Title page

Title: Effects of the microbial secondary metabolite benzothiazole on the nutritional physiology and enzyme activities of *Bradysia odoriphaga* (Diptera: Sciaridae)

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Abstract

Bradysia odoriphaga (Diptera: Sciaridae) is the major pest that damages Chinese chive production. As a volatile compound derived from microbial secondary metabolites, benzothiazole has been determined to possess fumigant activity against *B. odoriphaga*. However, the mechanism of action of benzothiazole is not well understood. In the present study, fourth-instar larvae of *B. odoriphaga* were exposed to LC₁₀ and LC₃₀ of benzothiazole. Sublethal concentrations (LC₁₀ and LC₃₀) of benzothiazole significantly reduced the food consumption of the larvae on the second day after treatment (2 DAT). However, no significant changes in pupal weight among the different treatments. We also measured the protein, lipid, carbohydrate, and trehalose contents and the digestive enzyme activities of the larvae, and the results suggest that benzothiazole reduced the nutrient accumulation and decreased the digestive enzyme activities of *B. odoriphaga*. In addition, the activity of glutathione S-transferase was significantly decreased at 6 h after treatment with benzothiazole, whereas general esterase activities were significantly increased at 6 and 24 h after treatment. The results of this study indicate that benzothiazole interferes in the normal food consumption and digestion process by decreasing the activities of digestive enzymes. These results provide valuable information for understanding the toxicity of benzothiazole and for exploring volatile compound for the control of this pest.

Keywords: *Bradysia odoriphaga*; benzothiazole; nutritional indices; enzymes; detoxification; volatile organic compounds

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43 **1. Introduction**

44 Chinese chive (*Allium tuberosum* Rottle ex Spreng) is a hardy perennial
45 vegetable with a high economic value in southeastern and eastern Asia [1,2]. The
46 chive maggot, *Bradysia odoriphaga* (Diptera: Sciaridae), is the major pest that
47 restricts Chinese chive production [3]. Its larvae feed on the roots and stems of chives
48 and cause more than 50% of production losses in the absence of insecticide treatment
49 [4]. The primary management practice for controlling *B. odoriphaga* is the application
50 of synthetic insecticides (such as organophosphates, carbamates, and neonicotinoids)
51 in China and elsewhere [5]. However, the control efficacy is unsatisfactory due to the
52 overlapping generations of the insect, and most of its life stages occur in sheltered
53 circumstances. This challenge has led to the excessive use of chemical insecticides,
54 which causes pollution in the environment and leaves high residues on marketed
55 Chinese chives. Thus, novel effective insecticides are necessary to be developed as an
56 alternative to conventional ones.

57 Nature-derived compounds have shown biological activity against pests [6-9].
58 Benzothiazole is one component of the volatile organic compounds (VOCs) derived
59 from *Aspergillus clavatus* [10] and ligninolytic Basidiomycetes [11], and this
60 compound has being investigated as a fumigant against *Sclerotinia sclerotiorum* [12],
61 *Ditylenchus destructor* [13] and *Tribolium castaneum* [14]. Our previous study
62 indicated that benzothiazole exhibited fumigation toxicity against all stages of *B.*
63 *odoriphaga*, thus demonstrating its potential as a fumigant for the control of this pest

[15]. We also found that benzothiazole significantly increased the respiratory rate of *B. odoriphaga* 0.5 and 1 h after treatment. With extended fumigation times, the respiratory rate was significantly reduced [15]. These results indicate that benzothiazole is not a common respiratory inhibitor. As a broad spectrum against pest, benzothiazole maybe act as a novel mechanism of action, and understanding it will be helpful for development new pesticide in the future.

In this study, we investigated the effects of benzothiazole on the consumption of food, the accumulation of nutrients, and the activities of digestive and detoxifying enzymes in *B. odoriphaga*. The results may help us to elucidate the biochemistry and physiology of benzothiazole toxicity and provide valuable information to research the mode of action of this natural compound.

