

## Characterization of digestive $\alpha$ - and $\beta$ -glucosidases in the midgut of *Plagioderia versicolora* Laicharting (Coleoptera: Chrysomelidae)

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### Abstract

The imported willow leaf beetle, *Plagioderia versicolora* is one of the main pests of forest trees worldwide including Iran. Activities of  $\alpha$ - and  $\beta$ -glucosidases in the midgut of *P. versicolora* adults were studied in this research. The results showed a higher  $\alpha$ -glucosidase activity of adult than  $\beta$ -glucosidase. The optimal pHs for  $\alpha$ - and  $\beta$ -glucosidases activities were found to be 6 and 9, respectively. Optimal temperatures for the enzymatic activities were recorded at 30 and 25 °C, respectively. Concentration of 0.5 mM  $K^+$  significantly minimized the activity of  $\alpha$ -glucosidase and maximized the activity of  $\alpha$ -glucosidase in presence of  $Ca^{2+}$ . The activity of  $\beta$ -glucosidase was decreased by adding  $Mg^{2+}$  and  $Zn^{2+}$ . 5 mM concentration of  $K^+$  had the most inhibitory impact on  $\beta$ -glucosidase activity, while 0.5 mM concentration of  $Na^+$  showed the most positive effect on the enzyme activity. The  $K_m$  of midgut  $\alpha$ - and  $\beta$ -glucosidase was 0.36 and 2.03 mM and the  $V_{max}$  value was 5.46 and 18.51  $\mu\text{mol}/\text{min}/\text{mg}$  protein, respectively.

**Key words:** *Plagioderia versicolora*,  $\alpha$ -glucosidase,  $\beta$ -glucosidase, Iran, midgut

چکیده

*Plagioderia versicolora* Laicharting (Coleoptera: Chrysomelidae) میانی معده در گلوکوزیداز

میسره شابراری و بهرام ناصری

سوسک برگخوار بید *Plagioderia versicolora* یکی از آفات مهم درختان جنگلی در دنیا از جمله ایران می باشد. در پژوهش حاضر، فعالیت آلفا و بتا- گلوکوزیداز در معده میانی حشره کامل *P. versicolora* بررسی شد. نتایج نشان داد که فعالیت آلفا- گلوکوزیداز بیش تر از بتا- گلوکوزیداز بود. pH بهینه برای فعالیت آلفا و بتا- گلوکوزیداز به ترتیب 6 و 9 به دست آمد. هم چنین دمای بهینه فعالیت این دو آنزیم به ترتیب 30 و 25 درجه سلسیوس محاسبه شد. غلظت 0/5 میلی مولار یون  $K^+$  بیش ترین تأثیر را در کاهش فعالیت آنزیم آلفا- گلوکوزیداز داشت و بهینه فعالیت آلفا- گلوکوزیداز در حضور یون  $Ca^{2+}$  مشاهده شد. فعالیت آنزیم بتا- گلوکوزیداز با اضافه کردن یون های  $Mg^{2+}$  و  $Zn^{2+}$  کاهش یافت. علاوه بر این، غلظت 5 میلی مولار یون  $K^+$  بیش ترین تأثیر بازدارندگی را بر فعالیت بتا- گلوکوزیداز داشت و یون  $Na^+$  (غلظت 0/5 میلی مولار) بیش ترین تأثیر مثبت را بر فعالیت آنزیم داشت. مقدار  $K_m$  آلفا و بتا گلوکوزیداز به ترتیب 0/36 و 2/03 میلی مولار و مقدار  $V_{max}$  به ترتیب 5/46 و 18/51 میکرومول بر دقیقه بر میلی گرم پروتئین به دست آمد.

واژگان کلیدی: *Plagioderia versicolora*، آلفا- گلوکوزیداز، بتا- گلوکوزیداز، ایران، معده میانی

### Introduction

The imported willow leaf beetle, *Plagioderia versicolora* (Laicharting) (Coleoptera: Chrysomelidae) is an important insect pest of willows in many countries of the world (Kimoto & Takizawa, 1994). Both larvae and adults are known to feed on leaves of the host trees. Adults utilize young leaves through making holes in them but the larvae intensively are interested in older ones (Çanakçıoğlu & Mol, 1998, 2000). Urban (2005) reported that the adults of *P. versicolora* are more destructive than their larvae. Several authors reported economic damages of *P. versicolora* to willows (Kokanova, 1992; Bogatko, 1993; Czerniakowski, 2000, 2002; Aslan, 2001).

