://doi.org/10.1006/meth.2001.1262>.

508 Ma K, Tang Q, Liang P, Xia J, Zhang B, Gao X (2019) Toxicity and sublethal effects of two plant
 509 allelochemicals on the demographical traits of cotton aphid, *Aphis gossypii* Glover (Hemiptera:
 510 Aphididae). PLoS One 14(11): e0221646. <https://doi.org/10.1371/journal.pone.0221646>.

511 Mahajan E, Singh S, Diksha Kaur S, Sohal SK. The genotoxic, cytotoxic and growth regulatory effects of
 512 plant secondary metabolite β -caryophyllene on polyphagous pest *Spodoptera litura* (Fabricius)

(Lepidoptera: Noctuidae). *Toxicon* 219: 106930. <https://doi.org/10.1016/j.toxicon.2022.09.016>.

Marciniak P, Kolińska A, Spochacz M, Chowański S, Adamski Z, Scrano L, Falabella P, Bufo SA, Rosiński G (2019) Differentiated effects of secondary metabolites from Solanaceae and Brassicaceae plant families on the heartbeat of *Tenebrio molitor* Pupae. *Toxins* (Basel) 11(5): 287. <https://doi.org/10.3390/toxins11050287>.

Méndez-Ortíz FA, Sandoval-Castro CA, Vargas-Magaña JJ, Sarmiento-Franco L, Torres-Acosta JFJ, Ventura-Cordero J (2019) Impact of gastrointestinal parasitism on dry matter intake and live weight gain of lambs: A meta-analysis to estimate the metabolic cost of gastrointestinal nematodes. *Vet Parasitol* 265: 1–6. <https://doi.org/10.1016/j.vetpar.2018.11.008>.

Nenaah G (2011) Individual and synergistic toxicity of solanaceous glycoalkaloids against two coleopteran stored-product insects. *J Pest Sci* 84: 77–86. <https://doi.org/10.1007/s10340-010-0329-y>.

Pei X, Bai T, Luo Y, Zhang Z, Li S, Fan Y, Liu TX (2024) Acetyl coenzyme A carboxylase modulates lipogenesis and sugar homeostasis in *Blattella germanica*. *Insect Sci* 31(2): 387–404. <https://doi.org/10.1111/1744-7917.13245>.

Pour SA, Shahriari M, Zibae A, Mojarab-Mahboubkar M, Sahebzadeh N, Hoda H (2022) Toxicity, antifeedant and physiological effects of trans-anethole against *Hyphantria cunea* Drury (Lep: Arctiidae). *Pestic Biochem Physiol* 185: 105135. <https://doi.org/10.1016/j.pestbp.2022.105135>.

Sanford L, Domek J, Cantelo W, Kobayashi R, Sinden S (1996) Mortality of potato leafhopper adults on synthetic diets containing seven glycoalkaloids synthesized in the foliage of various *Solanum* species. *American Potato Journal* 73: 79–88. <https://doi.org/10.1007/BF02854762>.

Senthil-Nathan S (2013) Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. *Front Physiol* 4: 359. <https://doi.org/10.3389/fphys.2013.00359>.

536 Shen CH, Peng LJ, Zhang YX, Zeng HR, Yu HF, Jin L, Li GQ (2022) Reference genes for expression
537 analyses by qRT-PCR in *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Insects* 13(2): 140.
538 <https://doi.org/10.3390/insects13020140>.

539 Spochacz M, Chowański S, Szymczak-Cendlak M, Marciniak P, Lelario F, Salvia R, Nardiello M, Scieuzo
540 C, Scrano L, Bufo SA, Adamski Z, Falabella P (2021) *Solanum nigrum* extract and Solasonine
541 affected Hemolymph metabolites and ultrastructure of the fat body and the midgut in *Galleria*
542 *mellonella*. *Toxins* (Basel) 13(9): 617. <https://doi.org/10.3390/toxins13090617>.

543 Sudhakar SR, Pathak H, Rehman N, Fernandes J, Vishnu S, Varghese J (2020) Insulin signalling elicits
544 hunger-induced feeding in *Drosophila*. *Dev Biol* 459(2): 87–99.
545 <https://doi.org/10.1016/j.ydbio.2019.11.013>.

546 Waizumi R, Hirayama C, Tomita S, Iizuka T, Kuwazaki S, Jouraku A, Tsubota T, Yokoi K, Yamamoto K,
547 Sezutsu H (2024) A major endogenous glycoside hydrolase mediating quercetin uptake in *Bombyx*
548 *mori*. *PLoS Genet* 20(1): e1011118. <https://doi.org/10.1371/journal.pgen.1011118>.

549 Wheeler AD, Isman BM (2001) Antifeedant and toxic activity of *Trichilia americana* extract against the
550 larvae of *Spodoptera litura*. *Entomol Exp Appl* 98(1): 9–16.
551 <https://doi.org/10.1023/A:1018755203570>.

552 Winkiel MJ, Chowański S, Gołębiowski M, Bufo SA, Słocińska M (2023) Solanaceae glycoalkaloids
553 disturb lipid metabolism in the *Tenebrio molitor* Beetle. *Metabolites* 13(12): 1179.
554 <https://doi.org/10.3390/metabo13121179>.

555 Wu CY, Xiao KR, Wang LZ, Wang J, Song QS, Stanley D, Wei SJ, Zhu JY (2022) Identification and
556 expression profiling of serine protease-related genes in *Tenebrio molitor*. *Arch Insect Biochem*
557 *Physiol* 111(3): e21963. <https://doi.org/10.1002/arch.21963>.