2. Materials and Methods

2.1 Insect culture and test compound

The larvae and pupae of *B. odoriphaga* were collected from a Chinese chive greenhouse in Liaocheng, Shandong Province, China (36°02'N, 115°30'E) in 2013. The insects were placed in Petri dishes and reared on fresh chive rhizomes (1 cm in length). The plates were maintained at 25 ± 1 °C with $70 \pm 5\%$ RH and a photoperiod of 14:10 h (L:D) [16]. The test compound benzothiazole (purity of 99%) was purchased from Beijing Lark Technology Co., Ltd., Beijing, China.

2.2 Fumigation bioassay

Newly emerged fourth-instar larvae of *B. odoriphaga* were used in a fumigation

bioassay. Seven concentrations of benzothiazole (0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 $\mu\text{L/L}$) and one control with distilled water were used. Thirty newly emerged fourth-instar larvae were transferred from the colonies maintained in the laboratory to a fresh Petri dish (90-mm diameter, with a 90-mm diameter moist filter paper and a piece of fresh Chinese chive rhizome) with a moist brush. Different doses (2, 3, 4, 5, 6, 7 and 8 μL) of benzothiazole were deposited on a piece of filter paper with a micropipette. The filter paper with the test compound was quickly fastened to the inner wall of the fumigation aquarium (10 L, 20 cm $\times$ 20 cm $\times$ 25 cm) with double-sided adhesive tape, and Petri dishes with larvae were placed into these aquariums concurrently. Then, the aquarium sealed with Parafilm. During exposure, the cultures were maintained at 25 ± 1 °C. This bioassay was performed with four replicates for each concentration. Mortality was assessed after 24 h of exposure, and larvae were considered dead if they were unable to move when disturbed with a moist brush.

2.3 Effects of benzothiazole on food consumption and pupal weight

To measure the effects of benzothiazole on the food consumption and pupal weight of *B. odoriphaga*, 50 newly emerged fourth-instar larvae were treated with LC_{10} and LC_{30} of benzothiazole and a distilled water control in each replicate using the bioassay method described above, and 5 replicates were conducted for each concentration. After a 24-h exposure, 30 surviving larvae were transferred to fresh Petri dishes (as described above) for each replicate, and fresh Chinese chive rhizomes were weighed and used to feed the larvae. After 24 h (2 days after treatment, 2 DAT),

the remaining rhizomes were dried in an oven at 60 °C (48 h) and reweighed. The food consumption experiment was performed in 5 replicates for each concentration, and the experiment was repeated 24 h later (3 DAT). The dry weights of the rhizomes at the outset of this test were calculated according to a dry weight/fresh weight conversion measured using extra rhizomes. After the food consumption experiment, the larvae were cultured with fresh Chinese chive rhizomes using the conventional method. After the larvae pupated, the weights of the female and male pupae were measured. In each treatment, 10 pupae were weighed together, and 5 replicates were performed.

2.4 Sample preparation for the biochemical assay

A number of biochemical indices were measured in the present study to evaluate the physiological and biochemical effects of benzothiazole on the larvae of *B. odoriphaga*. The larvae were treated with LC₁₀ and LC₃₀ of benzothiazole and distilled water; after 6 and 24 h, the surviving larvae were used to assay biochemical indices. In each biochemical index experiment, five larvae were used, with 4 replicates for each treatment.

2.5 Measurement of nutrient contents

Total protein content was measured according to the method described by Bradford [17]. First, 5 larvae were homogenized in 100 µL of 50 mM Tris-HCl (pH 7.1) containing 0.5% Triton x-100 and 20% sucrose. The sample was then centrifuged at 12,000 rpm for 10 min at 4 °C. Then, 30 µL of the supernatant were mixed with 150 µL 0.01% Coomassie Brilliant Blue G-250 (Solarbio, Beijing, China). Bovine serum

albumin (Solarbio) was used to establish the protein standard curve. The absorbance was measured at 595 nm using a BioTek Synergy™ 2 Multi-Mode Reader (BioTek Instruments, Inc. Winooski, Vermont, USA).

