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Effects of chlorpyriphos on the alpha- amylase activity in the *in vivo* and *in vitro* conditions: Case study: *Sitophilus oryzea*

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ABSTRACT

Purpose: Insect-insecticide interaction, as a dynamic system, increases our knowledge to improve chemical application. Although modes of action in the most insecticides is obvious (direct action) but the other targets which may be affected after treatment are not determined (indirect action). In this study digestive enzymes were considered as potential targets for insecticides. **Research method:** Some of (three sample) adults that were exposed to the different insecticide concentrations were selected for *in vivo* analysis. In this part, internal organs (midgut) were homogenates and enzyme activity was determined. On the other hand, in the *in vitro* assays, nontoxic adults were selected and after dissection of the guts, incubated to the different insecticide manually. Fifty microliters enzyme solution with 450 µl toxic solutions incubated 30 min in the room temperature before enzyme assay. **Findings:** Twenty hours after exposure to the insecticides, gut of adults were dissected and used for *in vivo* experiment. Our data showed that there was a significant difference in the enzyme activity among different concentrations of chlorpyriphos. The highest and lowest level of enzyme inhibitory was occurred in the 2000 and 0 ppm (control). There was no significant difference between control (0 ppm) and 300 ppm. Data in the *in vitro* experiment showed that enzyme activity was reduced in the toxic concentrations. Trend of enzyme inhibiting that occurred with chlorpyriphos was regular as the highest and lowest inhibiting were observed in the maximum (99.2 %) and minimum concentrations (7.4 %), respectively. **Limitations:** There is a problem in which how to correlate *in vivo* and *in vitro* results to practical toxicology. **Originality/Value:** Using of new insecticides with new and widespread mode of action can be recommended against postharvest pest in the practical entomology.

INTRODUCTION

The rice weevil, *Sitophilus oryzae*, is a critical stored pest in the world. It is a polyphagous insect that causes severe damage to rice, wheat, and other grains. The traditional methods of store management such as physical, biological, and chemical practices were used for several decades, and their results were not satisfied with farmers (Baker, 1988). Hence, finding new approaches to rice weevil control is needed to more profound studies about physiological systems in this pest.

The digestive system of the coleopteran varies in the form and function, particularly in the stored pest, that feeding process occurs in the environment with low humidity. Although most curculionids' species are phytophagous, some of them are carnivorous and zoophagous. Weevils, Curculionidae, comprising more than 60000 species and 5800 genera (Hernandez-Vera et al., 2019).

Usually, stored grain insects live in a relatively stable environment where changes in abiotic and biotic conditions occur very slowly (Perez-Mendoza et al., 2004). Oral digestion as a standard method in feeding has happened in the stores with dangerous insects (Boyd, 2003; Habibi et al., 2008; Saxena, 1963). In this way, qualitative and quantitative damages significantly reduce the economic value of a commodity (Liu et al., 2010; Barbehenn, 2002; Saadati et al., 2012a; b; c). Different proteins from various categories are responsible for in the take food materials. Many carbohydrates were reported from the various insects (Saadati et al., 2008; 2012c). Endopeptidase, exopeptidase, and carbohydrates as critical proteins in the digestive system were reported from the midgut of the *Sitophilus granarius* (Baker et al., 1983). Biotic and abiotic disruptors have key roles in the normal and abnormal activities many of digestive proteins. Insecticides, viral and bacterial proteins, animal and plant metabolites and environmental factors are famous disruptors in the enzyme engineering.

The high carbohydrate content in the diet of *Sitophilus* weevils would indicate that there is an active complex of carbohydrases in guts of these species (Araujo et al., 2006; Baker et al., 1983; Omar, 2012; Horne et al., 2009). Degradation of starch needs the α-amylase (EC 3.2.1.1) activity that breakdowns α-(1, 4) glycosidic bond in the starch and its derivations (Kunieda et al., 2006). This enzyme was proper target to inhibit with different disruptors (Saadati et al., 2007). Inhibiting of gut amylase in some pests like *Callosobruchus maculates* is in the final stage and used in the transgenic plants (Jouanian et al., 1998). To now, many isozyme were reported from digestive canal in the vertebrate and invertebrate animals (Saadati et al., 2012c). This viability in the form and function converted these enzymes to appropriate targets in the biotechnology and gene transforming to crop plants.

