

**EVALUTION THE EFFECT OF HEMAZOLE PESTICIDE ON THE VITALITY,  
GROWTH AND TISSUE CHANGES OF EARTHWORMS**

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**ABSTRACT**

This study was conducted to show the effect of Hemazole fungicide on the growth and vitality of earthworms when treated with different concentrations of the pesticide (1000,200,40) mg.kg<sup>-1</sup> and for periods of (7,14,21,and28) days, a clear decrease was observed in the growth rate of worms treated with the pesticide with the increase in the time period, and the highest decrease in growth was recorded at the concentration of 40 mg.kg<sup>-1</sup> amounted to 83.23% during the third period of rearing (21 days), as for the survival of worms, it was noted that young worms were affected by the three concentrations, and the time period for killing worms 100% was observed after (7, 15 and 21)minutes, respectively, while the time period for the death of adult worms was much higher, reaching (72, 30 and 13) minutes. As for the effect of the pesticide on the thickness of the body wall and the occurrence of tissue abnormalities, it was noted that the thickness of the body wall was significantly affected, especially at the concentration of 40 mg.kg<sup>-1</sup>, where the lowest thickness was 28,333, 170, 39.67 micrometers compared to the control group, which recorded the thickness of the body wall amounted to 56.667, 532.67, 124 Histological abnormalities were also observed, including necrosis in the epidermal layer, a decrease in the thickness of the longitudinal muscles, and flattening or necrosis in the epithelium of the alimentary canal.

**Key words:** Earthworms, Hemazole, Fungicide, Growth rate.

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تقييم تأثير مبيد الهيمازول على حيوية ونمو وتغيرات نسيج دودة الأرض

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باحث

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المستخلص

اجريت هذه الدراسة لبيان تأثير مبيد الهيمازول الفطري على نمو وحيوية ديدان الارض عند معاملتها بتركيز مختلفة من المبيد (40، 200 و1000) ملغم.كغم<sup>-1</sup> ولفترات (7،14، 21 و28) يوم ، اذ لوحظ انخفاض واضح في معدل نمو الديدان المعاملة بالمبيد مع زيادة الفترة الزمنية وان اعلى انخفاض في النمو سجل عند التركيز 40 ملغم.كغم<sup>-1</sup> بلغ 83.23% خلال الفترة الثالثة من التربية (21يوم)، اما بالنسبة لبقاء الديدان فلو حظ ان الديدان الفتية تاترت بالتركيز الثلاثة وكانت المدة الزمنية لهلاك الديدان 100% هي (7،15 و21) دقيقة على التوالي، في حين كانت المدة الزمنية لهلاك الديدان البالغة هي اعلى بكثير اذ بلغت (72، 30، 13) دقيقة، اما تاثير المبيد على سمك جدار الجسم وحدوث تشوهات نسيجية فلو حظ ان سمك جدار الجسم تاتر بشكل كبير وخاصة عند التركيز 40 ملغم.كغم<sup>-1</sup> اذ سجل اقل سمك هو 28.333، 170، 39.67 مايكروميتر مقارنة بمجموعة السيطرة التي سجلت سمك جدار الجسم بلغ 56.667، 532.67، 67،124 مايكروميتر كما لوحظ حدوث تشوهات نسيجية منها نخر في طبقة البشرة وقلة في سمك العضلات الطولية وتسطح او نخر في ظهارية القناة الهضمية

الكلمات المفتاحية: دودة الارض، هيمازول، مبيد فطري، معدل النمو.