The lipid content was measured according to the method described by Yazdani et al. [18]. Five larvae were homogenized in 100 µL 2% Na₂SO₄ and extracted with 750 µL methanol:chloroform (1:2). Then, the samples were centrifuged for 10 min at 8,000 rpm at 4 °C. Next, 500 µL of the supernatant from each sample were transferred to new tubes and dried at 40 °C. Each sample was dissolved with 500 µL H₂SO₄ and incubated at 90 °C (water bath) for 10 min. Then, 30 µL of each sample were mixed with 270 µL vanillin (Aladdin Industrial Inc., Shanghai, China), and the absorbance was read at 530 nm 30 minute later. Cholesterol (Solarbio) was used to establish the standard curve.

Carbohydrate content was measured using the method described by Roe [19]. Briefly, 5 larvae were homogenized with 100 µL 10% trichloroacetic acid (TCA) and centrifuged at 12,000 rpm for 10 min at 4 °C. Ten microliters of the supernatant were mixed with 200 µL 0.2% anthrone (200 mg of anthrone dissolved in 100 mL H₂SO₄) and heated at 100 °C (water bath) for 10 min. The absorbance was read at 630 nm, and glucose was used to generate the standard curve to calculate the carbohydrate content of each sample.

Trehalose content was measured using the procedure of Ge et al. [20]. Five larvae were homogenized on ice with 100 µL of a 20-mM phosphate buffer (pH 5.8) and centrifuged for 10 min at 12,000 rpm at 4 °C. Then, 30 µL of the supernatant were

152 mixed with 30 μ L 1% H_2SO_4 and incubated at 90 °C (water bath) for 10 min. After 3
153 min cooling on ice, 30 μ L 30% KOH was added to each sample, and the mixture was
154 incubated again for 10 min at 90 °C. After cooling for 3 min on ice, 600 μ L 0.2%
155 anthrone was added, and the mixture was incubated again at 90 °C for 10 min. After
156 cooling on ice, the absorbance was read at 630 nm, and trehalose (Solarbio) was used
157 to generate the standard curve. The amount of trehalose was determined according to
158 the standard curve. In addition, the trehalose fraction of the total carbohydrates was
159 calculated.

160 *2.6 Measurement of digestive enzyme activities*

161 Protease activity was determined using the method of Sandhya et al. [21]. Casein
162 (Solarbio) was used as the substrate. Larvae were homogenized with 100 μ L of a
163 20-mM phosphate buffer (pH 7.0) and centrifuged for 10 min at 12,000 rpm at 4 °C.
164 Next, 100 μ L 1% casein was added to 30 μ L of the supernatant, and the reaction was
165 incubated in a water bath at 37 °C for 15 min. Then, this reaction was arrested by
166 adding 100 μ L 0.4 M trichloroacetic acid (TCA), and the solution was centrifuged at
167 12,000 rpm for 10 min at 4 °C. One hundred microliters of the supernatant were
168 transferred to a new tube and mixed with 500 μ L 0.4 M Na_2CO_3 and 100 μ L
169 Folin-Ciocalteu reagent (Solarbio). The sample was incubated at 37 °C for 30 min,
170 and the absorbance was read at 660 nm. Tyrosine (Solarbio) was used to establish the
171 standard curve. One unit of protease activity was defined as the amount of enzyme
172 that produced 1 μ M tyrosine per minute under the assay conditions.

173 Lipase activity was determined using the method of Tsujita et al. [22]. The crude

174 enzyme was extracted as described above for protease activity determination. Larvae
175 were homogenized with 100 μ L of a 20-mM phosphate buffer (pH 7.0). Next, 10 μ L
176 of the enzyme solution were mixed with 18 μ L 4-nitrophenyl butyrate (50 mM) and
177 172 μ L phosphate buffer (pH 7.0). Then, the mixture was incubated for 15 min at 37
178 $^{\circ}$ C and transferred to -20 $^{\circ}$ C for 5 min to stop the reaction. The absorbance was read at
179 405 nm, and *p*-nitrophenol (Aladdin) was used to generate the standard curve.

