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Original Article

Biochemical Changes in *Spodoptera Frugiperda* (J.E. Smith) Larvae Response to Zinc Stress

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Abstract

This study sought to determine the efficacy of metal salt ($ZnSO_4$) against fall armyworm, Spodoptera frugiperda (J.E. Smith) Zn caused an extension in larval development time, and caused a shortening in the life spans of both females' and males' at all concentrations. Additionally, several body malformations were observed throughout the life cycle of the insect. The fecundity of the females was greatly affected, with 1045.11, 900.01, 838.58,738.58, and 619.34 eggs/female, for 150, 300, 450, 600, and 750 ppm of Zn, respectively, compared to the control of 1210.11 eggs/female. Notably, Zn caused a significant decrease in protein and glycogen levels. The activities of α-amylase, protease, and lipase were also decreased compared with the control. Conversely, SOD and CAT activities significantly increased at all concentrations of Zn. It was determined that the effects of Zn intensified depending on the increase in concentration.

Keywords: Antioxidant Enzymes, Digestive Enzymes, Growth, Fecundity, Zinc Exposure.

Introduction

Zn is a trace mineral essential for cell development and differentiation. It serves as a structural, catalytic, or regulatory component of many Zn-containing proteins, and it is important in almost all aspects of biology [1]. Additionally, Zn is a crucial element for the proper action of over 300 enzymes and controls the architecture of protein complexes [1,2]. It is also critical for maintaining the structure and function of biomembranes. Furthermore, it aids the activity of several antioxidant enzymes involved in cellular defense [2]. Thus, any alteration of Zn homeostasis may disrupt proper morphogenesis and growth of an organism [2]. Zn has beneficial effects at very low concentrations, while it can be potentially toxic when its concentration in an organism significantly exceeds physiological limits [3-5]. It can induce a broad range of physiological, biochemical, and behavioural dysfunctions [3], which have been studied in some insect species. When insects inhabit a Zn-contaminated environment, it causes bodily abnormalities [4], retards development and reproduction [6,7] and even reduces population growth [8].

Every living organism regulates its homeostasis, and metals are key players in the transport processes, and many enzymatic activities. Zn is a cofactor for thousands of metalloenzymes and proteins. It is also a crucial element, forming active sites in a wide range of enzymes, particularly metalloproteinases and peptidases, in carbonic anhydrase, and superoxide dismutase [8,9]. Zn is also required for their enzymatic antioxidants, which include SOD

and CAT activity. SOD and CAT need metals such as Zn for maximum effectiveness, but they can also cause oxidative stress by inducing free radical formation when taken in excess [9].

Maize, Zea mays L.is tack space L. is an important cereal crop that combines food and feed in over 170 countries, including Egypt. It is rich in nutrients and serves as an important crop for land use and intercropping, playing a vital role in the adjustment of planting structures. It is also one of the economic crops in Egypt [10]. However, the population of *S. frugiperda* causes huge losses to this crop [10].

S. frugiperda is a voracious pest of maize, potentially threatening the food security and incomes of millions of smallholder farmers [10]. The larvae of this pest primarily prefer maize crop, so maize is their primary food and adult moths are also highly fecund [11]. Hence, *S. frugiperda* can quickly colonise maize fields over an enormous area after the seedlings emerge, and then rapidly build up populations [10].

In this context, high levels of Zn can slow down the growth and development of insects and may even cause them to die by damaging their cells. However, the data on the effect of Zn on life cycle and its deleterious mechanisms are still scarce for insects. The aim of this study was to determine the toxic effects of Zn on *S. frugiperda* during its life cycle and investigate whether Zn affected the survival, development, antioxidant enzyme activities, digestive enzyme activities, and protein and glycogen content of *S. frugiperda* when larvae fed on maize leaves treated with various concentrations of Zn under laboratory conditions. This study can be a prelude to using this metal against *S. frugiperda* as one of the alternatives to chemical insecticides.