558 Yan H, Dong WB, Wang YQ, Ning HH, Gao YL, Li F, Wu SY. Transcriptomic analyses revealed detoxification

genes responsive to solanine treatment in *Phthorimaea operculella*. Journal of Applied Entomology 147(7): 542–558. <https://doi.org/10.1111/jen.13145>.

Ye S, Zhao L, Qi Y, Yang H, Hu Z, Hao N, Li Y, Tian X (2023) Identification of azukisapogenol triterpenoid saponins from *Oxytropis hirta* Bunge and their aphicidal activities against pea aphid *Acyrtosiphon pisum* Harris. Pest Manag Sci 79(1): 55–67. <https://doi.org/10.1002/ps.7172>.

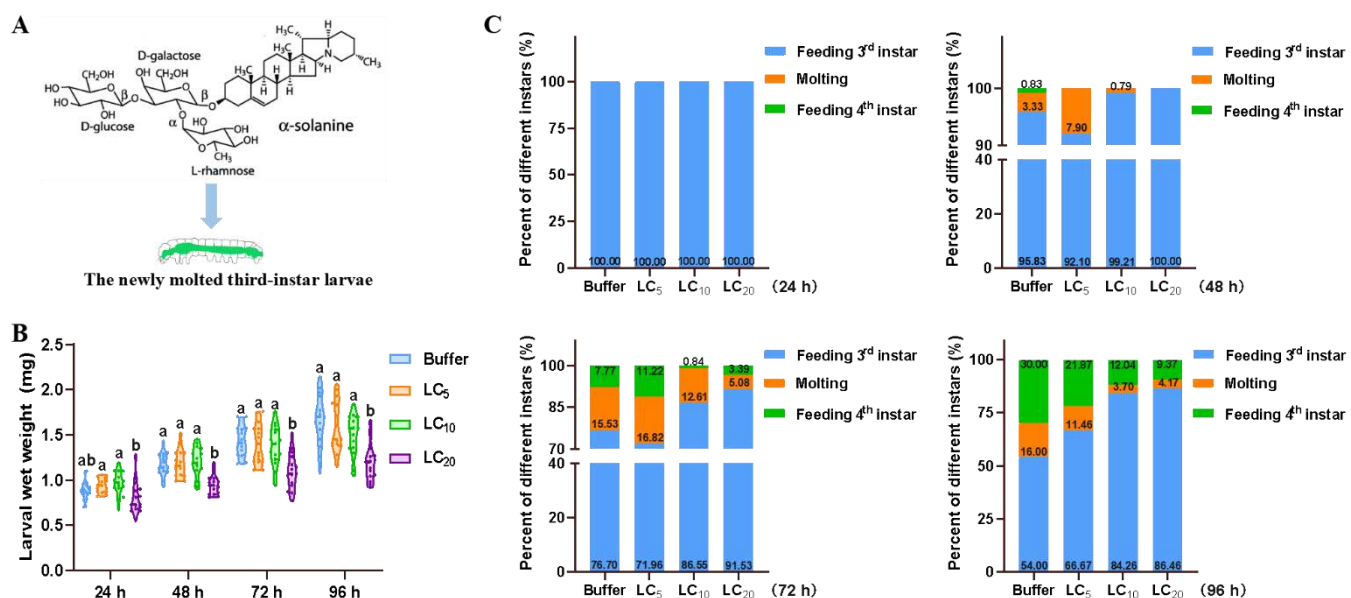
Yue Z, Liu XG, Zhou ZJ, Hou GM, Hua JP, Zhao ZW (2016) Development of a novel-type transgenic cotton plant for control of cotton bollworm. Plant Biotechnol 14 (8): 1747–1755. <https://doi.org/10.1111/pbi.12534>.

Zhang DW, Xiao ZJ, Zeng BP, Li K, Tang YL (2019) Insect behavior and physiological adaptation mechanisms under starvation stress. Front Physiol 10: 163. <https://doi.org/10.3389/fphys.2019.00163>.

Zhang L, Lu Y, Xiang M, Shang QL, Gao XW (2016) The retardant effect of 2-tridecanone, mediated by cytochrome P450, on the development of cotton bollworm, *Helicoverpa armigera*. BMC Genomic 17(1): 954. <https://doi.org/10.1186/s12864-016-3277-y>.

Zhao T, Lai D, Zhou Y, Xu H, Zhang Z, Kuang S, Shao X (2019) Azadirachtin A inhibits the growth and development of *Bactrocera dorsalis* larvae by releasing cathepsin in the midgut. Ecotoxicol Environ Saf 183: 109512. <https://doi.org/10.1016/j.ecoenv.2019.109512>.