180 The activity of α -amylase was determined using the method of Bernfeld [23].
181 The crude enzyme was extracted with a 20-mM sodium phosphate buffer (pH 6.9).
182 Ten microliters of the supernatant were mixed with 40 μ L 1% soluble starch and 50 μ L
183 sodium phosphate buffer. Then, the mixture was incubated for 30 min at 35 $^{\circ}$ C. The
184 reaction was stopped by the addition of 100 μ L dinitrosalicylic acid (DNS) and heated
185 in boiling water for 10 min. After cooling on ice, the absorbance was read at 540 nm,
186 and maltose was used to generate the standard curve. One unit of α -amylase activity
187 was defined as the amount of enzyme that produced 1 μ M maltose per min at 35 $^{\circ}$ C.

188 Trehalase activity was measured as described by Lei et al. [24]. The crude
189 enzyme was extracted with a 20-mM phosphate buffer (pH 5.8). In each tube, 30 μ L
190 of supernatant were mixed with 60 μ L 40 mM trehalose (Solarbio) and incubated at
191 37 $^{\circ}$ C for 30 min. Then, the reaction was stopped by heating in boiling water for 3
192 min and cooling on ice. Then, 90 μ L of a dye indicator (5 g 3,5-dinitrosalicylic acid, 5
193 g NaOH, 1 g phenol and 0.25 g anhydrous sodium sulfite dissolved in 500 mL
194 distilled water) were added to each tube, and the reaction was incubated at 90 $^{\circ}$ C
195 (water bath) for 5 min. After cooling on ice, 30 μ L potassium sodium tartrate were

added, and the absorbance was read at 550 nm. The standard curve was established using glucose.

2.7 Measurement of detoxifying enzyme activities

The activity of glutathione S-transferase (GST) was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate [25]. The crude enzyme was extracted with a 66-mM phosphate buffer (with 2 mM EDTA, pH 7.0). The reaction solution contained 10 μ L of the enzyme solution, 5 μ L 30 mM CDNB, 15 μ L 50 mM reduced glutathione (GSH) and 120 μ L phosphate buffer. The absorbance was recorded at intervals of 30 s for 5 min at 27 °C and 340 nm with a 96-well microplate reader. The activity of GST was corrected for non-enzymatic metabolism in the controls and determined using an extinction coefficient of 9.6/mM/cm [6].

The general esterase activity was measured using α -naphthyl acetate (α -NA, Aladdin) as the substrate [26]. The larvae were homogenized with 100 μ L of a 0.1-M phosphate buffer (pH 7.0) and centrifuged for 10 min at 12,000 rpm at 4 °C. Then, 25 μ L of the supernatant and 100 μ L α -NA (10 mM, initially dissolved in ethanol and brought up to volume with phosphate buffer) were incubated at 37 °C for 15 min. The reaction was stopped by the addition of 75 μ L 10 mM Fast Blue RR salt (Sigma) and 1.5% sodium dodecyl sulfate. The absorbance was read at 630 nm, and α -naphthol (Aladdin) was used to establish the standard curve.

2.8 Statistical analysis

The data obtained from the toxicity experiments were subjected to probit analysis using the SPSS program. Statistically significant mean values were

determined using ANOVA, and the significant differences among the treatments were determined using the Student-Newman-Keuls test (SPSS v. 13.0 for Windows) ($P < 0.05$).

3. Results

3.1 Toxicity of benzothiazole on the fourth-instar larvae of *B. odoriphaga*

The toxicity of benzothiazole on the fourth-instar larvae of *B. odoriphaga* is given in Table 1. The LC_{50} was 0.5945 $\mu\text{L/L}$. The concentrations that caused 10 and 30 percent mortalities were 0.3398 and 0.4729 $\mu\text{L/L}$, respectively.