Materials and Methods

Bioassays

Various concentrations were prepared by diluting the $ZnSO_4$ with distilled water to achieve 150, 300, 450, 600, and 750 ppm. Larvae were fed on leaf discs dipped in the tested concentrations. Pupae that shrank, softened, or displayed body rigidity were considered deformed. All pupae were individually transferred to glass jars until adults emerged. Adults that exhibited deficiencies and asymmetries in their wings were considered deformed. Newly emerged adults were sexed and paired (one female with one male) in oviposition container. All eggs were counted using a magnifying glass. Eggs that were dehydrated and absent of pedicel were considered deformed.

Biochemical Assays

Larvae were homogenized in a phosphate buffer solution (50 mM, pH 7). After centrifugation at 15,000 rpm for 10 min at 4 °C, the supernatant was collected and used to determine glycogen levels by the method of Van Handel and protein levels by the method of Bradford. In addition the activity of CAT and SOD was measured using the methods of Aebi [12] and Sun *et al.* [13], respectively. The activity of α-amylase (Bernfeld [14]), lipase (Ranjbar *et al.* [15]), and protease (García-Carreño and Haard [16]). One larva was tested per replicate, and 20 replicates were examined for each treatment [17,18].

Results

Significantly shorten the development time of first to fourth instar larvae. The larval duration of fifth/sixth-instar was significantly prolonged compared to the control. A significant difference was observed in the emergence of adults compared to the control, which had over 96% of the eggs successfully completing through the cycle until adult emergence. There was a significant increase the number of deformed pupae, adults, and offspring eggs compared to the control. However, the pupation rate and adult emergence were significantly decreased. No

significant effect was observed on pupal weight and sex ratio. APOP and TPOP were significantly affected. In addition, the 150 ppm concentration treatment did, to some extent, lengthen the oviposition period compared to that of the control group, while the 300-750 ppm concentration treatments shortened the oviposition period. The 150, 300, 450, 600, and 750 ppm treatments significantly reduced fecundity (1054.11, 900.01, 838.58,738.58 and 619.34 eggs/female, respectively) compared with the control treatment (1210.11 eggs/female). After treatment with Zn, the development time of pupae and adult longevity were lengthened after exposure to the 150 ppm concentration; however, there was no significant difference between the treatment and the control (see Table 1).

