

**COMPARING CARBOHYDRATE ENZYMES ACTIVITY IN
EURYGASTER INTEGRICEPS PUTTON (HEMIPTERA:
SCUTELLERIDAE), *RHOPALOSIPHUM PADI* L.
(HOMOPTERA: APHIDIDAE) AND *HAPLOTHRIPS TRITICI*
KURD. (PHLAEOTHRIPIDAE: THYSANOPTERA)
AS A COMPLEX PEST ON WHEAT**

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ABSTRACT: Sunn pest (*Eurygaster integriceps* Putton), Bird cherry-oat aphid (*Rhopalosiphum padi* L.) and wheat thrips (*Haplothrips tritici* Kurd.) are the most important pests which damage on wheat. They are dependent on their carbohydrate enzymes because of their nutrition diet. The weakness of wheat is caused because of their feeding on plant's carbohydrate materials. In this study the activity of three enzymes including: α -amylase, α and β -glucosidase were assayed in the midgut, salivary glands of sunn pest, the gut of wheat aphid and wheat thrips. Levels of these enzymes activities were different. In sunn pest's midgut the highest activity was found for β -glucosidase and the lowest was for α -amylase whereas in sunn pest salivary gland the highest activity was detected for α -glucosidase and the lowest in α -amylase. In the Aphid gut enzyme activity was different so that the highest activity was detected in α -amylase but the lowest in β -glucosidase. In wheat thrips the highest enzyme activity was detected in β -glucosidase and no α -amylase activity was observed. Comparing these four samples, the alpha amylase activity had the highest level in sunn pest's midgut and the lowest in wheat thrips. The highest activity of α , β -glucosidase enzyme was as the same as α -amylase and in sunn pest midgut was the highest. The lowest activity of α , β -glucosidase was found in wheat thrips and wheat aphid, respectively. So the most important pest is *E.integriceps* because of its carbohydrate enzymes.

KEY WORDS: α -Amylase, α -Glucosidase, β -Glucosidase, Enzyme assay, *Eurygaster integriceps*, *Ropalosiphum padi*, *Haplothrips tritici*.

Sunn pest, *Eurygaster integriceps* Putton (Hemiptera: Scutelleridae), is the key pest of wheat and barley in the wide area of the Near and Middle East to Eastern and South Europe and South Africa, which causes extreme damage to the vegetative growth stage of wheat in the early season (Popov et al. 1996; Paulin & Popov, 1980). Sunn pest feeding is like other heteropteran, which penetrates and cuts tissues with its stylets in piercing-sucking mouthparts while injecting amylases and proteases through the salivary canal in order to liquefy the tissues into a nutrient-rich slurry. The liquefied food is ingested through the food canal and passed into the gut where it is digested and engorged later (Cohen, 2000; Boyed et al., 2002). Sunn pest feeds on different stages of developing grains and

sucks milky nutrients from immature grains. Its enzymes, which are injected into the grains degrade gluten proteins, cause rapid relaxation of dough and poor volume and texture of bread (Rajabi, 2000).

Aphids are also one of the principal pests of cereal crops (Minks & Harrewijn, 1987; Leszczynski et al., 1994; Yu, 1992; 1996; Roiditakis et al., 2000). In summer, aphid populations on wheat can reach damaging levels, particularly in clement regions of the world (Carter et al., 1980; Leather et al., 1989). The Bird cherry-oat aphid (*R. padi* L.) is one of the most important pests in cereal fields and the infestation of *R. padi* in cereal fields varies greatly between years (Leather et al., 1989). Heavy aphid infestation in earlier growth stages can kill the young wheat plants, but normally, aphid feeding results in a poor root growth and ultimately reduction in cultivator number and grain yield (Russell, 1978).

Thrips (Thysanoptera: Thripidae) are serious pests of ornamental, vegetable, and fruit crops both in the open field and greenhouses throughout the world (Tommasini and Maini, 1995). They belong to Hemimetabola, and are considered as the sister group of Hemiptera that includes bugs, plant hoppers and aphids (Heming, 1973). Feeding by thrips can cause deformity, discoloration, silvering and bronzing of leaves and fruits on vegetable crops reducing their market value.

In Italy *Eurygaster integriceps* like other grain pests live on a polysaccharide-rich diet and are dependent on their α -amylases and glucosidases for survival (Mendiola-Olaya et al., 2000; Boyed et al. 2002). As many species of insect depend on the effectiveness of their amylases for survival, characterization studies of insect amylases are not only of interest for comparative investigations, but they can also contribute to clarify the compatibility of some natural diets with insect development (Buonocore et al., 1976).

α -Amylases (α -1,4-glucan-4-glucanohydrolases; EC 3.2.1.1) are one group of the hydrolytic enzymes which are widespread in animals, plants and microorganisms. These enzymes are purified from different origins including bacteria, nematodes and insects because of their important biochemical role in growth and development, also their physical and chemical properties have been characterized (Mendiola-Olaya et al., 2000; Oliveira-Neto et al., 2003; Mohamed, 2004; Erthal et al., 2007).

High activities of α -glucosidases (EC 3.2.1.20) are common in many insect species and are probably involved in the intermediate and final digestion of starch and glycogen (Terra, 1988). Insects secrete many types of α -amylases enzymes which catalyze the degradation of starch and glycogen, which can be characterized by the type of residue generated (Terra and Ferreira, 2005). α -Amylase converts starch to maltose which is then hydrolyzed to glucose by an α -glucosidase. In insects, only α -amylase has been found to hydrolyze long α -1,4-glucan chains such as starch or glycogen (Terra et al., 1996).

The α -amylase and glucosidases activity of salivary glands and gut of the sunn pest and other pests were undertaken to gain a better understanding of their digestive physiology, which we hope will lead to new strategies of control.

MATERIAL AND METHODS

Insects Samples:

Adult insects (*Eurygaster integriceps* Putton, *Rhopalosiphum padi* L. and *Haplothrips tritici* Kurd.) were collected from the Pakdasht wheat farm of Tehran Province, Iran and maintained on wheat plants in the laboratory at 27 ± 2 c under a 14th light: 10h dark (LD 14:10) photoperiod but wheat thrips which were maintained in refrigerator for further usages.

Sample preparation:

Enzyme samples from the salivary glands and midguts of adults of sunn pest and aphid were prepared by the method of Cohen (1993), with slight modification. Transiently, adults were randomly selected and their midguts and salivary glands complexes were took off by dissection under a light microscope in ice-cold distilled water. We used the whole body of wheat thrips. Samples were then placed in a pre-cooled homogenizer and ground in 1 ml of distilled water. The homogenates from each preparation (midguts, salivary glands complexes and wheat thrips) were transferred separately to 1.5 ml centrifuge tubes and centrifuged at 15000 x g for 20 min at 4 c. The supernatants were pulled and stored at -20 c for following analysis.

Amylase activity assay:

The alpha amylase activity was assayed by the dinitrosalicylic acid (DNS) procedure (Bernfeld,1955) using %1 soluble starch (Merck, Darmstadt, Germany) as the substrate. Ten micro liters of enzyme were incubated for 30 min at 35 c with 500 µl universal buffer containing succinate, glycine and 2-morpholinoethanesulfonic acid (PH 6.5) (Hosseinkhani & Nemat Gorgani, 2003) and 40 µl soluble starch. The reaction was stopped by addition of 100 µl DNS and heated in boiling water for 10 min. DNS is a color reagent: the reducing group released from starch by α-amylase action are measured by the reduction of DNS. Boiling water stops the activity of α-amylase and catalyzes the reaction between DNS and the reducing groups of starch. Reaction transferred into Elisa plates. The absorbance was read at 540nm with Elisa reader (Model ELX800) after cooling in ice for 5 min. One unite of α-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min at 35 c. A standard curve of absorbance against amount of maltose released was produced to enable calculation of the amount of maltose released during α-amylase assay. Serial dilutions of maltose (Merck) in the phosphate buffer PH (6.8) were made to give concentration of 1,2,3,4,5 mg ml⁻¹. They repeated four times.

A blank without substrate but with α-amylase extracts and a control containing no α-amylase extracts but with the substrate were run at the same time with the reaction mixtures. All assays were repeated two times. The procedure was repeated for each sample (midguts and salivary glands of sunn pest and wheat aphids).

α- and β- Glucosidase activity assays:

Glucosidases activity was evaluated from salivary glands of sunn pest extracts, midguts of both sunn pest and wheat aphid extracts and wheat thrips. α and β-glucosidase were assayed to estimate the hydrolysis of the P-nitrophenyl substrate (P-nitrophenyl-α-D- glucopyranoside and P-nitrophenyl-β-D- glucopyranoside for α- and β- glucosidase, respectively) by Elisa reader according to the method of Silva and Terra (1995) and Yu (1989). Assays were performed at 40 c in 0.04 m citric acid-phosphate (Na₂Hpo₄) buffer (PH=5.0) containing 10 mµ Nacl and 10 mµ CaCl₂. Reaction mixtures consisted of enzyme extract (10 µl) and substrate (10, 5 µl for α- and β-glucosidase, respectively) and buffer (Total mixture up to 50 µl). Reactions were terminated after 10 min by adding 150 µl 0.25 µ NaOH, as stop buffer. The production of p-nitrophenol was measured at 405 nm by Elisa reader (Model ELX800). All experiments were repeated at least two times in order to calculate mean activity. The procedure was repeated separately for midguts and salivary glands complexes for each pests.

Protein determination:

Protein concentration was measured according to the method of Bradford (1976), using bovin serum albumin (Bio-Rad, Germany) as a standard.

RESULTS

The amylolytic activity per milligram was almost identical in the midgut and salivary glands of sunn pest, *E. integriceps* Putton, wheat aphid, *R. padi* L. but not in *H. tritici* Kurd. The α -amylase specific activity of sunn pest midgut and salivary gland complexes were detected 1.167 and 0.0028 mU mgProtein⁻¹, respectively, although the specific activity of the midgut of the wheat aphids was 0.037 mU mgProtein⁻¹ and no α -amylase activity was detected in wheat thrips. So the alpha amylase activity was the highest in the midgut of sunn pest but very low in its salivary glands and midgut of wheat Aphid (Fig. 1).

The specific activity of α -glucosidase from midguts and salivary glands of sunn pest was found 0.28 and 0.0316 mU mgProtein⁻¹, respectively but that was 0.0065 and 0.0045 mU mgProtein⁻¹ in the midgut of the wheat aphids and wheat thrips, respectively. This result showed α -glucosidase activity in the midgut of sunn pest was so higher than its activity in its salivary glands also higher than its activity in other samples (Fig. 1).

β -Glucosidase activity was disclosed from all samples. β -glucosidase specific activity from the midgut of sunn pest and wheat aphids was contemplated 2.4 and 0.00091 mU mgProtein⁻¹ respectively, whereas it was 0.028 and 0.0081 mU mgProtein⁻¹ in salivary gland complexes of sunn pest and wheat thrips, respectively. So we found that the β -glucosidase activity in the midgut of sunn pest was very high, whereas it was very low in other samples (Fig.1).

All results of comparing these enzymes activity among sunn pest, wheat thrips and wheat aphid are showed in Table 1.

Comparing enzymes activity in the sunn pest salivary gland complexes, wheat thrips and wheat aphid midgut showed α -amylase had the most activity in aphid midgut but was different in the others and no activity was observed in wheat thrips. The most activity of alpha and beta glucosidase was detected in salivary glands of sunn pest (Fig.2).

DISCUSSION

α -Amylases are one of the most important enzymes in *E. integriceps* which digest gluten of wheat. The presence of amylase activity in salivary glands of many phytophagous heteroptans has also been reported (Boyed et al., 2002; Boyed, 2003). Insects secrete many types of α -amylases which catalyze the degradation of starch and glycogen. These can be characterized by the type of residue generated (Terra & Ferreira, 2005). This research indicated that the α -amylase activity is present in the salivary gland complexes and midgut of *E. integriceps* Putton and the midgut of *R. padi* L. but not in wheat thrips. Specific activity of this enzyme is high in the midgut, whereas its activity is very ignoble in salivary gland complexes. As Boyed et al. (2002) showed sunn pest can digest polysaccharides partially by salivary secretions, which would be ingested along with partially digested starches to be used in the midgut. But complete breakdown of starch should take place in the midgut where large amounts of amylases exist. The activity of this enzyme in salivary gland complexes and midgut of sunn pest confirmed that. Therefore when pest complexes attack wheat, their alpha amylase importance is different so that wheat thrips has no alpha amylase and doesn't damage on gluten which it differs in other pests. Alpha amylase is one of the most of the active enzymes in the midgut of a wheat aphids but in contrasting with the midgut of sunn pest is not high. α -amylase of *R. padi* can damage on wheat but

not as much as sunn pest midgut. However, this enzyme in *R. padi* is much more important than sunn pest salivary glands and can damage on wheat more.

High activities of α -glucosidase is common in many insect species and is probably involved in the digestion procedure of starch and glycogen (Terra, 1988). Comparing specific activity of the α -glucosidases of midgut and salivary glands of sunn pest, indicated both have this enzyme activity also this enzyme activity is present in the midgut of the wheat aphids and wheat thrips. Activity of α -glucosidase was very high in the midgut but low in salivary gland complexes of sunn pest and was the highest in the midgut of sunn pest but lowest in wheat thrips. We found that the damage of *R. padi* and *H. tritici* alpha glucosidase on wheat are the same because their enzyme activity has no significant differences with each other although it is lower in *H. tritici*.

Midgut of sunn pest and wheat aphid showed the highest and lowest activity of β -glucosidase, respectively. This enzyme activity in sunn pest salivary gland complexes is much more than wheat thrips and *R. padi*. So this enzyme damage on wheat is more important than wheat aphid and thrips.

Comparing enzymes activity in sunn pest salivary glands showed the lowest activity was in α -amylase, whereas α -glucosidase and β -glucosidase activity were high and the same.

Enzymes assay in the midgut of *E. integriceps* showed the highest activity was in β -glucosidase and the lowest in α -glucosidase. α -amylase activity was lower than β -glucosidase but more than α -glucosidase. Enzymes activity in *R. padi* proved that α -amylase activity was the highest in contrast with other enzymes. But in *H. tritici* the highest activity of enzymes was observe in β -glucosidase and no α -amylase activity was detected.

As a result we found that when complex of pests attack wheat field, the α -amylase which exist in salivary glands and midgut of sunn pest damage on wheat carbohydrates then wheat aphid α -amylase damage on it although wheat thrips doesn't secrete this carbohydrate enzyme. Then α , β -glucosidase enzymes dehydrate the production and damage on wheat.

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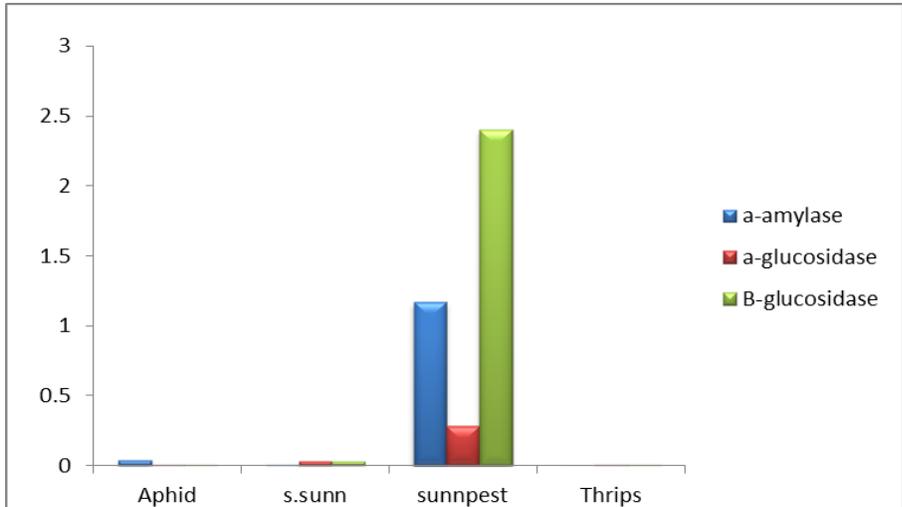


Figure 1. Comparing enzymes in the sunn pest midgut and salivary glands, wheat aphid midgut and wheat thrips showed all enzymes had the most activity in sunn pest midgut.

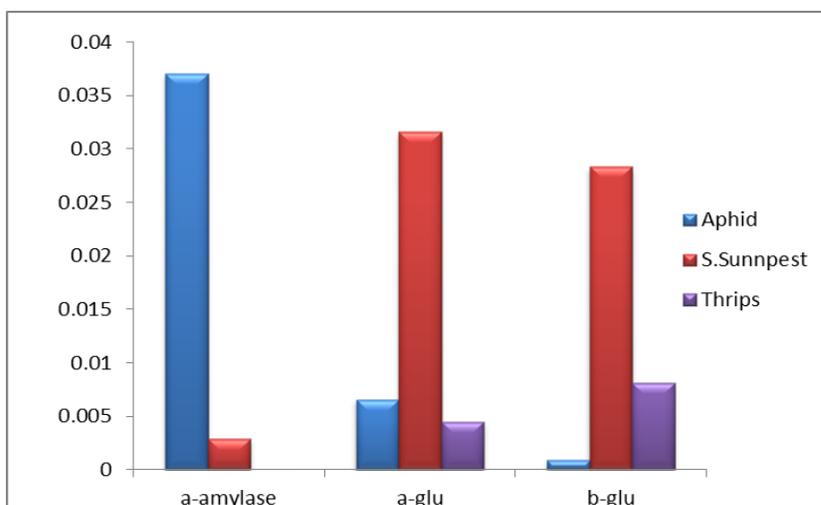


Figure 2- Comparing enzymes in the sunn pest salivary glands, wheat aphid midgut and wheat thrips.

Table 1. Specific activity of aphid midgut, sunn pest midgut and salivary glands and wheat thrips (mili Unit of activity/mgProtein).

Specific activity	Aphid	Sunn pest salivary gland	Sunn pest midgut	Thrips
α -amylase	0.037	0.0028	1.167	0
α -glucosidase	0.0065	0.0316	0.28	0.0045
β -glucosidase	0.00091	0.028	2.4	0.0081