3.2 Effects of benzothiazole on food consumption and pupal weight

After being fumigated with LC_{10} and LC_{30} of benzothiazole, the relative food consumption levels of *B. odoriphaga* fourth-instar larvae were significantly decreased compared with the control on the second day (2 DAT) ($F_{2,12} = 79.69$, $P < 0.0001$). However, on the third day (3 DAT), food consumption levels were not significantly different from the control ($F_{2,12} = 2.51$, $P = 0.1229$) (Fig. 1). After being exposed to LC_{10} and LC_{30} of benzothiazole, no significant differences were observed in the weights of both the female and male pupae compared with the control (Female, $F_{2,12} = 0.70$, $P = 0.5167$; Male, $F_{2,12} = 1.17$, $P = 0.3442$) (Fig. 2).

3.3 Effects of benzothiazole on nutrient contents

The effects of benzothiazole on the total protein content of *B. odoriphaga* are shown in Figure 3 A. The protein content was not significantly changed in both treatments compared with the control 6 h after treatment ($F_{2,9} = 0.63$, $P = 0.5571$), but

protein content was significantly reduced in both treatments compared with the control 24 h after fumigation ($F_{2,9} = 119.18$, $P < 0.0001$). Similarly, the lipid content was not significantly different at 6 h ($F_{2,9} = 0.61$, $P = 0.5658$), but it was significantly decreased after 24 h ($F_{2,9} = 13.90$, $P = 0.0018$) in the LC₁₀ and LC₃₀ treatments compared with the control (Fig. 3 B). The carbohydrate content was significantly decreased at both 6 and 24 h after benzothiazole treatment (6 h: $F_{2,9} = 5.75$, $P = 0.0247$; 24 h: $F_{2,9} = 19.92$, $P = 0.0005$), but there was no significant difference between the LC₁₀ and LC₃₀ treatments (Fig. 3 C). The amount of trehalose in the treated larvae was not significantly different both at 6 and 24 h after benzothiazole treatment compared with the control (6 h: $F_{2,9} = 0.073$, $P = 0.9301$; 24 h: $F_{2,9} = 0.829$, $P = 0.4674$). However, the trehalose fraction of the total carbohydrates increased 6 and 24 h after benzothiazole treatment (Fig. 3 D).

3.4 Effects of benzothiazole on digestive enzymes

Protease activity was significantly decreased by both benzothiazole concentrations compared with the control 6 h after treatment ($F_{2,9} = 30.63$, $P < 0.0001$). Protease activity was significantly decreased 24 h after the LC₃₀ treatment ($F_{2,9} = 17.73$, $P = 0.0008$) (Fig. 4 A).

Lipase activity was significantly decreased after treatment with the LC₃₀ of benzothiazole compared with the control at 6 h ($F_{2,9} = 7.37$, $P = 0.0127$). However, no significant difference was observed in either benzothiazole treatment compared with the control at 24 h ($F_{2,9} = 0.24$, $P = 0.7897$) (Fig. 4 B).

α -Amylase activity was significantly decreased at both 6 and 24 h after treatment

with the LC₃₀ of benzothiazole compared with the control (6 h: $F_{2,9} = 8.09$, $P = 0.0098$; 24 h: $F_{2,9} = 37.15$, $P < 0.0001$). However, no significant difference was found after treatment with the LC₁₀ of benzothiazole (Fig. 4 C).

The activity of trehalase was significantly decreased at both 6 and 24 h after benzothiazole treatment (6 h: $F_{2,9} = 11.43$, $P = 0.0034$; 24 h: $F_{2,9} = 24.39$, $P = 0.0002$), and the activity decreased in a concentration-dependent manner (Fig. 4 D).

3.5 Effects of benzothiazole on detoxifying enzymes

Glutathione S-transferase (GST) activity was significantly decreased at 6 h after benzothiazole treatment ($F_{2,9} = 12.79$, $P = 0.0023$). After being fumigated with the LC₃₀ of benzothiazole for 24 h, the activity of GST was significantly decreased compared with the control ($F_{2,9} = 9.58$, $P = 0.0059$) (Fig. 5 A). However, the general esterase activity was significantly increased after treatment with benzothiazole compared with the control (6 h: $F_{2,9} = 4.89$, $P = 0.0366$; 24 h: $F_{2,9} = 40.48$, $P < 0.0001$) (Fig. 5 B).

