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RESEARCH REPORT

Lipase and invertase activities in midgut and salivary glands of *Chilo suppressalis* (Walker) (Lepidoptera, Pyralidae), rice striped stem borer

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Abstract

The rice striped stem borer, *Chilo supprressalis*, was introduced to Iran in 1973 where it is now widely distributed and causes severe damages. Lipases, which catalyses the hydrolysis of fatty acid ester bonds, are widely distributed among animals, plants and microorganisms. Invertases (*β*-fructofuranosidase) are glycosidehydrolases that catalyze the cleavage of sucrose (*β*-D-glucopyranosyl-S-D-fructofuranoside) into the monosaccharides glucose and fructose. Laboratory-reared 4th instar larvae were randomly selected, their midgut and salivary glands were removed by dissection under a light microscope and lipase and invertase activities were assayed. The activity of lipase/invertase in the midgut and salivary gland were 0.49/0.27 and 0.35/0.23 µmol/min/mg protein, respectively. The optimum pH and temperature for both the two enzymes were determined to be 10-11 and 37-40 °C, which is consistent with pH and temperature values already observed in Lepidoptera. The enzyme activity was reduced by addition of NaCl, KCl, $MgCl_2$, SDS, urea and plant extracts from *Artemisia annua*, but not by $CaCl_2$ which enhanced enzyme activity. Pest control with usage of resistant varieties of plants is one of the most important practices that are dependant on inhibitors already present in nature. Hence, characterization of insect digestive enzymes, especially examination of inhibition effects on enzyme activity, could be useful in developing new strategies for pest control.

Key words: *α*-amylase; rice striped stem borer; midgut; salivary glands

Introduction

The rice striped stem borer (*Chilo supprressalis*, Walker) is a cosmopolitan and destructive pest in rice fields of the world (Zibaee *et al.*, 2008). This pest was introduced in Iran in 1973 and since then has been widely distributed in the country rice fields. It causes severe damages in all rice fields and its present density is superior than the economic injury level (EIL) (Dezfoulian and Moustofipoor, 1972). The chemical control by using organophosphorus compounds, has been a common control procedure, although other methods based on agricultural practices such as ploughing, usage of resistant varieties of plants, weed control as overwintering sites and biological control with *Trichogramma* spp. have been incorporated. In recent years, resistant varieties and pheromones also have been added to control the diffusion of *C. suppressalis* like in other places of the world (Muralidharan and Pasaalu, 2006). A study on 78 different varieties of rice showed that Binam with 15 % white head is the most resistant variety. Germplasts studies showed that Khazar variety is resistant to the first generation of rice striped stem borer. However, it is susceptible to the second generation.

Lipases (triacylglycerol–acyl-hydrolase EC 3.1.1.3), which catalyzes the hydrolysis of fatty acid ester bonds, are widely distributed among animals, plants and microorganisms (Naumff, 2001). It has been showed that lipases can also hydrolyze a variety of esters in organic solvent systems and thus they can be widely used in many industrial areas, e.g., dairy, food, detergent and biofuel industries (Ishaaya and Swirski, 1970; Henrissat and Bairoch, 1993; Grillo *et al*., 2007). The most characteristic property of lipases is that they act on substrate at the interface between the aqueous and the lipid phase (Grillo *et al.*, 2007).

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To date, many research groups have carried out the isolation and purification of lipases from various sources, mainly microorganisms, fish, fungi, milk and plants (Cherry and Crandall, 1932; Henrissat and Bairoch, 1993; Degerli and Akpinar 2002; Grillo *et al.*, 2007). However, lipid biochemistry studies in insects is time-consuming and moved on very slowly due to high diversity of insects and changes in the lipid composition and lipophorin present in hemolymph during metamorphosis from larva to pupa (Degerli and Akpinar, 2002). Recently, lipid mobilization and transport in insects is under investigation, especially lipases and lipophorin (a reusable lipoprotein particle in insect systems) because of their roles in energy production and transport of lipids at flying activity (Ayre, 1967). Although stored lipids in vertebrate adipose tissue are released as free fatty acids, in insects most fatty acids are released as 1,2-diacylglycerols and mobilization of lipid reserves from insect fat body is under the control of adipokinetic hormone (Grillo *et al.*, 2007).

Invertases (*β*-fructofuranosidase, EC3.2.1.26) also termed fructosidase, saccharase, or sucrase, are glycosidehydrolases (EC 3.2.1) that catalyze the cleavage of sucrose (*β*-D-glucopyranosyl-S-D-fructofuranoside) into the monosaccharides, glucose and fructose (Henrissat and Bairoch, 1993; Sturm and Tang, 1999; Naumoff, 2001). Invertase, thus, appears to be a particularly important enzyme for plants and animals. Given this general importance, a surprisingly limited number of studies have tried to quantify invertase activity in ants (Ayre, 1963, 1967; Ricks and Vinson, 1972) or other animals (Martinez del Rio, 1990; Zhang *et al.*, 1993). This might be due to the particular methodological problems arising from the quantification of invertase in animals whose carbohydrate metabolism is highly active.

Digestion is a phase of insect physiology on which little research has been performed, despite the economic importance of the food of insects and the fact that the most important control measures involve the action of digestive juices on poisons taken into the digestive tract. A better understanding of enzyme catalysis is essential in order to develop methods of insect control (Bandani *et al.*, 2001; Ghoshal *et al.*, 2001; Maqbool *et al.*, 2001). The purpose of the present study is to identify and characterize the lipase and invertase activities from midgut and salivary glands (SG) of rice striped stem borer larvae to gain a better understanding of the digestive physiology. This understanding will hopefully lead to new management strategies for control of this pest.

