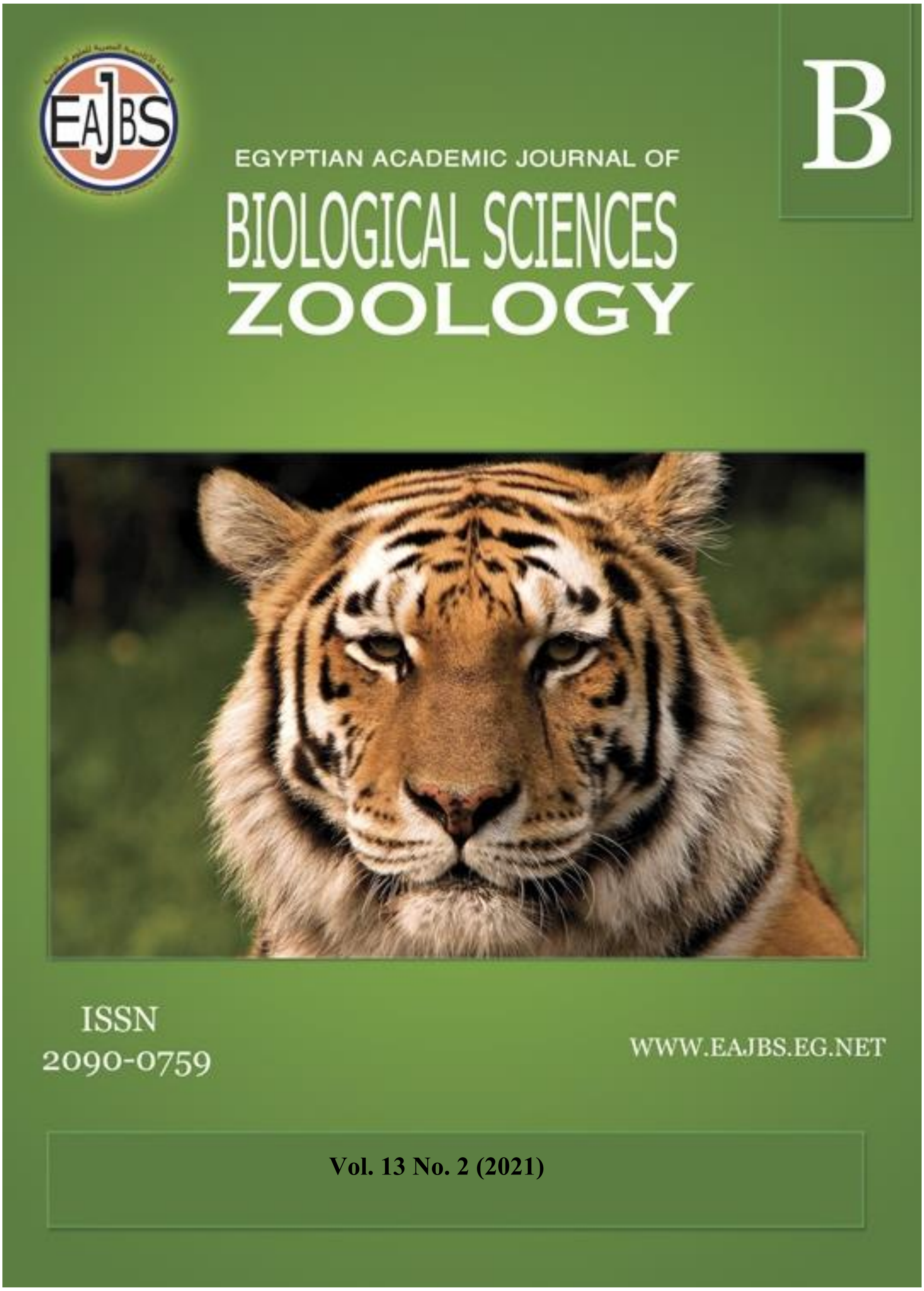

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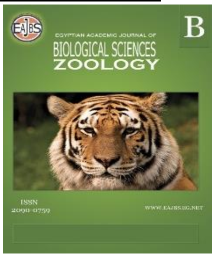

Individual and Combined Toxicity of Chromium and Remilitine Fungicide on The Growth of Earthworm, *Eisenia fetida*

