Effects of a lectin from *Polygonum persicaria* L. on *Pieris brassicae* L. (Lepidoptera: Pieridae)

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Abstract: In the current study, the entomotoxic effects of a lectin, extracted from *Polygonum persicaria*, were determined on *Pieris brassicae* larvae. The purified molecule significantly decreased larval survival, weight, and mass protein. Different concentrations of *P. persicaria* agglutinin (PPA) (0.5, 1 and 2 mg/ml) significantly decreased nutritional indices including: the efficiency of conversion of ingested food (ECI), the efficiency of conversion of digested food (ECD), and approximate digestibility (AD) in a dose-dependent manner. There was a significant decrease in larval α-amylase activity after larval fed on PPA. The decreased activity was found in both the biochemical assessment and gel electrophoresis; sharpness of one isozyme decreased and another completely disappeared. Meanwhile, PPA significantly decreased activities of glucosidases mainly β-glucosidase. Fed of *P. brassicae* larvae on different concentrations of PPA, significantly decreased activity of TAG-lipase in both the biochemical assessment and gel electrophoresis. It was found that alkaline phosphatase (ALP) and acid phosphatase (ACP) were also affected by PPA but concentrations of 1 and 2 mg/ml showed statistical differences compared to the control. General and specific proteolytic activities statistically decreased after larvae fed on different concentrations of PPA. Results of our study could bring attention to this molecule, showing it to be a safe and efficient control of the pest worldwide.

Key words: entomotoxic effect, lectin, *Pieris brassicae*, *Polygonum persicaria*

Introduction

Lectins are heterogeneous proteins of non-immune origin and with at least one non-catalytic domain. Lectins are able to specifically recognise carbohydrates (Carlini and Grossi-de-Sa 2002). These molecules could reversibly bind to carbohydrates without altering their covalent structure (Pusztai and Bardocz 2009). Lectins have been extensively distributed in nature. Their presence has been noted in plants, viruses, bacteria, invertebrates, and vertebrates (Carlini and Grossi-de-Sa 2002). These molecules may have several functions in living organisms but the entomotoxic properties of plant lectins are important in pest control strategies (Michiels *et al*. 2010). In fact, the majority of plant lectins bind to O- and N-glycans of animal glycoconjugates. This means that lectins are supposed to play a part in plant defense against plant-eating (phytophagous) invertebrates or higher vertebrates (Peumans and Van Damme 1995). Certain plant tissues, such as seeds, bark, and bulbs, contain high lectin concentrations which might indicate that lectins play a role as storage proteins (Michiels *et al*. 2010). Lectins extracted from different plant sources exhibit a considerable degree of structural similarity but also considerable differences in their carbohydrate-binding specificities (Carlini and Grossi-de-Sa 2002). Decades of research have led to the classification of plant lectins into twelve lectin families (Van Damme *et al*. 2008). Several phenomena induce expression of lectin including: salt stress, pathogen infection, jasmonic acid treatment, and insect herbivory (Michiels *et al*. 2010).

Comprehensive studies have been carried out demonstrating entomotoxic properties of plant lectins toward a wide range of insects such as Lepidoptera, Hemiptera, and Coleoptera (Carlini and Grossi-de-Sa 2002). A lectin extracted from the snowdrop, *Galanthus nivalis* L., has been shown to be very effective against several species of Lepidoptera and Hemiptera (Tinjuangjun *et al*. 2000; Vasconcelos and Oliveira 2004). Although their mode of action has not been fully understood, studies have shown binding of lectins to epithelial cells and the peritrophic membrane of insects. Even, immunohistochemical analyses revealed the presence of lectins in the hemolymph, ovaries, and fat bodies of insects that were fed lectins (Sadeghi *et al*. 2008). Because of these considerable findings, the current study was conducted to extract and purify a lectin from *Polygonum persicaria* L. to determine its effects on the survival and nutrition of *Pieris brassicae* L. (Lepidoptera: Pieridae). *P. persicaria* is a summer annual weed of horticultural and nursery crops that is originally native to Europe (Simmonds 1945). *P. persicaria* has various medicinal effects on fever, blood sugar, and diarrhea (Simmonds 1945). Additionally, *P. brassicae* known as the large cabbage white butterfly, is a major agricultural pest throughout Europe, North Africa, and Asia causing severe damage to Brassicaceae as well as many other fami-

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lies of plants. The objectives of the current study were (i) extraction and purification of lectin from *P. persicaria*, (ii) to find out the effects of lectin on larval survival and weight, and (iii) to determine the effects of lectin on the digestive physiology of *P. brassicae*.

Materials and Methods

Insect rearing

Eggs of *P. brassicae* were collected from Canola fields of Baboland, and transferred to a laboratory. The laboratory had the following conditions: a temperature of 25°C, 85% humidity, and 16 : 8 h light : dark.

