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Alpha-Amylase Enzymes of *Tribolium castaneum* (Herbst) and *Callosobruchus maculatus* (F.) And Their Response to A-Amylase Inhibitor from Rice

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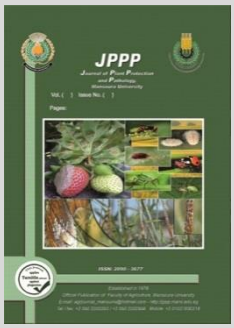

ABSTRACT

Insect α-amylase has recently become a target of insect control strategies using plant-derived α-amylase inhibitors especially for the control of stored product insect pests. The first step to establish such strategy is to characterize the α-amylase enzymes in the target pest. In the current study, α-amylase was biochemically characterized in the larval and adult stages of *Tribolium castaneum* and *Callosobruchus maculatus*. *T. castaneum* was found to have higher α-amylase activity with lower temperature stability compared to that of *C. maculatus*. The optimum pH for α-amylase activity was 5-6. Zymogram pattern revealed the presence of two α-amylase isoforms with high molecule weight in *T. castaneum*. *C. maculatus* has two α-amylase isoforms in the adult stage and three isoforms in the larval stage. Rice α-amylase inhibitors was purified and found to have strong inhibitory effect against α-amylase of both insects and also negatively affected their life parameters.

Keywords: *Tribolium castaneum, Callosobruchus maculatus*, α-amylase, α-amylase inhibitors

INTRODUCTION

About 25-30% of the world grain crop is lost each year during storage process due to the attack by different insect species that are associated with stored products. Stored product insect pests are categorized based on their feeding behaviour as primary and secondary pest. The cowpea weevil, *Callosobruchus maculatus* is one of the most important primary pest that known to attack legume (Franco *et al.*, 2000). One of the most important secondary pests is the red flour beetle, *Tribolium castaneum* which is responsible for sever losses of stored grain (Chen *et al.*, 1992).

Carbohydrate metabolism is most important for the nutrition activities of stored product insect pest. Complex carbohydrates need to be broken down into their sugar component to enter different metabolic cycle to yield ATP as a source of energy. Alpha-amylase enzyme is responsible for the breakdown of the bond between sugar molecules in the polysaccharides (Henrissat *et al.*, 2002). Stored product insect pests depend mainly in their life on α-amylase enzymes (Pereira *et al.*, 1999). Alpha-amylase was reported in different insect species including *C. maculatus* (Mendiola-Olaya *et al.*, 2000, Wisessing *et al.*, 2008) and *T. spp.* (Bandani & Balvasi, 2006) .

The use of α-amylase inhibitors to reduce insect growth by interfering with carbohydrate absorption as a strategy to control stored product pests was discussed by many authors (Mehrabadi *et al.*, 2011 and Neeta & Kamble, 2016). Characterization of α-amylase in any insect species is the first step to develop a control strategy using α-amylase inhibitors. In the current study, α-amylase enzyme were partially purified and characterized in *T. castaneum* and *C. maculatus* and the antimetabolic effect of α-amylase inhibitor isolated from rice (Sakha 101 variety) was also studied.

MATERIALS AND METHODS

1. Insect culture

Populations of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Callosobruchus maculatus* (Coleoptera: Chrysomelidea) were obtained from cultures maintained at Insect Physiology Laboratory, Faculty of Agriculture, Sohag University. *T. castaneum* population was maintained on wheat grains while, *C. maculatus* was maintained on cowpea seeds at 25±2°C, R.H. (50-70%) and photoperiod at (14:10) D:L hours.

2. Preparation of larval gut homogenate

The method of Cohen (1993) is used for extraction of α-amylase from *T. castaneum* and *C. maculatus* with some modifications. The larvae and the adult of both insects were weighted and homogenized separately in one ml of cooled sodium acetate buffer, 20 mM, pH 7 containing (10mM NaCl, and 20mM $CaCl_2$). The larvae and the adults of both insects were placed in a pre-cooled homogenizer and grounded into previous buffer. The homogenates were centrifuged at 10.000 rpm at 4 °C for 20 min. The resulting supernatants were collected and transferred to a new tube and stored at -20 °C for further use as a source of α-amylase enzyme.