$\alpha$ -Glucosidase is classified as one of the  $\alpha$ -D-glucoside glucohydrolases (E.C. 3.2.1.20), a group of

typical exo-type carbohydrases, which hydrolyzes 1, 2- $\alpha$ -glucosidic linkages in the non-reducing terminal of substrate (Chiba, 1997). Terra and Ferreira (1994) argued that  $\alpha$ -glucosidases hydrolyze oligosaccharides to maltopentaose, even though there are some exceptions. Silva & Terra (1995) mentioned that *Dysdercus peruvianus* Guerin-meneville (Hemiptera: Pyrrhocoridae) preferred oligosaccharides from perimicrovillar  $\alpha$ -glucosidase more than maltotetraose. The hydrolyzing of 1,4- $\alpha$ -glucosidic linkages and releasing alpha-glucose are catalyzed by  $\alpha$ -glucosidases (Ghadamyari *et al.*, 2010).

$\beta$ -Glucosidases (EC 3.2.1.21) are the enzymes which catalyze the hydrolysis of terminal, non-reducing  $\beta$ -linked monosaccharide residues from the corresponding glycoside (Terra & Ferreira, 2005). The  $\beta$ -glycosidase is named  $\beta$ -glucosidase (glucose),

$\beta$ -galactosidase (galactose),  $\beta$ -xylosidase (xylose) and others depends on the monosaccharide which is removed. The same  $\beta$ - glycosidase is able to hydrolyze numbers of various monosaccharide remains from glycosides regularly (Terra & Ferreira, 1994). Marana *et al.* (1995) noted that  $\beta$ - glucosidases may have an imperative role in hydrolyzing glycolipids. Furthermore, Terra & Ferreira (1994) reported that it has an important role in terminal digestion of cellulose and hemicelluloses and also in the cleavage of the carbohydrate moieties of glycoproteins.  $\alpha$ - and  $\beta$ -glucosidase have been isolated and characterized from many insects such as *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Marana *et al.*, 2000), *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Ferreira *et al.*, 2001), and *Rhynchophorus palmarum* Linnaeus (Coleoptera: Curculionidae) (Yapi *et al.*, 2009).

To date no studies have been made to characterize the  $\alpha$ - and  $\beta$ -glucosidases activity in the midgut of *P. versicolora*. Since carbohydrate digestion has an important role in physiological process of insects, the carbohydrate hydrolyzing enzymes need more consideration as a target to control *P. versicolora*. Therefore, any disturbance in insects' carbohydrates digestion and absorption can adversely affect their nutrition, growth and reproduction. Many studies have evaluated insect carbohydrates as a control method for their toxic proteins as inhibitors. Due to the importance of glucosidases in breaking down of secondary metabolites of plants, they have critical role in plant-herbivore interactions (Hemmingi & Lindroth, 2000). To provide new knowledge for a better understanding of the digestion biochemistry of *P. versicolora*, the present work is intended to study the enzymatic profiles and biochemical characteristics of  $\alpha$ - and  $\beta$ -glucosidases activities in its digestive tract.

## Materials and methods

### Insect rearing

Adults of *P. versicolora* were collected from infested leaves of *Salix aegyptica* L. (Salicaceae) in the

city of Rasht (37.2682° N, 49.5891° E), Guilan province, Iran, in summer 2013. They were reared in transparent plastic jars (6 × 8 cm) with a hole covered by muslin cloth for the aeration at a controlled condition (22 ± 2 °C, 70 ± 10% RH, and a photoperiod of 16:8 (L: D) hours). The plastic jars contained a piece of moist cotton to provide moisture and the insect was fed with a diet set on *S. aegyptica* leaves. The rearing continued for at least one generation to have a unified group of adults for the biochemical experiments.

### Sample preparation and enzyme assays

Since the adult stage of *P. versicolora* is more harmful (Urban, 2005), the experiment targeted the adult stage. Adults were randomly selected and their midguts removed through dissection under a stereo microscope in ice-cold distilled water and later placed in an Eppendorf tube containing 1 mL of distilled water. Tissues were ground by a homogenizer and centrifuged in 13000 rpm for 20 min at 4 °C. Supernatant was carefully removed and transferred to new tubes and stored at -20 °C for the experiments.

