In Vitro and In Vivo Effects of α-Amylase Inhibitor From Avena sativa Seeds on Life History and Physiological Characteristics of Sitotroga cerealella (Lepidoptera: Gelechiidae)

Ehsan Borzou, Gadir Nouri-Ganbalani, and Bahram Naseri

1Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran and 2Corresponding author, e-mail: bnaseri@uma.ac.ir

Subject Editor: Christos Athanassiou

Received 26 April 2017; Editorial decision 15 September 2017

Abstract

The inhibitory effects of Avena sativa L. seed extract were studied on life history and some physiological aspects of Sitotroga cerealella (Olivier; Lepidoptera: Gelechiidae). The inhibition of α-amylase activity in vitro by A. sativa proteinaceous extract suggested its potential antinutritional effects on S. cerealella larvae. Although, chronic ingestion of A. sativa inhibitor (I10: 0.108 mg protein/artificial seed) did not show significant reduction of the growth and development of S. cerealella. However, a delay in the developmental time of immature stages was detected when S. cerealella larvae were continuously fed on I50 and I10 concentrations (0.429 and 1.11 mg protein/artificial seed, respectively) of the inhibitor. The highest realized fecundity was recorded for females which came from larvae fed on I10 concentration (102.46 ± 2.50 eggs/female), and the lowest fecundity was observed for females which came from larvae fed on I30 and I50 concentrations (0.429 and 1.11 mg protein/artificial seed, respectively) of the inhibitor. The lightest weight of pupae of S. cerealella was observed on I50 concentration (50.00 ± 3.53 and 289.57 ± 29.00 µg/pupa, respectively). The lower survival rate of pupae at low temperature indicated that S. cerealella fed on I10 concentration of the inhibitor was less cold tolerant than control insects. The inhibitory studies indicated that A. sativa proteinaceous extract is a good candidate as an inhibitor of the α-amylase of this pest. This inhibitor can be expressed in genetically engineered plants to confer resistance to S. cerealella.

Key words: α-amylase inhibitor, cold tolerance, energy reserve, life span, the Angoumois grain moth

The Angoumois grain moth, Sitotroga cerealella (Olivier; Lepidoptera: Gelechiidae), is a gelechiid moth active in throughout the world and has become a serious pest of wheat (Triticum aestivum L.; Poales: Poaceae) and other grains (Shukle and Wu 2003, Hamed and Nadeem 2012). Initial infestation of grain seeds to S. cerealella occurs in the field just before harvest and it is carried into store where the population builds up rapidly (Weston and Rattlingourd 1999). The larvae of S. cerealella cause serious damage to kernals by feeding on them and producing feces (Shukle and Wu 2003).

The main method for control of stored-product insect pests is based on the use of environmentally toxic agrochemicals (Hagstrum and Subramanyam 1996, Haq et al. 2004). The use of insecticides to control of insect pests can lead to environmental risks, contamination of operators during handling period, and selection of resistant insects (Pereira et al. 2006, Fouad et al. 2013). Therefore, the development of environmentally-friendly agriculture is one of the main goals of researchers in integrated pest management programs.

Alpha-amylase (α-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) is an important digestive enzyme distributed in plants, animal tissues and micro-organisms. This enzyme converts starch to maltose, which is then hydrolyzed to glucose by α-glucosidase (Kazzazi et al. 2005, Mohammadzadeh and Izadi 2016). Stored-product insects like Trogoderma granarium (Everts; Coleoptera: Dermentidae), Tenebrio molitor (L.; Coleoptera, Tenebrionidae), Prostephanus truncates (Horn; Coleoptera: Bostrychidae) and S. cerealella are extensively starch dependent species and require α-amylase for their development and survival (Mendiola-Olaya et al. 2000; Dastrandj et al. 2013; Borzou et al. 2015, 2017). Therefore, the inhibition of this enzyme can be targeted in the development of new insecticidal technologies (Silva et al. 2001).