3. Enzyme assay

Dinitrosalicylic acid (DNS) method was used to determine α-amylase like-activity using 1% soluble starch as substrate. The larval and adult α-amylase crude extracts of both insect species was incubated with 500µL sodium phosphate buffer (pH 7, 0.1 M) and 40 µL soluble starch

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for 30 min at 35°C. Before boiling for 10 minutes in water bath, 100µL of DNS was added to stop the reaction. After cooling the absorbance was read at 540 nm. Maltose standard curve was constructed to enable calculation of the amount of maltose released during α-amylase assays and one unit of α-amylase activity was defined as the amount of enzyme required to produce 1 mg maltose in 30 min.

4. Protein determination

The protein content of α-amylase crude extracts of *T. castaneum* and *C. maculatus* was determined by Lowery's method (Lowery *et al.,* 1951), using bovine serum albumin fraction V (Sigma) as the standard.

5. Stability of α-amylase at different temperature and pH.

The stability of α-amylase crude extracts of *T. castaneum* and *C. maculatus* at different temperatures and pHs was determined in adult stages. The effect of temperature on α-amylase activity was determined by pre-incubating of the enzyme crude extract at 20, 30, 35, 40, 50, 60, 70, 80, 90 and 100°C for 45 minutes. After incubation, the reaction was cooled and the enzyme activity was assayed as described above.

To study the stability of α-amylase at different pHs ranged from 2 to 12. The α-amylase enzyme was incubated for 45 minutes in different pHs using the following buffers; 0.1 M: glycine-HCl for pH 2 and 3; Na-acetate-acetic acid for pH 4 and 5; phosphate buffer for pH 6 and 7; Tris-HCl for pH 8; glycine-NaOH for pH 9 and 10 and CAPs buffer for pH 11and 12. After incubation the enzyme activity was assayed as described above.

6. Diffusion assay for analysis of *T. castaneum* and *C. maculatus* α-amylases

Diffusion assay developed by Fossum and Whitaker (1974) was used with slight modification, 1% starch agar gel (pH 6.8) was prepared containing **(**10g of potato starch, 5g gelatin , 3.0g of beef extract and 15.0g Agar). All contents of starch agar gel were mixed and boiled for 20 minutes in water bath before poured into clean grease free glass Petri plates. After solidification, the wells were made in the starch-agar gel using a cork borer and 30µL of α –amylase crude extract (from Larvae and adults of both insects) was poured into the wells. The plates were covered with a tight fitting glass plate and incubated for 10 hours at room temperature. After incubation, the starch agar plates were flooded with iodine solution and the excess solution poured off out of plates. The presence of α-amylase activity was indicated by clear zone around the well as a result of hydrolysis of starch.

7. In gel visualization of T. castaneum and C. maculatus *α-amylases*

A constant volume of α-amylase crude extract for larvae and adults of *T. castaneum* and *C. maculatus* were loaded in 10% native polyacrylamide gel (PAGE). After electrophoresis, the gel was transferred to a tray containing 1% (V/V) Triton –X-100 in phosphate buffer (0.01 M) containing 2mM Cacl2, and 10mM NaCl for 90 minutes. The gel was incubated with starch (1%) in phosphate buffer for 1.5 hour, and then the gel was rinsed with water and treated with a solution containing (1.3% I_2 and 3% KI) to stop the reaction. The undegraded starch stained dark blue and white bands indicated the position of α-amylase, then the gel was photographed under lighter.