Integrated pest management of stored pest is related to chemical control in the world and particularly in Iran (Critchley, 1998; Javaheri et al., 2009). Insecticide and fumigant application are preliminary recommendation *S. oryzea* control. Two generation of pesticides like Organophosphate, pyrtheroids and acid cabamic derivations are first selection of farmers because of fast and efficient ability. Widespread of traditional insecticides has created serious concerns in the Eco toxicological risks like soil and water ground pollution, change in the natural balance and unknown effects on the non-target organism (Gunes & Yerli, 2011). Hence, reducing/removing ecological risks of common insecticides are main strategy to current studies. One of the dynamic systems that need to address in this field is Insect-insecticide interaction. Mode of action is important subjects of insecticides that contain direct effects. Nowadays, molecular toxicology proved that many targets in the body may be affected after entrance insecticides as known indirect effects (Saadati & Allahyari, 2018). To elucidate the effects of insecticides on the nutrition process, digestive enzyme-insecticide interaction experiments should be examined in *in vivo* and *in vitro* system. Determination of

the digestive enzymes-insecticide interaction can improve our understanding of molecular toxicology, particularly in the animal world. Furthermore these data can be included in biochemical models developed to predict enzyme responses to different concentrations of typical insecticides. In this study, for the first time, the interaction of α-amylase from the midgut of adult rice weevil with chlorpyrifos was studied in *in vivo* and *in vitro* conditions. The goal of this research was to increase our current information of enzyme behaviors after interaction with xenobiotic like insecticide. We expected that our data from this study will be helpful in the physiological and molecular systems to more identify potential targets in the mode of action studies.

MATERIALS AND METHODS

Insects

Adult *S. oryzae* used in this study were obtained from a laboratory strain reared on whole kernel, hard red winter wheat, *Triticum aestivum* L. Insect rearing were begins after adding 500 adults into 800 g of wheat (12.5% moisture content) in 2500 ml glass dish. Pots were held at 27 C and 75% r.h. with a 12:12 L: D photoperiod. All founding adults were removed from the initial culture and then collected adults after 72 hours.

Sample preparation

Using ice- cold phosphate buffer (4 ºC, pH=6.9) was separated the alimentary canal of adults. At first midut were cuts from main canal and then rinsed with same buffer and pooled in the micro tube (three). The midguts were homogenized using a handle homogenizer and then holed on the ice dish. Centrifuging at 12000 rpm for 10 minutes at 4 ºC was performed to take crude proteins for storing (Baker et al., 1983).

Enzyme purification

The purification process was performed in three steps, intermittently. At the first step crude extract was treated with ammonium sulfate at the 30 to 70 percent. The precipitated material was collected by centrifugation at 6000 rpm for 15 min, and then diluted with Tris-HCl buffer, before overnight dialyzing at 4 °C against the same buffer. The enzyme solution was applied to a Sepharyl G-100 column, equilibrated with the same buffer. The column was run at a flow rate of 0.5 $mL.min^{-1}$. Amylase activity was measured as an enzyme assay part. Fractions containing higher enzymatic activity were pooled and applied to a diethylaminoethyl (DEAE) - cellulose column, equilibrated with Tris-HCl (pH 8.8). The enzyme was eluted at a flow rate of 0.5 $mL.min^{-1}$ with a linear NaCl gradient (0–0.6 mol). Fractions (1.5 $Ml.tube^{-1}$) were collected, and their protein concentration and α-amylase activity were determined as previously described. In the final step, fractions containing the highest enzymatic activity were pooled and used as the enzyme source (Sorkhabi-Abolmaleki et al., 2014).

LC50 determination

Chlorpyriphos (40.8 EC, Aria Shimi Co., and Iran) was ordered for this experiment. After preliminary test, 2000, 1245, 775, 482, 300 and 0 ppm concentrations (active ingredient) were selected for final analysis. Three replicate were performed and LC50 were calculated using Poloplus software.