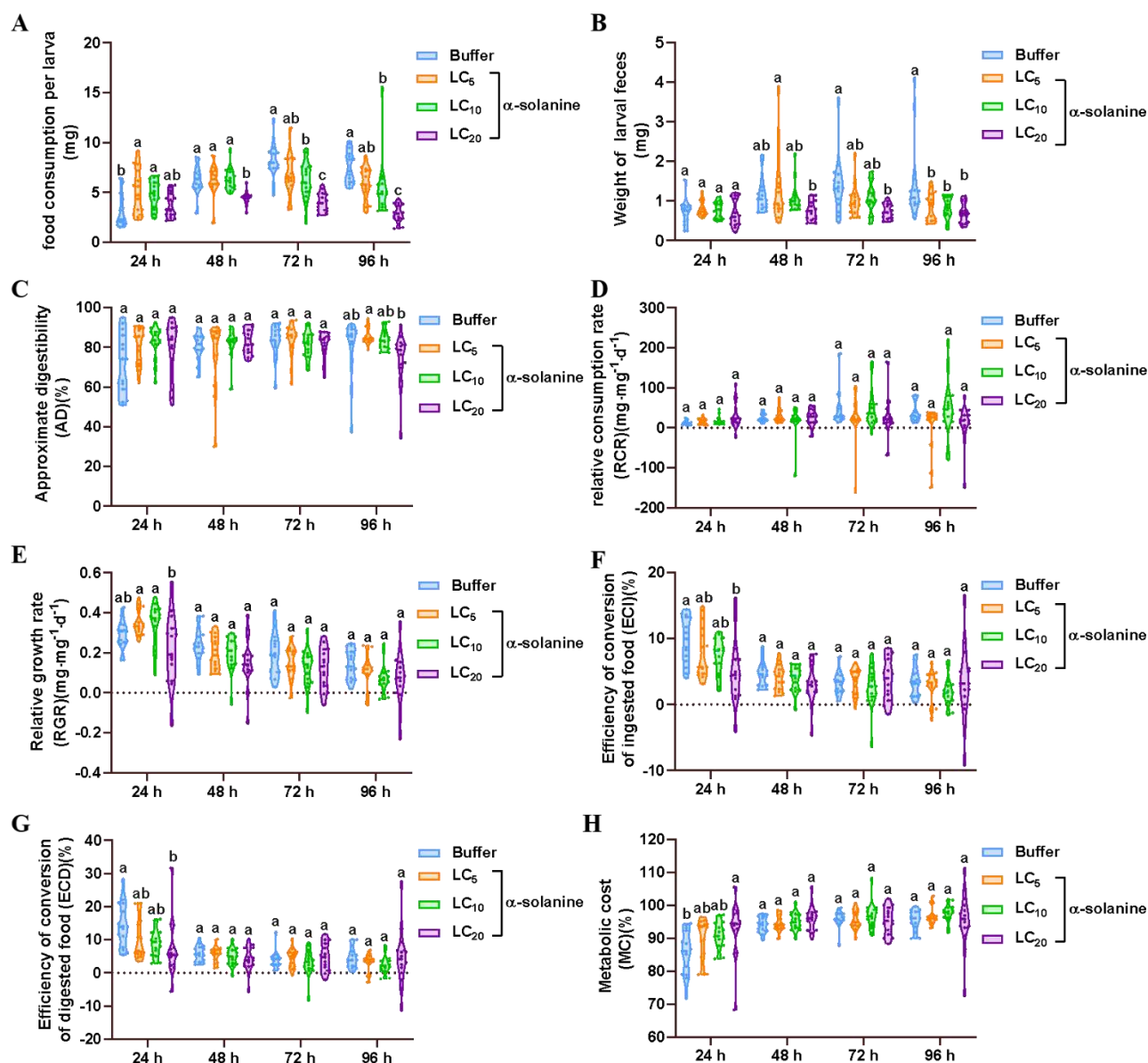
Zhou Y, Li HG, Huang Q, Liang S, Huang Q, Zuo M, Bao M, He B (2025) Toosendanin inhibits the growth of *Spodoptera litura* by inducing metabolic dysfunction in the midgut. Pestic Biochem Physiol 208: 106249. <https://doi.org/10.1016/j.pestbp.2024.106249>.



579

580 Fig. 1 The wet weight and percentage of different instars of *P. operculella* larvae treated with sublethal
 581 concentrations of α -solanine. (A) A simplified scheme of α -solanine-based larval treatment. (B) The wet
 582 weight of larvae after α -solanine treatment. Differences among different groups were indicated with lower-
 583 case letter based on One-way analysis of variance (ANOVA). (C) The percentage of different larval instar
 584 of *P. operculella* larvae after α -solanine treatment.

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Fig. 2 The nutritional indicators of *P. operculella* larvae treated with sublethal concentrations of α -solanine.

(A) Food consumption per larva after α -solanine treatment. (B) Larval feces weight following α -solanine

exposure. (C) Approximate digestibility (AD) per larva following treatment. (D) Relative consumption

