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Effect of allyl isothiocyanate on ultra-structure and the activities of four enzymes in adult *Sitophilus zeamais*

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ABSTRACT

Rarefaction and vacuolization of the mitochondrial matrix of AITC-treated (allyl isothiocyanate-treated) adult *Sitophilus zeamais* were evident according to the ultra-structural by TEM. Four important enzymes in adult *S. zeamais* were further studied after fumigation treatment with allyl isothiocyanate (AITC) extracted from *Armoracia rusticana* roots and shoots. The enzymes were glutathione S-transferase (GST), catalase (CAT), cytochrome c oxidase, and acetylcholinesterase (AChE). The results indicated that the activities of the four enzymes were strongly time and dose depended. With prolonged exposure time, treatment with 0.74 μg/mL AITC inhibited the activities of cytochrome c oxidase, AChE, and CAT, but induced the activity of GST. The activities of cytochrome c oxidase, AChE, and CAT were remarkably induced at a low AITC dosage (0.25 μg/mL), but were restrained with increased AITC dosage. The activity of GST was inhibited at a low AITC dosage (0.5 μg/mL), but was induced at a high AITC dosage (1.5 μg/mL). According to the results of TEM, toxic symptoms and enzymes activities, it suggested that mitochondrial maybe the one site of action of AITC against the adult *S. zeamais* and it also suggested that cytochrome c oxidase maybe one target protein of AITC against the adult *S. zeamais,* which need to further confirmed by protein function tested.

1. Introduction

The maize weevil, *Sitophilus zeamais* Motsch, is a stored-grain pest [1] that causes serious harm to grain storage and container transport worldwide. The control of this pest in stored grain is difficult [2].

At present, fumigants and preventive chemical protectants are used to control stored-grain pests in China. However, this method of control leads to increased *S. zeamais* resistance [3]. Thus, fumigant producers are facing tough challenges, and the search as well as development of new fumigants is imperative.*Armoracia rusticana* Gaertn is a type of cruciferous horseradish that is widely distributed in Europe and Asia. This horseradish releases a strong, pungent, lachrymatory odor when it is being cut, shredded, or grated, or when in contact with water. The odor comes from compounds such as isothiocyanates that are enzymatically degraded from sinigrin and thioglucosides, which naturally exist in *A. rusticana*. Recent studies show that horseradish extract exerts strong fumigation and repellant effects against *S. zeamais, Rhizopertha dominica, Tribolium ferrugineum* [4]. However, research on horseradish extract is mainly focused on the separation of ingredients, structural identification, and biological activity [5–8]; the mechanism of action of horseradish extract against the *S. zeamais* adult is rarely studied.

Different fumigants have different modes of insecticidal action. It was shown that a primary effect of halogenated hydrocarbons is to serve as alkylating agents. The sulfhydryl groups of proteins, in particular, are labile to methyl bromide-induced methylation [9]. In insects and other organisms, the mitochondria are organelles referred to as the "powerhouse of the cell" because they act as the site for the production of high-energy compounds (e.g., ATP). Mitochondria produce large amounts of energy through oxidative phosphorylation of organic molecules during cellular respiration and are capable of using glucose and oxygen to produce energy for use in many metabolic processes. This research aims to verify mitochondrial alteration in the integument and different stegma epithelial cells of adult *S. zeamais* fumigated with AITC.

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Studies show that the behavioral response of adult *S. zeamais* to allyl isothiocyanate (AITC) treatment has four stages: excitement, convulsions, paralysis, and death or recovery. The excitement period is characterized by intense activity, motor incoordination, and rapid knockdown. The convulsion period is characterized by abnormal movement, foot and antenna twitching and trembling, as well as absence of crawling. The paralysis period is characterized by immobility and slight foot-antenna trembling. Death is characterized by a dry body surface as well as stiffly extended feet and antennae. Biochemical analysis and polyacrylamide gel electrophoresis results indicate that different esterase activities may be some of the mechanisms of AITC against adult *S. zeamais* [10].

Glutathione S-transferase (GST) and catalase (CAT) are two important detoxifying enzymes of insects. GST is a complex, multigene family of enzymes [11–13]. The important function of GST is detoxification by conjugating reduced glutathione with a large number of electrophilic metabolites derived from a variety of xenobiotics, including carcinogens, toxins, and drugs [14–16]. CAT constitutes the most efficient and elaborate system available in both plants and animals for controlling the H_2O_2 concentration. CAT catalyzes the dismutation of H_2O_2 to one molecule of H_2O and a half molecule of O_2. H_2O_2 is a normal by-product of mitochondrial electron transport, β-oxidation of fatty acids, and photorespiration [17]. Cytochrome c oxidase is the terminal enzyme in the eukaryotic mitochondrial inner membrane and aerobic membrane electron transport chain. The function of cytochrome c oxidase is directly related to ATP synthesis, and its dysfunction often leads to some energy metabolism-related diseases, even death [18]. In the central nervous system, acetylcholinesterase (AChE; acetylcholine hydrolase, EC 3.1.1.7) is a pivotal enzyme responsible for the final stages of impulse transmission at cholinergic synapses involving the rapid hydrolysis of the neurotransmitter acetylcholine [19].