Preparation of Sepharose 4B-Galactose column

Sepharose 4B-Galactose column was prepared based on a method described by Bulgakov *et al*. (2004). Briefly, 20 ml of Sepharose 4B was suspended in 40 ml of 0.5 M Na_2CO_2 (pH of 11.0), 2 ml divinylsulphone was added to the suspension. The mixture was then incubated for 70 min at room temperature and during this time the mixture was gently shaken. An addition of 500 mg galactose in 50 ml 0.5 M Na_2CO_2 (pH of 11.0) was added to the mixture and incubation continued for 12 h more. The prepared sorbent was washed with water, the unbound arm was blocked with β-mercaptoethanol-containing buffer, and finally it was packed into a 1.5 × 30 cm column. The sorbent was equilibrated with Tris-HCl (pH of 7; 0.1 M) and used for the affinity purification of *P. persicaria* agglutinin (PPA).

Purification of PPA

For approximately 72 h, the stems of *P. persicaria* were incubated in phosphate buffer (0.1 M, a pH of 7.1), at 4°C. Then, the stems were ground in the buffer to completely destroy the tissue prior to an additional 24 h incubation. The mixture was filtrated using a layer of cheesecloth, then it was centrifuged at 5,000 rpm for 20 min. The remaining debris was removed by passing the supernatant through filter paper (Whatmann No. 4) (Hamshou *et al*. 2010). Supernatant was precipitated by a 0–60% concentration of ammonium sulfate and centrifuged at 5,000 rpm for 20 min. Debris was eluted in Tris-HCl buffer (0.1 M, a pH of 7) and dialysed in the same buffer overnight (de Oliveira *et al*. 2011). Dialysed samples were loaded into Sepharose 4B-Galactose column equilibrated with Tris-HCl buffer (0.1 M, a pH of 7) as affinity chromatography. The affinity column was washed with Tris-HCl buffer, and buffer containing 20 mM 1,3-diaminopropane (DAP) (Hamshou *et al*. 2010). Fractions showing the highest protein content were pooled and used for the forthcoming step. The lectin fractions obtained after the first affinity chromatography, were loaded on DEAE-Cellulose fast flow equilibrated with DAP (Hamshou *et al*. 2010). Finally, the lectin was eluted using Tris-HCl (0.1 M, a pH of 7.0) containing 0.5 M NaCl after DAP washing. Again, the samples showing the highest protein content were dialysed against water, and purity was analysed by SDS-PAGE stained with commassie brilliant blue (Laemmli 1970).

Hemagglutinin assay

The experiment was performed in microtiter plates, according to Correia and Coelho (1995). The peaks from chromatography (50 µl) were two-fold serially diluted in 150 mM of NaCl before adding a 50 µl 2.5% (v/v) suspension of cow erythrocytes. The titer was expressed as the highest dilution exhibiting hemagglutination.

Feeding treatment

Leaves of *Raphanus sativus* L. were soaked in three concentrations of PPA (0.5, 1 and 2 mg/ml as suggested in various papers). The control leaves were immersed in distilled water. Then, newly hatched larvae of *P. brassicae* were allowed to be fed on leaves until the fifth larval instar to measure biomass, survival nutritional indices, and activity of digestive enzymes. Several nutritional parameters were compared in the treated and control larvae. These indices were calculated as described by Scriber and Slansky (1982) as: the efficiency of conversion of ingested food (ECI) which estimates the percentage of ingested food that is converted to biomass and it was calculated as:

$$[\text{biomass gained (mg fresh mass)}/ \\ /\text{food ingested (mg dry mass)}] \times 100.$$

The efficiency of conversion of digested food (ECD) is an estimate of the efficiency which digested food is converted to biomass, and was calculated as:

$$\text{biomass gained (mg fresh mass)}/ \\ /[\text{food ingested (mg dry mass)} - \text{feces (mg dry mass)}] \times 100.$$

Approximate digestibility (AD) is an estimate of the amount of ingested food that is digested, and it was calculated as:

$$[\text{food ingested (mg dry mass)} - \text{feces (mg dry mass)}]/ \\ /\text{food ingested (mg dry mass)} \times 100.$$

Finally, metabolic cost (MC) was calculated as: 100 – ECD.

Fifteen larvae in three replicates were used for each treatment, and living larvae were dissected for digestive enzyme measurements.

Sample preparation

Larval bodies were separately cut. The midgut appeared after the fat bodies and other organs were removed. The midguts were separated from the insect body in saline solution (NaCl, 10 mM), rinsed in ice-cold distilled water, placed in a pre-cooled homogeniser, and ground before centrifugation. Equal portions of larval midgut and distilled water were used so as to have a desirable concentration of the enzymes (w/v). Homogenates were separately transferred to 1.5 ml centrifuge tubes and centrifuged at 13,000 rpm for 20 min at 4°C. The supernatants were pooled and stored at −20°C for subsequent analyses.