8. Extraction and purification of α-amylase inhibitor

Based on the germplasm screening conducted in our laboratory through our project (STDF, 26601), the rice (Sakha 101, variety) showed high α-amylase inhibitory activity against standard α-amylase enzyme for which it was selected. Crude extracts were obtained according to Baker (1987) with some modification. Finely grounded seeds of rice were defatted with ice-cold acetone for 2 hours and the solvent was removed using a filter paper and the powder was air-dried for overnight.The defatted powders were extracted in 0.01 M sodium phosphate buffer (1:10 W/V), containing 0.15 M NaCl at pH 7.0. The buffer containing the powder was stirred for 2 hours and kept overnight at 4°C for extraction with intermittent shaking by taking care no foam was formed. After 24 hours, the homogenate was centrifuged at 10.000 rpm for 20 mintues at 4 °C. The pellet was discarded, and the clear supernatant (crud extract) was collected. The crude extract was subjected to ammonium sulphat fractionation at concentrations of (0-30, 30-60 and 60-90%). As the fraction $F_{60\text{-}90}$ showed the highest α-amylase inhibitory activity, it was selected for inhibitory activity bioassay.

9. Inhibitory effect and kinetics of rice α-amylase inhibitor against α-amylase crude extracts from *T. castaneum* and *C. maculatus*

Different concentrations of rice α-amylase inhibitor (AI) were used to determine the IC_{50} (concentration of inhibitor required for 50% inhibition) values against α-amylase extracts of larvae and adult of both insects. Rice AI were incubated with α-amylase crud extracts for 20 minutes at 37°C before addition of 10% starch solution as a substrate to start the reaction. Remaining α-amylase activity was measured as described above and results were expressed as IC_{50}.

The mechanism of inhibition against α- amylase of *T. castanum* and *C. maculatus* (competitive or non-competitive) was determined at different substrate concentrations (1%, 0.5% and 0.25% starch) and a fixed concentration of α-amylase inhibitors using Lineweaver–Burk plots in which the inverse of enzyme activity were plotted versus the inverse of substrate concentration in the absence and in the presence of an inhibitor. The maximum velocity (*V*max), Michaelis constant (*K*m) and *K*i were calculated.

10. In Vivo effect of α-amylase inhibitors on *T. castaneum* and *C. maculatus*

T. castanum

Different concentrations (w/w) of (0.25, 0.5 and 0.1%) of rice AI (ammonium sulfate saturated fraction) were mixed with wheat flour and kept in plastic Petri dishes. Ten newly hatched larvae of *T. castanum* were introduced in the Petri dishes and kept in incubator condition in dark to maintain temperature and humidity. The bioassays were completely randomized with three replicates. The mean larval weight, larval mortality, number of pupa and number of emerged adults were recorded at 2 days intervals until 40 days after treatment.

C. maculatus

Artificial seeds was made for feeding studies contained chickpea flour and different concentrations (w/w) of (0.25, 0.5,and 0.1%) of rice AI which partially purified by ammonium sulfate saturation as described by

Macedo *et al*. (1993). Firstly, the mixture was conditioned at 28 ºC and 70% RH for 3 days, and then the mixture was filled completely in gelatinous capsules (0.5gm per capsules). After 48 hours period for adjustment in the growth chamber, 2-3 days old fertilized females were presented for oviposition and incubated for 3 days at 27 ºC and 70% R.H.The excess eggs were removed from the artificial seeds and only 3 eggs /seed were left. Five replicates were performed for each treatment. Artificial seeds carrying the eggs were kept in the incubator at the same condition and after 20 days, the artificial seeds were broken apart and number and weight of surviving larvae were recorded.

RESULTS AND DISCUSSION

1. Enzymes activity

The α-amylase specific activity was calculated in the crude extracts of larval and adult stages of *T. castanum* and *C. maculatus*. The larval stages in both insects were found to have higher α-amylase specific activity (0.0347 and 0.0224 U/mg protein for *T. castanum* and *C. maculatus*, respectively) compared to the adult stages (0.0182 and 0.0168 U/mg protein for *T. castanum* and *C. maculatus*, respectively). The α-amylase specific activity was found to be higher in *T. castanum* compared to *C. maculatus* in both stages. Alpha-amylase activity was identified to be the most important digestive enzyme in coleopteran species including *T. castaneum* (Sivakumar *et al.*, 2006), *C. maculatus* (Wisessing *et al.*, 2008), *Sitophilus oryza* (Celniska *et al.*, 2014) and *Rhynochophorus ferrugineus* (Abd El-latif, 2020).