The objectives of this study are (i) a preliminary study on the site of action of AITC against adult *S. zeamais* through TEM, (ii) to study the induction of AITC against cytochrome c oxidase, AChE, CAT, and GST in adult *S. zeamais*, (iii) to determine the relationships between the toxicity of AITC and these four enzymes in adult *S. zeamais*.

2. Materials and methods

2.1. Insects

The laboratory-adapted adult *S. zeamais* strain was obtained from the Research & Development Center of Biorational Pesticide, Northwest A&F University. *S. zeamais* was reared in the laboratory on cracked wheat with a moisture content of 13 ± 1%. The temperature in the insectary was set at 29 ± 1 °C, and the relative humidity was maintained at 75 ± 5%. Adult (2–3 weeks old) *S. zeamais* was used in the toxicity assays.

2.2. Plant materials

Dry roots of *A. rusticana* (moisture content, <11%; storage conditions, dry and shaded) were collected from Pengshi, Sichuan Province, China in 2011. The roots were pulverized and passed through a 40–60 mesh uniform sieve before extraction.

2.3. Chemicals

The AITC standard (>94%) was obtained from Sigma–Aldrich Chemical Co., St. Louis, MO, USA. CAT, AChE, and GST enzyme assay kits were purchased from Nanjing Jiancheng Bioengineering Company. A cytochrome c oxidase kit was purchased from GenMed Scientifics, Inc., USA. A Coomassie brilliant blue protein assay kit was purchased from Bio-RAD Laboratories, Inc. All other analytical grade reagents were purchased from Sigma–Aldrich Chemical Co., St. Louis, MO, USA.

2.4. Extraction and purification of AITC

A supercritical carbon dioxide fluid extraction (SFE-CO_2) instrument HA121-50-01 (Huaan Inc., Nantong, China) was used to extract active components from *A. rusticana*. Distilled water (pH 4; 40 mL)was used as modifier and added to the prepared hydrolysis powders in the initial SFE-CO_2 extraction experiments. The flow rate of carbon dioxide was set at 5 g min^{-1}. The extraction process was carried out for 1 h at a temperature of 45 °C with a pressure of 25 MPa. The oily residue containing AITC was collected and filtered into a 1 mL sample vial through a syringe filter of 0.2 μm pore size prior to GC analysis. The oily residue containing AITC was purified by dephlegmator, and oily AITC was collected at boiling 151–152 °C, then high purified AITC(94%) was obtained by GC detection.

2.5. Bioassay

The fumigation concentrations of AITC were set to 0.25, 0.5, 0.75, 1, 1.25, and 1.5 μg/mL (vapor concentration of AITC in air). A filter paper-lined, 300 mL tapered bottle containing AITC was used for each treatment. Insects and food were placed at the bottom of the bottle, which was exposed to AITC for 72 h before removing the insects and examining mortality by probing their abdomen with a sharp object. Approximately 150 insects (50 insects per species) were used for each treatment, with three replications. A control without any pesticide was also prepared. After treatment for 72 h, the corrected mortality, regression equation, and median lethal concentration (LC_{50}) of virulence were calculated.

Another experiment was designed with an AITC fumigation concentration of 0.74 μg/mL (vapor concentration of AITC in air). The same fumigation method was used to treat the insects. After treatment for 12, 24, 36, 48, 60, and 72 h, all *S. zeamais* specimens were subjected to enzyme assays.

2.6. Effects of AITC on ultrastructure adult S. zeamais

In order to analyze and compare the effect of different concentration AITC oil on the morphology of the mitochondria in the stigmata tissues from treated and untreated adult *S. zeamais*, the stigmata tissues of treated groups with LC_{50} and LC_{90} of AITC and non-treated groups were sampled. Dead adult from each experimental group were dissected in plate of Petri; the tissue was removed from the part of stigmata cut into 2 mm^2 segments with a scalpel, and then transferred into 4% (W/V) paraformaldehyde (Sigma–Aldrich, Shanghai), 1% (V/V) glutaraldehyde buffered with 0.1 M sodium phosphate (PB: 19 mL of 0.2 M NaH_2PO_4, 81 mL of 0.2 M Na_2HPO_4) at 4 °C for 4 h. This was followed by post-fixation in 1% (V/V) OsO_4 at 4 °C for 4 h. After five washes in phosphate-buffered saline (PBS: 8.0 g of NaCl, 0.2 g of KCl, 2.9 g of $Na_2HPO_4{\cdot}12H_2O$, 0.2 g of K_2HPO_4 in 1000 mL of distilled water, pH 7.4) the tissues were dehydrated in a graded series of alcohol (30%, 50%, 70%, 80%, 90% a, 95% and 100% for 15 min each) up to an absolute acetone for 30 min each, then the material was embedded in ERL [20], as generally described [21].The mitochondria in the stigmata sections (200 nm thickness) were prepared on carbon-coated copper grids and then examined and photographed with a TEM (TEM-123) at 80 KV.