α-Amylase assay

The method described by Bernfeld (1955) was used to assay α-amylase activity. Ten microlitres of the enzyme were incubated for 30 min at 35°C with 50 µl of phosphate buffer (0.02 M, pH 7.1) and 20 µl of 1% soluble starch as substrate. The reaction was stopped by the addition of 80 µl dinitrosalicylic acid (DNS) and heated in boiling water for 10 min prior to reading the absorbance at 545 nm. One unit of α-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min at 35°C. The negative control contained all reaction mixtures with the pre-boiled enzyme (for 15 min) to prove the presence of the enzyme in the samples.

TAG-lipase assay

The enzyme assay was carried out as described by Tsujita *et al*. (1989). Twenty µl of gut extract and 40 µl of *p*-nitrophenyl butyrate (27 mM) as substrate were incorporated using 100 µl of universal buffer (10 mM, pH 11), mixed thoroughly and incubated at 37°C. After 1 min, 100 µl of NaOH (1 M) were added to each tube (the control and treatment) and absorbance was read at 405 nm. One unit of enzyme will release 1.0 nmol of *p*-nitrophenol per min at a pH of 7.2 and 37°C when *p*-nitrophenyl butyrate is used as the substrate. A standard curve of *p*-nitrophenol was used to calculate the specific activity of the enzyme.

General proteases assay

Cohen's method (1993) was used to assay the general proteolytic activity in the midgut of *P. brassicae* fed on different concentrations of *Rhizoctonia solani* agglutinin (RSA), by using hemoglobin (20 mg/ml) as the substrate. The hemoglobin solution (50 µl) was added to 100 µl of universal buffer solution (pH of 9), and incubation at 30°C was initiated after the addition of 20 µl of enzyme solution, for 120 min. For the termination of proteolysis, 150 µl of 10% trichloroacetic acid (TCA) was added to the reaction mixture. Precipitation was achieved by cooling at 4°C for 45 min. The reaction mixture was centrifuged at 13,000 rpm for 10 min. The blank solution contained all the mentioned portions except for the enzyme. The peptides liberated from hemoglobin were estimated using Folin-phenol reagent at 630 nm (Folin and Ciocalteu 1927).

Determination of the specific proteases presence

Serine proteinases

Trypsin-, chymotrypsin- and elastase-like activities (as three subclasses of serine proteinases) were assayed using a concentration of 1 mM of BApNA (Nα-abenzoyl-L--arginine-*p*-nitroanilide), 1 mM SAAPPpNA (N-succinyl--alanine-alanine-proline-phenylalanine-*p*-nitroanilide), and 1 mM SAAApNA (N-succinyl-alanine-alanine-alanine-*p*-nitroanilide) as substrates, respectively. The reaction mixture included 35 µl of Tris-HCl buffer (20 mM, pH 8 as the literature-recommended pH for saerines), 5 µl of each mentioned substrate and 5 µl of enzyme

solution. The reaction mixture was incubated at 30°C for 0–10 min before adding 30% TCA to terminate the reaction. The absorbance of the resulting mixture was then measured spectrophotometrically at 405 nm by *p*-nitroaniline release. To prove the specific proteolytic activity, a negative control was provided for each substrate separately, containing all the mentioned components except for the enzyme pre-boiled at 100°C for 30 min (Oppert *et al*. 1997).

Exopeptidases

Activities of the two exopeptidase in the midgut of *Andrallus spinidens* Fabricius were obtained by using hippuryl-L-arginine and hippuryl-L-phenylalanine for amino- and carboxypeptidases, respectively. The reaction mixture consisted of 35 µl of Tris-HCl buffer (a pH of 7 as the literature recommended pH for exopeptidases), 5 µl of each mentioned substrate, and 5 µl of the enzyme solution. The reaction mixture was incubated at 30°C for 0–10 min before adding 30% TCA to terminate the reaction, and was read at 340 nm. To prove the specific proteolytic activity, a negative control was provided for each substrate separately, containing all the mentioned components except for the enzyme pre-boiled at 100°C for 30 min (Oppert *et al.* 1997).

Zymogram analyses of digestive enzymes

Zymogram analyses were carried out to find the effect of different concentrations of PPA on digestive enzymes. The enzyme activities were performed by non-denaturing polyacrylamide gel electrophoresis (PAGE) described by Laemmli (1970). Native-PAGE was performed in a 12% (w/v) separating gel and a 4% stacking gel. The electrode buffer was prepared based on the method of Laemmli (1970). The sample buffer contained 25% stacking buffer [0.5 M/l Tris-HCl (pH 6.8)], 20% glycerol, and 0.005% (w/v) bromophenol blue. Electrophoresis was conducted at room temperature under voltage (100 V) until the blue dye reached the bottom of the slab gel.

α-Amylase zymogram

The method described by Campos *et al*. (1989) was used to visualise the amylolytic activity in samples. To prepare the gel for α-amylase assay, the gel was rinsed with water and washed by shaking gently with 1% (v/v) Triton X-100 in phosphate buffer containing 2 mmol/l CaCl$_2$ and 10 mmol/l NaCl, for 1.5 h. The gel was then rinsed with water and treated with a solution of 1.3% I2, 3% KI to stop the reaction and to stain the unreacted starch background. Zones of α-amylase activity appeared as light bands against a dark background.