Materials and Methods

Insects

To decrease the side effects of laboratory mass culture, 400 pupae were collected from fields and reared on the same variety seedling (Taroum) as sampling sites. Insects were reared based on the method mentioned by Kammano and Sato (1985) in 28 ± 1 °C, light cycle 16L:8D and RH > 80 %. When the larvae grow up to 4th instar larvae, 30 larvae were randomly selected for biochemical analysis. For 4th instar determination, Dayer's formula was used which had been described by Majidi *et al.* (2002).

Sample preparation and enzyme assays

Briefly, larvae were randomly selected and total midgut and SG were removed by dissection under a stereo microscope in ice-cold saline buffer (6 µM NaCl). The midgut and SG were separated from the insect's body, rinsed in ice-cold buffer, placed in a pre-cooled homogenizer and ground in 1 ml of universal buffer containing succinate, glycine and 2-morpholinoethanesulfonic acid (pH 7.2) (Hosseinkhani and Nemat-Gorgani, 2003). The homogenates from both preparations (midgut and SG) were separately transferred to 1.5 ml centrifuge tubes and centrifuged at 20,000 x g for 20 min at 4 °C. The supernatants were pooled and stored at -20 °C for subsequent analyses.

Lipase activity

The enzyme assays were carried out as described by Tsujita et al. (1989). Thirty µl of gut and salivary glands tissue extracts and 100 µl of *p*-nitrophenyl butyrate (50 mM), as substrate, were incorporated, mixed thoroughly and incubated at 37 °C. For negative control tubes, samples (midgut and salivary glands) were placed in a boiling water bath for 15 min to destroy the enzyme activity and then cooled. After 1 min, 100 µl distilled water were added to each tube (control and treatment) and absorbance was read at 405 nm. One unit of enzyme

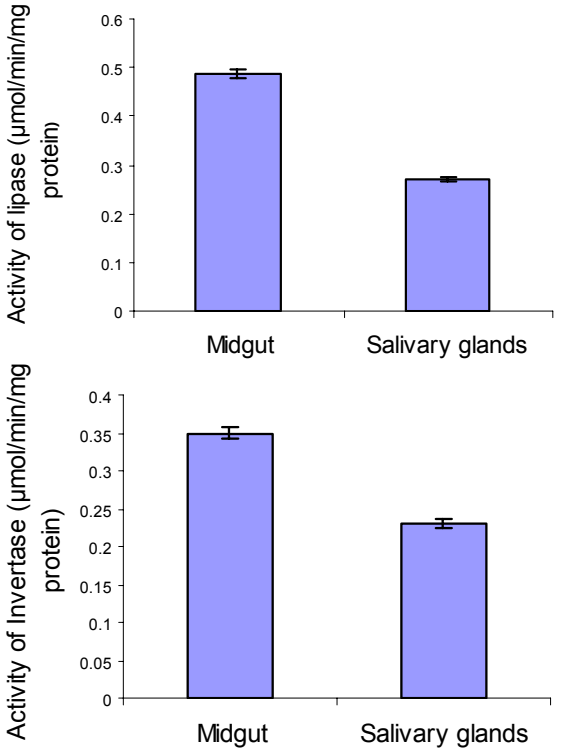

Fig. 1 Activity level of lipase (up) and invertase (down) in 4th instar larvae midgut and salivary glands of rice striped stem borer.

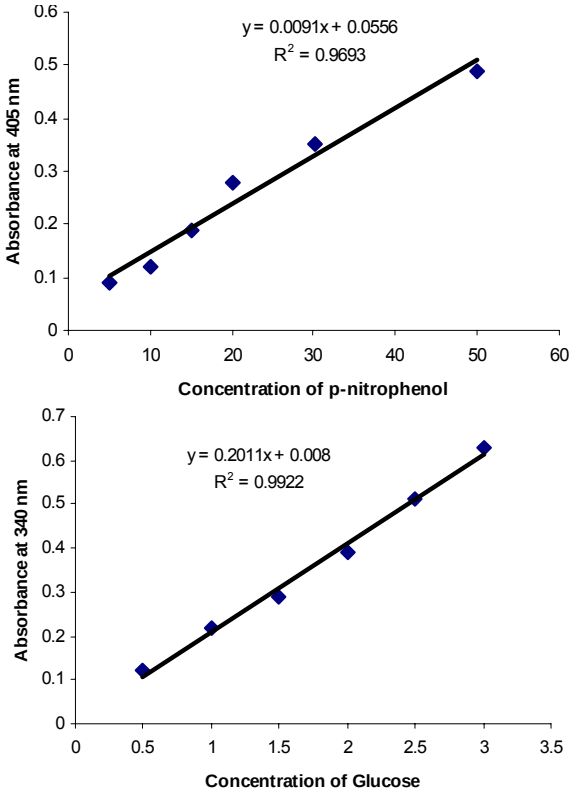

Fig. 2 Standard calibration curve for the determination of p-nitrophenol and glucose released in the lipase (up) and invertase (down) assay.

will release 1.0 nmol of p-nitrophenol per min at pH 7.2 at 37 °C using p-nitrophenyl butyrate as substrate. Standard curve was used to calculate the specific activity of enzyme.

Invertase activity

Samples were transferred to 100 ml flasks and 1 ml toluene was added to arrest the enzyme activity. After 15 min, 6 ml of 0.2 M glycine buffer (pH 7.2) containing 18 mM sucrose was added to the samples and the flasks were closed with cotton plugs then held for 24 h at 30 °C. Samples were passed through Whatman filter paper and glucose in the filtrate was assayed at 340 nm (Nelson, 1994).

