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Toxicity and physiological effects of an extracted lectin from *Polygonum persicaria* L. on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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ABSTRACT

A lectin extracted from *Polygonom persicaria* (PPA) was mixed with the artificial diet provided to the larvae of *Helicoverpa armigera* at various concentrations. Although the PPA significantly prolonged larval duration but, decreased pupal weight and larval survival. Nutritional indices such as approximate digestibility, efficiency of conversion of digested food and efficiency of conversion of ingested food were significantly decreased in the PPA-fed larvae. Activities of digestive enzymes in the PPA-fed larvae, such as α-amylase, α-glucosidase, lipase, trypsin, elastase and exopeptidases, were significantly lower than control except for β-glucosidase and chymotrypsin. PPA-fed larvae showed lower amounts of total protein and glycogen compared to control.

1. Introduction

Plants adopt several defense strategies including morphological and structural barriers or synthesize chemical compounds to conquer damages of phytophagous insects [31]. Chemical defenses of plants include secondary metabolites, peptides and proteins which affect behavior and physiology of insects [31]. Plant lectins are considered as specific plant proteins that help in defending them against attacks by phytophagous insects. These lectins possess at least one non-catalytic domain in their structure which bind to specific carbohydrates from simple monosaccharides to more complex glycans. They cause direct either direct toxicity or ecological and physiological disruptions on insects [16,29,30]. Plant tissues, such as seeds and bulbs, contain large amounts of lectins upholding their defensive roles against insect herbivores and pathogens as storage proteins [16]. Several studies have clearly demonstrated toxicity of plant lectins to a wide range of insects belonging to various orders such as Lepidoptera, Hemiptera, and Coleoptera [3,5,15,16,27,29,30,32]. The lectins can affect fecundity, growth and development of insects. They have the potential of binding to peritrophic membrane and/or chitinous structures in insect midgut thus impairing digestive, protective or secretory functions of the intestine via cell death [5,16,23,32]. Apoptosis or programmed cell death is a cellular process for elimination of unwanted and damaged cells in organisms which is regulated by a group of proteases called Caspases. Recent studies have shown that the lectins cause apoptosis in insect body via binding to the carbohydrate component of cell surface [23]. Morphological characteristics of cells treated by lectins are known as blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentation [11,23,33].

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae), is a polyphagous insect pest causing severe damages to various economically important crops including cotton, corn, tomatoes, sorghum, soybeans and groundnuts around the world [19,26]. Lack of effective and safe control methods and larval resistance to many insecticides are the main constraints to sustainable suppression of *H. armigera* outbreaks. In the current study, we investigated the toxicity of an extracted lectin from *Polygonum persicaria* L. (PPA) on development, physiology and caspase-3 dependent apoptosis on *Helicoverpa armigera* (Hübner). The present effort may be considered as an initial step to prove insecticidal properties of PPA and a recommendation for plant breeding programs assisting resistant varieties.

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2. Materials and methods

2.1. Insect rearing

Larvae of *Helicoverpa armigera* (Hübner) were collected from tomato fields of Astane-Ashrafieh in Guilan Province, Northern Iran. The larvae were reared on artificial diet containing cowpea (204 gr.), yeast (30 gr.), wheat germ (30 gr.), ascorbic acid (3.5 gr.), sorbic acid (1.3 gr.), formalin (2.7 mm), oil (4 mm), agar (14 gr.), and water (600 mm) [24] in laboratory condition at 25 ± 2 °C, 70% relative humidity and 16:8 light: dark photoperiod.

2.2. Purification of PPA

Purification of *P. persicaria* agglutinin was carried out using Sepharose 4B-galactose and DEAE-Cellulose fast flow columns washed by 1,3-diamino propane. The stems of *P. persicaria* were incubated in Tris-HCl buffer (0.1 M, pH 7.1) at 4 °C for approximately 72 h. The wet stems were ground in the buffer and further incubated for 24 h. Then, the mixture was filtrated using a filter paper (Whatman No. 1) and it was centrifuged at 6000 rpm for 20 min. The obtained supernatant was passed through the filter paper precipitated in 0–60% concentrations of ammonium sulfate. After centrifugation at 6000 rpm for 20 min, the pellet was suspended in Tris-HCl buffer (0.1 M, pH 7) and dialyzed in the same buffer overnight [6]. Dialyzed samples were loaded into affinity chromatography column of Sepharose 4B-Galactose equilibrated with Tris-HCl buffer and the column was washed by the buffer containing 20 mM of 1,3-diaminopropane (DAP) [10]. Fractions showing the highest protein contents were mixed and loaded onto DEAE-Cellulose fast flow equilibrated with DAP [10]. The fractions were gathered after column elution by Tris-HCl containing 0.5 M NaCl. Prior to freeze-drying, the sample was dialyzed against NaCl (0.5) to get the highest purity rate.