TAG-lipase zymogram

Zymogram analysis of lipase was carried out by using a 12% resolving and 4% stacking gel. After loading the samples, the gel was run at 4°C and with a constant voltage of 100 V. The gel gently separated from the glasses

and the gel was immediately immersed in 5 mM of 4-methylumbellipheryl-butyrate solution as fluorescent substrate. After 30 min, the gel was put on a UV trans-illuminator to observe the white bands in the dark background (Zibaee 2011).

Proteolytic zymogram

Electrophoretic detection of proteolytic enzymes was performed according to the method described by Garcia-Carreno *et al*. (1993). Non-reducing sodium dodecyl sulphate PAGE (SDS-PAGE) was carried out at 4°C by using gels containing 0.5% gelatin. When the dye reached the end of the glass, the gel was gently removed, washed with distilled water, and immediately fixed and stained overnight with 0.1% Coomassie brilliant blue R-250 in methanol-acetic acid-water (50 : 10 : 40). Destaining was done in methanol-acetic acid-water (50 : 10 : 40).

Protein assay

Protein concentrations were assayed according to the method described by Lowry *et al*. (1951). In this method, oxidation of peptide bonds are made using Folin-Ciocalteu reagent. Cu^{2+} is a recruit in the reaction. The absorbance is read at 545 nm (recommended by Ziest Chem. Co., Tehran-Iran).

Statistical analysis

All data obtained from a complete randomised design were compared by one-way analysis of variance (ANOVA) followed by Tukey's test when significant differences were found at $p \leq 0.05$. Differences between samplings were considered statistically significant at a probability less than 5%. The differences are marked in the figures and tables by letters.

Results and Discussion

Effects of PPA on larval survival and larval mass

Different concentrations of PPA significantly decreased larval survival and larval mass in a dose-dependent manner (Fig. 1). Larval survival was reduced to lower than 30% by feeding on 2 mg/ml of PPA (Fig. 1; Pr > F: 0.0001, F = 65.08). Also, the weight of the treated larvae decreased 12.5% compared to the control larvae. The amount of mass protein was found to be half of that of the control larvae (Fig. 1; Pr > F: 0.0002, F = 32.19). These results were accompanied by the morphology of the treated larvae versus the control (Fig. 2). Macedo *et al*. (2003) reported toxicity of a lectin from *Koelreuteria paniculata* Laxm that reduced larval weight by 84%. Coelho *et al*. (2007) observed that larvae of *Anagasta kuehniella* Zeller (Pyralidae) fed on *Annona coriacea* Mart. lectin had up to a 50% lower mass. In a comprehensive study, Macedo *et al*. (2007) found that leaf lectin from *Bauhinia monandra* Kurz had anti-insect activity against *A. kuehniella*, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelliade). The details are: a diet containing 1% of lectin decreased larval weight of *A. kuehniella* to 40% while concentrations of 0.5% and 0.4% caused a 20% and 50% mass loss of *Z. subfasciatus* and *C. maculatus*, respectively. De Oliveira *et al*. (2011) demonstrated a 0.25 mg de-
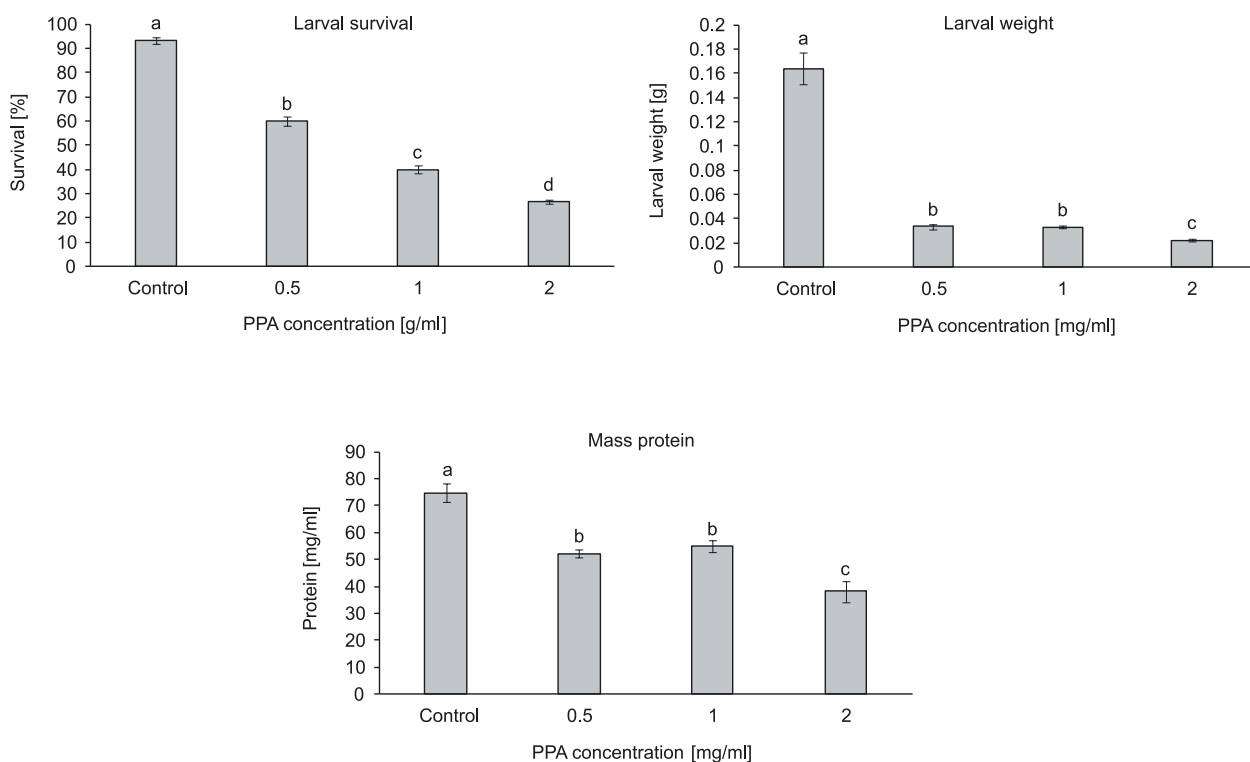