## INTRODUCTION

Earthworms are an environmentally important invertebrate through their important role in the ecosystem, as they facilitate the decomposition of plant and animal materials, break down the soil, release nutrients and make them available to plants (2, 8). Earthworms are also an important part of the food chain and are at the same time highly susceptible to pesticides that are sprayed directly into the fields Earthworms were considered an indicator of the percentage of soil pollution (27). because the increased presence of chemical pollutants in the soil ecosystem has negatively affected the population density of soil animals, including earthworms, and that chemical residues can have a great impact that cannot be ignored to a large extent (26), as recent global studies are directed towards the proper disposal of chemical wastes to maintain the health of the soil ecosystem (28). In addition, pesticides affect earthworms, their reproduction and the formation of young birds, and earthworms are food for many birds, so the pesticides will have an impact on the food chain and the death of many living organisms that depend on them when they are contaminated with pesticides (7). Since earthworms are non-target soil invertebrates that are affected by the use of pesticides because it has direct contact with the soil and thus its tissues are rapidly exposed to chemicals and accumulate in their tissues (25). Thus, earthworms response to applying pesticides to soil can provide information about their tolerance limits and their ability to treat the toxic effects of pesticides pests (10).

## MATERIALS AND METHODS

### Sample collection

The collection of earthworms began in September-November 2020 from the home garden, as the samples were collected by digging the soil 15-30 cm deep for the soil and more. They were preserved and bred in plastic boxes with dimensions (20×27×38 cm) These boxes contained a piece of cloth that was placed at the bottom of the box and extended to its sides, and soil and bird droppings were placed in these boxes according to the method (9,10).

Also, dilute concentrations of this pesticide were prepared. The prepared concentrations were as follows

40,200 and 1000 mg.kg<sup>-1</sup>, which was used to study its effect on the survival of earthworms for a period of 28 days, and the time required to kill the worms was recorded 100%.

Several preliminary experiments were conducted for the purpose of obtaining these concentrations.

### Effect of the fungicide Hemazole on the relative growth rate of earthworms

After taking the weights of the worms treated with the aforementioned pesticide, and for the purpose of knowing the effect of this, you are used on pesticide these pesticide on the growth rate of worms, their growth rate was calculated according to the method (22).

**Table 1. Shows the characteristics of fungicide Hemazol 30%**

<b>Chemical group</b>	<b>Oxazoles</b>
<b>The active substance in the pesticide</b>	<b>%30w/v</b>
<b>Trade Name</b>	<b>Hymazol Hemexazole</b>
	<b>Complementary Solvents 100%</b>

### Histological analysis

For the purpose of knowing the effect of the pesticide used in the experiments on the tissue composition of some areas in the bodies of earthworms treated with the pesticide, histological tests were conducted (13,24).

### Estimation of the protein content in the bodies of earthworms.

The amount of protein in the bodies of earthworms treated with pesticides was estimated using (15) method.

### Statistical analysis

Statistical analysis of the results was carried out using the SAS program by an automated computer in a completely randomized design; Because it is a factorial experiment (C.R.D) Differences between the arithmetic mean were selected using Duncan's multiple range test at a 5% probability level (5).

## RESULTS AND DISCUSSION

### Effect of different concentrations of the fungicide Hemazol on the relative growth rate of earthworms

Table (2) shows a decrease in the growth rate of earthworms treated with different concentrations of Hemazole (40,200 and 1000)

mg.kg<sup>-1</sup> for a period of four weeks (7,14,21 and 28) days, it was noted from the table that the effect of the concentration recorded the lowest growth rate of the worms was at a concentration of 200 mg.kg<sup>-1</sup> that reached 92.592%. While the lowest growth rate of worms recorded during the time periods was at the same concentration (200 mg.kg<sup>-1</sup>) reaching 83.23% during a period of 28 days, while the control group recorded a growth rate in the same time period of 111.805%. We conclude from this that the growth rates of worms treated with pesticides were less than the growth rate of worms in the normal condition in soils free of pesticides, and therefore the pesticides affect the number of worms in agricultural soils. The results of the study were also similar with what (12, 16, 23) mentioned

that the weight and growth of earthworms was significantly affected when exposed to pesticides for a period of four weeks, as a difference in weight was observed from the worms of the control group, which means that the growth and reproduction factors of earthworms exposed to pesticides appear as useful biological indicators for contamination. This explains the negative impact of pesticides on the growth and reproduction of earthworms. The results of the current study converge with what was shown by the scientist (19) that the accumulation of pesticide residues in the soil affects the number of worms in areas where pesticides are frequently used, and thus has an impact on the food chain and living organisms.