Plant α-amylase inhibitors (α-AIs) have been extensively studied in the past as they may play an important role in host-plant resistance to insect herbivores and pathogens (Pelegreti et al. 2008, Mehrabadi et al. 2012). They are defensive molecules produced by
plants both constitutively and in response to wounding (Franco et al. 2002, Oppert et al. 2004). The potential direct detrimental effects of α-AIs on insect herbivores are from their role as ‘digestibility reducers’ (Franco et al. 2005). α-AIs are capable of interfering with carbohydrate digestion by binding tightly to the active site of α-amyloses, complex formation being essentially irreversible. When the ability to utilize the ingested starch and to recycle the digestive enzymes is decreased by plant inhibitors, nutrition of the insect is impaired affecting its growth, survival, and fecundity (Piasek-Światkowska et al. 2007).

Feeding assay, where α-AI is incorporated in a diet, provides valuable information about the type of the inhibitor that has potential as resistance factors in genetic manipulation and plant breeding programs. Morton et al. (2000) showed that the introduction and expression of the bean α-AI gene into transgenic peas significantly reduced damage caused by Bruchus pisorum (L.; Coleoptera: Bruchidae). Naseri and Borzouei (2016) showed that wheat α-AIs have some detrimental effects on larval development of T. granarium at concentration occurring naturally in seeds. Borzouei et al. (2017) reported α-amylase inhibition of S. cerealella in vitro by proteinaceous extracts of different grains including barley, maize, rye, sorghum, triticale and wheat.

In the present study, we described the bioinsecticidal activity of Avena sativa L. (Poaceae) proteinaceous extract towards the growth and development of S. cerealella when incorporated in artificial seeds, as well as physiological reactions of this pest such as digestive enzymes activity and cold tolerance. The results could provide the basis for the selection of α-AI and present an optimized value for developing transgenic grains resistant to S. cerealella.

Materials and Methods

Insect Culture

A culture of S. cerealella was originally obtained from stored maize seeds from Ardabil, Iran, and was maintained at 25°C, 65% relative humidity with a 16-h day length as described by Borzouei and Naseri (2016). The insects were reared into plastic containers (diameter 15 cm, depth 6 cm) with a hole covered by a 50 mesh net for ventilation, containing the wheat seeds (cultivar Bam) for five generations. In each generation, S. cerealella adults were transferred to plastic funnel that was covered by a net cloth for collecting eggs. The sixth generation was used for the experiments.

Preparation of Enzyme Sample

Fourth instar larvae of S. cerealella were anaesthetized on ice; their guts were dissected over ice and homogenized immediately in ice-cold solution of 10 mM NaCl using a pre-cooled homogenizer (Teflon pestle). The crude midgut homogenate was centrifuged at 12,000 x g at 4°C for 15 min. The clear supernatant was transferred to a pre-chilled Eppendorf tube and stored at −20°C for subsequent use.

Extraction of A. sativa α-AI

α-AI from seeds of A. sativa was extracted according to Baker (1987) and Melo et al. (1999). In brief, dry mature seeds were ground for 5 min using mixer-blender to make a fine powder, and passed through a 60 mesh sieve. Thereafter, 30 g of powdered seeds was mixed with a solution of 0.1 M NaCl and stirred for 2 h, followed by centrifugation at 8,000 x g at 4°C for 30 min. The ammonium sulphate concentration was increased to 70% followed by centrifugation at 8,000 x g for 30 min. Pellet, containing the rich fractions of α-AI, was dissolved in ice-cold phosphate buffer (0.02 M, pH 7) and dialyzed overnight against distilled water. The dialyzed solution was then incubated at 70°C for 20 min to inactivate major endogenous enzymes and centrifuged at 7,500 x g for 15 min. Supernatant was taken and used as a source of inhibitor for the inhibition assays.

The In Vitro Effect of A. sativa α-AI

The ability of A. sativa inhibitor to inhibit the larval amylolytic activity of fourth instar S. cerealella was quantitatively estimated by incubating midgut extract with different concentrations of the semi-purified proteinaceous inhibitor (1.8, 0.90, 0.45, 0.22 and 0.11 mg/ml). Twenty microliters of proteinaceous inhibitor were pre-incubated with 20 μl enzyme at 37°C for 10 min. Then, amylolytic activity was measured by the Bernfeld method in 20 mM glycine-NaOH buffer, pH 11.5, containing 20 mM NaCl and 0.1 mM CaCl₂ (Bernfeld 1955). For the control group, the same enzyme extract was used without inhibitor. The inhibition percentage of the amylolytic activity was calculated according to Borzouei and Bandani (2013). The experiment was performed in five replicates for control and each inhibitor concentration.