Table 1. Growth and development

Biological characteristics	0.00	150 ppm	300 ppm	450 ppm	600 ppm	750 ppm
First-instar period (days)	3.92 ±0.05a	3.88 ±0.07a	3.73 ±0.01a	3.59 ±0.08a	3.21 ±0.02b	3.00 ±0.01b
Second-instar period (days)	3.51 ±0.08a	3.45 ±0.02b	3.39 ±0.07b	3.21 ±0.01b	3.05 ±0.08b	2.81 ±0.01c
Third-instar period (days)	3.24 ±0.01a	3.15 ±0.01a	2.93 ±0.03b	2.67 ±0.05b	2.45 ±0.06c	2.26 ±0.01c
Fourth-instar period (days)	3.01 ±0.03a	2.90 ±0.03ab	2.84 ±0.08b	2.55 ±0.09b	2.34 ±0.07c	2.11 ±0.02c
Fifth-instar period (days)	5.86 ±0.07a	5.95 ±0.08a	6.00 ±0.03ab	6.35 ±0.07b	6.53 ±0.09c	6.77 ±0.11c
Sixth-instar period (days)	7.49 ±0.08a	7.52 ±0.02b	7.65 ±0.04b	7.72 ±0.06b	7.92 ±0.11bc	8.02 ±0.08bc
Prepupa (days)	**1.29 ± 0.05a**	1.36 ± 0.01a	1.45 ± 0.03**a**	1.60 ± 0.04b	1.81 ± 0.08b	2.14 ± 0.-4b
Pupae period (days)	13.05 ±0.10a	13.57 ±0.13b	13.82 ±0.23b	14.09 ±0.35c	14.61 ±0.16c	15.23 ±0.23d
Pupation rate (%)	97.45 ±0.95a	95.44 ±0.78b	94.54 ±0.74c	90.65 ±0.87d	88.91 ±0.67e	81.07 ±0.53f
Pupae with deformities (%)	2.71 ±0.03a	2.96 ±0.05a	3.85 ±0.04b	4.12 ±0.02c	4.64 ±0.01d	5.56 ±0.04e
Pupae weight (g)	0.39 ±0.06a	0.35 ±0.07a	0.34 ±0.03a	0.32 ±0.09a	0.29 ±0.01b	0.27 ±0.09b
Adult emergence (%)	96.58 ±1.03a	96.06 ±1.02b	95.74 ±0.32c	94.43 ±0.84d	91.30 ±0.87e	86.23 ±0.99f
Sex ratio (♀/(♀+♂)) (%)	50.65 ±0.40a	50.86 ±0.78a	50.33 ±0.59b	49.79 ±0.73b	49.18 ±0.39c	48.34 ±0.46d
Adults with deformities (%)	3.44 ±0.01a	4.00 ±0.08b	4.47 ±0.08b	4.75 ±0.01c	5.65 ±0.02d	6.45 ±0.01e
Female adult longevity (days)	14.13 ±0.18a	14.46 ±0.25a	13.49 ±0.15b	13.27 ±0.23b	12.95 ±0.22d	12.73 ±0.23d
Male adult longevity (days)	13.27 ±0.26a	13.33 ±0.17a	12.92 ±0.22b	12.27 ±0.21c	11.560 ±0.16d	11.38 ±0.1Se
APOP[a] (days)	3.03 ±0.07a	3.28 ±0.03a	3.52 ±0.04b	3.81 ±0.02b	4.37 ±0.01c	4.87 ±0.04d
TPOP[b] (days)	53.75 ±0.78a	54.85 ±0.69b	55.60 ±0.60c	56.14 ±0.53d	56.54 ±0.36e	57.53 ±0.68f
Oviposition period (days)	9.58 ±0.20a	8.78 ±0.09b	7.83 ±0.10c	7.13 ±0.08d	6.96 ±0.04de	6.83 ±0.11e
Fecundity (eggs/♀)	1210.11 ±28.09a	1045.11 ±46.16b	900.01 ±20.21c	838.58 ±17.82d	738.58 ±18.40e	619.34 ±12.39f

Eggs with deformities (%)	4.05 ±0.08a	4.12 ±0.18a	4.31 ±0.17a	6.16 ±0.10b	9.07 ±0.11c	13.56 ±0.20d

[a]Adult preoviposition period. [b]Total preoviposition period. Means marked with different letters in the same row are significantly different (P <0.05).

Catalase and Superoxide Dismutase Activity

High concentrations of Zn caused an increase in the activities of antioxidant enzymes SOD and CAT, while 150 ppm of $ZnSO_4$ did not alter antioxidant activities when compared to control (Figure 1A-B). The activity of CAT was 0.172 µmoles/min/mg protein in the control and it was significantly increased 0.187, 0.217, 0.294, 0.479, and 0.753 µmoles/min/mg protein at the Zn concentrations of 300, 450, 600, and 750 ppm, respectively (Figure 1A). SOD activity was significantly increased at 600 ppm (0.686 U/min/mg protein) and 750 ppm (0.976 units/min/mg protein) of Zn concentrations compared to control (0.174 units/min/mg protein) (Figure 1B).

The activity of α-amylase, lipase, and protease in larvae fed with 150 ppm concentrations of Zn, did not show significant differences compared to the control, but the 300-750 ppm concentrations significantly decreased the activity of these enzymes (Figure 2A-C). The results showed that the activity of *α*-amylase sharply decreased to 0.036, 0.027, 0.019, 0.014, and 0.011 nmole/min/mg protein at 150, 300, 450, 600, and 750 ppm Zn concentrations, respectively (Figure 2A). There was no significant change in the lipase activity of Zn-fed larvae compared to the control (Figure 2B). However, significant decline in protease activity was measured in all Zn fed larvae at all concentrations (Figure 2C).