Fig. 1. Effects of PPA on larval survival, weight, and mass protein of *P. brassicae*. Statistical differences have been marked by different letters (Tukey's test, $p \leq 0.05$)
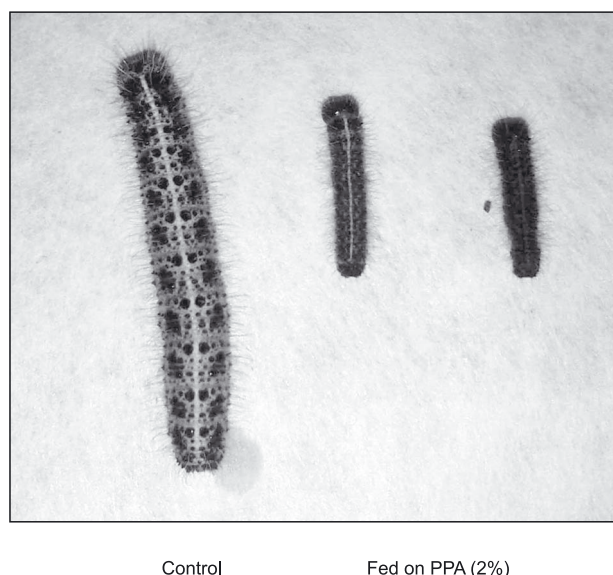
Control   Fed on PPA (2%)

Fig. 2. Effect of PPA (2%) on larvae of *P. brassicae*

crease in the mass of *A. kuehniella* after feeding on *Moringa oleifera* Lam. lectin. Ramzi *et al.* (2013) reported that feeding of *Ectomyelois ceratonia* Zeller (Lepidoptera: Pyralidae) in different concentrations of a lectin from *Citrullus colocynthis* L. caused morphological abnormality in the pupae and decreased survival of larvae to 66.6%. It has been shown, that plant lectins have severe effects on fecundity, development, and survival of insects (Michiels *et al.* 2010). Although significant differences have been obtained, a peculiar specificity of lectins as insect pest management molecules can be illustrated. Powell *et al.* (1995) showed a dose-dependent effect of a lectin from *Galanthus nivalis* L. (GNA), on honeydew production of *Nilaparvata lugens* Stal (Hemiptera: Delphacidae). Sauvion *et al.* (1996) reported a higher mortality of *Myzus persicae* (Schulzer) nymphs after feeding on artificial diets containing GNA, *Narcissus pseudonarcissus* L. lectin (NPA), *Allium sativum* L. agglutinin (ASA, garlic), or Concanavalin A (ConA, jack-bean *Canavalia ensiformis*) in comparison with the control. Caterpillars of *Lacanobia oleracea* (Linnaeus, 1758) fed on a GNA-containing artificial diet or transgenic plant showed decreased gains in biomass and longer instar durations (Fitches *et al.* 1997). Since, lectins have different specificities, they could interact with various glycoproteins or glycan structures of tissues. This means that lectins could interfere in the physiological processes of insects leading to a reduction in larval survival and biomass.

Effects of PPA on nutritional indices

Larvae fed on different concentrations of PPA significantly affected nutritional indices. In treated larvae, ECI statistically decreased so that all treatments gained the same statistical level (Table 1; Pr > F: 0.0084, F = 8.08). Although the same results were obtained in the case of ECD, the highest statistical difference of AD was observed between the control and larvae fed on PPA treated leaves (Table 1; Pr > F: 0.0001, F = 83.08; Pr > F: 0.0001, F = 43.96). The results of MC revealed a two-fold metabolic cost imposed on larvae fed on PPA in all of the concentrations (Table 1; Pr > F: 0.0001, F = 81.92). These results showed that loss of larval mass and survival may be attributed to the reduction of nutritional parameters. Moreover, AD results indicate a longer retention of food in the gut, more demand for nutrients, and compensation for the deficiency in the food stuff conversion by diverting energy from biomass production into detoxification. As the proportion of digested food metabolised for energy increases, there was also a decrease in ECD (de Oliveira *et al.* 2011). The lower values of ECI and ECD along with increased concentrations of PPA, indicate chronic toxicity of PPA and the reduction in ECD. These findings are probably the result of an increase in the proportion of assimilated energy diverted from growth, to cover MC associated with detoxification and excretion of RSA (de Oliveira *et al.* 2011).