In vivo and in vitro experiment

Some of (three samples) adults that were exposed to the different insecticide concentrations were selected for *in vivo* analysis. In this part, internal organs (midgut) were homogenates, and enzyme activity was determined. On the other hand, in the *in vitro* assays, nontoxic adults were selected and, after dissection of the guts, incubated to the different insecticide manually. Fifty microliters enzyme solution with 450 µl toxic solutions incubated 30 min in the room temperature before enzyme assay.

Enzyme assay

A) α-Amylase activity assay

Amylase activity in the midgut was determined using a diagnostic kit (Amylase kit®, Pars Azmoon Co., Iran). The substrate was ethylidene-*p*nitrophenyl maltoheptaoside (EPS-G7). Absorbance, which is directly related to α-amylase activity, was measured at 405 nm and 37 ºC using an auto analyzer (Alcyon 300® Plus, Molecular Devices Corporation, Sunnyvale, CA). Before application, the auto analyzer calibrated with the control sera N and P (TrueLab N® and TrueLab P®, respectively; Pars Azmoon Co., Iran) and a calibrator solution (TrueCal U®, Pars Azmoon Co., Iran). After calibration, the auto analyzer mixed 4 µl of enzyme sample with 300 µl of the substrate solution, automatically, and calculates the enzyme activity ($IU.L^{-1}$) after a reaction delay of 1 minute and 36 seconds. Optimized pH was eight for all treatments. The assays were replicated three times. Finally, the specific α-amylase activity calculated as $U.mg^{-1}$ protein that known Specific activity (Saadati & Mirzaei, 2016).

B) Total protein assay

Total protein was assayed according to Biuret test using diagnostic kit (Total protein kit®, Pars Azmoon Co., Iran). In this procedure, copper sulfate ions react with the peptide bonds to produce purple (or pink) color. The final amount of proteins in the samples is estimated by comparing their intensity color with a standard solution that contains defined bovine serum albumin (BSA) concentration (Saadati & Mirzaei, 2016).

Determination of kinetic parameters

Kinetic parameters of the purified alpha-amylase were carried out using of starch as substrate. The substrate concentrations were 0.1, 0.2, 0.4, 0.6, 0.8 and 1. The enzymatic assay was done as above mentioned part and maximum velocity (Vmax) and constant of Michaelis-menton (Km) were estimated by Sigma plot software, version 5.

Statistical analysis

Data were compared by one-way analysis of variance (ANOVA) and factorial design that followed by Duncan's test at $p< 0.05$ (Mstat-C and SPSS ver. 15). Differentially activities were shown (as different letters) in figures.

RESULTS

Direct toxicity of chlorpyriphos to rice weevil

The median lethal concentration of chlorpyriphos to *Sitophilus oryzea* was calculated as 810.35 ppm. These results suggest that chlorpyriphos can be considered as a potential candidate for rice weevil and the other stored pest management (Fig. 1).

In vivo experiment

Twenty hours after exposure to the insecticides, the gut of adults were dissected and used for *in vivo* experiment. Our data showed that there was a significant difference in the enzyme activity among different concentrations of chlorpyriphos (Fig. 2). The highest and lowest level of enzyme inhibitory was occurred in 2000 and 0 ppm (control). There was no significant difference between control (0 ppm) and 300 ppm.