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ABSTRACT

Earthworms are a type of invertebrates that contributes to the growth of plants and fertility improvement of soil. Remilitine fungicide is commonly used by farmers in agriculture in the Benghazi region, whereas Chromium is one of the main soil pollutants, however, more studies are needed concerning its toxicity in soil organisms. Consequently, chromium was selected to be used in this research. Along with Remilitine fungicide and Chromium as well as Remilitine fungicide separately and in combination the body growth of the earthworm, *Eisenia fetida* in the laboratory. All worms were adults and fully clitellated. The adult worms were exposed to 70 days of sublethal concentration of Remilitine500ppm and Chromium200 ppm as well as the mixture of Remilitine - Chromium (200+ 500ppm). The worms of control treatment reported significantly greater body weight ($F= 5.4$, $P < 0.05$), as compared to that of Remiltine treated worms Furthermore, significant differences in worm were also revealed body weight between the control and chromium treated worms after 70 days ($F=10.33$, $P<0.05$). These results also reveal statistically significant differences in body weight between the Remilitine - chromium mixture and the control ($F=14.57$ $P<0.05$). From these results, it is concluded that the interaction type of Chromium and Remilitine combined toxicity is antagonistic.

INTRODUCTION

Earthworms are considered vital species within terrestrial food webs that execute a large number of critical functions for example enhancement of microbial activity, aeration of the soil, tillage and decomposition of organic litter (Fragoso *et al*.,2004). Chemicals possibly will influence the functions of the soil by directly disturbing one or more of these routes or by indirectly reducing the activity and number of soil engineers like the earthworms(*Fragoso et al*.,2004). The scope of this study is on the assessment of the interactions of Remilitine and Chromium mixture in the earthworms *Eisenia fetida* and the resulting impacts.

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Charles Darwin (1809–1882) deliberated that earthworms are one of the earth's most significant caretakers. He was the origin that described earthworms tilling the soil, swallowing and ejecting soil as castings, otherwise worm manure. He expected that an acre of soil may possibly hold an excess of 50,000 earthworms and produce 18 tons of organic castings yearly .As earthworms swallow great quantities of soil or specific portions of soil (i.e., organic matter), are constantly exposed to pollutants through their alimentary surfaces (Morgan and Morgan, 1988). Furthermore, numerous readings have revealed that earthworm skin is also an important method of contaminant uptake (Saxe., et al 2001) and (Vijver et al., 2003). Extra nutrients and toxic materials are mounted up and consequently exert adverse effects by a range of interactive modes of action, with regard to the mechanisms of uptake and the mechanisms of toxicity.

The Oligochaete *Eisenia fetida* worms abstain a very thick layer of mucus that surrounds the epidermis of the skin (Laverack, 1963), where respiration and elimination of waste products take place. This mechanism makes the earthworms quite sensitive to the loss of water. The digestive system of oligochaete species is well studied (Wallwork, 1983). There is an indication that the digestion of food via the gut is not a heterogeneous procedure thru the gut route. During digestion, in the first part of the digestive system of an oligochaete, mucus is mixed with the food and calciferous glands actively release Ca_2C in the gut contents. The crop is used for storing the gut components, before grinding and digestion in the gizzard which opens in the intestine. The intestine forms the principal part of the alimentary canal. Earthworms have the ability to mount up organics to a great extent, and the capability to deal with high levels of accumulated organics can be credited to the capability of organics to bind to adipose tissues (Loonen, 1997) and (Beyer, 1996). Earthworms are furthermore capable to store metals to a great magnitude. The capability to deal with high levels of accumulated metals can be credited to the slower turnover of the tissues in which metals are stored.

The reproduction, change of body weight and endpoints mortality are standardized and described clearly in widely recognized guidelines for testing of chemicals ISO (1993) and OECD (1984). Other endpoints like morphological changes, behavior and physiological alterations are reported infrequently, but they are not assessed in a standardized approach.

Several researchers including Khan, *et al*., (2007) stated a substantial reduction in earthworm biomass after exposure to different concentrations of copper chloride and concluded unusual functioning of the major functional systems such as absorption and digestion. The combined effect of pesticide cyperkill with heavy metal on the growth and reproduction of earthworm *Eisenia fetida* was studied by (Aebeed and Amer, 2018). Aebeed and Mohamed (2018), who reports the effects of heavy metals on cocoon production, juvenile number and the growth, of the earthworm *Eisenia fetida*. Others reported the effect of metals and the pesticides chlorpyrifos, carbofuran, mancozeb and their formulations on the tropical (De Silva, *et al*., 2010). Furthermore, others studied the effects of the organophosphates, chlorpyrifos and diazinon were reported by (Booth, *et al*., 2000).On the other hand, Xiao, *et al*., (2006) proposed that the earthworm's growth can be considered a sensitive parameter to evaluate the toxicity of acetachlor on earthworms.