Effect of PPA on digestive enzyme activity

Digestive enzyme activities significantly decreased in the larvae fed on PPA, in both biochemical assays and native-PAGE. Activity of α-amylase significantly decreased in the larvae fed on 1 and 2 mg/ml of PPA so that significant changes were observed in isoenzyme presence and sharpness (Fig. 3; Pr > F: 0.0006, F = 18.83). Although activities of both α- and β-glucosidases statistically decreased in the treated larvae, reduction of β-glucosidases was more obvious than α-glucosidase (Fig. 4; Pr > F: 0.0008, F = 16.9; Pr > F: 0.0004, F = 20.87). PPA had the lowest effects on the enzymes involved in lipid digestion including TAG-lipase, acid phosphatase (ACP) and alkaline phosphatase (ALP), so that a concentration of 2 mg/ml had the highest effect on the enzymatic activities (Fig. 5 and 6; Pr > F: 0.0018, F = 18.34; Pr > F: 0.0002, F = 25.93; Pr > F: 0.0008, F = 23.72). General and specific proteolytic activities in the midgut of *P. brassicae* larvae were significantly decreased by PPA (Fig. 7; Table 2). In all assayed

Table 1. Effects of PPA concentrations on nutritional indices of *P. brassicae* larvae

Treatment	ECI	ECD	AD	MC
The control	51.63±12.09 a	62.95±1.02 a	93.36±0.047 a	37.04±0.34 b
PPA (0.5%)	15.86±2.67 b	25.98±2.79 b	91.46±1.97 a	74.01±1.22 a
PPA (1%)	16.63±1.75 b	31.59±7.51 b	83.88±2.81 b	68.40±7.30 a
PPA (2%)	15.02±1.30 b	24.20±6.14 b	78.84±3.33 c	75.79±3.39 a

Statistical differences are shown by various letters (Tukey's test, p ≤ 0.05)

ECI – the efficiency of conversion of ingested food; ECD – the efficiency of conversion of digested food; AD – approximate digestibility; MC – metabolic cost
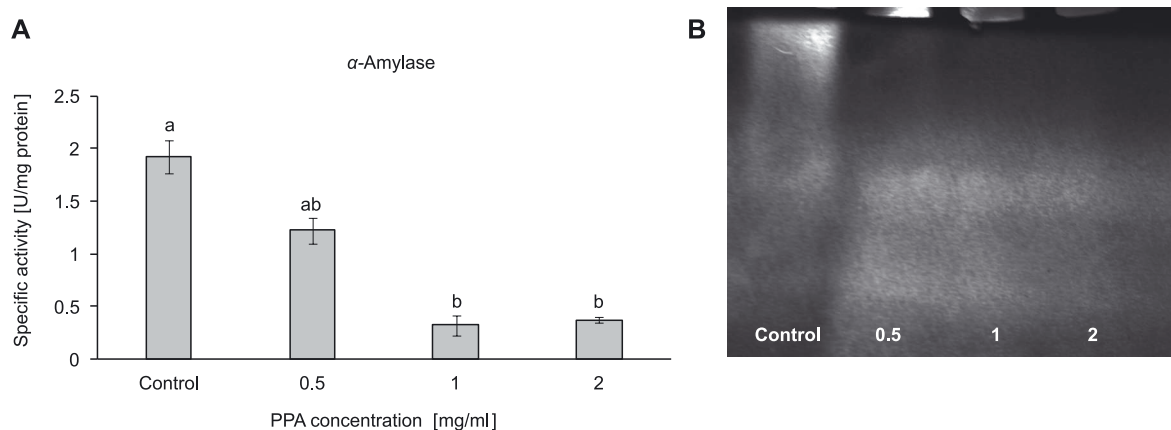
Fig. 3. Effects of PPA on *α*-amylase activity in *P. brassicae* larvae, by biochemical assessment (A) and native-PAGE (B). Statistical differences are shown by various letters (Tukey's test, $p \leq 0.05$)
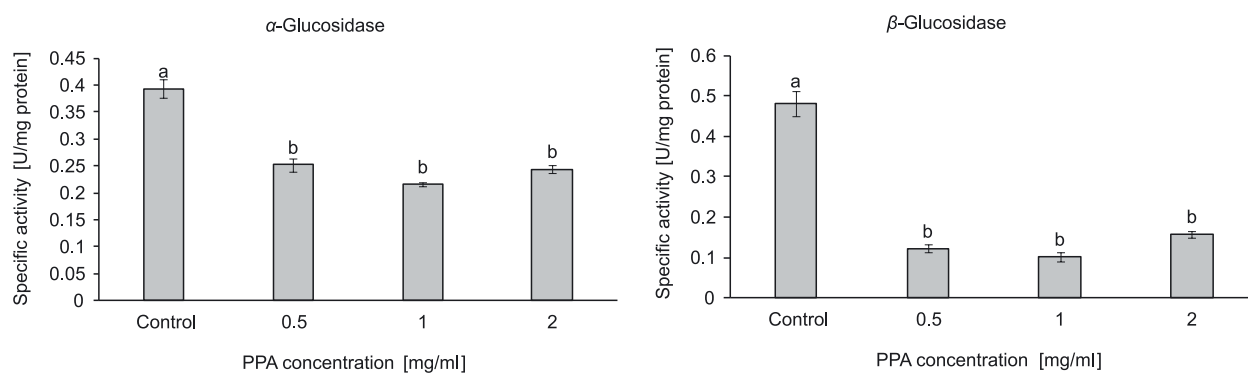