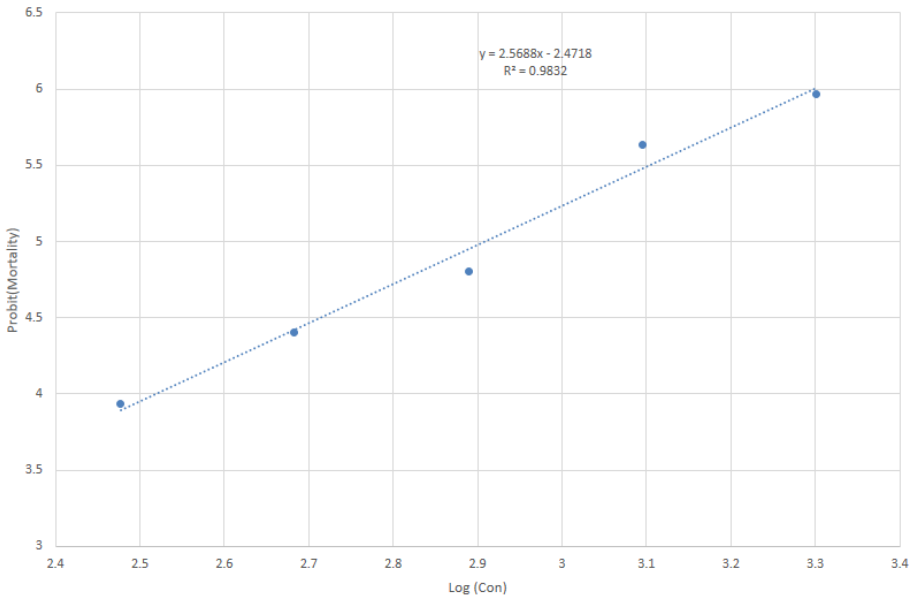

Fig. 1. Dose- response graph. The value of concentrations was considered as Antilog against mortality as Probit.

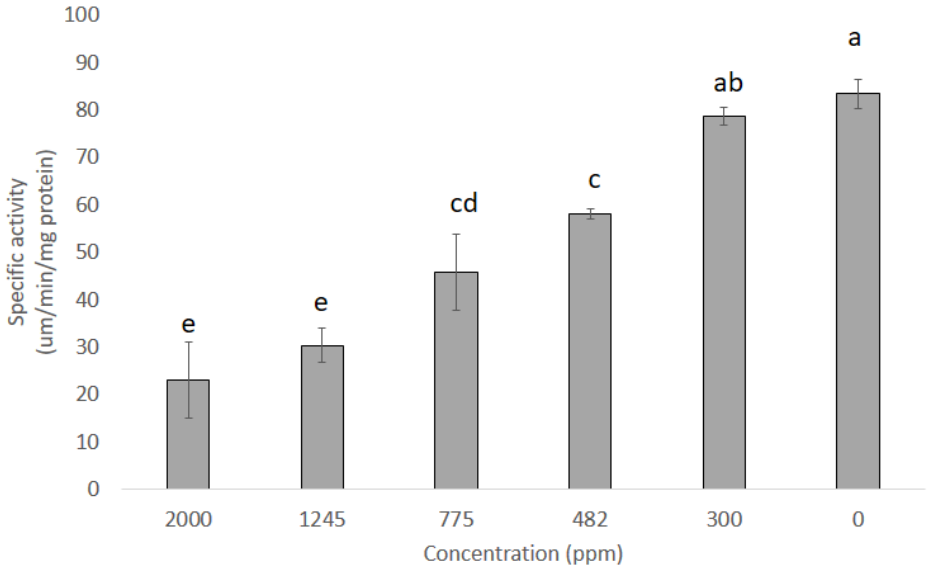

Fig. 2. In vivo assay: alpha- amylase activity after incubation with different concentrations of chlorpyriphos in the Adult rice weevil. Values are the average specific activity± SE from three independent experiments. Different letters showed significant difference among concentrations (P< 0.05).

