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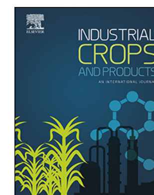

Ginsenosides from the stems and leaves of *Panax ginseng* show antifeedant activity against *Plutella xylostella* (Linnaeus)

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ABSTRACT

The diamondback moth, *Plutella xylostella*, is one of the most serious insect pests of cruciferous crops all over the world. Although chemical insecticides are used to control this pest, it develops resistance to almost all kinds of insecticides. In recent, triterpenoid saponins have been reported to show potent antifeedant or synergize insecticidal activity on several pests. Therefore, to assess the antifeedant activity of ginsenosides against *P. xylostella* is benefit to biological control of this pests. Total ginsenosides were extracted from ginseng leaves and stems, and the total content of 9 ginsenosides was 72.91 mg/mL measured by high-performance liquid chromatography (HPLC) method. Total ginsenosides extracted from ginseng leaves and stems showed significant antifeedant activity on *P. xylostella* larvae in both non-choice and choice assays. The concentration of median antifeedant (AFC_{50}) of total ginsenosides for the second instar larval of *P. xylostella* were 4.98 and 5.03 mg/mL at 24 h and 48 h respectively in non-choice assay, and they were 2.74 and 4.14 mg/mL respectively in choice assay. The residue rate of 9 ginsenosides in the pest became gradually higher as applied concentration increased. Feeding with ginsenosides generally resulted in decrease of glutathione S-transferase (GST), acetylcholine esterase (AChE), and carboxylesterase (CarE) activities, but increase of mixed-functional oxidase (MFO) activity in *P. xylostella.* These results indicate that ginsenosides are suitable for developing it into a botanical pesticide.

1. Introduction

The diamondback moth, *Plutella xylostella* (Linn.), is one of the most serious insect pests of cruciferous crops all over the world (Thorsteinson, 2011; Ahmad et al., 2009). Therefore, many chemical insecticides are widely used on crops to control *P. xylostella* throughout the growing season. However, chemical control of this pest remains problematic because high tolerance to most of the insecticides and adverse effects on natural enemies may result in outbreak of thepest (Liang et al., 2003; Xu et al., 2004). Also, excessive use of chemicals has led to environmental contamination, created pesticide residue, and even threatened human beings' health seriously (Wang et al., 2015). Among current alternative methods aiming at decreasing the use of chemical insecticides, natural insecticides originating from plants have been suggested as a priority source for insect control because botanical products are often selective to insect pests and have no or little harmful effect on non-target organisms and the environment. Furthermore, they are easily available (Charleston et al., 2005).

Ginseng (*Panax ginseng* Meyer) root has been usually used as a traditional herbal medicine in Asian countries for thousands of years. Numerous studies have reported that ginseng has many pharmacological effects on anti-fatigue, anti-carcinogenic, anti-oxidant, anti-aging, neuroprotective, as well as on regulating of the endocrine, immune, and cardiovascular systems (Zhang et al., 2018; Attele et al., 1999; Lü et al., 2009; Ong et al., 2015). Ginsenosides are the major bioactive constituents of ginseng (Yang et al., 2014). Up to now, about 70 ginsenosides with diversified sapogenins are isolated from ginseng (Shin et al., 2015). However, a lot of studies on ginsenosides have mainly focused on the roots and rhizomes of *P. ginseng* because the aerial parts, including the stems and leaves, are usually discarded. In recent, many kinds of ginsenosides have been isolated from ginseng leaf, and their similar medical effects to ginseng root were also reported (Li et al., 2012a,b).

The production of secondary metabolism may lie in the ways that

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plants protect themselves against pests and diseases. It is interesting that the stems and leaves of ginseng were hardly affected by pest problems (Beyfuss, 1992). This may relate to ginseng plant accumulates large amount of ginsenoseds during its development. In previous studies, triterpenoid saponins have been reported to show potent antifeedant or synergize insecticidal activity on several pests. For example, a mixture of triterpenoid saponins from stem bark of *Catunaregam spinose* and an oleanane-type saponin from *Barbarea vulgaris* are repellent or deterrent to *Plutella xylostella* (Gao et al., 2011; Shinoda et al., 2002). The objective of this study was to assess the antifeedant activities of ginsenosides from ginseng leaf against *Plutella xylostella* and their influences on detoxifying enzymes of the pest. Results from this study provide the scientific basis for using ginsenosides as a new tool for protecting plants from insect pests.

2. Materials and methods

2.1. Insect rearing

The *P. xylostella* tested were obtained from Institute of Plant Protection in Chinese Academy of Agricultural Sciences, the established laboratory of stock culture maintained for > 15 generations at 25 ± 1 °C temperature, 75 ± 10% RH and a 14: 10 (L: D) photoperiod. Second instar *P. xylostella* larvae were selected at random for biological and enzyme activity assays.

2.2. Plant materials and extraction

The fresh stems and leaves of ginseng were collected from a ginseng field at the Botanical Garden, Jilin Agricultural University, China. Total ginsenosides were isolated using the method described by Wu et al. with slight modification (Wu et al., 2001). The stems and leaves were oven dried at 50 °C for 3 days. Then the leaves and stems were grinded using mill (Janke & Kunkel KG. IKA-werk, Germany) and pass through sieves (60 mesh). 500 g powder was extracted three times for 30 min in 70% methyl alcohol with ultrasound-assisted. After filtration, the filtrate was concentrated in vacuo to evaporate the solvents. The concentrate was dissolved with distilled water, then extracted by water-saturated butanol for three times. Finally, the extract was concentrated in vacuo to evaporate the solvents and dissolved with 500 mL ultrapure water containing 1 g/L Triton X-100. The ginsenoside concentration of stock solution was determined using HPLC method. The stock solution was diluted to six different concentrations (1, 2, 5, 10, 20 and 50 mg/mL) by ultrapure water containing 1 g/L^{-1} Triton X-100 according to HPLC result.