Observed a concentration dependent decrease in protein and glycogen contents in larvae (Figure 3A-B). A significant decline in protein amount at all concentrations of Zn when compared to control. Moreover, high concentrations of Zn caused a significant reduction in protein content (3.27 and 3.01 mg, respectively) when compared to the control (4.21 mg) (Figure 3A). Additionally, the glycogen level of the larvae fed with 150, 300, 450, 600, and 750 ppm (2.58, 2.26, 1.92, 1.74, and 1.60 µmol, respectively) of Zn, showed a significant decrease compared to the control, 2.73 µmol/L (Figure 3B).

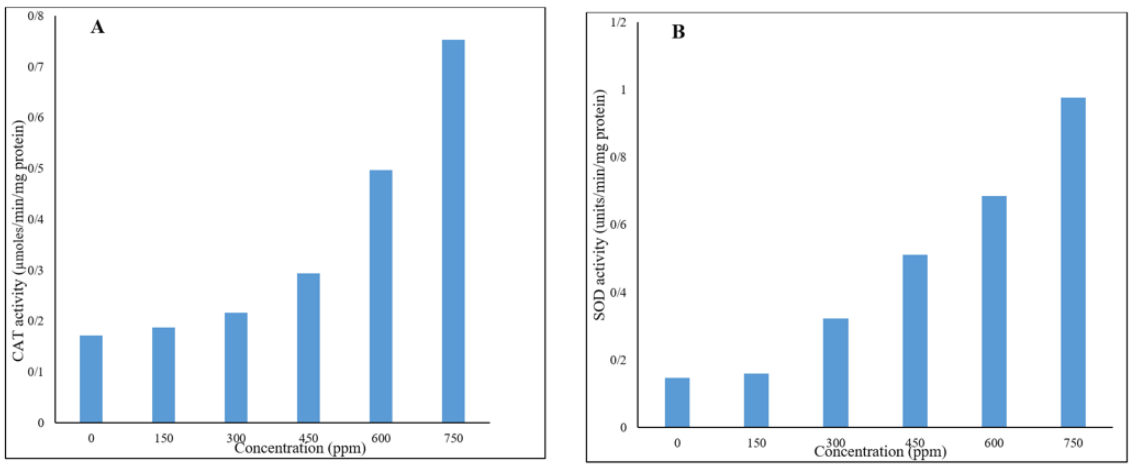

Figure 1. (**A**) CAT and (**B**) SOD activity

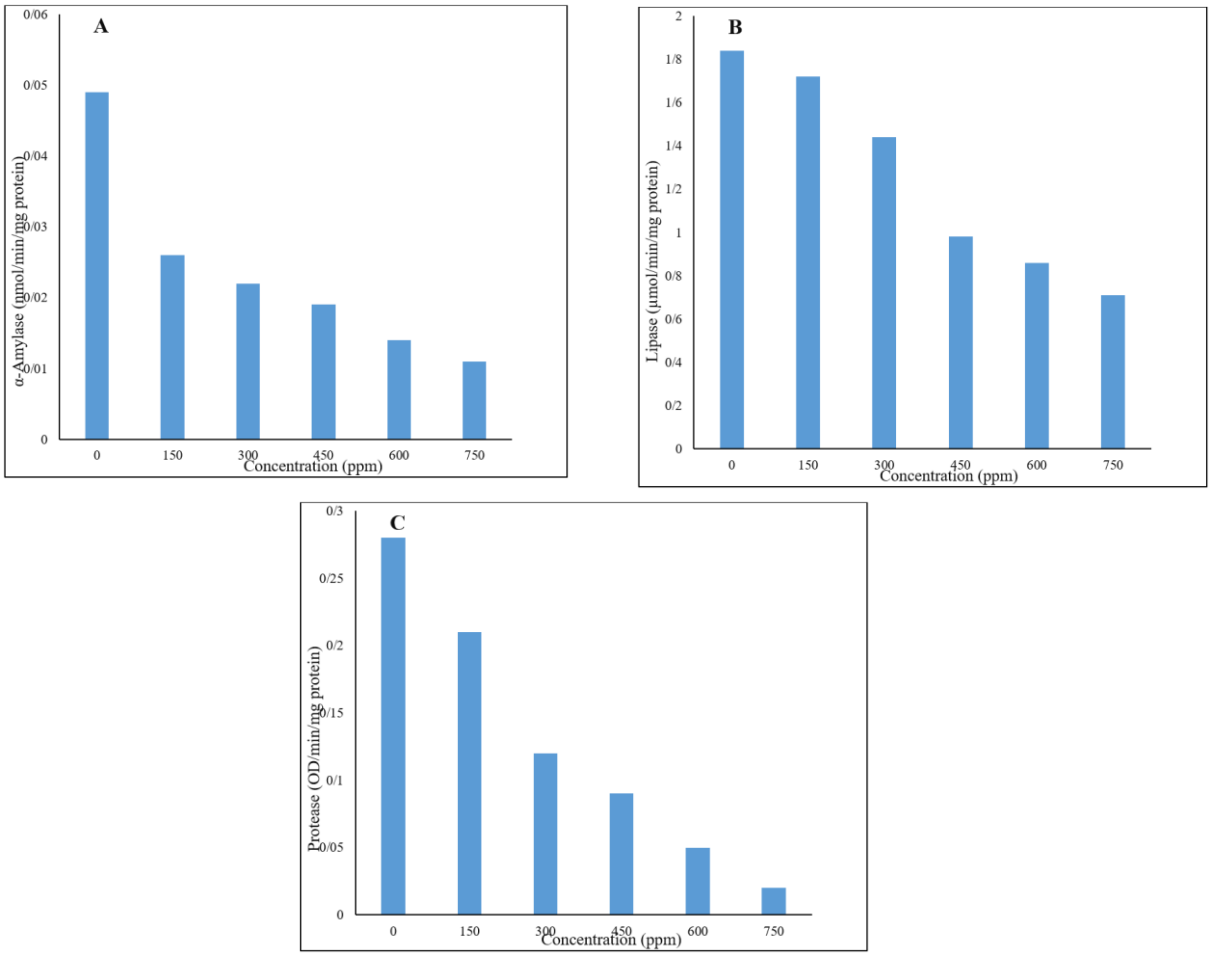

Figure 2. Activity of (**A**) α-amylase, (**B**) lipase, and (**C**) protease

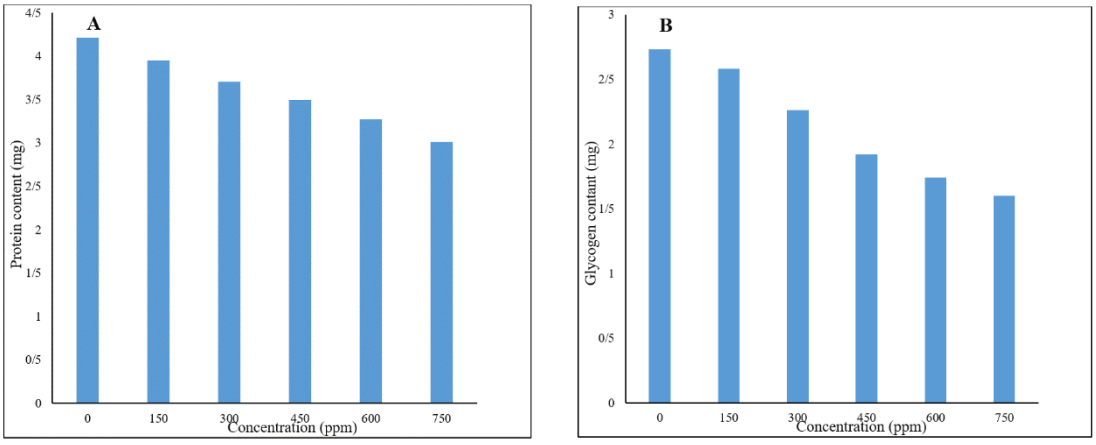

Figure 3. (**A**) protein and (**B**) glycogen content

Discussion

A negative impact of Zn salt on the survival of larvae was observed in this study. According to the results, increased concentrations of Zn salt have prolonged the length of the larval period (the fifth and sixth stages). Consequently, when larvae feed on zinc-treated leaves, they divert more energy from food nutrients to detoxification processes, resulting in decreased body weight and extended larval development.

