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Effects of tea saponin on growth and development, nutritional indicators, and hormone titers in diamondback moths feeding on different host plant species

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ABSTRACT

The diamondback moth (DBM) is an important worldwide pest. This insect has been studied for several decades; however, its control remains problematic. Numerous chemical insecticides have become ineffective and chemical residues constitute an important problem. In the present study, we determined the mortality of 3rd instar DBM larvae feeding on three different host plant species and exposed to various concentrations of tea saponin (TS). In addition, we evaluated growth and development parameters, nutritional indicators, and juvenile hormone (JH) and molting hormone (MH) titers in 2nd instar larvae exposed to LC_{20} and LC_{50} doses of TS. We found that treatment of DBM larvae with LC_{20} and LC_{50} doses of TS led to lower growth rates, decreased feed consumption, reduced frass production, lower pupal weights, reduced percentage pupation, slower adult emergence percentages, and diminished fecundity, but prolonged durations of the larval and pupal periods. The efficiency of conversion of ingested and digested food increased, but the approximate digestibility did not differ significantly between treatments and controls. JH and MH titers were higher after TS treatment; these increases varied according to the host species and TS concentration. Our results indicate that TS represents a potential new alternative insecticide based on its natural origin, low cost, and minimum environmental impact.

1. Introduction

The diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is a major pest of cruciferous crops worldwide [1,2,3]. The global annual cost for managing *P. xylostella* is estimated to be more than one billion US dollars because of high fecundity and generation overlap [2,4,5,6]. DBM populations have been suppressed by using a wide range of methods. Since the 1980s, the development of insect resistance has led to increasing interest in natural pest control agents [7], especially plant extracts. These natural compounds mainly comprise phenols, terpenoids, essential oils, alkaloids, polypeptides, xavonoids, and other substances [8] with the capacity to disrupt feeding, retard growth and development, and hinder host finding. Tea saponin (TS) is extracted from the seeds of plant species belonging to the genus *Camellia*, in the family Theaceae. TS has been investigated for use as an alternative pest insecticide [9,10] and/or acaricide, and also for its ability to protect plants from bacterial, fungal, yeast, and viral pathogens [11]. In addition, TS can enhance the efficiency, solubilization, and attenuated poison capacity of wettable powder pesticides [11]. Hence, TS has been widely used as an environmentally friendly pesticide additive, to improve the solubilization of active compounds, thereby reducing the required dosage and delaying the development of pest resistance [12].

The physiological and metabolic functions of insects have frequently been reported to be influenced by host plant variety. Wei et al. (2006) investigated the recession of resistance to insecticides and demonstrated significant differences in acetylcholinesterase (AChE) and carboxylesterase (CarE) activities in DBM populations feeding on different host plant varieties [13]. The development and reproduction of the peach fruit borer, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), is strongly influenced by host plant variety [14]. In the present study, we exposed DBM larvae feeding on three different host plant species to various TS concentrations. We evaluated the effects of TS on (1) larval growth and development, (2) larval food utilization and food conversion ratios, and (3) larval titers of juvenile hormone (JH) and molting hormone (MH).

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2. Materials and methods

2.1. Insects

The diamondback moths (*P. xylostella*) used in this study were collected from laboratory fields at the Institute of Plant Protection, Fujian Academy of Agricultural Sciences, Fuzhou, China (26°07′N, 119°18′E). These fields had not been sprayed with insecticides. The populations were maintained in a rearing room at 25 ± 1 °C and 70–80% relative humidity, under a 14-h light:10-h dark cycle.

2.2. Host plants

Three cruciferous species, namely, cabbage (*Brassica oleracea* L. var. *capitata*), radish (*Raphanus sativus* L. var. *radiculus* Pers), and rape (*Brassica campestris* L.) were planted in greenhouse (26°13′09″N, 119°33′32″E). The plants were not sprayed with insecticides.

2.3. Reagents

Tea saponin (90% saponin) was purchased from HanQing Biological Technology Co. Ltd., Huaihuai, Hunan Province, China. Juvenile hormone (93%) was purchased from Sigma Chemical Co., USA. Molting hormone [20-hydroxyecdysone (20E) 95%, MH] was purchased from Sigma.

2.4. Toxicity bioassay

We conducted toxicity tests to determine the mortality of 3rd instar larvae exposed to TS as the active ingredient. For each host plant, we evaluated five TS concentrations (200 mg·L^{-1}, 1000 mg·L^{-1}, 5000 mg·L^{-1}, 25,000 mg·L^{-1}, and 125,000 mg·L^{-1} for larvae feeding on cabbage; 64 mg·L^{-1}, 320 mg·L^{-1}, 1600 mg·L^{-1}, 8000 mg·L^{-1}, and 40,000 mg·L^{-1} for larvae feeding on radish; 125 mg·L^{-1}, 250 mg·L^{-1}, 500 mg·L^{-1}, 1000 mg·L^{-1}, and 2000 mg·L^{-1} for larvae feeding on Chinese cabbage). The dilutions were made by using distilled water (pH, 6.8) based on preliminary mortality tests. Newly cut leaf disks (diameter, 3.4 cm) from each of the three host plant species were immersed in TS solution for 15 s and were then transferred into plastic jars (diameter, 3.4 cm; two leaf disks per jar). Each plastic jar was lined with moistened filter paper to prevent the leaves from withering. Thirty 3rd instar larvae were starved for 4 h and were then transferred into the plastic jars (30 larvae per jar). Each treatment was replicated three times. Larvae were considered to be dead if they did not respond when lightly prodded with forceps. Larval mortality (%) was quantified after 24 h of treatment.