Fig. 4. Effects of PPA on *α*- and *β*-glucosidase activities in *P. brassicae* larvae. Statistical differences have been shown by various letters (Tukey's test, $p \leq 0.05$)
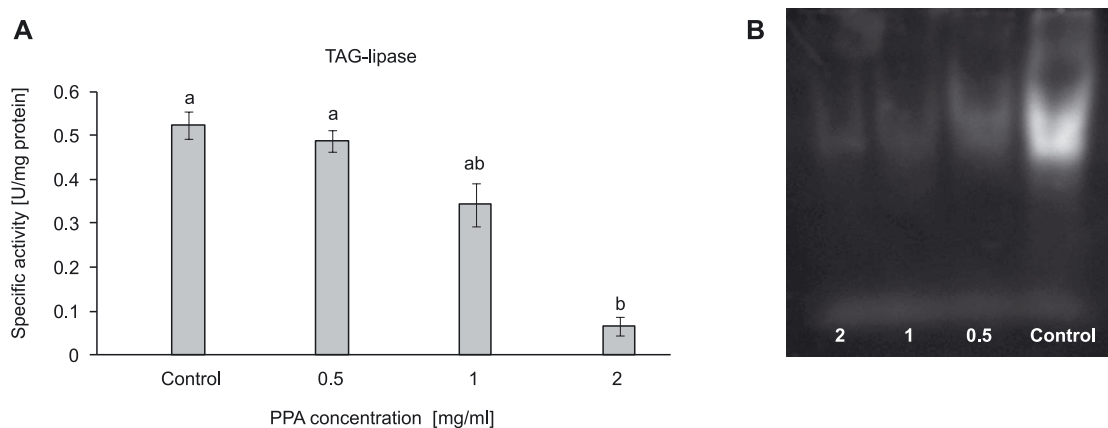

Fig. 5. Effects of PPA on TAG-lipase activity in *P. brassicae* larvae, by biochemical assessment (A) and native-PAGE (B). Statistical differences are shown by various letters (Tukey's test, $p \leq 0.05$)

specific proteases, all concentrations of PPA significantly decreased specific proteolytic activities except for aminopeptidase (Table 2). Among the treated values, no statistical differences were observed except for chymotrypsin in which concentrations of 1 and 2 mg/ml showed more of a reduction (Table 2). Ramzi *et al*. (2013) reported that *E. ceratoniae* larvae fed diets containing *C. colocynthis* lectin, decreased digestive enzymatic activities in the treated larvae in comparison with the control, except for TAG-lipase. The authors reported that higher concentrations