4. Discussion

Food consumption and pupal weight are important insect physiological parameters that can be affected by internal and external factors [27]. In the present study, food consumption by the surviving *B. odoriphaga* larvae was significantly reduced on the second day after benzothiazole treatment (2 DAT); however, no significant difference was found in pupal weight compared with the control. This result may be partly due to the observation that the amount of food consumed on the

third day (3 DAT) was not significantly different, and the larval developmental time of *B. odoriphaga* was prolonged 1-2 days after benzothiazole treatment (Zhao et al. unpublished data), which means the need for more time to accumulate nutrients. These results were consistent with Liu et al. [6], reported that food consumption significantly decreased when the fourth-instar larvae of *Ostrinia furnacalis* were exposed to naturally derived fraxinellone. Similar low food consumption was also observed when *Glyphodes pyloalis* larvae were treated with the natural essential oil of lavender [7].

Proteins, lipids and carbohydrates are vital chemical constituents of the insect body and are involved in the regulation of many physiological and biochemical reactions. In our study, the protein, lipid and carbohydrate contents were significantly reduced when treated with both concentrations of benzothiazole. The reduction of proteins may interfere with physiological and biochemical processes by degrading proteins into amino acids and using the tricarboxylic acid cycle to compensate for insufficient energy and stress [28]. Protease activity was reduced after treatment with benzothiazole, which is consistent with another report that the digestive protease of *Leptinotarsa decemlineata* Say was inhibited by clitocypin [29]. After treatment with insecticidal chemicals, the secretion and function of insect digestive enzymes becomes imbalanced, and normal consumption and digestive processes are affected [30]. In our study, the carbohydrate content was decreased, which may be due to the reduction of food consumption. In addition, the observed reduction in α -amylase activity indicates that the conversion of hydrolyzing starch to maltose and glycogen to

glucose was decreased after benzothiazole treatment, which may be another reason for the reduction in carbohydrate content. This result is similar to the reports of Yazdani et al. [7] and Etebari et al. [31]. Lipid content and lipase activity were affected when *B. odoriphaga* was treated with benzothiazole, which may be explained by the disruption of hormone secretion and lipid metabolism after treatment with insecticidal chemicals [32]. This result is consistent with the report that *Eurygaster integriceps* lipase activity was decreased after treatment with *Artemisia annua* extract [33].

Trehalose is the primary blood sugar in the hemolymph of insects and controls the utilization of glucose as an important energy source in flight or in other physiological activities [34]. We observed no significant difference in the trehalose content among the benzothiazole and control treatments. However, the trehalose fraction of the total carbohydrates increased after benzothiazole treatment compared with the control. Trehalose can effectively prevent protein denaturation and inactivation under adverse conditions [35]. Hence, more trehalose may have been needed to regulate the development and growth of the insect after benzothiazole treatment. Trehalase, which can catabolize one mole of trehalose into two moles of glucose, also plays an important role in the regulation of insect biochemistry and physiology [36]. The activity of trehalase was reduced significantly after treatment with benzothiazole, which may explain why the trehalose fraction increased while the carbohydrate content decreased. A similar trend was observed in the content of trehalose and trehalase activity when *Apolygus lucorum* was treated with hexaflumuron [27].