2.3. Effect of PPA on survival, pre-adult duration and pupal weight

Four concentrations of PPA (i.e. 0.1, 0.5, 1 and 2%) were mixed with the larval diet. A diet without PPA was used as control. Thirty third instar larvae (in five replicates) of *H. armigera* were separately allowed to feed on PPA treated and control diets until adult. Certain biological characteristics were recorded including mortality, developmental time, malformation of larvae/pupae and pupal weight.

2.4. Nutritional indices

Artificial diet containing 1% of PPA were provided to 20 (four replicates) third instar larvae to find out possible effects of the lectin on nutritional indices of *H. armigera* larvae. The experiments were continued until sixth instar larvae and compared with the control (also 20 larvae). Nutritional indices were calculated as described by Scriber and Slansky [22] as: Approximate digestibility (AD): [food ingested (mg dry mass) − feces (mg dry mass)]/food ingested (mg dry mass) × 100. The efficiency of conversion of digested food (ECD): biomass gained (mg fresh mass)/[food ingested (mg dry mass) − feces (mg dry mass)] × 100. The efficiency of conversion of ingested food (ECI): [biomass gained (mg fresh mass)/food ingested (mg dry mass)] × 100 and metabolic cost (MC): 100 − ECD.

2.5. Digestive enzyme assays

2.5.1. Sample preparation

Sixth larval instars from control and PPA-fed diets (1%) (10 larvae from each diet) were individually selected and their midguts were dissected out in an ice-cold saline solution (NaCl, 10 mM). The midgut samples were homogenized in 1 ml of distilled water by a glass pestle, and then were transferred to 1.5 ml of microtubes prior to centrifugation at 13,000 rpm for 20 min at 4 °C. The supernatants were pooled and stored at −20 °C for subsequent analyses.

2.5.2. α-Amylase assay

α-Amylase assay was performed according to the method of Bernfeld [2] using 1% soluble starch as substrate. Reaction mixture containing 10 μl of the enzyme, 50 μl of Tris-HCl buffer (20 mM, pH 7.1) and 20 μl of 1% soluble starch that was left at 30 °C for 30 min. Then, amylolytic activity was stopped by adding100 μl of dinitrosalicylic acid (DNS) and then placed in boiling water for 10 min. The absorbance was read at 540 nm after cooling on ice for 5 min. The blanks contained the reaction mixture without enzyme (Distilled water instead).

2.5.3. α- and β-glucosidase assay

The activities of α- and β-glucosidases were determined based on the method of Silva and Terra [25] with slight modifications. Reaction mixtures contained universal buffer (pH 7.00, 50 μl), enzyme extract (15 μl), and substrate (30 μl; *p*-nitrophenolα-glucopyranoside forα-glucosidase and *p*-nitrophenol-β-glucopyranoside for β-glocusidase). The absorbance was read at 405 nm after 10 min. The blanks contained the reaction mixture without enzyme (Distilled water instead).

2.5.4. Triacylglycerol (TAG)-lipase

The method of Tsujita et al. [28] was used to assay TAG-lipase activity. Briefly, 20 μl of enzyme, 40 μl of *p*-nitrophenyl butyrate (27 mM) and 100 μl of Tris-HCl buffer (20 mM, pH 7) were incubated together for 1 min at 30 °C. The reaction was stopped by adding 100 μl of NaOH (1 M) and absorbance was read at 405 nm. The blanks contained the reaction mixture without enzyme (Distilled water instead).

2.5.5. General protease assay

General protease assay was performed on the midgut of the larvae according to the method of Cohen (1993). Reaction mixture included 50 μl of hemoglobin (20 mg/ml) as substrate, 100 μl of universal buffer (pH 9) and 20 μl of enzyme extract. The mixture was incubated for 120 min at 30 °C. Then, 150 μl of 10% trichloroacetic acid (TCA) was added to the reaction mixture for termination of the reaction. Precipitation was achieved by cooling at 4 °C for 5 min and the mixture was centrifuged at 13,000 rpm for 10 min. The peptides liberated from hemoglobin were estimated using Folin-Phenol reagent at 630 nm [8].

2.5.6. Determination of the specific protease assay

2.5.6.1. Serine proteinases. Activities of the three subclasses of serine proteases including trypsin, chymotrypsin and elastase were determined using a 1 mM concentration of BApNA (Nα-abenzoyl-L-arginine-*p*-nitroanilide), 1 mM of SAAPFpNA (*N*-succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide), and 1 mM of SAAApNA (*N*-succinyl-alanine-alanine-alanine-*p*-nitroanilide) as the substrates, respectively. Briefly, 5 μl of enzyme, 5 μl of each substrate and 35 μl of Tris-HCl buffer (20 mM, pH 8) were incubated for 10 min at 30 °C. Then, the reaction was stopped by adding trichloroacetic acid (30%) and the absorbance was read at 405 nm. In this assay, a negative control (to prove specific proteolytic activity) was provided for each substrate separately including all the mentioned components except for the enzyme pre-boiled at 100 °C for 30 min [17].

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