$\alpha$ - and  $\beta$ -Glucosidases activities were assayed by incubating 5  $\mu$ L of enzyme solution with 10  $\mu$ L of p-nitrophenyl-  $\alpha$ -D-glucopyranoside (pNaG) (10 mM), p-nitrophenyl-  $\beta$ -D-glucopyranoside (pN $\beta$ G) (10 mM) and 30  $\mu$ L of 100 mM universal buffer (pH 7) containing glycine, succinate, 2-morpholinoethan sulfuric acid, 20 mM at 37 °C for 7 min (Ferreira & Terra, 1983; Zibaee, 2012). Absorbance was read at 405 nm. All experiments were repeated three times.

### Effect of pH and temperature on the enzyme activity

The effects of different temperatures on the enzyme activities were determined by incubating the reaction mixture at 15, 20, 25, 30, 35, 40, 45 and 50 °C for 7 min. Optimal pH was determined using universal buffer with pH set at 3-12. The enzyme assay was done accordingly.

### Effect of different cations on the enzyme activity

The effects of various cations on glucosidase activities were measured by adding different

concentrations of chloride salts of  $\text{Na}^+$  (0.5, 3 and 5 mM),  $\text{K}^+$  (0.5, 3 and 5 mM),  $\text{Ca}^{2+}$  (0.5, 3 and 5 mM),  $\text{Mg}^{2+}$  (0.5, 3 and 5 mM),  $\text{Mn}^{2+}$  (0.5, 3 and 5 mM) and  $\text{Zn}^{2+}$  (0.5, 3 and 5 mM) to the assay mixture and the activity was measured after 7 min. A control was also measured (no compounds added) (Shabarari *et al.*, 2014).

#### Kinetic parameters ( $V_{\max}$ and $K_m$ ) of $\alpha$ - and $\beta$ -glucosidase in the midgut

5  $\mu\text{l}$  of appropriately diluted enzyme preparation were used in each assay. Final concentrations of substrate were 2, 4, 6, 8 and 10 mM for the enzymes. The Michaelis constant ( $K_m$ ) and the maximum velocity ( $V_{\max}$ ) were estimated by Sigmaplot software version 11.

#### Protein determination

Protein concentration of the midgut extract was measured according to the method of Lowry *et al.* (1951) by using bovine serum albumin (Bio-Rad, München, Germany) as a standard.

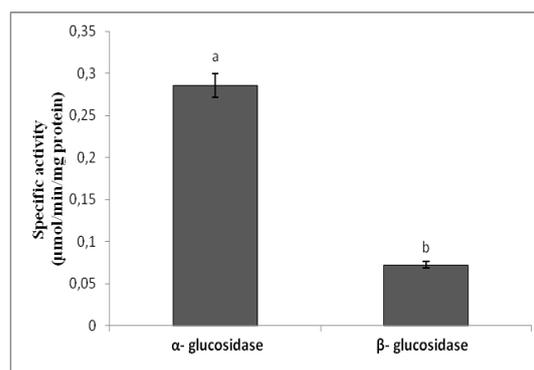
#### Statistical analysis

Data were compared by one-way analysis of variance (ANOVA) followed by Tukey's student test when significant differences were found at  $P \leq 0.05$  (SAS, 1997). Differences between the effect of ions on  $\alpha$ - and  $\beta$ -glucosidase activity were compared by the LSD (least significant difference) test. The comparison of  $\alpha$ - and  $\beta$ -glucosidase activity was done by *t*-test.

#### Results and discussion

Both  $\alpha$ - and  $\beta$ -glucosidases were present in the midgut of *P. versicolora*, even though the results showed higher activity of  $\alpha$ -glucosidase than  $\beta$ -glucosidase. The activities of  $\alpha$ - and  $\beta$ -glucosidases were 0.285 and 0.072  $\mu\text{mol}/\text{min}/\text{mg}$  protein, respectively (Fig. 1). Riseh *et al.* (2012) reported that the  $\alpha$ - and  $\beta$ -glucosidases activity in the male's digestive system of the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Col.: Curculionide) was 0.29- and 4.31- fold higher than the last larval instar, respectively. In comparison with the