It is well known that the detoxifying enzymes are the primary enzymes to metabolize xenobiotics in insect [37]. The glutathione S-transferase family is a major family of detoxifying enzymes that can catalyze the conjugation of glutathione (GSH) to the electrophilic centers of xenobiotics. This conjugation can increase the water solubility of toxic materials and decrease their toxicity [25]. However, the results of the current study show that benzothiazole reduced the activity of GST, which may be a result of the reduction of a specific protein, catalytic inhibition or a reduction in glutathione (GSH) availability [38]. This result suggests that GST activity is inhibited by benzothiazole and may not be involved in the detoxification or metabolism of benzothiazole. General esterases are major hydrolytic enzymes that hydrolyze ester bonds in diverse biomolecules [39]. Benzothiazole does not contain an ester bond in its molecular structure; however, our results indicate that esterase activity increased when the larvae of *B. odoriphaga* were treated with benzothiazole. This result indicates that in the process of benzothiazole metabolism and detoxification, the toxin may be transformed into an intermediate that contains an ester bond, thus inducing esterase activity. However, no information on the *in vivo* detoxification or metabolism of benzothiazole in insects is available, and this proposal needs to be further researched. In addition, detoxification consumes large amounts of energy and leads to reduced nutrient content, which was observed in this study and has been demonstrated in other reports [7,40].

In conclusion, the low dose effect of benzothiazole may involve disrupting digestive enzymes activities, reducing food consumption and decreasing nutrient

accumulation, eventually leading to an insufficient energy supply for normal biochemical and physiological processes. However, further studies are needed to confirm the mechanism of action on the genetic and protein levels. In addition, large-scale field application studies and the effects of benzothiazole on non-target organisms also need to be evaluated.

Acknowledgments

This study was supported by grants from the Special Fund for Agro-scientific Research in the Public Interest from the Ministry of Agriculture of China (201303027), and from National Science Foundation of Shandong Province, China (ZR2015CQ017).

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Figure legends

Figure 1. The amount of food consumption (dry weight of Chinese chive rhizome) in fourth-instar larvae of *B. odoriphaga* (Mean $\pm$ SE) after treatment with benzothiazole. Bars with the same letters mean no significant differences (Student-Newman-Keuls test, $p < 0.05$). DAT: day after treatment. The amount of food consumption at 2 DAT means the food consumption during 1 DAT and 2 DAT.

Figure 2. Body weight of female and male pupae of *B. odoriphaga* (Mean $\pm$ SE) after treatment with benzothiazole. Bars with the same letters mean no significant differences (Student-Newman-Keuls test, $p < 0.05$).

Figure 3. The amount of nutrient (A: total protein, B: lipid, C: carbohydrate, D: trehalose) and the trehalose fraction of the total carbohydrates in fourth-instar larvae of *B. odoriphaga* (Mean $\pm$ SE) after treatment with benzothiazole. Bars with the same letters mean no significant differences (Student-Newman-Keuls test, $p < 0.05$).

Figure 4. The activities of digestive enzymes (A: protease, B: lipase, C: α -amylase, D: trehalase) in fourth-instar larvae of *B. odoriphaga* (Mean $\pm$ SE) after treatment with benzothiazole. Bars with the same letters mean no significant differences (Student-Newman-Keuls test, $p < 0.05$).

503

504 Figure 5. The activities of detoxifying enzymes (A: glutathione S-transferase (GST),
505 B: general esterase) in fourth-instar larvae of *B. odoriphaga* (Mean $\pm$ SE) after
506 treatment with benzothiazole. Bars with the same letters mean no significant
507 differences (Student-Newman-Keuls test, $p < 0.05$).

1 Table 1 Toxicity of benzothiazole on the fourth-instar larvae of *B. odoriphaga*

2

Treatment	N ^a	Concentration $\mu\text{L/L}$ (95% CL) ⁻¹			Slope $\pm$ SE	χ^2 (df)
		LC ₁₀	LC ₃₀	LC ₅₀		
Benzothiazole	960	0.3398 (0.3178-0.3590)	0.4729 (0.4525-0.4949)	0.5945 (0.5637-0.6338)	5.275 $\pm$ 0.377	13.18 (6)

3

4 ^a:The number of subjects

5 χ^2 is significant ($p < 0.05$).

Figure(1)

Figure(2)

Figure(3)

Figure(4)

Figure(5)

Benzothiazole

Bradysia odoriphaga

Physiological and biochemical indexes