Kinetic parameters measurements

Twenty µl of appropriately diluted enzyme preparation was used in each assay. Final concentrations for substrate were 20, 30, 40, 50 and 60 mM for lipase and 0.09, 0.18, 0.36, 0.72 and 1.54 mM for invertase, respectively. The Michaelis constant (K_m) and the maximum velocity (V_{max}) were estimated by Sigmaplot software version 11 (Systat Software Inc., Chicago, IL, USA) and the results of K_m and V_{max} were the means ± SE of three replicates for every population.

Effect of pH and temperature on enzyme activity

The effect of temperature and pH on lipase and invertase activity were examined using enzyme extractions from the larval midgut and SG. The effect of temperature on enzymes activity was determined by incubating the reaction mixture at 20, 25, 30, 35, 37, 40, 45, 50, 55, 60 and 70 °C for 24 h, followed by measurement of activity. Optimal pH for their activities was determined using universal buffer with pH set at 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13.

Effect of activators and inhibitors on enzyme activity

To test the effect of different ions on the enzymes, midguts and SG were dissected in distilled water. Enzyme assays were performed in the presence of different concentrations of chloride salts of Na^+ (5, 10, 20 and 40 mM), K^+ (5, 10, 20 and 40 mM), Ca^{2+} (5, 10, 20 and 40 mM), Mg^{2+} (5, 10, 20 and 40 mM), and ethylenediaminetetraacetic acid (EDTA; 0.5, 1, 2 and 4 mM), sodium dodecylsulfate (SDS; 1, 2 and 4 mM), urea (0.5, 1, 2, 4, 6 and 8 mM) and *Artemisia annua* extract (10, 15 and 25 % concentrations). These compounds were added to the assay mixture, and activity was measured after 30 min incubation. A control was also measured (no compounds added).

Effect of A. annua extract on enzymatic parameters of Invertase and lipase

For this experiment, 20 µl of appropriately diluted enzyme preparation was used in each assay. Final concentrations for substrate were 20, 30, 40, 50 and 60 mM for lipase and 0.09, 0.18, 0.36, 0.72 and 1.54 mM for invertase, respectively. Finally, 20 % of plant extract added to each well. K_m and V_{max} were estimated by Sigmaplot software version 11 (Systat Software Inc.) and the results of

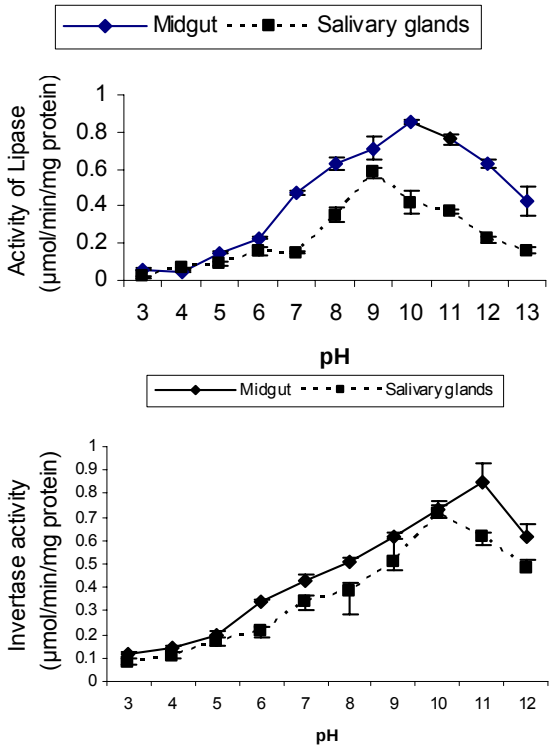

Fig. 3 Effect of pH on activity of lipase (up) and invertase (down) extracted from midgut and salivary gland of rice striped stem borer.

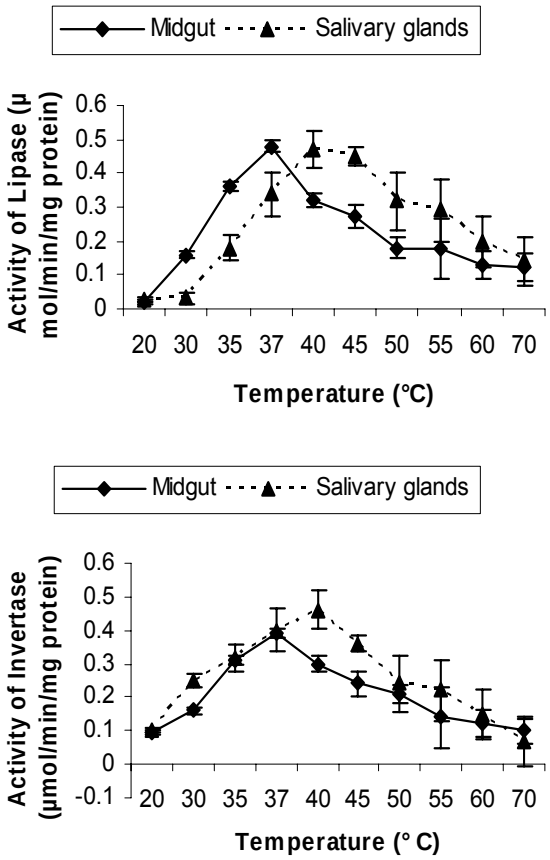

Fig. 4 Effect of temperature on activity of lipase (up) and invertase (down) extracted from midgut and salivary gland of rice striped stem borer.

K_m and V_{max} were the means ± SE of three replicates for every population. For determination of *A. annua* extract effect on enzymatic parameters of lipase and invertase, 20 % of plant extract was added to each well.