**Table 2. Effect of treating earthworms with Hemazole concentrations on growth rate.**

Exposure period Conc.	growth rate%			28 day	Impact rate the Concentration
	7day	14day	21day		
0	108.703 a	111.29 3	111.356 a	111.805 a	110.789 a
1000 mg.kg <sup>-1</sup>	78.822 e	a 64.224 f	* 94.109 a	* 92.173 b	35.762 c 92.592 b
200 mg.kg <sup>-1</sup>	100.857 a	a 94.109 a	92.173 b	83.23 d	98.031 b
40 mg.kg <sup>-1</sup>	103.327 a	100.592 a	96.742 a	91.463 c	
time period effect	98.883 a	92.554 a	75.068 b	71.624 b	

\*Numbers with different letters(a,b,c) differ from each other statistically at a probability level of 5%, according to Duncan's test

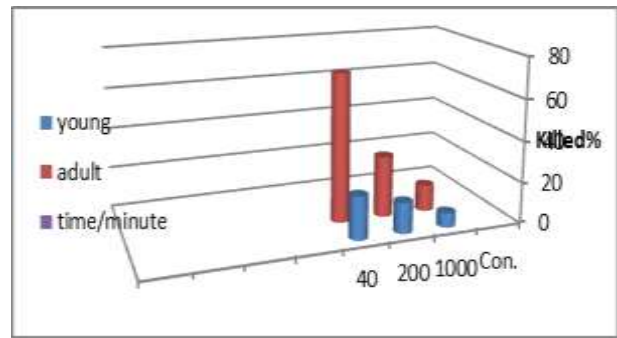
#### Effect of concentrations of the fungicide Hymazol on the survival of worms

This experiment was conducted on adult and young worms using the same concentrations used (40, 200, 1000 mg.kg<sup>-1</sup>) of pesticide Hemazole on worms in the experiments of soil treatments and from the observation of figure (1) it was found that the survival period of young worms differs from those of large worms when exposed to these concentrations, as the period required to kill 100% of small individuals, as it was different from those of individuals or adult worms. It was found that the period required to kill 100% of the young individuals treated with the pesticide concentrations Hemazole was 7,15 and 21, minutes, respectively. Where it took to kill 100% of adult worms and for the same

pesticide was 72, 30, and 13 minutes, respectively. From this it is clear that the time required to kill 100% of the adult worms took twice as long or more in all concentrations than the time required for the death of young individuals. While the time period for killing 100% of the adult worms when treated with the same concentrations of the pesticide Hemazole, but after 14 days of the pesticide's preparation for the first time to clarify the effect of the intensity of the pesticide's survival in the soil after exposure to environmental influences, the period required to kill the worms was 42, 30 and 19 minutes on straight. The results of the current study agreed with what (18) found that a mortality rate of (100%) in earthworms exposed to herbicides at concentrations. The results of the

current study converged with what (14) found when using flubendiamide, widely used against pests, which had a long-term stability in the soil, affecting non-target organisms, including the earthworm *Eudrilus Eugeniae*, as its toxic effect on these worms was observed during the period of time. (7 and 14) days at concentrations (332.27 and 238.31 mg.kg<sup>-1</sup>) and they killed worms by 50%. 1000 and 500 mg.kg<sup>-1</sup>.

**Effect of different concentrations of the fungicide Hymazol on body wall thickness of earthworms:** Table (3) shows the effect of different concentrations ( 40,200 and 1000 mg.kg<sup>-1</sup>) of the fungicide Hymazol on the body wall thickness of earthworms for a period of (28) days ,it was noticed from the (Table 3 ) that the thickness of the epidermis was significantly affected at the concentrations (40, 200 and1000 mg.kg<sup>-1</sup>) and there was a significant difference compared to the control group. Also, all concentrations had a significant effect and caused a decrease in the thickness of the body wall when compared with the control group, as it was the lowest recorded thickness (170) μm at the concentration (40 mg.kg<sup>-1</sup>), and the results also showed that all concentrations had a significant effect on the