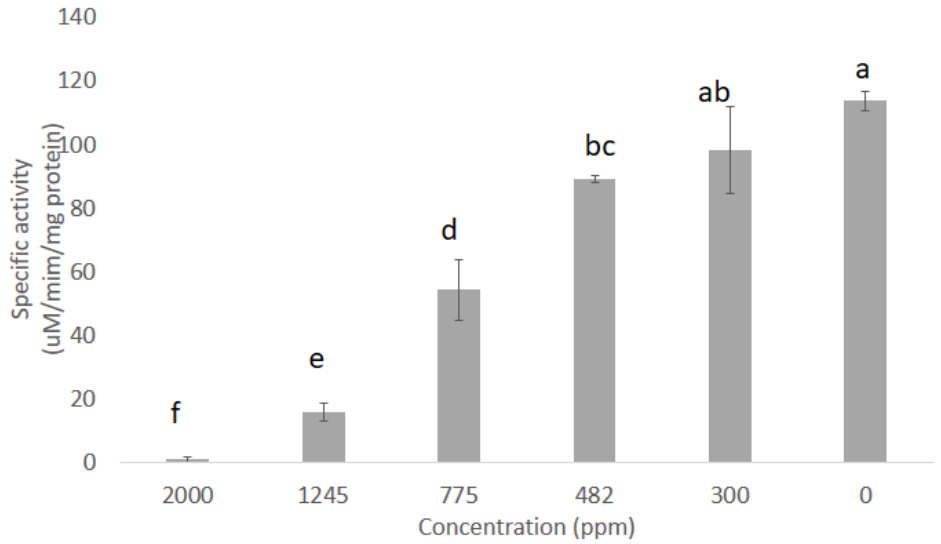

Fig. 3. *In vitro* assay: alpha amylase activity after incubation with different concentration of chlorpyriphos in the Adult rice weevil. Values are the average specific activity ± SE from three independent experiments. Different letters showed significantly difference among concentrations ($P< 0.05$).

In vitro experiment

Mixture of enzyme solution and different concentrations of insecticides were incubated for 30 min in 27±1ºC before the determination of enzyme activity. In this part, the gut of nontoxic adults was dissected and exposure to the different concentration of chlorpyriphos, manually. Our data showed that enzyme activity were reduced in the toxic concentrations (Fig. 3). The trend of enzyme inhibiting that occurred with chlorpyriphos was regular as the highest and lowest inhibiting were observed in the maximum (99.2 %) and minimum concentrations (7.4 %).

Determination of kinetic parameters

Maximum velocity (Vmax) and constant of Michaelis-menton (Km) were estimated by Sigma plot software. Results of analysis showed that Vmax and Km in the *in vivo* and *in vitro* experiment were 18.75; 38.65 and 68.03 109.3 respectively.

DISCUSSION

Direct and indirect effects of some insecticides are currently not well known. The role of pesticides in human life is well understood and more advantage has concluded from using of pesticides in the different ecosystems (Saadati & Allahyari, 2018). Our knowledge about the mode of action and the final fate of insecticides is not complete, and should be improve in the future. Previous knowledge of toxicology showed that organophosphate pesticides like chlorpyriphos affected acetylcholinesterase enzyme in the central nervous system, but Saadati and Allahyari (2018) showed the other targets like digestive systems may be affected. The proteome analysis of the alimentary canal in the *Euryagster integriceps* were studied to identify changed proteins after occurrence different life stages (Saadati et al., 2012a; b). Sharma et al. (2004) showed that effective proteins in the carbohydrates metabolism in the brown plant hoppers were down-regulated after exposure to the some of carbamate insecticides.

Table 1. Inhibitory effects of five concentrations of chlorpyriphos on the α-amylase activity in the midgut of rice weevil 30 minutes after incubation. Values are average of Inhibitory effects and calculated based to the control treatment (% inhibitory).

Concentration (ppm)	Inhibitory %	
	In vivo	*In vitro*
0	0	0
300	5.6	13.5
482	30.3	21.4
775	45	52.3
1245	63.6	86
2000	72.5	99.2

Widespread use of traditional insecticides against stored insects caused to appear new risks in the human and non-target organisms life's. Hence, finding safe properties of common insecticide or try to find new chemicals may be reduce health risks in the near future (Saadati & Allahyari, 2018). Indirect effects of insecticides on the physiological process like nutrition can be considered as a new agent to help us for choosing of efficient drugs in store management. One of the best theories in the insect- plant interaction is this point that nutrition processes disrupt or suppress. Digestive enzymes as biological catalyzer are ideal candidate in this process (Pauchet et al., 2008; Quistad et al., 2006). Alpha- amylase enzyme plays vital role in the carbohydrates metabolism, and in this study this enzyme was incubating with different concentrations of chlorpyriphos, which recommended for rice weevil control to study amount of changes in its activities.