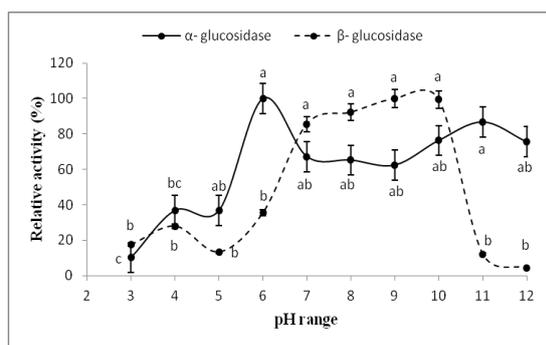
males, the  $\alpha$ - and  $\beta$ -glucosidases activity in the female's digestive system was 6.09- and 2.55- fold higher than the last larval instar, asexg production requires more energy and subsequently higher level of  $\alpha$ - and  $\beta$ -glucosidases activity in females.



**Fig. 1.** Comparison of glucosidase activity in the midgut of *P. versicolora* adult. Different letters show statistical differences between values (*t*-test).

The pH range of 5 to 7.5 exists in the midgut of most insect species (Terra & Ferreira, 1994). However, high acidic conditions (pH 3.1 to 3.4) have been observed in the midguts of cyclorrhaphous Diptera and pH range of 9 to 12 (high alkaline conditions) recorded for scarab beetles, lepidopteran and nematoceran Diptera larvae (Terra & Ferreira, 1994; Clark, 1999). Our results indicated a wide-ranging pH of 3 to 12 for  $\alpha$ -glucosidase activity (Fig. 2). The optimal  $\beta$ -glucosidase activity was observed at pH 9 and then the activity dropped rapidly and reached zero at pH 11. Blake *et al.* (1971) stated that the high pH of midgut may has an important role in the action of releasing hemicelluloses from plant cell walls ingested by insects and also preventing tannin from being bound with proteins. According to Sharifi *et al.* (2011) the optimal  $\alpha$ - and  $\beta$ -glucosidase activity in the midgut extracts of larval *Lasioderma serricorne* (Fabricius) (Col.: Anobiidae) was at pH 5 and 6, respectively. Riseh *et al.* (2012) found the optimal pH 5 for the activities of  $\alpha$ - and  $\beta$ -glucosidases in the midgut of *R. ferrugineus*. Sajjadian *et al.* (2012) noted that  $\alpha$ - and  $\beta$ -glucosidase in the midgut of larval *L. serricorne* were optimally

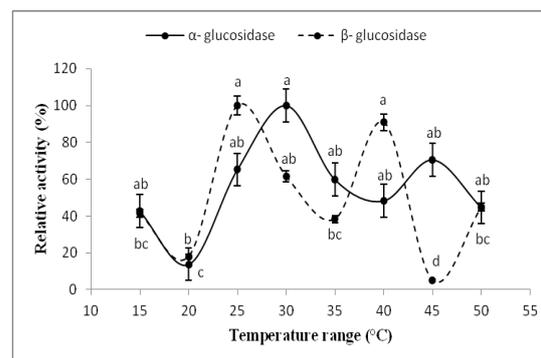
active in pHs 5 and 6, respectively. They also stated that  $\alpha$ -glucosidase was stable in acidic conditions (pH 4) more than highly acidic or alkaline environments.  $\beta$ -Glucosidase was also stable in acidic conditions (pH 5) and its maximum stability occurred at pH 6.