of lectin decreased band sharpness and some isoenzymes of all assayed enzymes disappeared (Ramzi *et al*. 2013). It has been shown, that lectins are highly resistant to proteolysis so they can bind to insect proteins (e.g. digestive enzymes). This mainly happens in the gut, which leads to being retained in the insect's body or excreted in honeydew or feces (Michiels *et al*. 2010). Since lectins were exposed to insects via feeding, receptors of the alimentary tract might be the first binding locations in the insect's body. Lectins are able to pass through the gut
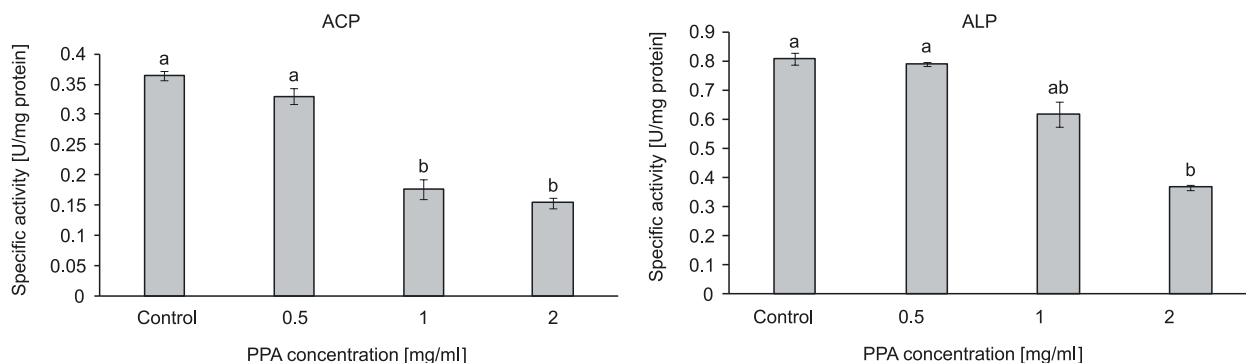
Fig. 6. Effects of PPA on acid phosphatase (ACP) and alkaline phosphatase (ALP) activities in *P. brassicae* larvae. Statistical differences are shown by various letters (Tukey's test, p ≤ 0.05)
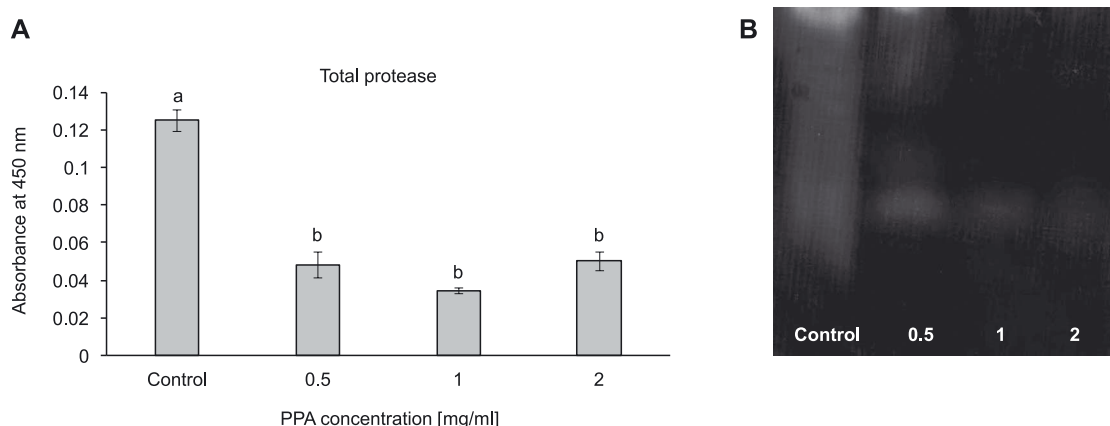

Fig. 7. Effects of PPA on general proteolytic activity in *P. brassicae* larvae, by biochemical assessment (A) and native-PAGE (B). Statistical differences are shown by various letters (Tukey's test, p ≤ 0.05)

Table 2. Effects of PPA concentrations on specific proteolytic activities of *P. brassicae* larvae

Treatment	Trypsin	Chymotrypsin	Elastase	Aminopeptidase	Carboxypeptidase
Control	0.38±0.013 a	0.25±0.011 a	0.30±0.008 a	0.50±0.011 a	0.68±0.013 a
PPA (0.5%)	0.22±0.007 b	0.134±0.036 b	0.104±0.023 b	0.43±0.031 a	0.481±0.044 b
PPA (1%)	0.186±0.014 b	0.031±0.008 c	0.016±0.005 b	0.50±0.016 a	0.477±0.007 b
PPA (2%)	0.19±0.016 b	0.064±0.008b c	0.041±0.017 b	0.51±0.022 a	0.35±0.008 b

Statistical differences are shown by various letters (Tukey's test, p ≤ 0.05)

epithelium barrier, hence, a whole new set of candidate receptors may come into focus (Michiels *et al*. 2010). Histofluorescence studies on the midguts of *Acyrthosiphon pisum* Harris, 1776 fed *Sclerotinia sclerotiorum* (Lib.) de Bary, (1884) lectin, revealed the brush border zone of the midgut as the primary target for *S. sclerotiorum* agglutinin (SSA). Meanwhile, exposure of insect midgut CF-203 cells to 25 mg/ml SSA, decreased cell viability. It means that lectin could bind to membrane proteins, and initiate signaling transduction cascade which can lead to death of the midgut epithelial cells (Hamshou *et al*. 2010). Since digestive enzymes are secreted by the epithelial cells of the midgut, any decrease in their number definitely negatively affects digestive enzyme secretions and activity. It is supposed that PPA also binds to these cells, kills them, and leads to a lower activity of the digestive enzymes due to their lower amounts in comparison with the control.

Conclusions

Although the effects of plant lectins on insects are somehow complex, they cause mortality and lower fecundity which are two crucial factors for the population growth of pests. If PPA showed its great potential to decrease the physiological performance of *P. brassicae*, a friendly environmental control procedure may be promoted. Before achieving this objective, it is necessary to determine the biochemical and genomic interactions between PPA and *P. brassicae*. As an example, gene expressions of digestive enzymes and proteins involved in reproduction must be elucidated to have a better perspective of the PPA effects.

Acknowledgements

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