The present results showed that amylase in the gut of rice weevil were inhibited by chlorpyriphos in both *in vivo* and *in vitro* experiments. Our data showed that more inhibition were occurred in the higher concentration in comparison to the low concentrations. Saadati and Allahyari (2018) showed that organophosphate pesticides like fenitrothion could decrease amylase activity in the gut of *E.integriceps* as significant differently. Chlorpyriphos and diazinon reduced amylase and lipase activity in the gut of sunn pest (Saadati & Mirzaei, 2016). If it is not clear the details of chlorpyriphos-amylase interaction, but also it is predictable tertiary conformation of alpha amylase was changed after interact with chemical. The fate and activity of active sites in the enzyme structure is not clear and need to more studies to determine how much of these sites be inactive.

The results of this study suggest that more inhibitory were occurred in the *in vitro* experiment in comparison to *in vivo* condition. Similar results were reported in the previous researches in the sunn pest (Saadati & allahyari, 2018; Saadati & Mirzaei, 2016). Removing/reducing of α-amylase activity in the gut of sun pest led to the accumulation of starch, and similar carbohydrates. This process may yield insect dying because of absence energy production mechanism (Saadati & Allahyari, 2018). Starch digestion is important reaction in the herbivorous insects that performed with carbohydrates. Any disruption in the enzymes like alpha- amylase can be lead to suppress of normal nutrition. Saadati et al. (2007) reported several different protein and non-protein inhibitors that act against alpha-amylase in the digestive system of *E.integriceps*. Deltamethrin as pyrtheroid compound had no effect on the alpha- amylase activity in the serum of the mice after mixing with their food as poison baits (Eraslan et al., 2007). On the other hand acetylcholinesterase activity was collapse in the serum immediately after take. In the similar study, cypermethrin had no effect on the alpha-Amylase activity in the serum of rat after take some toxicant (Veerapan et al., 2012). Any reduction of digestive enzyme particularly alpha- amylase were not observed in the serum of *Gallus domesticus* after oral treatment with cypermethrin (Anwar, 2004). Saadati and Mirzaei

(2016), and Saadati and Allahyari (2018) were mentioned that different major conditions like nature of insecticides, different doses, age and health of the organism, and environmental factors and minor conditions like the intrinsic difference of protein sequencing and structure were effective in the insecticide- enzyme infarctions

Although some researchers 100 percent inhibition of target proteins was considered as ideal goal, but partial inhibition was considered as proper tactic in the integrated pest management by practical entomologists (Saadati & Mirzaei, 2016). Data from this study indicated that chlorpyriphos could inhibit alpha-amylase activity, generally. This result proved that not only target point of chlorpyriphos was not limited to the acetyl cholineesterase, but also digestive enzyme like alpha-amylase can be reacted to different dose of this insecticide.

CONCLUSION

The linking between *in vitro* and *in vivo* studies is main challenge to take better conclusion for physiological systems in the crop and store pest. Currently, there are few studies investigating the effects of insecticides on digestive enzymes of *S.oryzea*, and this is an open area of study to unravel the mechanism of their inhibitory effects and their interaction with the assimilation process. The proteome analysis of target organs in the insect body can also be another strategy for molecular toxicology.

Conflict of interest

The authors at this moment hereby declare that there is no conflict of interest.

REFERENCES

Anwar, K. (2004). Toxic effects of Cypermethrin on the development of muscle in chick embryo of *Gallus domesticus*. *International Journal of Agriculture and Biology*, *6*, 1-7. https://doi.org/10.3923/jas.2003.432.445

Araujo, A. R., Ferreira, G. H., Oliveria, M. G. A., & Guedesm, R. N. C. (2006). Enzyme activity of the energy-metabolism of pyrethroid-resistant and -susceptible populations of the maize weevil (*Sitophilus zeamais*). *9th International Working Conference on Stored Product Protection.* PS 4-2-6211.

Baker, J. E. (1988). Development of four strains of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) on barley, corn (maize), rice, and wheat. *Journal of Stored Product Research*, *24*, 98-193. https://doi.org/10.1016/0022-474x(88)90018-5

Baker, J. E., Woo, S. M., & Byrd, R. V. (1983). Ultrastructural features of the gut of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) with notes on distribution of proteinases and amylases in crop and midgut. *Journal of Canadian Zoology, 62*, 1251-1259. https://doi.org/10.1139/z84-181. https://doi.org/10.1139/z84-181

Barbehenn, R. V. (2002). Gut-based antioxidant enzymes in a polyphagous and a graminivorous grasshopper. *Journal of Chemical Ecology, 28*, 1329-1347.