2.5. Larval growth and development

Newly cut leaf disks (diameter, 2.0 cm) from each of the three host plant species were immersed in two different TS concentrations [LC_{20} (lethal concentration required to kill 20% of the population) and LC_{50} (lethal concentration required to kill 50% of the population)] for 15 s and were then air dried at room temperature to remove excess water. For the control treatment, leaf disks were soaked in distilled water and were then subjected to the same procedure as described for the TS treatments.

Newly molted 2nd instar larvae from the stock culture were starved for 4 h and were then placed in Petri dishes (diameter, 4 cm) containing the treatment and control leaf disks. We replaced the leaf disks and 30 larvae from each treatment replicate every 2 days until the larvae became pupae. Freshly emerged male and females from these pupae were paired and maintained in glass tubes (30 mm × 200 mm) in a rearing room at 25 ± 1 °C and 70–80% relative humidity, under a 14-h light:10-h dark cycle. All the insects were provided with cotton soaked in 30% sugar solution as a food source, together with a fresh leaf of each of the three host plant species for oviposition.

For each TS concentration, we recorded the development times of 2nd, 3rd, and 4th instar larvae at daily intervals. In addition, we observed the duration of the pupal stage (days) (mean ± SE), pupal mortality (%), pupal weight (mg), duration of oviposition (days), and fecundity of adults (eggs per female). Each experiment was replicated three times.

2.6. Nutritional indices

The 2nd instar larvae were reared as described in Section 2.5. This rearing was continued until pupation. We recorded the larval weight, weight of food provided, weight of uneaten food, and mass of fecal matter produced at daily intervals. In order to compensate for the decrease in the weight of food provided to the larvae because of evaporation, we simultaneously conducted a control experiment by maintaining weighed leaves in a Petri dish and reweighing these leaves after 24 h. We calculated the food utilization rates according to the formulas of Viskari et al. (2000) and Waldbauer (1968) as follows [15,16]:

(i) Mean relative growth rate (MRGR) = $(\log W_2 - \log W_1) / t$
(ii) Efficiency of conversion of ingested food (ECI) = $[(\log W_2 - \log W_1)/Q)] \times 100$
(iii) Efficiency of conversion of digested food (ECD) = $[(W_2 - W_1)] / (Q - F)] \times 100$
(iv) Approximate digestibility (AD) = $[(Q - F) / Q] \times 100$

where W_1 = the weight of newly molted 2nd instar larvae (g); W_2 = the weight of larvae after the feeding period (g); t = the duration of the feeding period (days); Q = the fresh weight gain of larvae during the feeding period; and F = the mass of fecal matter produced by larvae during the feeding period.

2.7. Juvenile hormone extraction and analysis

We extracted juvenile hormones (JHs) following the procedure of Qian et al. (2006) [17], but with slight modifications. Each larva was weighed and placed in a glass homogenizer with 2 mL of extraction solution (methanol: ether = 1:1) for homogenization. Samples were centrifuged three times at 10,000 rpm for 10 min with *n*-hexane. The supernatant (upper) phase was collected and dried at 40 °C by using a pressure blowing concentrator. Next, we added 150 μL of standard solutions (11.63 ng·μL^{-1}) to 150 μL of mobile phase; the obtained solution was filtered using an organic phase membrane before being subjected to chromatographic analysis (W2695, Waters Co., USA).

Juvenile hormone was dissolved into 10 mL of ethanol (99.7%) as the mother liquor (930 ng·L^{-1}). Next, 1 mL of 930 ng·L^{-1} mother liquor was mixed into 10 mL of constant volume as the mother liquor (930 ng·L^{-1}) and the resulting solution was stored in a refrigerator at 4 °C.

A standard curve was prepared by diluting the mother liquor into a ratio equality series concentration (46.5 ng·L^{-1}, 23.25 ng·L^{-1}, 11.625 ng·L^{-1}, 5.81 ng·L^{-1}, 2.91 ng·L^{-1}, and 1.45 ng·L^{-1}) as the standard solutions. The standard solutions were used as the abscissa and the peak areas were used as the ordinate. The linear range was identified.

The peak areas were determined using the following chromatographic conditions: mobile phase, methanol plus water (70:30); flow rate, 1 mL·min^{-1}; detection wavelength, 218 nm; injection volume, 10 μL; and column temperature, 35 °C.

The precise concentration of juvenile hormone was analyzed by continuously injecting the JH standard sample five times under identical chromatographic conditions. The precise concentration was determined based on standard deviations and the variation coefficient.

The standard solutions were continuously injected during stable operation of the chromatographic equipment. When the difference between each adjacent peak area was <1.5%, the following solutions