The objective of this study was to study the growth of the earthworm *Eisenia fetida* under the effect of sublethal concentrations of Remilitine fungicide and Chromium as well as their mixture on the growth of the earthworm, *Eisenia fetida*, reared on the treated soils in an experimental study at the laboratory scale.

MATERIALS AND METHODS

The Strain*E. Fetida* (European) were transported from the Czech Republic as a study sample and cultivated in the zoology lab, the University of Benghazi for more than two years.*E. fetida* was selected for this research as it has a more reproductive perspective when compared with the local species. The worms were maintained at room temperature of approximately 21 ± 2°C, in a glass-aquaria on a culture media as described by Organization for Economic Cooperation and Development (OECD, 2004). The food was comprised of artificial soil mixed with barley grains powder as a food addition every seven days during the experiment period. The moisture circumstances of the rearing soil were at almost55 - 65% water holding capacity. The moisture, subsequently, was preserved by frequently sprinkling water on the soil. Fungal growth was removed continuously when detected on the soil surface. The soil was changed every two months until the worms requested for the experiment were with an average weight of 8 grams. In this experimentation, the adult worms were exposed to the synthetic soil polluted with sub-lethal concentrations of Remilitine 500ppm and Chromium200 ppm as well as the mixture of Remilitine and Chromium (200+ 500ppm). The artificial soil used OECD (OECD, 2004) consisted of quartz sand70%, kaolin clay20%, sphagnum peat10% and calcium carbonate to adjust the pH to 5-6.5. An amount of 250 grams of soil was transferred to glass containers (15 cm L, 12 cm W, 20 cm H) to which100ml of Remilitine500ppm and Chromium200 ppm as well as the combinations of Remilitine – Chromium mixture (200+ 500ppm) was added and mixed thoroughly. Each treatment was reproduced three times and the control was done with three repeats using plain water. Ten adults *E. fetida* were relocated into each test container, after their primary body weight as a whole duplicate were taken. The used dose was selected using a preliminary trial test while considering the findings of prolonged effects rather than acute direct mortality. The further worms' body weight was again taken after 28, 49 and 70 days after treatment. Five grams of barely grain powder was spread on the top of each examination container as food, supplement and soil moisture content was checked every seven days and water was added when required.

The parameters measured in this experiment were: Worm body weight change at 28, 49 and 70 days post-treatment. All data were endangered to SPSS, while, ANOVA was used for significant difference and T-test was for the mean differences.

RESULTS

Change in Body Weight:

The Fungicide Remiltine Treated Worms:

The fungicide Remilitne showed relatively different effects on the treated worms' body weight compared to that of the control Table (1) and Figure (1). The worms of the control treatment reported significantly greater body weight ($F= 5.4$, $P < 0.05$), than that of Remiltine treated worms. However, the change in worms body weight over time was almost comparable in control to that of the Remiltine treated worms with the means ± S.D were 9.43 ± 0.472, 9.13 ± 1.464, 9.167 ± 1.504, 9.50 ± 1.562 for Remilitne treated compared to 9.93 ± 0.75,9.93 ± 0.75,10.43 ± 0.61 and 10.86 ± 0.72, for control, 28, 49 and 70 days fig(1). The results clearly show that control worms gained greater weight after 70 days compared to Remiltine treated worms.

Table 1.The mean ± S.D of *E.fetida* body weight in grams of control and Remilitne treatments.

Time	control	Remilitne
Zero	9.93 ± 0.75	9.43 ± 0.472
After 28	9.93 ± 0.75	9.13 ± 1.464
After 49	10.43 ± 0.61	9.167 ± 1.504
After 70	10.86 ± 0.72	9.50 ± 1.562

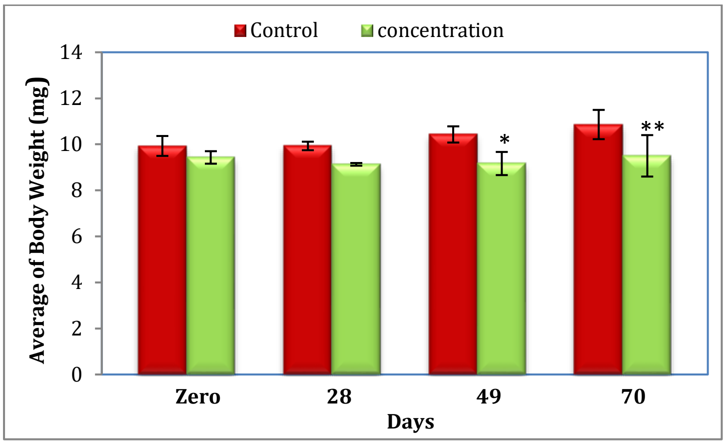

Fig. 1. The mean ± SD of worms' body weight in grams in control and Remilitne treatments.* Significantly different from controls at p < 0.05, ** p<0.01.

The Chromium Treated Worms:

Eisenia fetida treated with chromium has similarly revealed weight decrease compared to the control at zero, 28 days 49, 70 days. The results revealed significant differences in worm body weight between the control and chromium treated after 70 days (F=10.33, P<0.05). The mean ±S.D. of the body weight-related an increase in worms bodyweight along the time which mean that chromium excreted some effect on the worm at least as for as the time of the experiment, the mean ±S.D for chromium treated were 7.00±0.36, 8.30±1.17, 8.90±1.03 and 9.23±1.06 compared to 8.66±0.73, 8.56±0.32, 9.80±0.60 and 10.93±1.10 for control, 28, 49 and 70 days (Fig. 2).

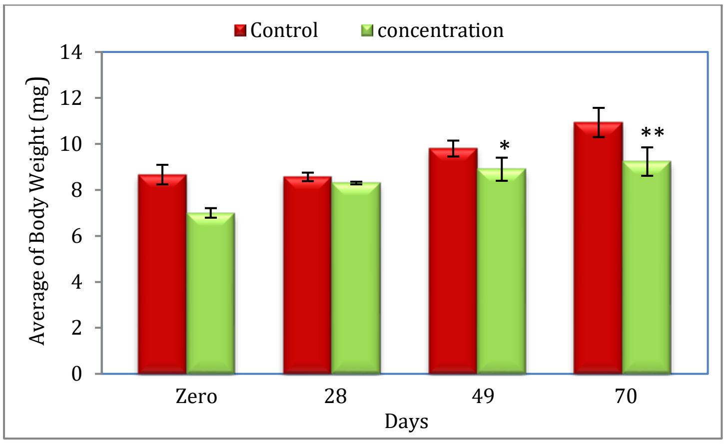

Fig. 2. The mean ± SD of worms' body weight in grams in control and chromium treatments. * Significantly different from controls at p < 0.05, ** p<0.01.

The Remilitne -Chromium Mixture Treated Worms:

The mean values of body weight of worms exposed to the Remilitne - chromium mixture (200+500ppm) Table (2) over 10 weeks. The result revealed a significant difference in body weight between the Remilitine - chromium mixture and control (F=14.57, P<0.05). Furthermore, there is a significant difference in the worms' body weight as time pars (F=6.59 p<0.05). The mean ± S.D of the bodyweight reveal an increase in worms' weight along time, thus the mean ± S.D was 6.86±0.51, 7.36±0.70, 8.36±1.15 and 9.13±1.94 for Remilitne - chromium treated compared to 8.66 ± 0.73, 8.56 ± 0.32, 9.80 ± 0.60 and 10.93 ± 1.10 for control at zero, 28, 49 and 70 days' Figure(3).

Table 2. The mean ± S.D of *E.fetida* body weight in grams of control and Remilitne + chromium treatments.

Time	control	Remilitne + chromium
Zero	8.66±0.737	6.86±0.51
After 28	8.567±0.321	7.36±0.70
After 49	9.800±0.600	8.36±1.15
After 70	10.933±1.101	9.13±1.94

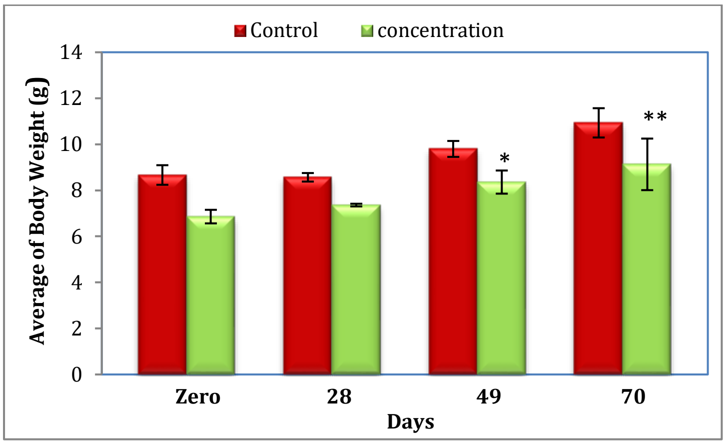

Fig. 3. The mean ± SD of worms' body weight in grams in control and Remilitne + chromium treatments.
*Significantly different from controls at p<0.05, ** p<0.01. ***p<0.001

DISCUSSION

Earthworms are regularly used as a fragment of sequences of indicator species to test the effects of pollutants on the ecosystems. A wide array of substrates (including artificial substrates like OECD soils), test designs, and endpoints are exploited and guidelines have been considered to regulate the assessment of adverse effects on earthworms. Apart from laboratory testing, terrestrial model ecosystems (TMEs) (Koolhaas *et al* ., 2004) The species most frequently tested in laboratory settings are the compost worms *Eisenia andrei* and *E. fetida* as more field-appropriate species unlike *L. rubellus* and *A. caliginosa*that are difficult to rear.

Chromium is a dangerous heavy metal, where it causes respiratory and digestive cancers, severe diarrhea and nausea. Moreover, the toxicity of chromium in soil organisms is not studied enough (Cefalu and Hu, 2004).

Morgan *et al*., (1990) found discrete alterations in the distribution of various metals throughout the earthworms' body, whereby the sequestration of chlorogocytes played a dominant role, causing changing patterns of tissue accumulation, on the other hand, different tolerances to metals were reported by (Morgan and Morgan, 1998). Metals such as Cu and Cd are mainly bound to metal-binding proteins (St¨urzenbaum *et al*., 2001)and with these proteins, the metal travels through the body to the organs and tissues in which it is deposited inorganic forms. Cd was retrieved in great amounts from the nephridia and to a lesser extent from the body wall of earthworms (Prinsloo *et al*., 1990), and Pb is located in waste nodules found in the coelomic fluid (Andersen and Laursen, 1982).

Other studies on the collaboration between heavy metals have been carried out, where Pan and Yu (2011) found that the combination of Cd and Pb had synergistic toxicological effects on enzyme activities in soil. Aebeed *et al*. (2019) established significant effects on the body weight and cocoon production, while the high significant juvenile number was described in Eisenia *fetida* worms. The combined toxicity tests on earthworms in this research displayed that the interaction of Remilitine fungicide and Chromium was antagonistic.

The proposed objectives of this study are in agreement with that of (Aebeed and Amer 2018) and (Aebeed *et al*., 2019) who stated that mortality is in general accepted to be a somewhat insensitive parameter, whereas sub change in body weight and reproduction were more vital to assess. However, *E. fetida* body weight decrease due to heavy metal was detected in some studies, (Berthelot *et al*., 2008). Whereas, no impact on body weight was detected in other studies (Van Gestal *et al*., 1993). The relationship between metals and body weight has been clarified by (Spurgeo and Hopkin, 1996), who specified that the worms living in metal-contaminated soil reach the lower weight or require more time to reach the maximum weight than in non-polluted sites.

Based on the statistical analysis performed in this study, it was found that, due to the high toxicity of Remilitine fungicide for the body weight, while the toxicity of Chromium was less on the body weight. In addition, This study showed that the toxicity of Remilitine - Chromium mixture during the time is moderate bodyweight which is greeted with other studies (Aebeed and Mohamed,2018), (Aebeed *et al*., 2019) and (Dou and Hu, 2014).

Conclusion:

The present study, indicated that the interaction of Remilitine - Chromiummixture is antagonistic., whereas the toxicity of Remilitine alone is more severe on body weight than chromium alone. The mechanisms of effects combined between Remilitine and Chromium are complex, which may have been influenced by the competitive adsorption of between Chromium and Remilitine in soil and biomembrane and their bioavailability. Further studies are required to intensively identify the mechanisms of the combined toxicological effects on soil animals.

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