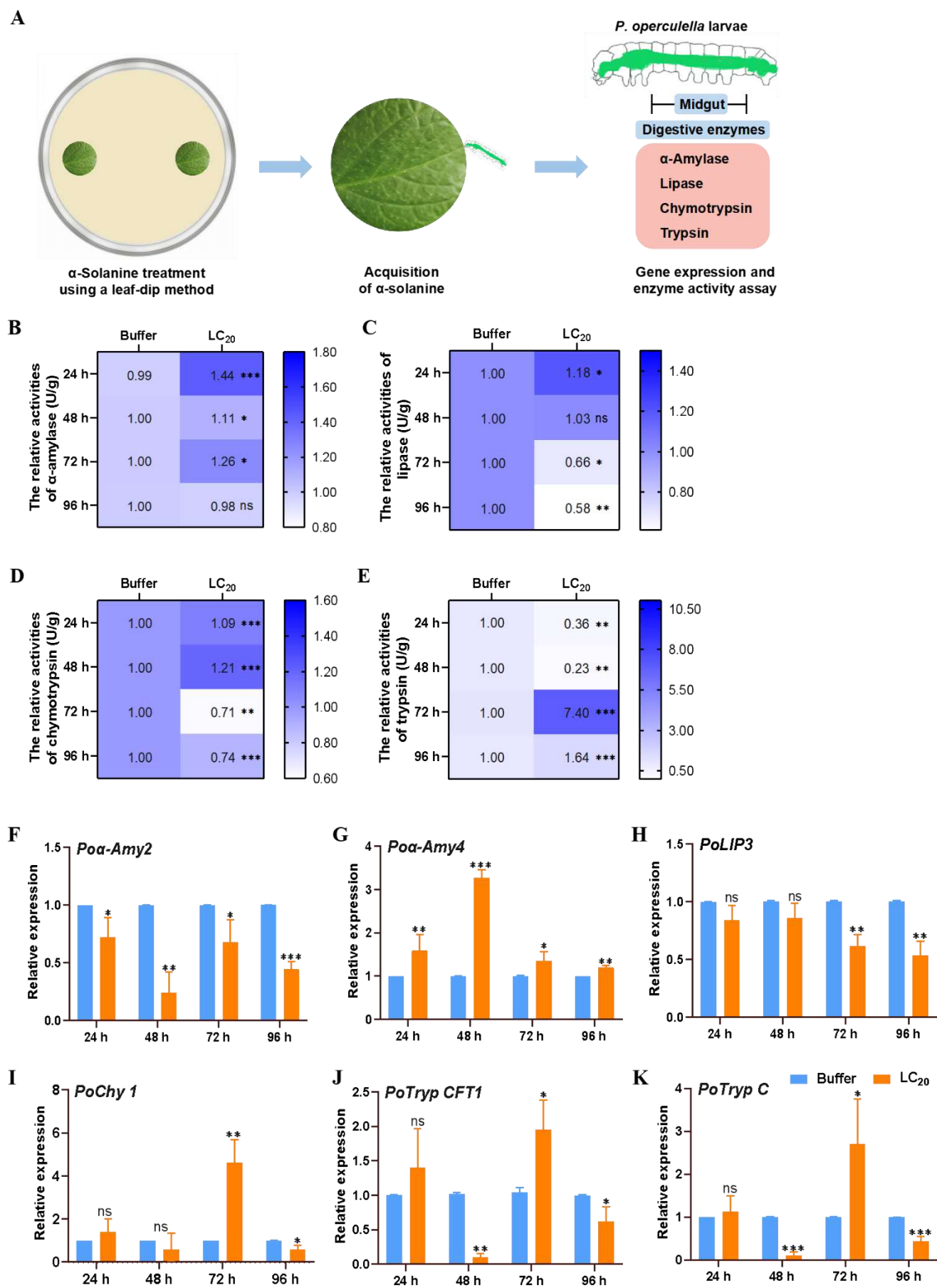
rate (RCR) per larva post-treatment. (E) Relative growth rate (RGR) per larva post α -solanine treatment.

(F) Efficiency of conversion of ingested food (ECI) per larva post-treatment. (G) Efficiency of conversion

of digested food (ECD) per larva following treatment. (H) Metabolic cost (MC) per larva after treatment.

Differences among different groups were indicated with lower-case letter based on One-way analysis of

variance (ANOVA).

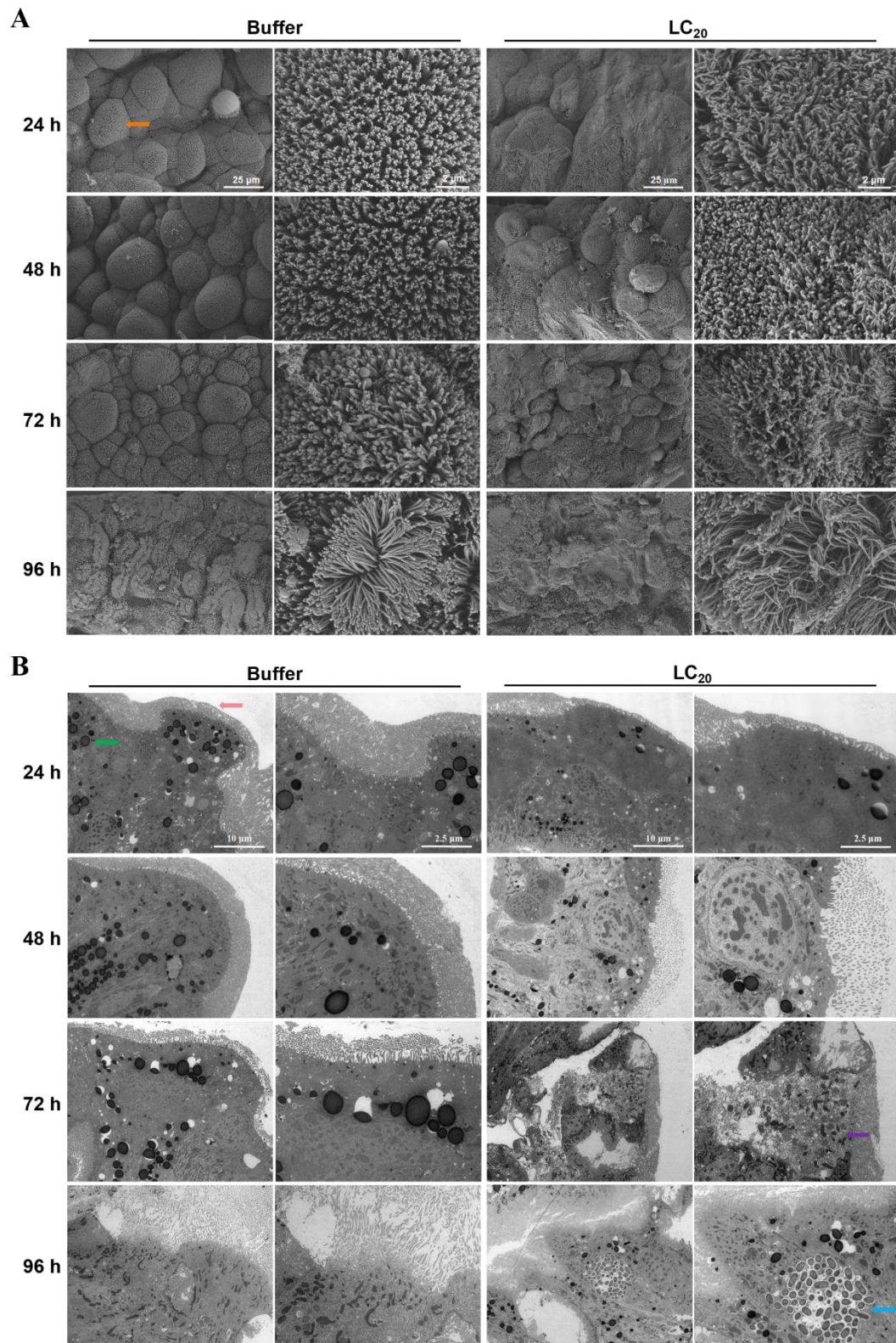


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597 Fig. 3 Sublethal effects of α-solanine on the expression of digestion-related genes and the activities of