**Figure 1. the effect of Hemazole on survival of young and adult worms**

thickness of the duct epithelium. peptic ulcer, although the lowest log thickness is (39.67) μm at concentration (40 mg.kg<sup>-1</sup>), than the above. It can be said that all concentrations were significantly affected by the decrease in the cross-sectional thickness of the insecticide-treated worms. The results of the study were also similar with (3) when treatment earthworm *A. caliginosa*, with neem’s leaves which a clear impact on the thickness of the epidermis and a decrease in the thickness of the epithelium of the duct epithelium. Somewhat similar to the results (17) which showed a decrease in the thickness of the body wall, subepidermal hemorrhage and the occurrence of ruptures in the longitudinal muscle layer with necrosis in other parts when treating earthworms *Aporrectodea caliginosa* with different concentrations of Zn

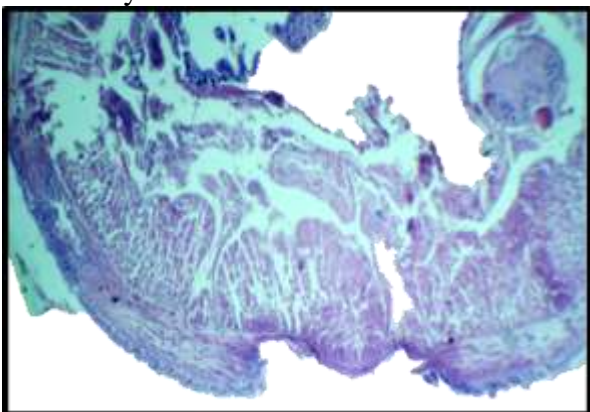
**Table 3. Effect of treating earthworms with different concentrations of Hemazole fungicide on body wall thickness**

Region Conc.	Epidermis thickness Mm	wall thickness μm	Gut channel epithelium thickness/μm
0	56.667 a	532.67 a	124.67 a
1000 mg.kg <sup>-1</sup>	25.5 B	229.5 c	130.33 a
200 mg.kg <sup>-1</sup>	25.5 b	385.33 b	93.5 b
40 mg.kg <sup>-1</sup>	28.333 b	170 c	39.67 c

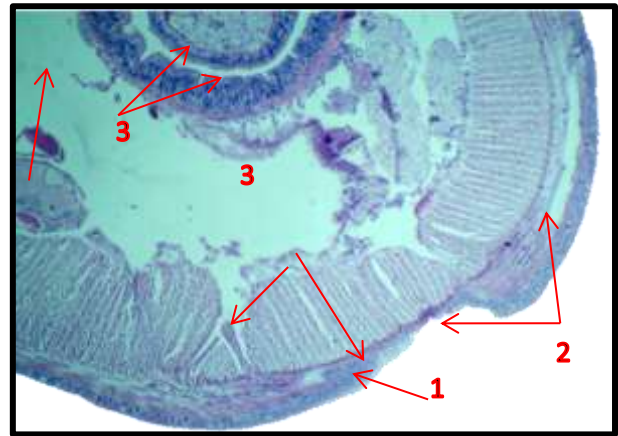
**Histological changes of earthworms, as a result of their treatment with the fungicide Hymazol:** The results of the current study of cross- sections under the microscope showed the presence of many histopathological changes in the bodies of earthworms treated with the fungicide Hymazol at concentrations (40, 200and 1000 mg.kg<sup>-1</sup>) for a period of 28

days, tissue changes occur. At concentration (1000 mg.kg<sup>-1</sup>) A clear necrosis of some parts of the epidermal layer was observed it extends to the circular muscle layer and a clear degeneration of the longitudinal muscle layer, as in Figure (2) as for treating worms with the same pesticide at the concentration (200 mg.kg<sup>-1</sup>) for the same period of 28 days