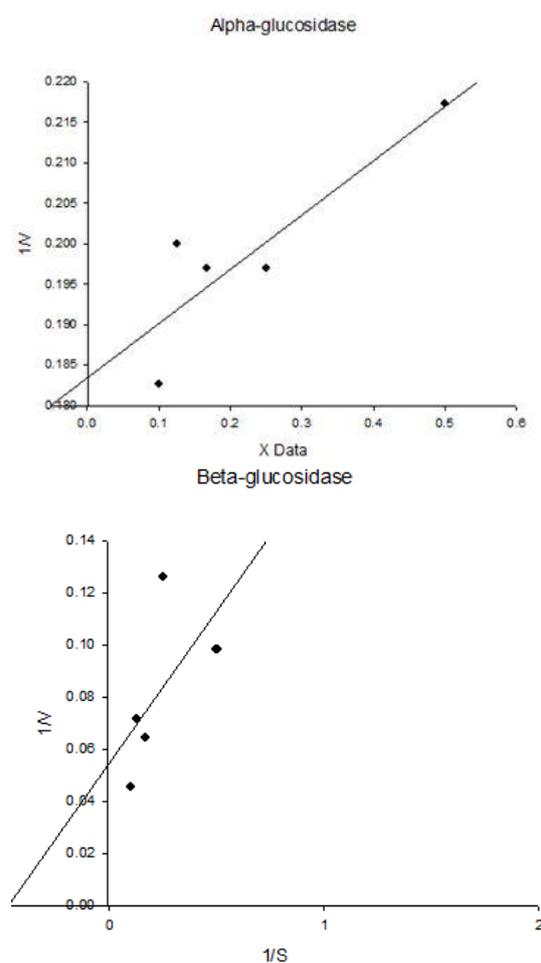
**Fig. 2.** Effect of pH on  $\alpha$ - and  $\beta$ -glucosidase activity in the midgut of *P. versicolora* adult. Different letters show statistical differences among values (Tukey's test;  $P < 0.05$ ).

The optimal temperature for  $\alpha$ - and  $\beta$ -glucosidase activities was 30 and 25 °C, respectively.  $\alpha$ - and  $\beta$ -Glucosidase showed significant activities at about 25 to 45 and 25 to 40 °C, respectively and increased afterward (Fig. 3). Riseh *et al.* (2012) found that the highest enzymatic activity of  $\alpha$ - and  $\beta$ -glucosidase of *R. ferrugineus* occurred at 50 and 50-60 °C, respectively. The maximum activity for  $\alpha$ - and  $\beta$ -glucosidase of *L. serricornis* was reported 35 and 40 °C, respectively, while at higher temperatures the enzymes activity declined rapidly (Sajjadian *et al.*, 2012).

The  $K_m$  and  $V_{max}$  values were respectively 0.36 mM and  $5.46 \mu\text{mol min}^{-1} \text{mg}^{-1}$  protein and 2.03 mM and  $18.51 \mu\text{mol min}^{-1} \text{mg}^{-1}$  protein for  $\alpha$ - and  $\beta$ -glucosidase. Analysis of Lineweaver-Burk plots (Fig. 4) provides information concerning glucosidase activities in *P. versicolora* adults. For saturating the active site of the enzyme, an optimal concentration of the substrate is needed that is disproportionate to  $K_m$  (Zibae *et al.*, 2011), as low  $K_m$  shows strong and high  $K_m$  shows weak bindings between the enzymes and substrates.



**Fig. 3.** Effect of temperature on  $\alpha$ - and  $\beta$ -glucosidase activity in the midgut of *P. versicolora* adult. Different letters show statistical differences among values (Tukey's test;  $P \leq 0.05$ ).



**Fig. 4.** Lineweaver-Burk plots of  $\alpha$ - and  $\beta$ -glucosidase in the midgut of *P. versicolora* adult.

The activity level of  $\alpha$ - and  $\beta$ -glucosidase was changed by using different ions (Tables 1 and 2). Among ions used for  $\alpha$ -glucosidase activity, 0.5 mM concentration of  $K^+$  showed the most inhibitory effect on the enzymatic activity. At 5 mM concentration of  $Mn^{2+}$  and 0.5 as well as 3 mM of  $Mg^{2+}$ , the activity level of the enzyme significantly increased. However, the most favorable effect on the  $\alpha$ -glucosidase activity was observed at 5 mM concentration of  $Ca^{2+}$  (Table 1). Although Vatanparast *et al.* (2013) reported that  $\alpha$ - and  $\beta$ -glucosidase activity of *Xanthogaleruca luteola* (Muller) (Coleoptera: Chrysomelidae) was inhibited by adding  $Ca^{2+}$  and  $Mg^{2+}$ , current study indicated that the level of  $\alpha$ -glucosidase activity heightened in presence of  $Ca^{2+}$  and  $Mg^{2+}$ . Terra & Ferreira (2005) also emphasized the role of calcium in the catalytic activity and stability of digestive glucosidases. Differences between our results and those reported by Vatanparast *et al.* (2013) could be attributed to the differences in experimental insect species. The activity level of  $\beta$ -glucosidase significantly decreased along with the enhancement of  $Mg^{2+}$  and  $Zn^{2+}$  concentrations. The lowest level of the enzyme activity was observed at 5 mM concentration of  $K^+$ . However, significant increase of  $\beta$ -glucosidase activity was recorded in presence of 3 mM of  $K^+$ .  $Mn^{2+}$  (at 3 mM) showed significant adverse effect on the enzyme activity (Table 2). The inhibitory effect of  $Cu^{2+}$ ,  $Cl^{2+}$ ,  $Zn^{2+}$ ,  $Cl^{2+}$ ,  $Fe^{2+}$  and  $Cl^{2+}$  on  $\beta$ -glucosidase activity of *R. palmarum* was reported by Yapi *et al.* (2009).