598 digestive enzymes in *P. operculella* larvae. (A) Schematic illustration of α -solanine-induced effects on
599 larval digestive enzymes. (B–E) Relative activities of α -amylase (B), lipase (C), chymotrypsin (D), and
600 trypsin (E) after α -solanine treatment. (F–K) The transcription levels of *Poa-Amy2* (F), *Poa-Amy4* (G),
601 *PoLIP3* (H), *PoChyl1* (I), *PoTryp CFT1* (J), and *PoTryp C* (K) after α -solanine exposure. The control group
602 was assigned a value of 1 at 24 h, 48 h, 72 h, and 96 h, with differences at each time were compared
603 separately. Asterisks indicate significant differences (Student's *t*-test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$),
604 and 'ns' indicates no significant difference.

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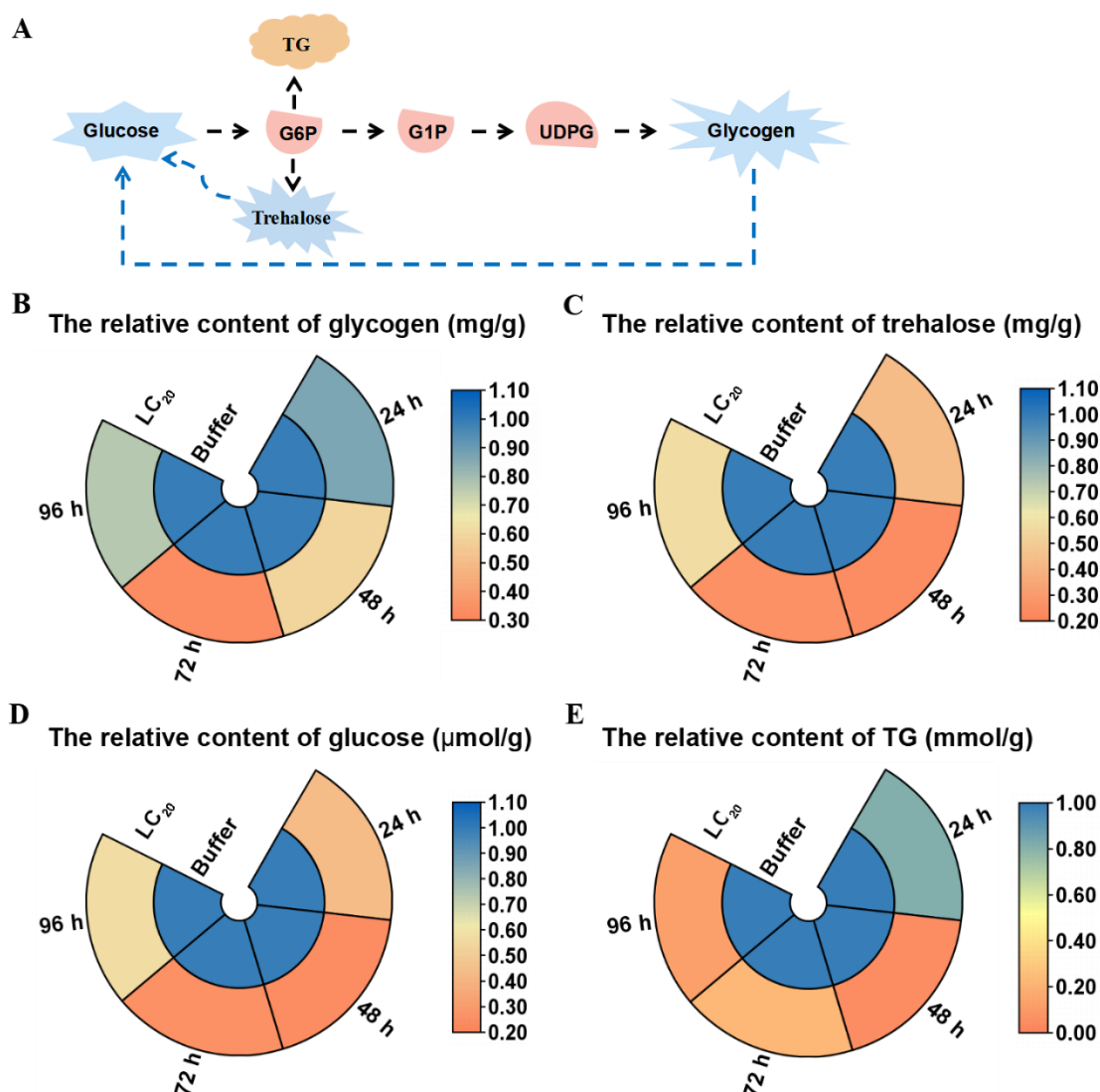


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607 Fig. 4 Midgut ultrastructure of *P. operculella* larvae following α -solanine treatment. (A) Morphology of
 608 midgut epithelium of *P. operculella* larvae treated with α -solanine for 24 h, 48 h, 72 h, and 96 h using
 609 SEM. The orange arrowhead indicates microvilli (Mi). (B) Morphology of the midgut columnar cells of *P.*

610 *operculella* larvae treated with α -solanine for 24 h, 48 h, 72 h, and 96 h using TEM. The pink, green,
611 purple, and blue arrowheads indicate Mi, intracellular lipid droplets, the area of damaged cells, and the
612 area of microorganisms distribution.