Twenty microliters of enzyme was pre-incubated with 20 μl A. sativa proteinaceous extract (1.80, 0.90, 0.45, 0.22, and 0.11 mg/ml) at 37°C for 10 min, followed by determination of the amylolytic activity from S. cerealella larval midgut preparations in the polyacrylamide gel electrophoresis (PAGE) (Laemmli 1970) as described by Mohammadzadeh et al. (2013). After electrophoresis for 3 h, the gel was rinsed with distilled water, left in a solution of 1% (v/v) Triton X-100 for 15 min, and then placed in a 1.0% soluble starch solution in 20 mM glycine buffer, pH 9.0, containing 20 mM CaCl₂ and 10 mM NaCl for 1.5 h at 37°C. After 1.5 h, zones of amylolytic activities appeared as light bands against dark background after staining with a solution of 1.3% I₂ and 3% KI for 15 min. The gels were washed, in order to remove the excess iodine solution, and then photographed.

Effect of pH on the inhibitory activity of A. sativa proteinaceous extract was determined using universal buffer as described by Mehrabadi et al. (2010). The highest concentration (1.80 mg/ml) of the seed extract was incubated with S. cerealella enzyme extract in pHs of 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, and 12.0. The experiment was performed in five replicates for each pH. Procedure for the percentage of α-amylase inhibition (%I) was conducted as described by Mehrabadi et al. (2010) as follows:

%I = 100 × [(A540 control – A540 Exp.)/A540 control]

Where, A540 control is the optical density in control, and A540 Exp. is the optical density in treatment.

Bioassay of A. sativa α-AI

The basic diet used was flour milled from wheat seeds (cultivar Bam). Initially, A. sativa proteinaceous extract was lyophilized, and then dissolved in 25 mM potassium phosphate buffer (pH 7.0). Inhibitors were dissolved at three concentrations and incorporated at 100 μl of solution per 100 mg of flour. Final concentrations of the inhibitor in the artificial seeds were 0.108, 0.429, and 1.11, mg/artificial seed. To determine the effect of A. sativa α-AI on life history of S. cerealella, 100 newly emerged larvae (within 24 h) were individually reared on artificial seeds (Shukle and Wu 2003) supplemented (0.108, 0.429, and 1.11, mg/artificial seed) or not (control) with A. sativa α-AI. Immature stages mortality, post-embryonic development, realized fecundity (eggs laid per female), and egg fertility were evaluated as described earlier (Borzouei and Naseri 2016). Also, pupae of control and inhibitor treatments (n = 20) were dug up 3 d after pupation, assessed to viability, and then weighed to determine their fresh weight.
Biochemical Analyses of Pupae

One S. cerealella pupa was weighed using a Sartorius balance with sensitivity of 0.0001 mg and used to prepare one sample for analysis of glycogen and lipid contents. Five replications (n= 5) were analyzed for each control and treated insects. The analytical procedures for glycogen and lipid assay were the same as described earlier (Borzouei et al. 2017).

Inducing Cold-hardening Insects Fed on Control and Inhibitor Treatments and Sampling Design

To determine the effect of A. sativa α-AI on cold hardiness of S. cerealella pupae, about 300 larvae in five replications (each replication included about 60 newly emerged first instar larvae) were initially reared on artificial seeds supplemented (0.108, 0.429, and 1.11 mg/artificial seed) or not (control) with A. sativa α-AI at 25°C, relative humidity 60% with a 16-h day length until the fourth instar. Then all containers were placed at a temperature of 17°C under the same humidity and photoperiod to induce diapause (Li and Xie 1981), and larvae were allowed to pupate inside seeds. Diapause was induced in nearly all pupae under these conditions. Pupae of control and inhibitor treatments were dug up 3 d after pupation, assessed to viability, and then placed separately into a 5 ml vial with the top end open. The vials were transferred to a programmable refrigerated test chamber and the temperature lowered at a rate of 0.5°C/min, from 17°C to −5, −10 and −15 ± 0.5°C. The pupae were held at these temperatures for 24 h and then slowly (0.5°C/min) heated to 25°C and held at that temperature for 24 h. After that, live and dead pupae were counted and the pupae showing no abdominal movement in response to stimulation were classified as dead (Bemani et al. 2012). Five replicates, each containing 15 pupae, were used for control and inhibitor treatments for each temperature.