Boyd, D. W. (2003). Digestive enzymes and styletmorphology of *Deraecoris nigritulus* (Uhler) (Hemiptera: Miridae) reflect adaptions for predatory habits. *Annual Entomological Society of America, 96*, 667-671. https://doi.org/10.1603/0013-8746(2003)096[0667:deasmo]2.0.co;2

Critchley, B. R. (1998). Literature review of sunn pest *Eurygaster integriceps* Put. (Hemiptera, Scutelleridae). *Crop Protection*, *17*, 271-287. https://doi.org/10.1016/s0261-2194(98)00022-2

Eraslan, G., Bilgili, A., Essiz, D., Akdogan, M., & Shahindokuyucu, F. (2007). The effects of deltamethrin on some serum biochemical parameters in mice. *Pesticide Biochemistry and Physiology*, *87*, 123-130. https://doi.org/10.1016/j.pestbp.2006.07.001

Gunes, E., & Yerli, S. V. (2011). Effects of Deltamethrin on lipase activity in guppies (*Poecilia reticulate*). *Turkish Journal of Fisheries and Aquatic Sciences*, *11*, 473-476.

Habibi, J., Courdon, T. A., Backus, E. A., Brandt, S. L., Wagner, R. M., Wright, M. K., & Huesing, J. E. (2008). Morphology and histology of the alimentary canal of *Lygus hesperus* (Heteroptera: Cimicomorpha: Miridae). *Annual Entomological Society of America*, *101*, 159-171. https://doi.org/10.1603/0013-8746(2008)101[159:mahota]2.0.co;2

Hernandez-Vera, G., Toseveski, I., Caldara, R., & Emerson, B. C. (2019). Evolution of host plant use and diversification in a species complex of parasitic weevils (Coleoptera: Curculionidae). *Journal of life and Enviromental Science*, *PeerJ*: 7-e6625. https://doi.org/10.7717/peerj.6625

Horne, I., Haritos, V. S., & Oakeshott, J. G. (2009). Comparative and functional genomics of lipases in holometabolous insects. *Insect Biochemistry and Molecular Biology*, 39, 547-567. https://doi.org/10.1016/j.ibmb.2009.06.002

Javaheri, M., Scheafer, C. W., & Lattin, J. D. (2009). Shield bugs (Scutelleridae). In *Heteroptera of economic importance* (Scheafer C Panizzi W ed.). Washington, USA: CRC Press. 457-503. https://doi.org/10.1201/9781420041859.ch14

Jouanian, L., Bonade-Bottino, M., Giard, C., Morrot, G., & Giband, M. (1998). Transgenic plants for insect resistance. *Plant Science*, *131*, 1-11. https://doi.org/10.1016/s0168-9452(97)00239-2

Kunieda, T., Fujiyuki, T., Kucharski, R., Foret, S., Ament, S. A., Toth, A. L., Ohashi, K., Takeushi, H., Kamikuochi, A., Kage, E., Morioka, A., Bey, M., Kubo, T., Robinson, G. E., & Malaeszka, R. (2006). Carbohydrate metabolism genes and pathways in insects: insights from the honey bee genome. *Insect Molecular Biology*, *15*, 563-576. https://doi.org/10.1111/j.1365-2583.2006.00677.x

Liu, J., Zheng, S., Liu, L., Li, L., & Feng, Q. (2010). Protein profiles of the midgut of *Spodoptera litura* at the sixth instars feeding stage by shutgun ESI-MS approach. *Journal of Proteome Research*, *9*(5), 2117-2147. https://doi.org/10.1021/pr900826f

Omar, Y. M. M. (2012). Morphological studies on some external and internal structures of rice weevil, *Sitophilus oryzea*, a major pest pf the stored cereals in Egypt. *Journal of Plant Protection and Pathology*, *3*, 843-863. https://doi.org/10.21608/jppp.2012.84169

Pauchet, Y., Muck, A., Heckel, D. G., & Preiss S. (2008). Mapping the larval midgut lumen proteome of *Helicoverpa armigera*, a generalist herbivourous insect. *Journal of Proteome Research*, *7*, 1629-1639. https://doi.org/10.1021/pr7006208

Perez-Mendoza, J., Throne, J. E., & Baker, J. E. (2004). Ovarian physiology and age-grading in the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). *Journal of Stored Product Research*, *40*, 179-196. https://doi.org/10.1016/s0022-474x(02)00096-6.