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615 Fig. 5 Sublethal effects of α -solanine on sugar-lipid metabolism-related biochemical indices in *P.*

616 *operculella* larvae. (A) Schematic representation of connecting sugars to lipogenesis. Glucose, glycogen,

617 trehalose, and TG are energy reserves of insects. Glucose is absorbed by the midgut, transported to the fat

618 body via hemolymph, sequentially converted into glucose-6-phosphate (G6P), glucose-1-phosphate (G1P),

619 and UDP-glucose (UDPG), followed by the synthesis of glycogen from UDPG. As a major energy storage

620 source, the branched macromolecular polysaccharide glycogen can be broken down into glucose to help

621 insects cope with stress conditions. Glucose also synthesizes trehalose that can protect cells against

622 environmental stressors. Trehalose is broken down into two glucose moieties to produce the energy

623 required for various physiological processes. Moreover, glucose is converted to TG, the dominant lipid

624 storage form, via several steps, including glycolysis, pyruvate metabolism, acetyl-CoA and fatty acids
625 synthesis. The black arrows denote the metabolic pathway underlying the bioconversion of glucose to
626 glycogen, trehalose, and TG. The blue arrows indicate the metabolic pathway for the conversion of
627 glycogen or trehalose to glucose. (B–E) The contents of glycogen (B), trehalose (C), glucose (D), and TG
628 (E) after α -solanine exposure. The control group was assigned a value of 1 at 24 h, 48 h, 72 h, and 96 h,
629 respectively.

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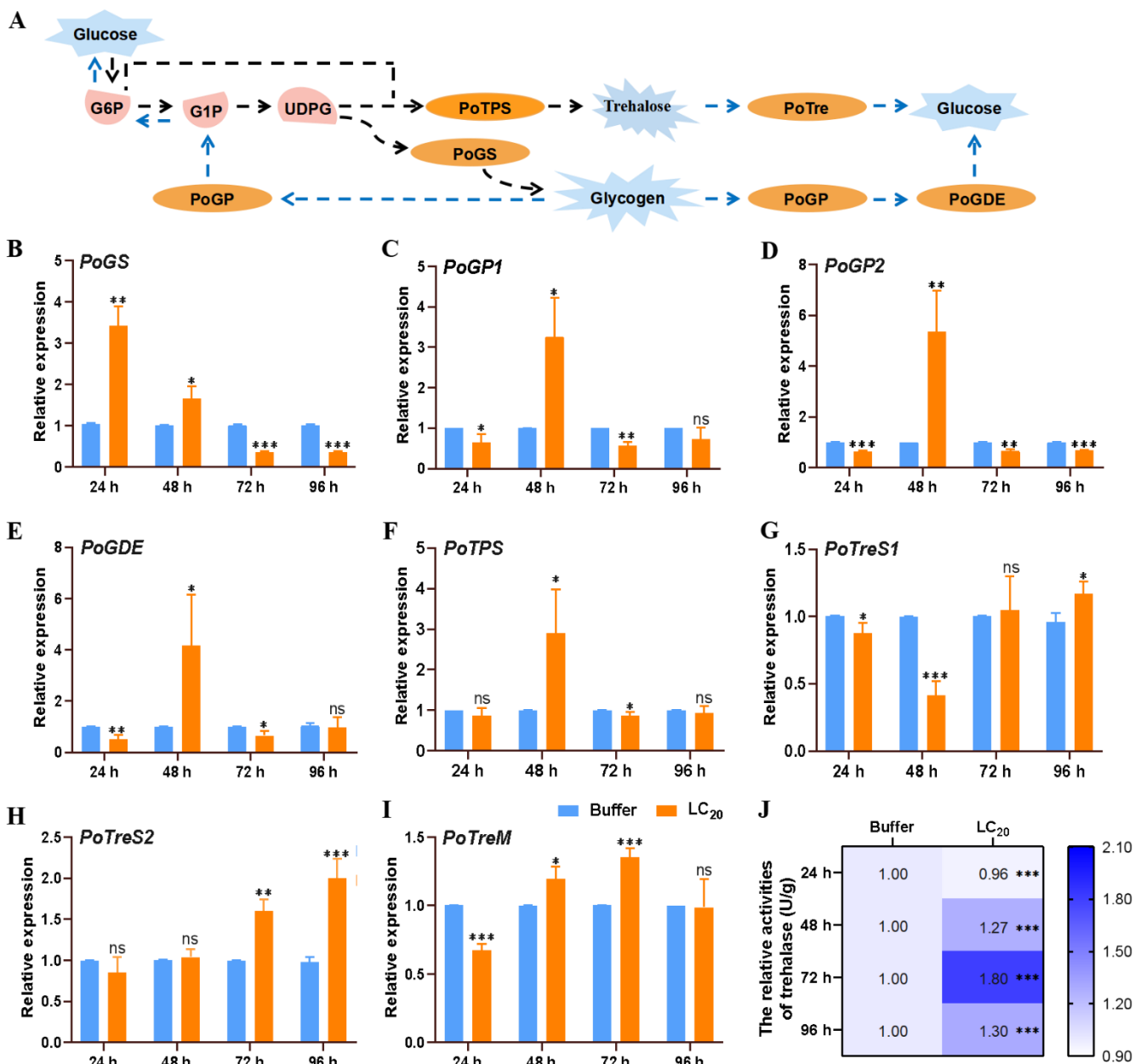
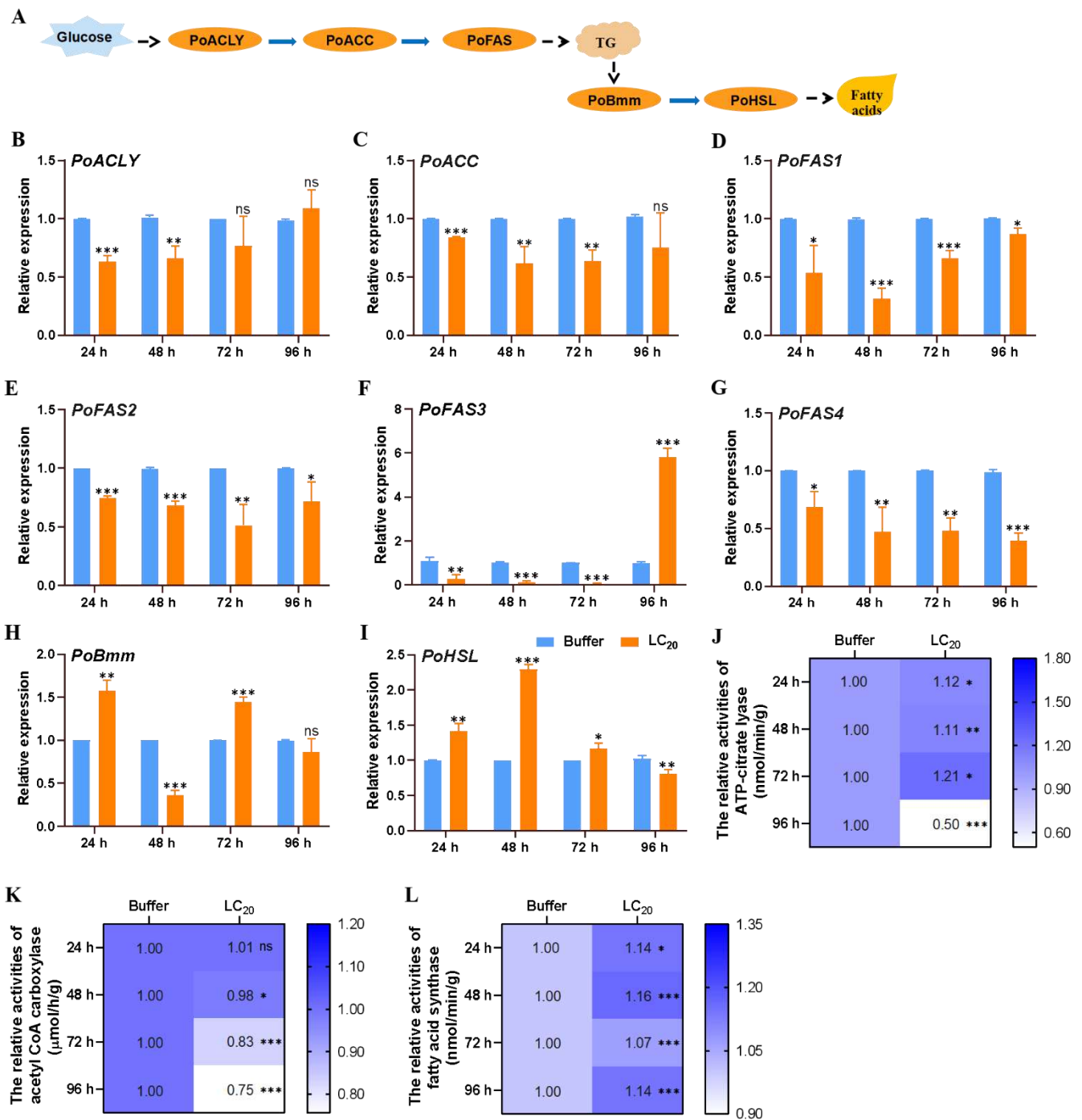