Statistical Analysis

The results of in vitro effect of A. sativa α-AI on cold hardiness of S. cerealella pupae, about 300 larvae in five replications (each replication included about 60 newly emerged first instar larvae) were initially reared on artificial seeds supplemented (0.108, 0.429, and 1.11 mg/artificial seed) or not (control) with A. sativa α-AI at 25°C, relative humidity 60% with a 16-h day length until the fourth instar. Then all containers were placed at a temperature of 17°C under the same humidity and photoperiod to induce diapause (Li and Xie 1981), and larvae were allowed to pupate inside seeds. Diapause was induced in nearly all pupae under these conditions. Pupae of control and inhibitor treatments were dug up 3 d after pupation, assessed to viability, and then placed separately into a 5 ml vial with the top end open. The vials were transferred to a programmable refrigerated test chamber and the temperature lowered at a rate of 0.5°C/min, from 17°C to −5, −10 and −15 ± 0.5°C. The pupae were held at these temperatures for 24 h and then slowly (0.5°C/min) heated to 25°C and held at that temperature for 24 h. After that, live and dead pupae were counted and the pupae showing no abdominal movement in response to stimulation were classified as dead (Bemani et al. 2012). Five replicates, each containing 15 pupae, were used for control and inhibitor treatments for each temperature.

Results

Inhibition of Midgut α-Amylase by A. sativa Proteinaceous Inhibitor

The effect of A. sativa proteinaceous extract on digestive amylolytic activity of S. cerealella was determined by enzyme assays (Fig. 1) as well as by PAGE (Fig. 2). Different concentrations of A. sativa proteinaceous extract had inhibition effects ranging from 16 to 66% (F1, 20 = 75.30; P < 0.01). When concentrations of 1.80, 0.90, 0.45, 0.22, and 0.11 mg/ml were used, they inhibited the enzyme activity by 65.68, 45.15, 31.63, 23.1, and 16.62%, respectively. One major isoform of α-amylase obtained from fourth instars fed on wheat in
Effect of Inhibitor on *S. cerealella* Life History

To estimate the in vivo effects of *A. sativa* proteinaceous extract on the life history of *S. cerealella*, developmental time, fecundity and fertility were studied with the appropriate control (Table 1). *S. cerealella* feeding on artificial seeds supplemented or not with *A. sativa* inhibitor showed a significant difference in larval and pupal period. Larvae fed on seeds containing an I\(_{10}\) concentration of the inhibitor showed the shortest larval and pupal period, and the longest period was at I\(_{50}\) concentration. Moreover, male and female longevity of *S. cerealella* that fed on artificial seeds containing I\(_{30}\) or I\(_{50}\) concentrations were significantly shorter than those of I\(_{10}\) concentration and control (Table 1).

The results showed that the highest realized fecundity and egg fertility of *S. cerealella* was recorded for female developed from larvae fed on artificial seeds containing an I\(_{10}\) concentration of the inhibitor. However, the lowest fecundity and fertility was for female developed from larvae fed on I\(_{50}\) concentration (Table 1).

### Table 1. Mean (±SE) duration (days) of larval and pupal period, adults longevity, realized fecundity (eggs laid per female), and egg fertility (percentage of hatched eggs per female) of *Sitotroga cerealella* fed on artificial seeds supplemented or not (control) with *Avena sativa* α-amylase inhibitor

| Treatment          | n   | Larval and pupal period | n   | Male longevity | n   | Female longevity | n   | Fecundity | n   | Fertility (%) |
|--------------------|-----|-------------------------|-----|----------------|-----|------------------|-----|-----------|-----|--------------|
| Control            | 75  | 30.8 ± 0.2 c            | 39  | 7.3 ± 0.1 a    | 36  | 7.9 ± 0.1 ab     | 36  | 93.5 ± 1.6 b| 36  | 81.1 ± 0.9 a  |
| I\(_{30}\) (0.108 mg/artificial seed) | 79  | 29.8 ± 0.2 d            | 40  | 7.3 ± 0.1 a    | 39  | 8.4 ± 0.2 a      | 39  | 102.4 ± 2.5 a| 39  | 82.5 ± 1.2 a  |
| I\(_{30}\) (0.429 mg/artificial seed) | 61  | 31.8 ± 0.2 b            | 35  | 6.2 ± 0.2 b    | 26  | 7.6 ± 0.2 b      | 26  | 75.4 ± 2.7 c| 26  | 71.0 ± 1.6 b  |
| I\(_{30}\) (1.11 mg/artificial seed) | 43  | 34.5 ± 0.3 a            | 26  | 5.6 ± 0.2 c    | 17  | 6.3 ± 0.3 c      | 17  | 31.6 ± 3.1 d| 17  | 50.0 ± 2.5 c  |
| df                | 3, 261 |                       | 3, 137 |                       | 3, 120 |                       | 3, 120 |                       | 3, 120 |                       |
| F                 | 48.14 |                       | 15.30  |                       | 10.48   |                       | 123.61  |                       | 81.41   |                       |
| P                 | <0.01 |                       | <0.01  |                       | <0.01   |                       | <0.01   |                       | <0.01   |                       |