Quistad, B. G., Liang, S. N., Fisher, K. J., Nomura, D. K., & Casida, J. E. (2006). Each lipase has a unique sensitivity profile for organophosphorus inhibitors. *Toxicological Science*, *91*, 166-172. https://doi.org/10.1093/toxsci/kfj124

Saadati, M., & Allahyari, H. (2018). Toxicology in vitro: The study of biochemical effects of Fenitrithion and Fenvalerate on the enzyme activity. *Journal of pesticides and Bio-fertilizers*, *1*, 1-5.

Saadati, M., Farshbaf Pourabad, R., Golmohammadi, G., & Sadeghi, H. (2008). Some properties of α-amylase in the salivary gland of *Eurygaster integriceps*. *Munis Entomology and Zoology*, *3*, 733-744.

Saadati, M., Farshbaf Pourabad, R., Toorchi, M., Zarghami, N., & Komatsu, S. (2012a). Protein patterns in salivary gland of sunn est, *Eurygaster integriceps* (put.) (Hem: Scutelleridae). *Turkish Journal of Entomology*, *36*, 71-80. https://doi.org/10.1002/arch.21047

Saadati, M., Farshbaf Pourabad, R., Valizade, M., & Yazdanian, M. (2007). Effects of some mineral compounds on the salivary α-amylase activity of the sunn pest, *Eurygaster integriceps*. *Turkish Journal of Entomology*, *31*, 163-173.

Saadati, M., & Mirzaei, M. (2016). Insecticide-enzyme interaction: cypermethrin, chlorpyrifos, diazinon and deltamethrin with α-amylase and lipase in the gut of sunn pest, *Eurygaster integriceps*. *Biological Systems*, *5*, 1-5. https://doi.org/10.4172/2329-6577.1000168

Saadati, M., Toorchi, M., Farshbaf Pourabad, R., & Zarghami, N. (2012b). Protein map of gut in adult sunn pest, *Eurygaster integriceps* (Put.) (Hem: Scutelleridae): two-dimensional electrophoresis technique. *Munis Entomology and Zoology*, *7*, 229-237.

Saadati, M., Toorchi, M., Farshbaf Pourabad, R., Zarghami, N., Nouri, M., & Komatsu, S. (2012c). Proteome analysis of gut and salivary gland proteins of fifth-instar nymph and adults of sunn pest, *Eurygaster integriceps*. *Archives of Insect Biochemistry and Physiology*, *81*, 105-119. https://doi.org/10.1002/arch.21047

Saxena, K. N. (1963). Mode of ingestion in a heteropterous insect *Dysdercus koenigii* (F.) (Pyrrohocoridae). *Journal of Insect Physiology*, *9*, 47-71. https://doi.org/10.1016/0022-1910(63)90084-2

Sharma, R., Komatsu, S., & Noda, H. (2004). Proteomic analysis of brown plant hopper: application to the study of carbamate toxicity. *Insect Biochemistry and Molecular Biology*, *34*, 425-432. https://doi.org/10.1016/j.ibmb.2004.01.004

Sorkhabi-Abdolmaleki, S., Zibaei, A., Hoda, H., & Fazeli Dinan, M. (2014). Purification and characterization of midgut α-amylase in a predatory bug, *Andralus spinidens*. *Journal of Insect Science*, *14*, 65-73. https://doi.org/10.1673/031.014.65

Veerapan, M., Hwang, I., & Pandurangan, M. (2012). Effect of cypermethrin, carbendazim and their combination on male albino rat serum. *International Journal of Experimental Pathology*, *93*, 361-369. https://doi.org/10.1111/j.1365-2613.2012.00828.x