2. Stability of α-amylase at different temperatures and pHs.

Temperature and pH are the major factors affecting enzyme activity. Figure (1) shows the optimum temperature for the activity of α-amylase in adults of *T. castanum* and *C. maculatus*. In *T. castanum*, α-amylase activity was in its highest at temperatures from 30-40 ˚C. When the temperature increased to 50 ˚C degree, the enzyme lost 55% of its activity and the enzyme activity was totally lost at 100 ˚C. The highest α-amylase activity of *C. maculatus* was observed at temperature between 50-60 ˚C which indicate that the α-amylase of *C. maculatus* has higher temperature stability compared to that of *T. castanum*. The α-amylase of *C. maculatus* lost 80.5% of its activity when the temperature reached 60 ˚C and totally inhibited at 100 ˚C. In colelopteran insects, the optimum temperature observed for α-amylase activity was 35-50 ºC. (Darvishadeh *et al.*, 2012, Delkash-Roudsari *et al.*, 2014 and Abd El-latif, 2020).

Darvishadeh *et al.*, 2012 revealed that α-amylase is active at acidic condition, pH 6-7 and even at pH 8 (Vale *et al.*, 2012). The pH stability of α-amylase in adults of *T. castanum* and *C. maculatus* is shown in figure (2). The optimum pH range for α-amylase activity was from 5-6 in both insect. At high alkaline pH (11, 12) the enzyme was almost inactive.

3. Diffusion assay for analysis of *T. castanum* and *C. maculatus* α-amylases

Diffusion assay method was used to confirm the activity of α-amylase on the starch agar. The presence of α-amylase activity was indicated by clear zone around the well because of hydrolysis of starch. Alpha-amylase activity of *T.castaneum* and *C. maculatus* was readily observed by the presence of a clear lysis zone around the well following the treatment of the starch-agar plate with Gram's iodine (Figure 3).

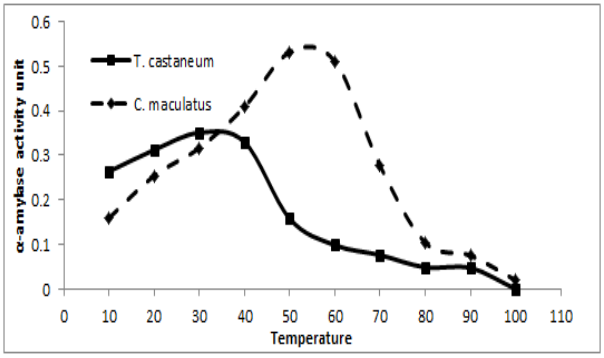

Figure 1. Effect of temperature on the activity of α-amylase in *T. castanum* and *C. maculatus*

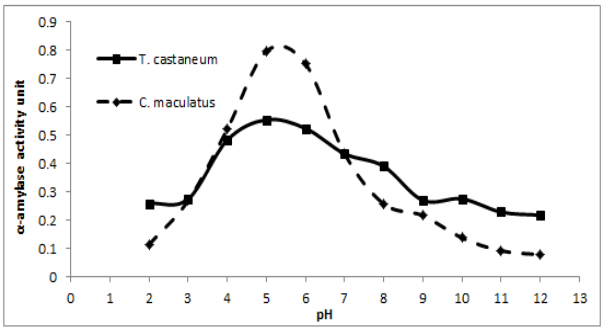

Figure 2. Effect of pH on the activity of α-amylase in *T. castanum* and *C. maculatus*

Figure. 3. Qualitative screening of α-amylase enzyme from *T. castaneum (a)* and *C. maculatus (b).*

4. In gel visualization of *T. castaneum* and *C. maculatus* α-amylases

Zymogram pattern in the native gel proved that both insects, *T. castanum* and *C. maculatus* have α-amylase activity (Figure 4).The larval and adult stages of *T. castaneum* found to have two α-amylase isoforms (bands) with the same relative motilities with relatively higher molecular weights compared to that present in *C. maculatus*. Adult and larval stages of *C. maculatus* share two α-amylase isoforms (bands) with lower molecular weight; however the larval stage has additional α-amylase isoform (band) with intermediate molecular weight that was not detected in the adult stage. Wiessing *et al.* (2008) reported the presence of single α-amylase isoform in *C. maculatus* with molecular weight of 50 kDa. One to eight α-amylase isoforms were reported in different insect species (Kazzaz *et al.*, 2005, Mehrabadi *et al.*, 2011 and Darvishadeh *et al.*, 2012).