Mean values in a column followed by different letters are significantly different on the basis of ANOVA with Tukey’s test (P < 0.05). The n value shows the number of insects tested for each parameter.

Effect of Inhibitor on Weight and Energy Reserves of Pupae

Pupal weight of *S. cerealella* demonstrated a significant difference among control and different concentrations of *A. sativa* proteinaceous inhibitor, being heaviest at I\(_{30}\) concentration and lightest at I\(_{50}\) concentration (Table 2).

The pupae of *S. cerealella* came from larvae that continuously fed on artificial seeds containing I\(_{30}\) and I\(_{50}\) concentrations of proteinaceous inhibitor showed significant reduction in energy reserves compared to control pupae. A significant difference, however, was not found for glycogen and lipid contents between control pupae and those fed on I\(_{30}\) concentration. Larval feeding on I\(_{30}\) concentration of the inhibitor resulted in 17, and 18% reductions in glycogen and lipid contents of pupae, respectively, compared to control. Larval feeding on I\(_{50}\) concentration of the inhibitor resulted in a greater magnitude in glycogen (32%) and lipid contents (36%) of pupae (Table 2).

Pupal Survival Rate Following Exposure to Sub-Zero Temperatures

Survival rate of *S. cerealella* pupae came from larvae fed on artificial seeds supplemented (0.108, 0.429, and 1.11, mg/artificial seed) or not (control) with *A. sativa* proteinaceous inhibitor differed significantly following exposure to sub-zero temperatures. In general, pupal survival rate was higher in the control and I\(_{10}\) concentration groups than the I\(_{30}\) and I\(_{50}\) concentrations for −10 and −15°C (Table 3).

Discussion

α-Als have been considered as natural control agents against insect pests, because they reduce amylolytic activity in vitro and affect larval development of a number of Lepidoptera and Coleoptera species (Sivakumar et al. 2006, Bonavides et al. 2007). These inhibitors can be expressed in genetically engineered plants to confer resistance to target pests. Carrillo et al. (2011) showed that the expression in Arabidopsis plant of the gene coding for a low-molecular-weight cysteine-proteinase inhibitor isolated from barley seeds reduced the performance of two aphid species when expressed at different levels.

Our study indicated that *S. cerealella* larvae have amylolytic activity that was inhibited in vitro by *A. sativa* proteinaceous inhibitor; although chronic ingestion (I\(_{10}\)) of this inhibitor did not significantly
The pupae from larvae fed on artificial seeds supplemented or not (control) with *Avena sativa* α-amylase inhibitor. Mean values followed by different lowercase letters in a column and by different uppercase letters in a row are significantly different on the basis of ANOVA with Tukey’s test (*P* < 0.05). The *n* value shows the number of pupae tested for each parameter.

Table 3. Survival rate of *Sitotroga cerealella* pupae following exposure to sub-zero temperatures

| Treatment | Survival rate (%) | F and P |
|-----------|-------------------|---------|
|           | −5°C/24 h | −10°C/24 h | −15°C/24 h | F<sub>1,5</sub> | P |
| Control   | 90.6 ± 3.3 a,A  | 74.6 ± 3.8 a,B | 41.3 ± 4.8 a,C | 33.65 | <0.01 |
| I<sub>10</sub> (0.108 mg/artificial seed) | 86.6 ± 5.9 a,A  | 77.3 ± 5.4 a,B | 40.0 ± 7.6 a,C | 54.76 | <0.01 |
| I<sub>20</sub> (0.429 mg/artificial seed) | 78.6 ± 5.7 a,A  | 48.0 ± 3.8 b,B | 14.6 ± 4.4 b,C | 42.64 | <0.01 |
| I<sub>50</sub> (1.11 mg/artificial seed) | 34.6 ± 4.4 b,A | 10.6 ± 5.1 c,B | 0.0 ± 0.0 b,B | 21.87 | <0.01 |
| df        | 3, 16         | 3, 16         | 3, 16         |        |    |
| F         | 26.79         | 43.17         | 16.05         |        |    |
| P         | <0.01         | <0.01         | <0.01         |        |    |

