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The toxicity and physiological effect of essential oil from *Chenopodium ambrosioides* against the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

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ABSTRACT

In this study, the bioactivities of the essential oil of *Chenopodium ambrosioides* L. and its two main components, α-terpinene and *p*-cymene, were evaluated against the diamondback moth (DBM), *Plutella xylostella* (L.). The contact and fumigant toxicity of the essential oil significantly decreased as the DBM larval instar increased. The essential oil had 30-fold more potent toxicity against third-instar larvae than either α-terpinene or *p*-cymene. With respect to antifeedant activity, treatment with *C. ambrosioides* essential oil dose-dependently decreased leaf consumption by third-instar larvae, and the median antifeedant concentration (AFC_{50}) was 66.81 mg/L at 24 h and 78.24 mg/L at 48 h after the treatment. Development of pupae was also inhibited, and the median concentrations of pupae weight inhibition ($PWIC_{50}$) and percentage inhibition of pupation (PIC_{50}) were 176.5 mg/g leaf and 111.6 mg/g leaf, respectively. In general, contact treatment with the essential oil significantly inhibited the activities of insecticide detoxifying enzyme, including carboxylesterase and glutathione-*S*-tranferases, whereas, fumigant exposure only altered carboxylesterase activity. At nearly all the tested concentrations, the essential oil induced the activities of superoxide dismutase, peroxidase, and catalase; however, peroxidase activity was inhibited by contact treatment. Thus, the essential oils from *C. ambrosioides* showed potential as new control products to combat field crop-infesting insect pests, and it may function as fumigant, insecticide synergist, antifeedant, or insect growth regulator.

1. Introduction

Indiscriminate utilization of synthetic chemical insecticides has led to insect resistance and caused widespread public concern about long-term health and environmental effects. Consequently, there is growing motivation to develop natural and environmentally derived alternatives for insect pest control. One potential alternative is plant derivatives, many of which have traditionally been used for crop protection, especially in the Mediterranean and in southern Asia regions (Isman, 2005). Interest in plant essential oils (EOs) was reinvigorated by the demonstration of their fumigant and contact insecticidal activities against a wide range of insect and mite pests in the 1990s (Isman, 2000, 2005).

One plant of potential interest for developing insecticides is *Chenopodium ambrosioides* L. (Chenopodiaceae), which originated in Central America and has been widely distributed in hot subtropical, tropical, and temperate regions. *C. ambrosioides* is an annual plant that has a strong aromatic odor and is known by several common names in different regions (Cavalli et al., 2004; Liu et al., 2013).

In the area of insect pest control, the toxicity of *C. ambrosioides* extracts, essential oils, and powder has been reported mainly in insects of medical importance (Denloye et al., 2009; Harraz et al., 2015; Zhu et al., 2012) and storage insect pests (Chu et al., 2011; Delobel and Malonga, 1987; Denloye et al., 2010; Ntonifor et al., 2011; Tapondjou et al., 2002, 2003).

One problematic agricultural pest is the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera; Plutellidae), which is a major cosmopolitan pest that afflicts *Brassica* species and other

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cruciferous crops all over the world (Talekar and Shelton, 1993). DBM has developed resistance to a wide range of insecticides (Talekar and Shelton, 1993; Wei et al., 2010b; You et al., 2013), resulting in the increased failure to successfully control DBM. In addition to resistance, concerns about potential adverse impacts of synthetic pesticides have prompted the development of alternative approaches for DBM control, including plant-derived products.

In this paper, we report the contact and fumigant toxicities of *C. ambrosioides* EO and its two main components toward DBM larvae. Antifeedant and pupation inhibition activities of the EO were the key parameters measured. We also measured the inhibitory activity of the EO toward some DBM larva endogenous enzymes, including carboxylesterase (CarE, EC 3.1.1.1), glutathione-*S*-tranferases (GSTs, EC 2.5.1.18), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and peroxidase (POD, EC 1.11.1.7).

2. Materials and methods

2.1. Insect stock

Insects used in these experiments were originally collected from a vegetable field in Fuzhou City, Fujian province, China. The adults were introduced into a chamber containing radish seedlings (*Raphanus sativus* L. var. *radiculus* Pers) and absorbent cotton with a 100 g/kg honey solution. Insects were then allowed to mate and lay eggs. Larvae in the experiment were reared continuously on the radish seedling for at least 5 generations. The rearing chamber was maintained at 25 ± 1.0 °C, 70 ± 5% relative humidity, and a 14:10-h light:dark photoperiod.

2.2. Extraction and characterization of the essential oil

Whole plants of *C. ambrosioides* were collected from a field located in Xindian, Fuzhou City, Fujian province, China (26° 07′ 30.74″ N , 119° 18′ 23.98″ E). The species was identified by Professor Changfang Wang from the Institute of Plant Protection, Fujian Academy of Agricultural Sciences. Whole plants were spread out on racks and dried naturally in the laboratory (25–32 °C , 20–60% relative humidity) for at least 20 days until they were crispy to the touch. A powder was obtained by grinding the dried leaves and stems in a pulverizer (Type FC130, Shanghai Traditional Chinese Medicine Machinery Factory) to a mesh size of less than 0.5 mm. The pulverized powder was subjected to hydrodistillation using an oil analyzer (100 g powder per analyzer) (Chinese Pharmacopoeia Commission, 2005). The collected oil was dried over anhydrous sodium sulfate and stored in closed glass vials at 4 °C. Chemical analysis of the oil was determined using GC–MS (Varian Saturn 2100) (Wei et al., 2010a). Compounds α-terpinene and *p*-cymene accounted for 26.81% and 49.60% of the total oil, respectively (Wei et al., 2010a).