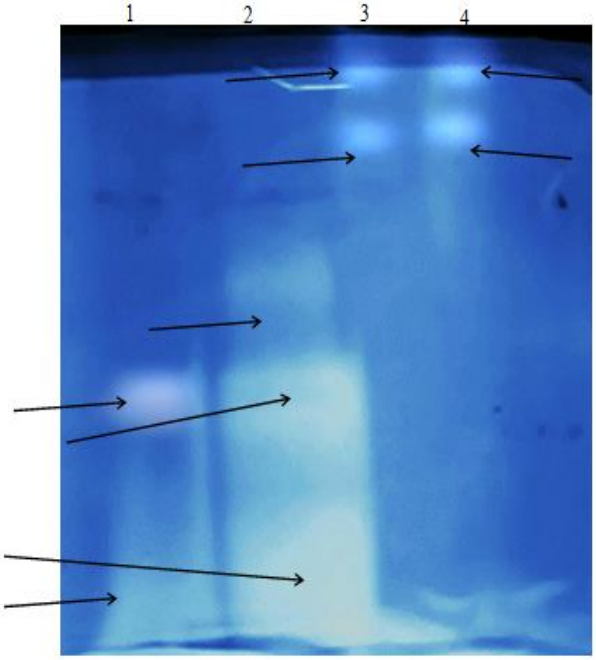

Figure 4. Native-PAGE gel electrophoresis of α-amylase of *T. castaneum* and *C. maculatus*

1= *C. maculatus* adult, 2: *C. maculatus* larva, 3: *T. castaneum* adult, 4: *T. castaneum* larva

- **The arrows point to α-amylase isoforms**

5. Kinetic studies

Kinetic analysis of α-amylase activity gave line reciprocal Michaelis-Menton (Lineweaver-Burk) plots, enable the estimation of *K*m , *V*max values (Table 1). The kinetic analysis of α-amylase in *T. castanum* revealed that the α-amylase of the adult stage has higher velocity (*V*max) of 0.4338 (µmol maltose released /min/mg protein) compared to the larval stage (0.2958). However the α-amylase of the larval stage has stronger affinity to its substrate with lower *K*i value (0.53 mM) compared to the adult stage (0.66 mM).

In case of α-amylase of *C. maculatus*, the kinetic analysis revealed that α-amylase of the larval stage has higher velocity with *V*max value of (0.4435 µmol maltose released /min/mg protein) compared to that of the adult stage (0.4103) and also lower affinity to its substrate with higher *K*m (0.83 mM) compared to the adult stage (0.67 mM). In general, the α-amylase of *T. castanum* in both larval and adult stages found to have stronger affinity with lower *K*m values compared to that of *C. maculatus*. When rice α-amylase inhibitor (rice AI) was used, the *Vmax* values were reduced with no change in *K*m values in both insects and both stages which indicate that rice AI is of non-competitive type of inhibitors. The *K*i and IC_{50} values in case of *T. castaneum*, revealed that rice AI is more potent and has stronger affinity against α-amylase of the adults stage compared to the larval stages. The same result was noticed in case of *C. maculatus*, as *K*i and IC_{50} values of the adult stage were lower than that of the larval stage.

6. In Vivo effect of rice AI on *T. castaneum* and *C. maculatus*

The use of α-amylase inhibitors to interfere with the metabolic pathway of carbohydrate in insect become the focus of attention (Mehrabadi *et al.*, 2011). In the current study, α-amylase inhibitor was isolated, partially purified from rice (Sakha 101 variety) and studied for its inhibitory activity against α-amylase of *T. castanum* and *C. maculatus*

Table 1. Kinetic analysis of α-amylase of larval and adult stages of *T. castanum* and *C. maculatus* against rice AI.