The pupae from larvae fed on artificial seeds supplemented or not (control) with *A. sativa* α-amylase inhibitor. Mean values followed by different lowercase letters in a column and by different uppercase letters in a row are significantly different on the basis of ANOVA with Tukey’s test (*P* < 0.05).

The effects of *A. sativa* inhibitor on α-amylase of *S. cerealella* indicated a dose dependent manner of inhibition. These findings are in agreement with other reports; for example, Dasrtranj et al. (2013) noted that 14 μg of crude protein of wheat (cultivar MV17) caused 71% inhibition of amylolytic activity in *T. molitor*, whereas the lowest concentration (0.87 μg of crude protein) inhibited only 18% of amylolytic activity. Electrophoretic resolution of the enzyme inhibition also showed that the activity of α-amylase decreased as a dose dependent manner of inhibition. However, *A. sativa* proteinaceous extract was not capable of inhibiting α-amylase isoform completely (Fig. 2). To our knowledge, this is the first study in which specific insect α-AI has been detected using an in-gel assay.

The effect of α-AIs from several grains was studied by Zoacettale et al. (2007) and Do Nascimento et al. (2011) on fitness-related parameters of insect pests. In agreement with these studies, the results of this study showed that *A. sativa* inhibitor was effective in reducing larval survival, growth, and development. In the present study, the feeding bioassay showed that *A. sativa* inhibitor incorporated artificial seeds delayed the development of larval and pupal stages, and increased their mortality. Probably, the reduction in growth and survival rate of *S. cerealella* was because of inhibition of the midgut digestive α-amylase by this inhibitor. However, the chronic ingestion of *A. sativa* inhibitor had not significant effect on the developmental time of this pest. This finding suggests that different concentrations of tested inhibitor did not necessarily retard larval development because of either synthesizing α-amylase isoforms insensitive to α-AIs or secreting digestive proteinases that have the capacity to degrade α-AIs (Lomate and Hivrale 2011, Borzoui et al. 2017). Overall, if the developmental time of larvae and pupae of *S. cerealella* is increased, the survival rate of immature stages would be reduced, leading to a consequent decrease in the pre-adult population, which grows exponentially with each advancing generation. This would result in a significant reduction in stored-product losses by this insect. Our finding is consistent with the results obtained by Shukle and Wu (2003), who reported 12 and 21% increase in the developmental time of *S. cerealella* fed on 1 and 5% concentrations of Kunitz soybean inhibitor.

The performance of lepidopteran adults, such as *S. cerealella*, can directly be influenced by the quality of diet eaten by larval stage (Borzoui and Naseri 2016). The results of this study showed that inhibitor-incorporated artificial seeds had significant effect on the longevity of *S. cerealella* adults. The reduced adult longevity of *S. cerealella* at I<sub>10</sub> and I<sub>50</sub> concentrations of the inhibitor demonstrated their efficacy against *S. cerealella* larvae as reducing amylolytic activity. Furthermore, the fecundity and fertility was lower in *S. cerealella* fed on inhibitor-incorporated artificial seeds because of the lower qualitative value of this food. Similarly, Masoumzadeh et al. (2014) reported potential toxic effects of acarbose inhibitor for *Plodia interpunctella* (Hubner; Lepidoptera: Pyralidae) including the increased developmental time and reduced activity of digestive α-amylase.