It is clear that  $\alpha$ - and  $\beta$ - glucosidases have fundamental impacts on the digestion of *P. versicolora*. The characterization of *P. versicolora* digestive glucosidases and examination of their inhibiting effects on the enzymatic activity of this pest could help improve safe control programs. Further experimental investigations are required to characterize  $\alpha$ - and  $\beta$ - glucosidase activity in *P. versicolora* larvae.

**Table 1.** Effects of ions on  $\alpha$ -glucosidase activity (mean  $\pm$  SE) from midgut extracts of *P. versicolora* adults.

Relative activity (%)	Concentration (mM)	Compound
100b <sup>*</sup>	Control	<b>Mg<sup>2+</sup></b>
151.96 $\pm$ 14.58a	0.5	
186.22 $\pm$ 10.42a	3	
143.3 $\pm$ 18.30ab	5	
100b	Control	<b>Mn<sup>2+</sup></b>
131.49 $\pm$ 11.09ab	0.5	
96.06 $\pm$ 21.02b	3	
154.72 $\pm$ 8.29a	5	
100b	Control	<b>Ca<sup>2+</sup></b>
516.92 $\pm$ 167.26a	0.5	
303.93 $\pm$ 45.49ab	3	
639.7 $\pm$ 108.19a	5	
100a	Control	<b>Na<sup>+</sup></b>
259.44 $\pm$ 73.20a	0.5	
122.30 $\pm$ 45.30a	3	
69.57 $\pm$ 3.81a	5	
100ab	Control	<b>K<sup>+</sup></b>
35.43 $\pm$ 13.79b	0.5	
121.65 $\pm$ 41.39a	3	
111.02 $\pm$ 7.59ab	5	
100b	Control	<b>Zn<sup>2+</sup></b>
401.57 $\pm$ 124.63a	0.5	
58.66 $\pm$ 12.9c	3	
109.05 $\pm$ 15.13b	5	

<sup>\*</sup>Different letters for each compound show that the relative activity of the enzyme is significantly different from each other (LSD;  $P < 0.05$ ).

**Table 2.** Effects of ions on  $\beta$ -glucosidase activity (mean  $\pm$  SE) from midgut extracts of *P. versicolora* adults.

Relative activity (%)	Concentration (mM)	Compound
100a <sup>*</sup>	Control	<b>Mg<sup>2+</sup></b>
30.76 $\pm$ 15.41b	0.5	
24.72 $\pm$ 7.29b	3	
12.63 $\pm$ 6.01b	5	
100ab	Control	<b>Mn<sup>2+</sup></b>
85.71 $\pm$ 17.31b	0.5	
32.41 $\pm$ 17.03c	3	
181.60 $\pm$ 94.2a	5	
100a	Control	<b>Ca<sup>2+</sup></b>
66.70 $\pm$ 19.7b	0.5	
117.50 $\pm$ 21.90a	3	
60.10 $\pm$ 21.70b	5	
100b	Control	<b>Na<sup>+</sup></b>
171.97 $\pm$ 10.10a	0.5	
159.34 $\pm$ 7.65a	3	
113.18 $\pm$ 12.3b	5	
100b	Control	<b>K<sup>+</sup></b>
87.36 $\pm$ 8.25b	0.5	
140.1 $\pm$ 26.70a	3	
7.84 $\pm$ 1.37c	5	
100a	Control	<b>Zn<sup>2+</sup></b>
32.42 $\pm$ 6.40b	0.5	
50.55 $\pm$ 13.70b	3	
47.25 $\pm$ 20.70b	5	

<sup>\*</sup>Different letters for each compound show that the relative activity of the enzyme is significantly different from each other (LSD;  $P < 0.05$ ).

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