Insect species	Stage		*V*max (µmol maltose released /min/mg protein)	*K*m (mM)	*K*i (µM)	IC_{50} (µg/ml)
T. castanum	Larva	Without inhibitor	0.2958	0.53		
		Rice AI	0.2258	0.53	1.70	1.925
	Adult	Without inhibitor	0.4338	0.66		
		Rice AI	0.3689	0.66	0.53	0.626
C. maculatus	Larva	Without inhibitor	0.4435	0.83		
		Rice AI	0.3468	0.83	0.77	0.758
	Adult	Without inhibitor	0.4103	0.67		
		Rice AI	0.2861	0.67	0.35	0.417

T. castaneum

The antimetabolic effects of rice AI were tested against *T. castaneum* by incorporating the F_{60-90} (NH4)2 SO4 proteins into the artificial diet at different concentrations. The mean larval weight, larval mortality, number of pupa and number of emerged adults were recorded at 2 days intervals until 40 days after treatment and presented in table (2). Noticeable decrease in the mean larval weight of rice AI treated larvae was observed two days after treatment compared to control. The highest reduction in mean larval weigh was recorded at highest rice AI concentration of (1:1).

Table 2. *In vivo* effects of rice AI on the mean larval weight, larval mortality, pupation and adult emergence of *T. castaneum*.

	D	2				4				6				8				10			
	Treatment	W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A
Control		0.0212	0	0	0	0.0212	0	0	0	0.0214	0	0	0	0.0214	0	0	0	0.0214	0	0	0
		12				14				16				18				20			
		W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A
		0.0032	0	8	0	00034	0	8	0	0.0035	0	4	4	0.0036	0	4	4	0.0076	0	0	8
		22				24				26				28				30			
		W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A
		-	0	2	8	-	0	2	8	-	0	2	8	-	0	2	8	0	0	0	10
		32				34				36				38				40			
		W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A
		-	0	0	10	-	0	0	10	-	0	0	10	-	0	0	10	-	0	0	10
Rice AI		2				4				6				8				10			
		W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A
	1:.25	0.0138	0	0	0	0.0121	30	0	0	0.0144	30	0	0	0.0157	30	0	0	0.0157	30	0	0
	1:0.5	0.0172	0	0	0	0.0136	30	0	0	0.0133	40	0	0	0.0107	40	0	0	0.0107	40	0	0
	1:1	0.0141	0	0	0	0.0064	60	0	0	0.0060	60	0	0	0.0053	70	0	0	0.0052	70	0	0
		12				14				16				18				20			
		W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A
	1:.25	0.0094	40	2	0	0.0040	40	5	0	0.0038	40	5	0	0.0026	40	3	2	-	40	1	5
	1:0.5	0.0037	40	4	0	0.0049	40	4	0	0.0048	40	3	1	0.0047	40	0	4	0.0040	40	0	4
	1:1	0.0055	70	1	0	0.0026	70	2	0	0.0023	70	2	0	-	70	2	1	-	70	1	2
		22				24				26				28				30			
		W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A
	1:.25	-	40	1	5	-	40	1	5	-	40	0	4	-	40	0	4	-	40	0	4
	1:0.5	0.0031	40		4	0.0030	40	0	4	-	40	1	4	-	40	1	4	-	40	1	4
	1:1	-	70	1	2	-	70	1	2	-	70	0	2	-	70	0	2	-	70	0	2
		32				34				36				38				40			
		W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A	W	M	P	A
	1:.25	-	40	0	4	-	40	0	4	-	40	0	3	-	40	0	3	-	40	0	3
	1:0.5	-	40	0	5	-	40	0	4	-	40	0	4	-	40	0	4	-	40	0	4
	1:1	-	70	0	2	-	70	0	2	-	70	0	2	-	70	0	2	-	70	0	2

W= Mean larval weight, M= larval mortality, P= Number of pupa, A= number of adults

LSD $_{0.05}$ (W) = 0.0047

Moderate larval mortality ranged from 30-60% was observed at the fourth day in the rice AI treated larvae while no mortality was recorded in the control treatment. The mortality reached its highest level (70%), eight days after treatment with rice AI at concentration of (1:1), however no additional mortality was observed after the 8th day.