Polyacrylamide Gel Electrophoresis (PAGE)

In order to determine the molecular mass of native lipase, native polyacrylamide disc-gel electrophoresis was carried out using the method of Parish and Marchalonis (1970) using 2.7 % and 7.7 % polyacrylamide for the stacking and resolving gels, respectively. The gel was stained with 1.5 % (w/v) Coomassie Brilliant Blue G-250 and distained in glacial acetic acid-methanol-water 7.5: 5.0: 87.5.

Protein determination

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

Statistical analysis

Data were compared by one-way analysis of variance (ANOVA) followed by Tukey's studentized test when significant differences were found at *P*=0.05. Enzyme kinetic parameters were analyzed by using the Sigmaplot software version 11 (Systat Software Inc.).

Results

Lipase and Invertase activities

Studies showed that lipase and invertase are present in the midgut and salivary glands of adult *C. suppressalis* (Fig. 1). The activity of lipase was 0.486 µmol/min/mg protein and 0.27 µmol/min/mg protein in midgut and SG, respectively. The invertase activity in midgut and SG was 0.35 and 0.23 µmol/min/mg protein, respectively. There was a significant difference in the degree of enzyme activity between midgut and salivary glands (Figs 1, 2).

Effect of pH and temperature on enzyme activity

The *in vitro* evaluation of *C. suppressalis* lipase and invertase from midgut and SG indicated that enzyme activity increased steadily from pH 3 to 11 and from 3 to 10, respectively. After reaching the threshold pH level, enzyme activity decreased with the increasing of pH and there were significant differences among measured values for each pH (Fig. 3). Both enzymes were considerably active over a broad range of temperatures. As the results show, the optimum temperature for lipase and invertase activities were 37 and 40 °C for midgut and SG, respectively (Fig. 4).

Effect of activators and inhibitors on enzyme activity

Several molecules and chemical compunds affects the activity of lipase and invertase in midgut and SG of rice striped stem borer, although they had a similar effect on both enzymes (Tables 1 and 2). Activity level of enzymes in midgut and SG elevated due to increasing of $CaCl_2$ and EDTA concentrations for lipase and just $CaCl_2$ for invertase (Table 1 and 2). Activity level of enzyme decreased in presence of NaCl, KCl, EDTA, $MgCl_2$, SDS, urea and *A. annua* extract in both midgut and SG (Tables 1 and 2).

Kinetic Parameters

As can be seen in Table 3, lipase V_{max} of midgut and SG were 0.5 and 0.35 µmol/min/mg protein, respectively. Lipase K_m of midgut and SG were 15 and 19 mM, respectively. Invertase V_{max} was 0.9 and 0.5 µmol/min/mg protein in midgut and SG, respectively, while invertase K_m was 0.31 and 0.39 mM in midgut and SG (Table 3, Fig. 5).

Effect of A. annua extract on enzymatic parameters of invertase and lipase

Enzymes parameters changed due to using of *A. annua extract*. As it is shown in Table 4, lipase kinetic parameter, V_{max}-K_m were 0.35 µmol/min/mg protein-37.5 mM in midgut and 2.12 µmol/min/mg protein-21 mM in SG. As well as invertase is concerned, V_{max}-K_m were 0.49 µmol/min/mg protein-0.30 mM in midgut and 0.81 µmol/min/mg protein-0.92 mM in SG (Table 4, Fig. 6).

Native PAGE

Analysis of midgut and SG lipase and invertase enzyme from homogenates of *C. suppressalis* by vertical slab electrophoresis on 8 % polyacrylamide gels indicated one band in all samples except for midgut's lipase (Fig. 7).

Table 1 Relative activity of *C. suppressalis* lipase toward different compounds

Compounds	Concentration (mmol/l)	Relative activity (Midgut)	Relative activity (salivary gland)
Control	**-**	100	100
NaCl	**5**	102.77*	189.47*
	10	77.77*	157.9*
	20	33.33*	89.47*
	40	6.38*	52.63*
$CaCl_2$	**5**	50*	57.89*
	10	77.77*	121.05*
	20	88.88*	173.68*
	40	119.44*	210.52*
KCl	**5**	97.3*	100*
	10	76.54*	87.36*
	20	55.89*	68.66*
	40	32.77*	45.25*
$MgCl_2$	**5**	247.22*	421.05*
	10	208.33*	363.15*
	20	91.66*	236.84*
	40	44.44*	131.57*
EDTA	**0.5**	38.88*	48.84*
	1	72.22*	73.68*
	2	105.55*	157.89*
	4	231.55*	207.89*
SDS	**2**	113.88*	194.73*
	4	80.55*	121.05*
	6	44.44*	52.63*
	8	26.66*	45.26*
Urea	**1000**	116.66*	205.23*
	2000	102.77*	142.1*
	4000	69.44*	115.78*
	5000	27.77*	89.47*
	6000	0.036*	31.57*
Plant extract	**10 %**	78*	86*
	15 %	59*	63*
	25%	34*	39*

*$P < 0.05$ vs control

Table 2 Relative activity of *C. suppressalis* invertase in presence different compounds

Compounds	Concentration (mmol/l)	Relative activity (Midgut)	Relative activity (salivary gland)
Control	-	100	100
NaCl	**5**	102.77*	189.47*
	10	77.77*	157.9*
	20	33.33*	89.47*
	40	6.38*	52.63*
$CaCl_2$	**5**	50*	57.89*
	10	77.77*	121.05*
	20	88.88*	173.68*
	40	119.44*	210.52*
KCl	**5**	97.3*	100*
	10	76.54*	87.36*
	20	55.89*	68.66*
	40	32.77*	45.25*
$MgCl_2$	**5**	247.22*	421.05*
	10	208.33*	363.15*
	20	91.66*	236.84*
	40	44.44*	131.57*
EDTA	**0.5**	38.88*	48.84*
	1	72.22*	73.68*
	2	105.55*	157.89*
	4	231.55*	207.89*
SDS	**2**	113.88*	194.73*
	4	80.55*	121.05*
	6	44.44*	52.63*
	8	26.66*	45.26*
Urea	**1000**	116.66*	205.23*
	2000	102.77*	142.1*
	4000	69.44*	115.78*
	5000	27.77*	89.47*
	6000	0.036*	31.57*
Plant extract	**10 %**	78*	86*
	15 %	59*	63*
	25%	34*	39*