Body weight is a primary fitness index when characterizing insect population dynamics (Liu et al. 2004). *S. cerealella* fed on I<sub>10</sub> concentration of the inhibitor showed higher pupal weight, followed by those consuming the larval base diet. At moderate (0.429 mg/
artificial seed) and high (1.11 mg/artificial seed) concentrations of A. sativa inhibitor, there was a marked reduction in pupal weight. Ingestion of potent α-Al adversely affected the carbohydrate intake, especially starch, by S. cerealella larvae, which caused developmental abnormalities and following reduced weight of pupae. Saadati et al. (2010) expressed that the quality of larval diet is the main factor influencing the energy reserves of herbivorous insects that consequently affect their cold hardiness (Puton; Hemiptera: Scutelleridae).

The insects can accumulate large amounts of nutrients, in the form of lipids and glycogen, during feeding period (Hokkanen 1993). In general, the larger pupae, the more energy are stored (Roder et al. 2008). We found that glycogen and lipid contents were significantly reduced in larvae that fed on artificial seeds containing I₃₀ and I₅₀ concentrations of A. sativa inhibitor. This reduction can be due to reduction in food digestion by S. cerealella larvae. Nouri-Ganbalani et al. (2016) reported that a decreased body energy contents can be achieved by the reduced digestive amylolytic activity. Lipids serve as the energy source for post-diapause development. While, glycogen is the major metabolic fuel during the inactive diapause period (Kostal et al. 1998) that its metabolism is linked to the production of cryoprotectants for the ability of cold hardiness (Li et al. 2002, Thompson 2003). In this study, it is clearly observed that the inhibitor incorporated into artificial seeds affected the cold hardiness capacity of S. cerealella. We presume that these effects might be related to the changing of energy reserve and cryoprotectants levels, but this question needs to be further explored. Several studies indicated that pre-overwintering feeding can affect energy reserves (Liu et al. 2007, Rochefort et al. 2011), survival and cold hardiness of the insects (Trudeau et al. 2010, Mohammadzadeh and Izadi 2016). Cheng et al. (2010) expressed that the quality of larval diet is the main factor influencing the energy reserves of herbivorous insects that consequently affect their cold hardiness.

It is concluded that, as compared to larval based diet, there were significant differences in the life history, pupal weight, energy reserves, and cold hardiness of S. cerealella when exposed to moderate and high concentrations of this inhibitor. Based on the data presented here, A. sativa proteinaceous extract has a strong inhibitory activity on midgut α-amylase and population dynamics of S. cerealella. However, the present work is the first study on inhibitory effects of A. sativa proteinaceous extract, so more survey should be conducted.

Acknowledgments

We thank the University of Mohaghegh Ardabili (Ardabil, Iran), for cooperation by support for the experiment.

References Cited

Baker, J. E. 1987. Purification of isoamylases from the rice weevil, Sitophilus oryzae L. by HPLC and their interaction with partially purified amylase inhibitor from wheat. Insect Biochem. 17: 37–44.

Bemani, M., H. Izadi, K. Mahdian, A. Khani, and M. A. Samih. 2012. Study on the physiology of diapause, cold hardness and supercooling point of overwintering pupae of the pistachio fruit hull borer, Armama comaroffi. J. Insect Physiol. 58: 897–902.

Bernfeld, P. 1955. Amylases, α and β. Methods Enzymol. 1: 149–158.

Bonavides, K. B., P. B. Pelegirini, R. A. Laumann, M. F. Grossi-De-Sa, C. Bloch Jr., J. A. T. Melo, B. F. Quinimino, E. F. Noronha, and O. F. Franco. 2007. Molecular identification of four different α-amylase inhibitors from barley (Diplotyx alata) seeds with activity toward insect enzymes. J. Biochem. Mol. Biol. 40: 1–7.

Borzouei, E., and A. R. Bandani. 2013. Wheat and triticale proteinaceous seed extracts inhibit gut α-amylase and protease of the carob moth, Ectomyelois ceratoniae. Molec. Entomol. 4: 13–21.

Borzouei, E., and B. Naseri. 2016. Wheat cultivars affecting life history and digestive amylolytic activity of Sitotroga cerealella Olivier (Lepidoptera: Gelechiidae). Bull. Entomol. Res. 106: 464–473.

Borzouei, E., B. Naseri, and F. R. Namin. 2015. Different diets affecting biological and digestive physiology of the Khapra beetle, Trogoderma granarium Everts (Coleoptera: Dermentidae). J. Stored Prod. Res. 62: 1–7.