Fig. 6 Sublethal effects of α -solanine on gene transcription levels and key enzyme activity related to sugar metabolism. (A) Model of sugar metabolism in *P. operculella*. Trehalose is synthesized from glucose-derived G6P and UDPG by sequential catalysis by TPS and TPP. Subsequently, trehalose is specifically hydrolyzed to glucose by trehalase. Excessive glucose is converted to UDPG, which is then converted into glycogen by GS. The reverse reaction, catalyzed by GP, forms G1P, which is eventually converted into glucose. And glycogen also is broken down to release glucose with the help of GP and GDE. The black arrows show the metabolic pathway of converting glucose to glycogen and trehalose. The blue arrows

639 show the metabolic pathway for converting glycogen or trehalose to glucose. (B–I) Expression of *PoGPI*,
640 *PoGP2*, *PoGDE*, *PoGS*, *PoTPS*, *PoTreS1*, *PoTreS2*, and *PoTreM* genes in *P. operculella* larvae after α -
641 solanine exposure. (J) Relative activities of trehalase in *P. operculella* larvae after α -solanine exposure.
642 The control group was assigned a value of 1 at 24 h, 48 h, 72 h, and 96 h, and the differences at each time
643 point were compared separately. Asterisks indicate significant differences (Student's *t*-test, **P* < 0.05, ***P*
644 < 0.01, ****P* < 0.001), and 'ns' indicates no significant difference.