2.3. Determination of ginsenosides

The reference standards of nine ginsenosides, Rg_1, Re, Rf, Rb_1, Rg_2, Rc, Rb_2, Rb_3, and Rd, were purchased from the Natural Medicinal Chemistry Laboratory of Jilin University, with the batch number of 201523, 201545, 201549, 201551, 201506, 201579, 201518, 201543 and 201501, whose purities were all higher than 98%. Analyses of ginsenoside concentration of extracts from ginseng leaves and stems as well as pests were carried out on a Shimadzu (Shimadzu Corporation, Tokyo) LC-2010 A HPLC system equipped with a quaternary gradient pump unit, a UV–vis detector (190–700 nm), an autosampler (0.1–100 μL), and a reversed-phase Nucleosil C18 (4.6 mm × 150 mm; film thickness, 5 μm; Shimadzu, Tokyo, Japan) column. Acetonitrile and water were used as mobile phase A and mobile phase B respectively. Separation was achieved using the following gradient program: 82–78% B for 0–24 min, 78–74% B for 24–26 min, 74–68% B for 26–30 min, 68–66.5% B for 30–50 min, 66.5–62% B for 50–55 min. The column temperature was set at 30 °C. The injection volume was 20 μl. The flow rate was 1 ml/min. The UV detection wavelength was 203 nm (Zhang et al., 2013).

2.4. Bioassay

The antifeedant activities were tested using the non-choice and choice leaf disk method according to Valladares et al. (1997) and Sengonca et al. (2006). Cabbage (*Brassica oleracea* L. var. capitata L.) leaf disks with a diameter of 1.5 cm were immersed in six concentrations of ginsenoside solutions respectively for 30 s. The leaves were left to air dry for 1.5 h (the solvent was removed quickly by air drying). For each concentration, six leaf disks of the same treatment were placed individually into a 9 cm diameter petri dish lined with filter paper, and 10 s instar *P. xylostella* larvae were released into the dish separately to assessed the antifeedant index (AI). The other Petri dish was putted with three leaf disks of the same treatment and three control leaf disks without treatment to assess the feeding index (FI). Each treatment was performed three times. The Petri dishes were placed in an incubator at 25 ± 1 °C with 75 ± 10% RH and a 14: 10 (L: D) photoperiod. Both antifeedant index (AI) and the feeding index(FI) were assessed after 24 h and 48 h of exposure to the total ginsenosides.

The consumed area was measured using a WinFOLIA systerm (Regent Instruments Canada Inc., Canada) and the antifeedant index (AI) rate was calculated by the following equation $AI\% = [(1 - T/C) \times 100]$, where T is the average area treated leaf extract consumed and C is the average area of leaf consumed without treatment (Carpinella et al., 2003). The Feeding Index (FI) was calculated as $[(C - T)/(C + T)] \times 100$. Log dosage-probit regression equation of total ginsenosides were established for two time spots, and the concentration of median antifeedant (AFC_{50}) and 95% confidence limit were calculated.

2.5. Enzyme activity assay

Four different concentrations of ginsenosides (5, 10, 20 and 50 mg/mL) were selected for enzyme activity analysis and the insects were treated according to non-choice leaf disk method described above. During the process of enzyme preparation, 5 s instar larvae were randomly picked from each petri dish at 24 h and 48 h respectively. The larvae of each treatment were put into 2 mL glass homogeniser with normal saline, and the weight of larva (g): the volume of normal saline (mL) was 1: 9. The homogenate was obtained under ice bath condition, and then centrifuged at 15,000 rpm at 4 °C for 30 min. After that, the supernatant was taken into a new centrifuge tube for the test of enzyme activity. For determination of glutathione S-transferase (GST), acetylcholine esterase (AChE), and carboxylesterase (CarE) activities, the assay kits (Nanjing Jiancheng Bioengineering Institute) were employed. The determination of mixed-functional oxidase (MFO) activity was conducted using an enzyme-linked immunoassay kit (R&D Systems Inc.). The total protein concentrations were determined using a total protein quantitative assay kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions.

2.6. Analysis of ginsenoside residue rate in insect

Four different concentrations of ginsenosides (5, 10, 20 and 50 mg/mL) were selected to assess the residual rate of ginsenosides in *P. xylostella*. The insect treatment was the same as non-choice leaf disk method except for spraying 100 μL of ginsenosides on each cabbage leaf instead of immersing the leaf. After 24 h, all the 10 larvae of each treatment were put into 2 mL glass homogeniser with 2 mL of methyl alcohol. The homogenate was soaked at room temperature for one day and then centrifuged at 15,000 rpm for 30 min. The supernatant was transferred to a new centrifuge tube for HPLC analysis. The Residue Index(RI) was calculated as $[(T/C) \times 100]$, where T is the total ginsenosides extracted from *P. xylostella* and C is the total ginsenosides eaten by *P. xylostella*.

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