*$P < 0.05$ vs control

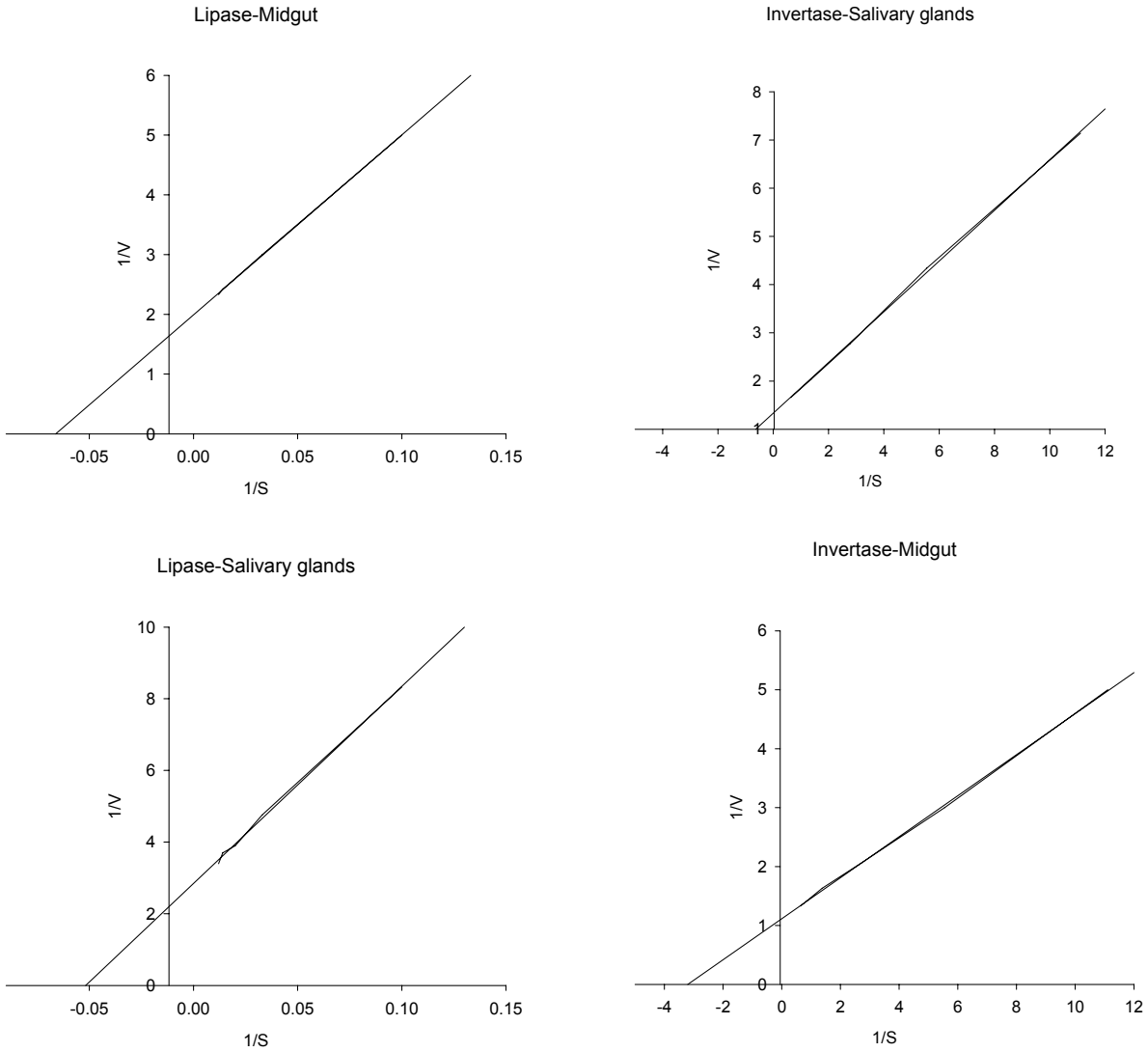

Fig. 5 Lineweaver-Burk plot (V_{max} and K_m) of lipase and invertase extracted from 4th instar larvae of rice striped stem borer.

Discussion

The present study shows that the larvae of *C. suppressalis* present lipase and invertase activities both in the midgut and in the SG. Reports concerning lipase characterization have been obtained from several species of insects. Metcalf (1945) found amylase, protease, and lipase to be absent from the SG of the mosquito *Anopheles quadrimaculatus* while he observed that invertase is present in both midgut and crop homogenates. Fisk and Shambaugh (1954) found no activity of lipase in the SG of *A. quadrimaculatus*. A total body lipase was partially purified from abdomen homogenate of *Gryllus campestris* L. (Orthoptera, Gryllidae) (Orscelik *et al.*, 2007).