2.3. Reagents

α-Terpinene (85%) and *p*-cymene (≥99.5%) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Eserine and Fast Blue RR salt were obtained from Fluka (Sigma–Aldrich). α-Napthyl acetate naphthalene-acetic acid (α-NA), 2,4-dinitrochlorobenzene (CDNB), coomassie brilliant blue G-250, pyrogallol, guaiacol, and H_2O_2 were purchased from Shanghai Chemical Industry Co., Ltd in China. All reagents were analytical grade.

2.4. Bioassays

All bioassays were performed at 25 ± 1.0 °C and 70 ± 5% relative humidity over a 14:10-h light:dark photoperiod.

2.4.1. Contact toxicity

C. ambrosioides EO, *p*-cymene, and α-terpinene were diluted with acetone to prepare 5–6 solutions of differing concentration gradients. EO concentrations were 225, 450, 900, 1800, 3600 mg/L for second-instar larvae, 1750, 3500, 7000, 14,000, 28,000 mg/L for third-instar larvae and 2250, 4500, 9000, 18,000, 36,000 mg/L for fourth-instar larvae. *p*-cymene concentrations were 18,000, 36,000, 72,000, 144,000, 288,000, 576,000 mg/L for third-instar larvae. α-terpinene concentrations were 32,000, 64,000, 128,000, 256,000, 512,000 mg/L for third-instar larvae. An Automatic Micro-applicator (Burkard, Ricksmanworth, England) was used to dispense and apply 0.5 μL aliquots of the prepared solutions to the dorsal thorax of individual larvae. Every treatment contained three replicates, with 30 larvae each. Acetone was applied to a group of larvae as a control. The treated larvae were fed fresh cabbage leaves within the chamber, and larval mortality was recorded after 48 h. The mortality data were subjected to probit analysis (Finney, 1971) to determine a median lethal dose (LD_{50}).

2.4.2. Fumigant toxicity

C. ambrosioides EO, *p*-cymene, and α-terpinene were diluted with acetone to prepare five gradient solutions of varying concentrations based on a preliminary assay. EO concentrations were 2500, 5000, 10,000, 20,000, 40,000 mg/L for second-instar larvae, 4375, 8750, 17,500, 35,000, 70,000 mg/L for third-instar larvae, and 6250, 12,500, 25,000, 50,000, 100,000 mg/L for fourth-instar larvae. *p*-cymene concentrations were 500,000, 700,000, 900,000, 1,100,000, 1,300,000 mg/L for third-instar larvae. α-terpinene concentrations were 500,000, 700,000, 900,000, 1,100,000, 1,300,000 mg/L for third-instar larvae. One hundred μL aliquots of the solutions were applied uniformly to filter paper strips (Whatman No. 1, 1.00 cm × 10.00 cm). Acetone was applied to another filter paper strip as control. Groups of 30 larvae were placed in a conical flask (300 mL) with a treated filter paper strip attached to the flask stopper. Every treatment contained three replicates. Insect mortalities were recorded daily up to 48 h. Mortality data were subjected to probit analysis (Finney, 1971) to determine a median lethal concentration (LC_{50}).

2.4.3. Antifeedant activity

The antifeedant activity of the EO was determined against third-instar DBM larvae using a non-choice leaf disc method (Guo et al., 2013). Cabbage (*Brassica oleracea* L. var. *capitata*) leaves were cleaned with distilled water, dried, and leaf discs (Ø = 1.40 cm) were made using an iron borer. We then serially diluted EO in acetone containing 0.2% Tween-80. Leaf discs for each treatment group were immersed in a test solution for 10 s and allowed to dry at room temperature. Leaf discs for control groups were only treated with solvent that contained no EO. Four treated discs were placed in a 9-cm petri dish with a moistened piece of filter paper (Whatman No. 1). Newly molted third-instar larvae were selected and food deprived for 4 h. One larva was added to each petri dish, with 30 larvae per treatment group. All treatments and controls were evaluated in triplicate. After 24 and 48 h of treatment, the larvae were removed from the petri dish using a fine hairbrush. Leaf discs were photographed, and the leaf area (mm^2) consumed was determined using Photoshop software (Xiao et al., 2005). An analysis of variance (ANOVA) was used to test for significant differences in the mean leaf area consumed per larvae (mm^2/larvae) across treatment groups, followed by Fisher's least significant difference (LSD) tests. Significance was set at $P < 0.05$. The antifeedant activity of compounds was calculated using an antifeedant index (% AFI) (Huang et al., 2008) as the formula: