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## Heavy metals stress on the growth parameters of the cotton leaf worm, *Spodoptera Littoralis* (Boisd.), (*Lepidoptera: Noctuidae*)

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**Key words:** Cotton leaf worm, *Spodoptera littoralis*, Heavy metals, Growth parameters.

### Abstract

A Large number of insects are influenced easily by a number of pollutants; such as cadmium (Cd) and lead (Pb). Cadmium has an increasing international concern because its toxicity is generally considered to be much higher than those of other heavy metals. In this research, we tried to evaluate the effects of both cadmium and lead on the larval and pupal durations together with adult longevity. From the obtained results, we found that cadmium (Cd) treatment disturbed larval development, metamorphosis and decreased the survival of both larvae and pupae. The mean total percentage survival of the larvae fed castor bean leaves treated with cadmium was significantly lower than that of the control and the lead treatments. It was observed that, percentage pupation was successful under lead treatment (93% percentage pupation) and the percentage larval survival recorded the highest value (94%) compared with those of the control and cadmium treatments. Our research assumed that lead does not affect the growth parameters of the insect.

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## Introduction

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a major pest of cotton and other cultivated crops in Egypt as well as Mediterranean and Middle East countries (Campion *et al.*, 1977; Nasr *et al.*, 1984; Ahmad, 1988; Adham *et al.*, 2005 a, b; Tiessen, 2012).

Many ecological factors such as heavy metals can affect the growth performance of the herbivorous insects (Bechard *et al.*, 2008; Haq *et al.*, 2012). Among the well known heavy metals, which could be considered as pollutants in the environment, were cadmium (Cd) and lead (Pb).

Cadmium exerts negative impacts on living organisms and accumulates in food chains (ROBERTS *et al.*, 1979). A general increase in the levels of cadmium threatens the health of terrestrial and aquatic organisms and therefore has become a major topic of ecotoxicological researches (Suzuki *et al.*, 2004). It is considered as an "end of the road" heavy metal pollutant, bioavailable and toxicologically active and its toxicity to men and some animals is well documented (Adriano, 1986 and Wagner, 1993).

Furthermore, Lead is considered a significant environmental pollutant (Haq *et al.*, 2012). Many studies have been carried out to demonstrate the relations concerning the biological effects of lead and its toxic potential against insects (Talbot, 2004; Margim, 2005).

From previous studies, it was assumed that, some physiological changes may underlie the reduced growth, longevity, fecundity and hatchability as recorded in many insects and other arthropods exposed to heavy metals (Gintenreiter *et al.*, 1993; Raymskeller *et al.*, 1998; Moe *et al.*, 2001; Cervera *et al.*, 2004; Cervera *et al.*, 2006; Van Ooik *et al.*, 2007; Bechard *et al.*, 2008).

The relationships between growth parameters of different instars of *S. littoralis* caused by metal

contamination were less studied in Egypt. Hence, the aim of this research is to investigate some impacts of Cd and Pb on growth parameters of the third, fourth, fifth and sixth instars of the cotton leaf worm, *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae).

## Materials and methods

### *Insect rearing*

All insects used in the bioassays were obtained from a standard laboratory colony maintained at the Department of Entomology, Faculty of Science, Cairo University.

The newly hatched larvae of *S. littoralis* (Boisd.) were placed in polyvinyl plastic boxes (12 W × 6 H × 18 L cm) lined with Whatman filter paper (no.1). Larvae were freely fed fresh castor bean leaves (*Ricinus communis* L.) until pupation. The rearing room was kept at constant temperature of  $25 \pm 2$  °C, relative humidity of  $65 \pm 5$  % and a photoperiod of 16 : 8 (L : D) hour as suggested by Adham *et al.* (2005 a, b). Uneaten and nibbled leaves were removed and newly fresh ones were provided.

Larvae were observed daily to record the larval duration and the percentage survival. Newly formed prepupae were weighed to record the average fresh weight of prepupae. Percentage pupation, average pupal fresh weight, pupal duration as well as adult emergence and sex ratio were also recorded. After successful eclosion, newly emerged moths were fed 10% sugar solution and were offered a small fresh twig of *Nerium Oleander* L. to serve as an ovipositional site. Mean adult longevity was determined in terms of days. Newly deposited egg patches were collected daily and then transferred in 100 cm<sup>3</sup> rearing cups.

### *Cadmium and lead treatments*

To study the effects of both cadmium (Cd) and lead (Pb) on the cotton leaf worm, *S. littoralis* (Boisd.), through food; three groups of *S. littoralis* (Boisd.) colonies were established. Each group consisted of 3 replicates of 20 newly hatched larvae per box. One of

these groups was the control one. While the other groups (cadmium and lead) were the treated groups. In this research, we adopted the leaf dipping method (Cuthbertson *et al.*, 2003). The larvae were allowed to feed freely on fresh castor bean leaves dipped in the heavy metal solution (100 ml g / kg) for 10 seconds and then allowed to dry in room air for 10 minutes. Left diets were replaced with freshly treated diets every 24 h. The experiment was started with larvae hatched in the same day and offered daily heavy metals treated leaves from hatching till pupation.

*Statistical analysis*

All data were presented as mean ± SE. Data were analyzed using one way analysis of variance (ANOVA) and Duncan’s multiple range test. All statistical computations were carried out using SAS program (Anonymous, 2000).

**Results and discussion**

*Larval stage*

Data presented in table (1) revealed insignificant difference ( $p > 0.05$ ) between the means of larval durations for the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> instars under each treatment and among treatments. It was observed that the larvae fed on cadmium treated castor bean leaves have comparatively prolonged larval duration compared to that of both the control and lead treatment which reach its highest value at the 6<sup>th</sup> instar. Our results agreed with findings of other authors who stated that a significant prolongation of the cotton leaf worm, *S. littoralis* (Boisd.), development after acute exposure to cadmium concentration of 100µg/g and higher (Ortel *et al.*, 1993; Gintenreiter *et al.*, 1993; Ilijin *et al.*, 2010). Generally, a high cadmium concentration increases the larval duration of holometabolous (Sildanchandra and Crane, 2000; Nascarella *et al.*, 2003; Wu *et al.*, 2006) and hemimetabolous insects (Cervera *et al.*, 2004). It has to be mentioned that, the survival rate of the cotton leaf worm *S. littoralis* (Boisd.), varied among treatments (table 1).

**Table 1.** Mean values of the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> larval duration and total percentage of larval survival of *Spodoptera littoralis* (Boisd.) fed on castor bean leaves treated with cadmium (Cd) and lead (Pb).

| Larval stage | Larval duration (days)   |                          |                          |                          | Total larval survival (%) |
|--------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
|              | 3 <sup>rd</sup> instar   | 4 <sup>th</sup> instar   | 5 <sup>th</sup> instar   | 6 <sup>th</sup> instar   |                           |
| Treatment    | Mean ± SE                |                          |                          |                          |                           |
| Control      | 3.5 ± 0.1 <sup>a</sup> * | 3.2 ± 0.122 <sup>a</sup> | 3.4 ± 0.187 <sup>a</sup> | 3.8 ± 0.2 <sup>a</sup>   | 92 ± 1.225 <sup>a</sup>   |
| Cd           | 4.3 ± 0.122 <sup>b</sup> | 4.3 ± 0.122 <sup>b</sup> | 4.5 ± 0.158 <sup>b</sup> | 5.8 ± 0.122 <sup>b</sup> | 78 ± 1.225 <sup>b</sup>   |
| Pb           | 3.1 ± 0.1 <sup>c</sup>   | 3.1 ± 0.1 <sup>c</sup>   | 3.2 ± 0.122 <sup>c</sup> | 3.8 ± 0.122 <sup>c</sup> | 94 ± 1.871 <sup>a</sup>   |

\* Means in columns followed by the same letters are insignificantly different ( $P > 0.05$ ).

The mean total percentage survival of the larvae fed castor bean leaves treated with Cd was significantly lower than that of the control and Pb treatments. Exposure to Cd during larval stage suggests that metal tolerance builds over time. The Cd treatment disturbs larval development, metamorphosis and decreases the survival of larvae and pupae. Chronic cadmium toxicity caused significant reductions in larval development and survival. While treatment with Pb did not inhibit their growth and decreased their mortality. Our results assumed that cadmium exerted a strong adverse impact on the cotton leaf

worm *S. littoralis* (Boisd.), growth and development. Such a decrease in performance in response to pollutant is characteristic for chewing insects (Koricheva *et al.*, 1998).

*Pupal stage*

Results presented in Table (2) showed that the mean prepupal fresh weight was highly significant ( $P < 0.01$ ) between the three treatments. The least value of the mean prepupal fresh weight was recorded for larvae fed castor bean leaves treated with Cd as shown in table (2).

**Table 2.** Mean values of the prepupal fresh weight, percentage pupation, pupal fresh weight and pupal duration (days) of *Spodoptera littoralis* (Boisd.) resulting from larvae fed castor bean leaves treated with cadmium (Cd) and lead (Pb).

| Pupal stage<br>Treatment | prepupal fresh weight (gm)  | pupation (%)            | pupal fresh weight (gm)     | pupal duration (days)     |
|--------------------------|-----------------------------|-------------------------|-----------------------------|---------------------------|
|                          | Mean ± SE                   |                         |                             |                           |
| Control                  | 1.878 ± 0.103 <sup>a*</sup> | 86 ± 1.871 <sup>a</sup> | 1.623 ± 0.049 <sup>a</sup>  | 10.4 ± 0.245 <sup>a</sup> |
| Cd                       | 1.128 ± 0.073 <sup>b</sup>  | 77 ± 1.225 <sup>b</sup> | 0.776 ± 0.023 <sup>b</sup>  | 12.8 ± 0.374 <sup>a</sup> |
| Pb                       | 1.511 ± 0.065 <sup>c</sup>  | 93 ± 1.225 <sup>c</sup> | 1.192 ± 0.0438 <sup>c</sup> | 10.8 ± 0.2 <sup>a</sup>   |

\* Means in columns followed by the same letters are insignificantly different (P > 0.05).

The mean percentage pupation was found to be lower (77 ± 1.225) for the larvae fed on castor bean leaves treated with Cd, while the highest value (93 ± 1.225) was recorded for the Pb treatment and a highly significant difference (P < 0.01) was found between the two treatments.

The mean pupal fresh weight was highly significant (P < 0.01) between treatments and the lowest value (0.776 ± 0.023) was observed for Cd treatment.

Obtained data showed that the mean pupal duration was highly insignificant (P>0.05) between treatments. The longest pupal duration (12.8±0.374)

was observed for Cd treatment as indicated in table (2).

*Adult stage*

Results presented in Table (3) showed insignificant difference (P > 0.05) between means of the adult emergence percentage among the treatments.

The mean percentage of males resulting from larvae fed on leaves treated with Pb was lower (40 ± 3.536) compared to that of control and Cd, while the mean percentage of females resulting from larvae fed castor bean leaves under control treatment was the lowest (52 ± 5.148) as shown in table (3).

**Table 3.** Mean values of percentage adult emergence, sex ratio, and adult longevity of *Spodoptera littoralis* (Boisd.) resulting from larvae fed castor bean leaves treated with cadmium (Cd) and lead (Pb).

| Adult stage<br>Treatment | adult emergence (%)      | Sex ratio (%)           |                           | Longevity (days)         |
|--------------------------|--------------------------|-------------------------|---------------------------|--------------------------|
|                          |                          | males                   | females                   |                          |
| Mean ± SE                |                          |                         |                           |                          |
| Control                  | 83 ± 3.391 <sup>a*</sup> | 48 ± 5.148 <sup>a</sup> | 52 ± 5.148 <sup>a</sup>   | 7.4 ± 0.245 <sup>a</sup> |
| Cd                       | 46 ± 4.301 <sup>a</sup>  | 42 ± 2.811 <sup>a</sup> | 58 ± 2.811 <sup>b,a</sup> | 5.2 ± 0.374 <sup>a</sup> |
| Pb                       | 80 ± 2.739 <sup>a</sup>  | 40 ± 3.536 <sup>a</sup> | 60 ± 3.536 <sup>b,a</sup> | 7.6 ± 0.245 <sup>a</sup> |

\* Means in columns followed by the same letters are insignificantly different (P > 0.05).

The mean adult longevity was insignificant (P > 0.05) between the three treatments and the lowest adult longevity (5.2 ± 0.374) was recorded under Cd treatment. Taking into account that there was a strong correlation between pupal mass and fecundity in the cotton leaf worm (Lazarević *et al.*, 1998), the reduced pupal mass in response to the highest cadmium concentration (Cd) had implications for

female reproductive success. It was assumed that larger male insects have a higher mating ability (Santos *et al.*, 1988) and, thus, the reproductive performance of the male moth could also be affected as a consequence of lower pupal mass. Accordingly, a reduced body size led to reduced longevity.

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