Current study showed that both lipase and invertase are present in *C. suppressalis* and that the optimal pH for both enzymes are in alkaline condition (around pH 10). Optimal temperatures for both enzymes are 37-40 °C in midgut and SG, respectively. Ishaaya and Swirski (1970) demonstrated that the optimum pH and temperature for invertase activity in the hemipteron *Chrysomphalus aonidum* are 5.5 and 30 °C, respectively. Degleri and Anuipor (2002) showed that the optimal pH of lipases activity in the teleost fish *Cyprinion macrostomus* is 7.5. Studies on lipase properties of yeasts revealed that optimum pH and temperature for lipases activity are 7.5-8.2 and 30-40 °C, respectively (Vakhlu and Kour, 2006). The utilization of dietary lipids was studied in adult females of the blood-sucking bug *Rhodnius prolixus* with the use of radiolabeled triacylglycerol (Grillo *et al.*, 2007). These researches indicates that lipase activity is affected by pH and shows an optimal activity at a pH of 7.0-7.5. The optimal pH generally reflects the pH of the environment in which the enzyme normally functions. One way in which pH affects reactions rates is by altering the charge state of the substrate or of the active site of the enzyme. Extreme pHs can also disrupt the hydrogen bonds

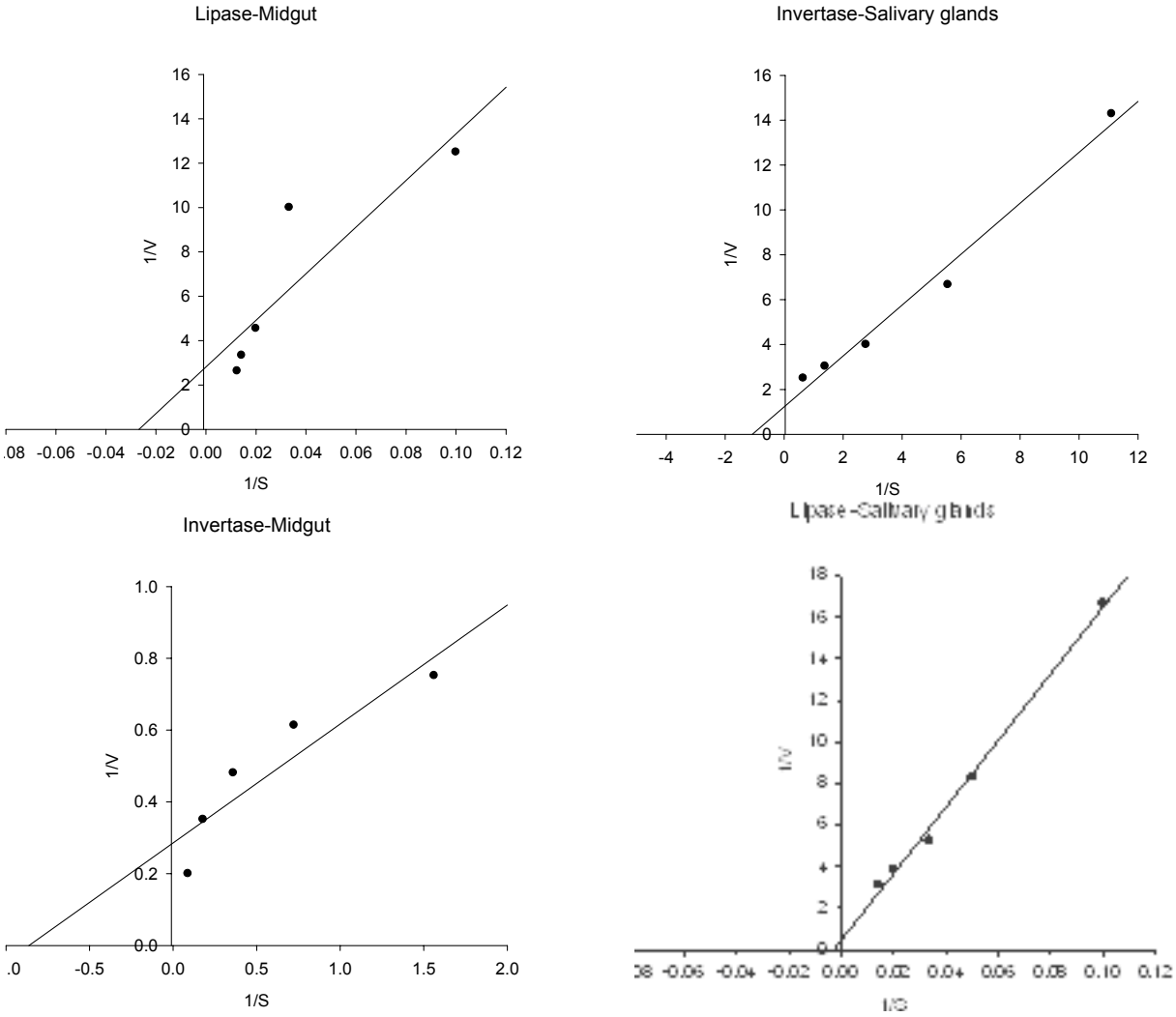

Fig. 6 Lineweaver-Burk Plot (V_{max} and K_m) of lipase and invertase extracted from 4th instar larvae of rice striped stem borer due to using *A. annua* extract.

that hold the enzyme in its three-dimensional structure, denaturing the protein (Zeng *et al.*, 2000). Biological reactions occur faster with increasing temperature up to the point of enzyme denaturation, above which temperature enzyme activity and the rate of the reaction decreases sharply (Applebaum 1985; Agblor *et al.*, 1994; Zeng *et al.*, 2002).

Current study results showed that Ca^{2+} ions have activatory effects on the lipase and invertase activities of rice striped stem borer. Podolor and Applebaum (1971) reported that Ca^{2+} ions have activatory effects on the lipase and invertase activities of the coleopteron *Callosobruchus chinensis*. These results showed that these enzymes are metalloproteins which require calcium for maximum activity.