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Fig. 7 Sublethal effects of α -solanine on gene transcription levels and key enzyme activities involved in lipid metabolism. (A) Model of lipid metabolism in *P. operculella*. ACLY is an important enzyme linking glucose metabolism to lipid metabolism by generating acetyl-CoA from glucose-derived citrate for fatty acid biosynthesis. ACC, the rate-limiting enzyme in fatty acid synthesis, catalyzes the carboxylation of acetyl-CoA into malonyl-CoA. Generated malonyl-CoA is then catalyzed by FAS, key and multifunctional enzymes, to form long-chain fatty acids that can be stored as TG in lipid droplets. The breakdown of TG

653 requires a series of enzymatic reactions induced by Bmm and HSL. (B–I) Expression of *PoACLY*, *PoACC*,
654 *PoFAS1*, *PoFAS2*, *PoFAS3*, *PoFAS4*, *PoBmm*, and *PoHSL* genes in *P. operculella* larvae after α -solanine
655 exposure. (J–L) The relative activities of ATP-citrate lyase (J), acetyl CoA carboxylase (K), and fatty acid
656 synthase (L) in *P. operculella* larvae after α -solanine exposure. The control group was assigned a value of
657 1 at 24 h, 48 h, 72 h, and 96 h, and the differences at each time point were compared separately. Asterisks
658 indicate significant differences (Student's *t*-test, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$), and 'ns' indicates
659 no significant difference.

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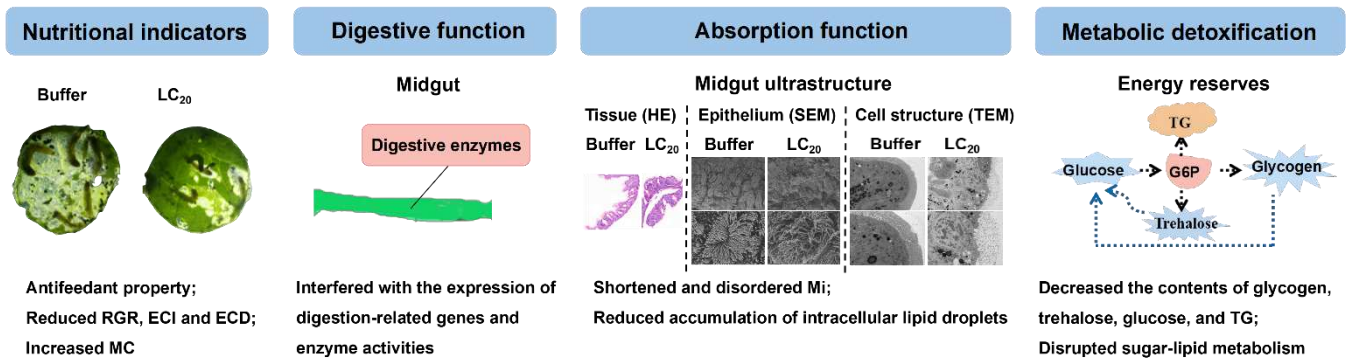
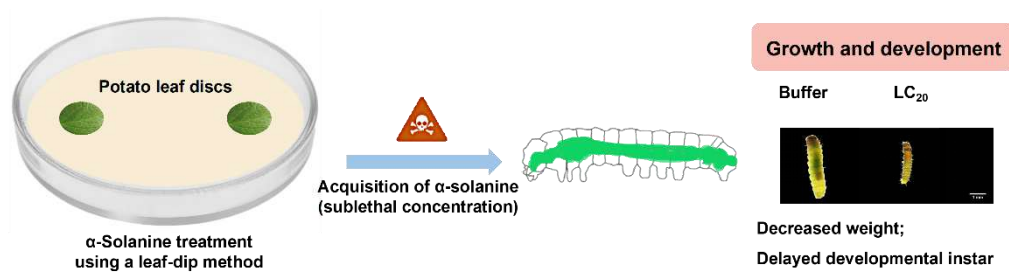
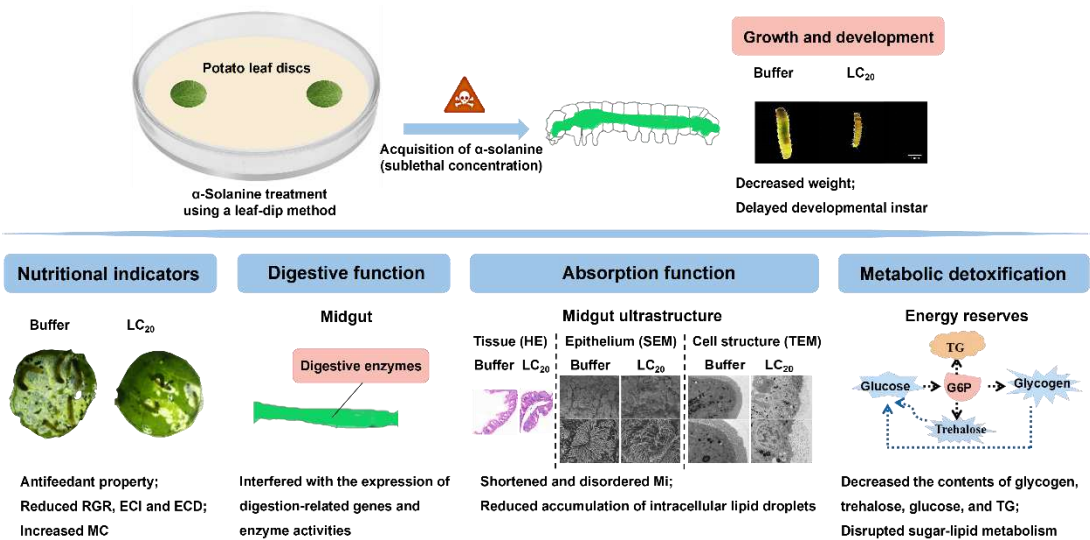


Fig. 8 Summary model diagram of sublethal concentration of α -solanine-driven inhibitory effects on *P. operculella* larvae.



Supplementary Files

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- [SupplementaryMaterials.docx.doc](#)