Additionally, results indicated that fecundity was significantly decreased after Zn treatment, largely because the mating times and oviposition period were greatly reduced. Moreover, Zn caused a decrease in lifetime in both of sexes. The reduction in lifetime may be due to reduced energy reserves (Jiang *et al.* [19]) or stress on the immune system (Kafel *et al.* [20]) and antioxidant system (Topkara *et al.* [21]) caused by heavy metal toxicity. Similar negative effects of metals were observed in other studies. For example, Qi *et al.* [8] found that the larval period of *S. litura* Fab. increases with the amount of Zn salts ($ZnCl_2$ and $ZnSO_4$) in the diet. Ranjbar *et al.* [7] showed that the maximum concentrations of Fe, Zn, and Cu salts in the *S. litura* larvae caused an increase in the length of the larval and pupal periods and, as a result, a delay in the emergence of adults. Rizwan *et al.* [22] demonstrated a significant effect of Zincol, added to diet, on larval mortality, pupation and adult emergence was demonstrated for *Tribolium castaneum* Herbest. Chen *et al.* [4] showed that adult aphids developing on high concentrations of Cd and Zn metals had lower adult fecundity. Also, Shaik *et al.* [5] reported that high concentrations of Zn and Fe metals adversely affected the growth and development of *S. littoralis* Boisd. larvae. Antioxidant enzymes are the first line of antioxidant defense in insects [23]. In this study, when larvae fed on zinc-treated leaves, there was an increase in the activity of SOD and CAT, with the highest increase occurring at the highest concentration. Coskun *et al.* [24] reported that different concentrations of Zn metal caused an increase in SOD and CAT activity compared to the control in *Galleria mellonella* L. Topkara *et al.* [21], who observed a significant increase in CAT activity in *Hyphantria cunea* Drury larvae fed an artificial diet treated with Cu, Ni, and Zn metals. This study showed a significant decrease in *α*-amylase, protease, and lipase activity in the larvae were fed on leaves treated with different concentrations of Zn. This depletion might be due to an energy demand and an increased metabolism as a result of the Zn effect, which, in turn, impairs the nutrition [25]. This result is supported by Ranjbar *et al.* [15] and Wang *et al.* [26], who reported that the decreased activity of midgut lipase in *Musca domestica* L. and *Ectomyelois ceratoniae* Zeller, exposed to heavy metals (Cd^{2+}, Cu^{2+}, and Zn^{2+}) may be due to the disturbance of digestion and absorption of processes. Pompka *et al.* [27] observed decreased levels of protein, carbohydrates, lipids, and glycogen in *S. exigua* Hübner larvae fed on a diet containing on different concentrations of Cd, as noted by Li *et al.* [28] in *Daphnia magna* Straus. In the present study, the results showed that the amounts of protein and glycogen decreased in larvae that were fed all the concentrations of Zn. This depletion might be due to an energy demand and an increased metabolism as a result of the Zn effect [24]. Also, depletion may be due to stress conditions imposed on this insect that require more energy, leading to the metabolism of the protein and glycogen to provide energy for the insect to survive. These results are supported by Qi *et al.* [8]; Jiang *et al.* [19]; and Kafel *et al.* [20].

Conclusion

In summary, this study provides the negative effects of *S. frugiperda* fed with maize leaves soaked in Zn salt. These findings show that Zn caused a significant alteration in the antioxidant enzyme activity, digestive enzyme activity, growth, and development. The data which was observed in *S. frugiperda* highlight its potential for determining the toxic effect of Zn. Therefore, this study will contribute to the knowledge and provide insights into the toxicological aspects of Zn, which will aid further research on populations of important agricultural pests.

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