Researches have shown a significant correlation between the activity level of digestive enzymes in the hemolymph and the midgut. Saleem and Shakoori (1987) showed that sublethal concentrations of pyrethroids decrease amylase activity in larval gut of the beetle *Tribolium castaneum*. Lee *et al.* (1994) showed that some insect growth regulators decreased the activity level of alpha-amylase and esterase in treated larvae of *C. suppressalis*. Ascher and Ishaaya (2004) showed that the activity level of this enzyme increased 30 % in the noctuid moth *Spodoptera littoralis* treated with phentine acetate compared with control. Shekari *et al.* (2008) suggest that its activity level decreases 24 h after treatment and sharply increases at 48 h.

Digestive enzyme inhibitors occur naturally in many food plants and are particularly abundant in cereals and legumes (Franco *et al.*, 2002). Insects gain access to food sources when they evolve enzymes that are not affected by inhibitors present in the food source, and plants become resistant when they evolve inhibitors effective against these

Table 3 Kinetic parameters of lipase and invertase enzymes extracted from midgut and SG of 4th instar larvae of *C. suppressalis*

Enzymes	Midgut*		SG*	
	V_{max} (µmol/min/mg protein)	K_m (mM)	V_{max} (µmol/min/mg protein)	K_m (mM)
Lipase	0.5 ± 0.06	15 ± 3.74	0.35 ± 0.09	19 ± 6.45
Invertase	0.9 ± 0.036	0.31 ± 0.047	0.5 ± 0.078	0.39 ± 0.77

*Means ± SE, N = 3

Table 4 Kinetic parameters of lipase and invertase enzymes extracted from midgut and SG of 4th instar larvae of *C. suppressalis* after exposure to *A.annua* extract

Enzymes	Midgut*		SG*	
	V_{max} (µmol/min/mg protein)	K_m (mM)	V_{max} (µmol/min/mg protein)	K_m (mM)
Lipase	0.35 ± 0.087	37.5 ± 15.24	2.12 ± 0.68	21 ± 5.8
Invertase	0.49 ± 0.087	0.30 ± 0.063	0.81 ± 0.021	0.92 ± 0.77

*Means ± SE, N = 3

insect enzymes. When the action of digestive enzymes is inhibited, insect's nutrition is impaired, growth and development are retarded and eventually death occurred due to starvation. Genes encoding for digestive enzyme inhibitors have been used to make transgenic crops by gene transfer technology. In transgenic pea expressing the α-amylase inhibitor, the expression of digestive enzyme inhibitors makes plants harmful to target insects and pests, interfering with their digestive and absorption processes, whereas neither anti-nutritional nor toxic effects were observed in rats (Pusztai *et al.*, 1999). The primary reason for producing insect-resistant transgenic crops is to reduce the use of chemical pesticides, which by one side lowers production costs and in the meantime reduces the insecticide loads in the environment. Making insect-resistant plants requires the characterization of α-amylase and other digestive enzymes of the target insect and the identification of suitable inhibitors from plants or other sources.

In our opinion, the purification and characterization of more insect digestive enzymes will greatly facilitate the understanding of the mechanisms responsible for this selectivity and will help to design new and more specific strategies for insect control.

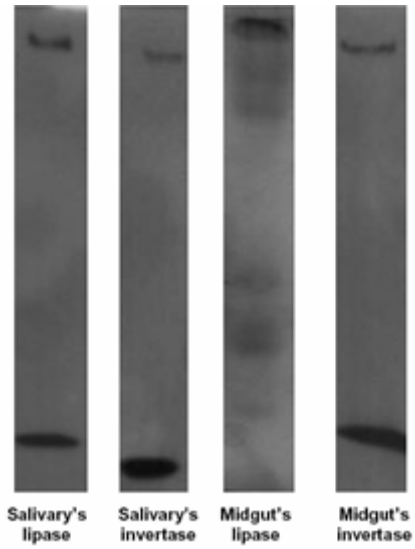

Fig. 7 Native-PAGE gel electrophoresis of midgut and SG from *C. suppressalis*.

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References

Agblor A, Henerson HM, Madrid FJ. Characterization of alpha-amylase and polygalacturonase from *Lygus spp.* (Heteroptera: Miridae). Food Res. Inter. 27: 321-326, 1994.

Applebaum S W. Biochemistry of digestion. In: GA Kerkut, LL Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol.4: Regulation, digestion, excretion. Pergamon Press., pp. 279-307, 1985.

Ascher KRS, Ishaaya I, Antifeeding and protease and amylase inhibiting activity of phentin acetate in *Spodoptera littoralis* larvae, Pestic. Biochem. Physiol. 75: 326-336, 2004.

Ayre GL. Feeding behaviour and digestion in *Camponotus herculeanus* (L.) (Hymenoptera: Formicidae). Entomol. Exp. Appl. 6: 165-170, 1963.

Ayre G L. The relationships between food and digestive enzymes in five species of ants (Hymenoptera: Formicidae). Canad. Entomol. 99: 408–411, 1967.

Baker JE. Interaction of partially-purified amylase with amylase inhibitors from wheat. Comp. Biochim. Physiol. 93B: 239-246, 1989.

Bandani AR, Amiri B, Butt TM, Gordon-Weeks R. Effects of efrapeptin and destruxin, metabolites of entomogenous fungi, on the hydrolytic activity of a vacuolar type ATPase identified on the brush border membrane vesicles of *Galleria mellonella* midgut and on plant membrane bound hydrolytic enzymes. Biochim. Biophys. Acta 1510: 367-377, 2001.

Bradford MA. rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254, 1976.

Da Silva MCM, Mello LV, Coutinho MV, Rigden DJ, Neshich G, Chrispeels M, *et al*. Mutants of common bean alpha-amylase inhibitor-2 as an approach to investigate binding specificity to alpha-amylases. Pesq. Agropec. Bras. 39: 201-208, 2004.