Borzouei, B. B. Naseri, and G. Nouri-Ganbalani. 2017. Effects of food quality on biology and physiological traits of Sitotroga cerealella (Lepidoptera: Gelechiidae). J. Econ. Entomol. 110: 266–273.

Carrillo, L., M. Martinez, F. Alvarez-Alfageme, P. Castanera, G. Smaghe, I. Diaz and F. Ortego. 2011. A barley cysteine-protease inhibitor reduces the performance of two aphid species in artificial diets and transgenic Arabidopsis plants. Transgenic Res. 20: 305–319.

Cheng, W. J., W. L. Quan, X. L. Zheng, C. L. Lei, and X. P. Wang. 2010. Effect of host-plants on cold hardiness of insects. J. Environ. Entomol. 32: 332–337 (in Chinese with English abstract).

Dastranj, M., A. R. Bandani, and M. Mehrabadi. 2013. Age-specific digestion of Tenebrio molitor (Coleoptera: Tenebrionidae) and inhibition of proteolytic and amylolytic activity by plant proteinaceous seed extracts. J. Asia Pac. Entomol. 16: 309–315.

Do Nascimento, V. C., H. C. Castro, P. A. Abreu, A. Elein, A. Oliveira, J. H. Fernandez, S. Araujo Jda, and O. L. Machado. 2011. In silico structural characteristics and α-amylase inhibitory properties of ric c 1 and ric c 3, allergenic 2S albumins from Ricinus communis seeds. J. Agric. Food Chem. 59: 4814–4821.

Foud, H. A., L. R. D. Farom, E. F. Vilela, and E. R. de Lima. 2013. Flight responses of Sitotroga cerealella (Lepidoptera: Gelechiidae) to corn kernel volatiles in a wind tunnel. Arthropod Plant Interact. 7: 651–658.

Franco O. L., D. J. Rigden, F. R. Melo, and M. F. Grossi-de-Sa. 2002. Plant α-amylase inhibitors and their interaction with insect α-amylases. Structure, function and potential for crop protection. Eur. J. Biochem. 269: 397–412.

Franco, O. L., F. R. Melo, P. A. Mendes, N. S. Paes, M. Yokoyama, M. V. Coutinho, C. Bloch, Jr. and M. F. Grossi-de-Sa. 2005. Characterization of two Acanthoscelides obtectus α-amylases and their inactivation by wheat inhibitors. J. Agric. Food Chem. 53: 1585–1590.

Hagström, D. W. and B. Subramaniam. 1996. Integrated management of insects in stored products. Marcel Dekker, Inc, New York.

Hamed, M. and S. Nadem. 2012. Effect of cereals on the development of Sitotroga cerealella (Lövibel): (Lepidoptera: Gelechiidae) and subsequent quality of the egg parasitoid, Trichogramma chilonis (Ishii) (Hymenoptera: Trichogrammatidae). Pakistan J. Zool. 44: 923–929.

Haq, S. K., S. M. Atif, and R. H. Khan. 2004. Protein protease inhibitor genes in combat against insects, pests, and pathogens: natural and engineered phytoprotection. Arch. Biochem. Biophys. 431: 145–159.

Hokkanen, H. M. T. 1993. Overwintering survival and spring emergence in Meligethes aeneus: effects of body weight, crowding, and soil treatment with Beauveria bassiana. Entomol. Exp. Appl. 67: 241–246.

Kazazia, M., A.R. Bandani, and S. Hosseilkhani. 2005. Biochemical characterization of α-amylase of Sunn pest Eurygaster integriceps. Entomol. Sci. 8: 371–377.

Kostal, V., J. Sula, and P. Simk. 1998. Physiology of drought tolerance and cold hardiness of the Mediterranean tiger moth Cynthocalphora pudica during summer diapause. J. Insect Physiol. 44: 165–173.

Laemmlli, U.K. 1970. Cleavage of structural proteins during the assembly of bacteriophage T4. Nature. 227: 680–685.

Li, C., and B. Y. Xie. 1981. Effects of temperature and photoperiod on diapause of cotton bollworm, Helicoverpa armigera. Entomol. Knowl. 18: 38–61.

Li, Y. P., L. Ding, and M. Goto. 2002. Seasonal changes on glycerol content and enzyme activities in overwintering larvae of the shonai ecotype of the rice stem borer, Chilo suppressalis Walker. Arch. Insect Biochem. Physiol. 50: 53–61.