Larvae started to pupate, 12 days after treatment in both control and rice AI treatments. Out of 10 live larvae in the control treatment pupated at the day 12. Out of six live larvae, five larvae pupated in rice AI (1:0.25) treatment and four in AI (1:0.25) treatment. While in case of rice AI (1:1) treatment, two out of 3 lived larvae pupated. The emergence of adults started at the day 16 in control and rice AI treatment however; at the end of the experiment (day 40) only 2-4 adults were recorded in rice AI treatment compared to 10 adults in the control.

It could be concluded that rice AI could efficiency reduced the larval weight, survival, pupation and adult emergence of *T. castaneum* at all concentrations, while the highest reduction was recorded at concentrations of (1:1).

C. maculatus

Table (3) shows the effect of rice AI on the larval weight and larval mortality of *C. maculatus* using artificial seed method. Rice AI significantly reduced the mean larval weight compared to control. The highest reduction (81.88%) was observed when rice AI was added at concentration of (1:1). Rice AI caused larval mortality percentage ranged from 13.33% at concentration of (1:0.25) to 80% at concentration of (1:1). There was no significant differences in mean larval weigh between the two concentrations, (1:0.25) and (1:0.5), however; the concentration (1:0.25) caused higher mortality (33.33%) compared to (1:0.25) concentration (13.33%). Our laboratory observation indicated that none of the live larvae in the treated diet could continue to the adult stage.

Table 3. *In vivo* effects of rice AI on the larval weight and mortality of *C. maculatus*

Treatment	Concentration W/W	Mean larval weight (g)	Larval mortality
control		0.00842^{a}	0.00^{a}
Rice (Local)	1:0.25	0.00212^{b}	13.33^{b}
	1:0.5	0.00232^{b}	33.33^{c}
	1:1	0.00152^{c}	80.00^{d}
LSD $_{0.05}$		0.00049	6.66

Means in a column followed by the same letter are not significantly different

CONCLUSION

The results of the present study strongly support the raised trend of using α-amylase inhibitors from different plant sources in the control of insect species via introducing resistant plant varieties using genetic modification techniques. The study also highlights the mandatory of characterization of digestive enzymes of the target insect as the first step to select the suitable inhibitor.

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أنزيم الألفا أميليز فى حشرتى خنفساء الدقيق الحمراء *Tribolium castaneum* (Herbst) وخنفساء اللوبيا *Callosobruchus maculatus* (F.) واستجابتهم لمثبط ألفا أميليز من الأرز

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لقد أصبح إنزيم الألفا أميليز أحد أهداف إستراتيجيات مكافحة الآفات بأستخدام مثبطات الألفا أميليز المشتقة من البناتات خاصة فى مكافحة حشرات المواد المخزونة. إن الخطوة الأولى لبناء مثل هذه الأستراتيجية هى توصيف إنزيم الألفا أميليز فى الأفة المستهدفة. فى هذه الدراسة تم توصيف إنزيم الألفا أميليز فى اليرقات والحشرات الكاملة لحشرتى خنفساء الدقيق الحمراء وخنفساء اللوبيا. احتوت خنفساء الدقيق الحمراء على نشاط أعلى لإنزيم الألفا أميليز بدرجة ثبات حرارى أقل مقارنة بخنفساء اللوبيا. درجة الحموضة من 5-6 هى درجة الحرارة المثلى لإنزيم الألفا أميليز. عند دراسة نمط مشابهات الإنزيم لوحظ أن خنفساء الدقيق الحمراء أحتوت على مشابهين لإنزيم الألفا أميليز ذات وزن جزيئى عالى بينما احتوت خنفساء اللوبيا على مشابهين لإنزيم الألفا أميليز فى طور الحشرة الكاملة وثلاثة مشابهات فى طور اليرقة. تم تنقية مثبط الألفا أميليز من حبوب الأرز وقد وجد أن له تأثير تثبيطى قوى ضد إنزيم الألفا أميليز فى كلا الحشرتين وأيضا أثر بصورة سلبية على الخصائص الحياتية لكلاهما.