Degerli N, Akpinar MA. Partial purification of intestinal triglycride lipase from *Cyprinion macrostomus* and effect of pH on enzyme activity. Turk. J. Biol. 26: 133-143, 2002.

Dezfoulian A, Moustofipoor P. Rice striped stem borer. Agri. Org. Iran. Pub., pp 149, 1972.

Fisk FW, Shambaugh GF. Invertase activity in adult *Aedes aegypti* mosquitoes. Ohio. J. Sci. 54: 237-240, 1954.

Franco OL, Riggen DJ, Melo FR, Bloch C, Silva C, Grossi de sa MF. Activity of wheat α-amylase inhibitors towards bruchid α-amylases and structural explanation of observed specificities. Eur. J. Biochem. 267: 2166-2173, 2002.

Ghoshal D, Sen S K, Goyal A. Introduction and expression of cowpea trypsin inhibitor (CpTI) gene in transgenic tobacco. J. Plant. Biochem. Biotech. 10: 19-25, 2001.

Grillo LA, Majerowicz D, Gondim KC. Lipid metabolism in *Rhodnius prolixus* (Hemiptera: Reduviidae): role of a midgut triacylglycerol-lipase. Insect Biochem. Mol. Biol. 37: 579-588, 2007.

Henrissat B, Bairoch A. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. 293: 781-788, 1993.

Hosseinkhani S, Nemat-Gorgani M. Partial unfolding of carbonic anhydrase provides a method for its immobilization on hydrophobic adsorbents and protects it against irreversible thermoinactivation. Enz. Micro. Tech. 33: 179-184, 2003.

Ishaaya I, Swirski E. Invertase and amylase activity in the armoured scales *Chrysomphalus aonidum* and *Aonidiella* aurantii. J. Insect. Physiol. 16: 1599-1606, 1970.

Ishaaya I, Moore I, Joseph D. Protease and amylase activity in larvae of the Egyptian cotton worm, *Spodoptera littoralis*. J. Insect. Physiol. 17: 945-953, 1971.

Lee HR, Kim JW, Lee I, Studies on the toxicity of insect growth regulators against the fall webworm (*Hyphantaria cunea* Drury) and the rice stem borer (Chilo suppressalis Walker) II. Comparison in enzyme activities. Korean, J. Appl. Entomol. 33: 88-95, 1994.

Maqbool SB, Riazuddin S, Loc NT, Gatehouse AMR, Gatehouse JA, Christou P. Expression of multiple insecticidal genes confers broad resistance against a range of different rice pests. Mol. Breeding 7: 85-93, 2001.

Martinez del Rio C. Dietary, phylogenetic, and ecological correlates of intestinal sucrase and maltase activity in birds. Physiol. Zool. 63: 987-1011, 1990.

Mendiola-Olaya E, Valencia-Jimenez A, Valdes-Rodriguez S, Delano-Frier J, Blanco-Labra A. Digestive amylase from the larger grain borer, *Prostephanus truncates* Horn. Comp. Biochem. Physiol. 126B: 425-433, 2000.

Muralidharan K, Pasalu IC. Assessments of crop losses in rice ecosystems due to stem borer damage (Lepidoptera: Pyralidae). Crop Prot. 25: 409-417, 2006.

Naumff DG. *β*-Fructosidase superfamily: Homology with some *α*-L-arabinases and *β*-D-xylosidases. Proteins: Struct. Funct. Genet. 42: 66-76, 2001.

Nelson N . A photometric adapatation of Somogyi method for determination of glucose. J. Biol. Chem. 153: 375-380, 1994.

Orscelk O, Akpinar MA, Gorgun S. Partial Purification of Total Body Lipase from *Gryllus campestris* L. (Orthoptera: Gryllidae). Fen Bilimleri Dergisi. 27: 1-10, 2007.

Parish SH, Marchalonis JJ. Polyacrylamide disc gel electrophoresis. Anal. Biochem. 34: 436. 1970.

Pusztai A, Bardocz GG, Alonso R, Chrispeels MJ, Schroeder LM, Tabe TJ, *et al*. Expression of the insecticidal bean alpha-amylase inhibitor transgene has minimal detrimental effect on the nutritional value of peas fed to rats at 30% of the diet. J. Nutrition. 129: 1597-1603, 1999.

Ricks BL, Vinson SB. Digestive enzymes of imported fireant, *Solenopsis richteri* (Hymenoptera: Formicidae). Entomol. Exp. Appl. 15: 329-334, 1972.

Saleem MA, Shakoori AR. Point effects of Dimilin and Ambush on enzyme activies of *Tribolium castaneum* larvae, Pestic. Biochem. Physiol. 29: 127-137, 1987.

Shekari M, Jalali Sendi J, Etebari K, Shadparvar A. Effects of *Artemisia annua* L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). Pestic. Biochem. Physiol. 91: 66-74, 2008.

Sturm A, Tang GQ. The sucrose-cleaving enzymes of plants are crucial for development, growth

and carbonpartitioning. Trends Plant Sci. 4: 401-407, 1999.
Tsujita T, Ninomiya, H, Okuda, H. p-nitrophenyl butyrate hydrolyzing activity of hormone-sensitive lipase from bovine adipose tissue. J Lipid Res. 30: 997-1004, 1989.
Vakhlu J, Kour A. Yeast lipases: enzyme purification. Biochem. Prop. Gene. Cloning. 9: 69-85, 2006.
Zeng F, Cohen A C. Partial characterization of α-amylase in the salivary glands of *lygus Hesperus* and *L. lineolaris*. Comp. Biochem. Physiol. 126B: 9-16, 2000.
Zibaee A, Jalali Sendi J, Alinia F, Etebari K. A study on biochemical differences among five different groups of rice striped stem borer *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). Inv. Surv. J. 5: 20-29, 2008.