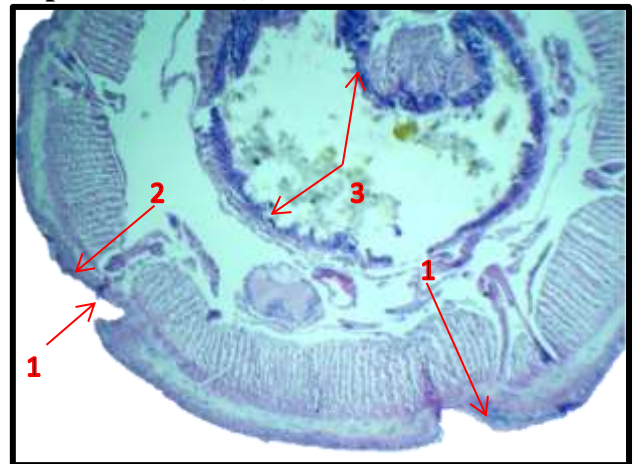
histological changes, such as clear necrosis of some parts of the epidermal layer, extending to the circular muscle layer were also observed and notice the flattening of the blind and epithelial cells as in Figure (3) . At the third concentration ( $40 \text{ mg.kg}^{-1}$ ) and when treated With the same pesticide for the same period of 28 days histological changes were observed in the transverse section. Also, clear necrosis of some parts of the epidermal layer extending to the circular muscle layer with degeneration and a decrease in the thickness of the epithelium of the alimentary canal as in Figure (4). The results of the current study are in agreement with what was found by (3,13) when they treated earthworms *E.fetidea* with the herbicide Butachlor, which caused a breakdown of the epithelium of the alimentary canal and changed its shape, as well as the destruction of bile cells It was also somewhat similar to the results (16,5) which showed a decrease in the thickness of the body wall, subepidermal hemorrhage and the occurrence of ruptures in the longitudinal muscle layer with necrosis in other parts when treating earthworms *A. caliginosa* with different concentrations of Zn. The results of the current study also matched somewhat (1) when treating earthworms with sewage water of hospitals in the governorates of Mosul and Dohuk, where necrosis was observed in the epithelium of the alimentary canal, blind and subepithelial canal, and its separation from the alimentary canal.



**Figure2.** A cross-section of the body of an adult earthworm treated with Hemazole at a concentration of  $1000 \text{ mg.kg}^{-1}$  for 28 days, a clear necrosis of some parts of the epidermal layer was observed (1) extending to the circular muscle layer (2) with a clear degeneration of the longitudinal muscle layer (3). Colored E. & H.. 40 X



**Figure 3.** A cross section of the body of an adult earthworm treated with Hemazole at a concentration of  $200 \text{ mg.kg}^{-1}$  for 28 days. A clear necrosis of some parts of the epidermal layer was observed (1) extending to the circular muscle layer (2), with a flattening of the cells of the blind and epithelial duct (3). Colored E. & H.. 40 X



**Figure 4.** A cross section of the body of an adult earthworm treated with Hemazole at a concentration of  $40 \text{ mg.kg}^{-1}$  for 28 days. A clear necrosis of some parts of the epidermal layer was observed (1) extending to the circular muscle layer (2) with degeneration and a decrease in the thickness of the epithelium of the alimentary canal (3). Colored E. & H.. 40 X

**Effect of concentrations of the fungicide Hymazol on the protein content of earthworms for a period of 28 days**  
Table (4) shows the effect of treating earthworms at concentrations ( $40, 200$  and  $1000 \text{ mg.kg}^{-1}$ ) of the fungicide Hemazole on the protein content of their bodies, which showed a significant difference in the decrease in the protein content of these worms, as the highest decrease was recorded at the concentration ( $40 \text{ mg.kg}^{-1}$ ), where the amount of protein was



equal to (0.084% mg.kg<sup>-1</sup>) compared to the control group, which recorded the amount of protein content equal to (0.1265 mg.kg<sup>-1</sup>). We conclude from the previous table that the protein content of worms treated with pesticides is lower than the protein content of the control group in which the protein concentration increased. This study is consistent with what was shown by the scientist (17) that when earthworms type *Aporrectodea caliginosa* were treated with Zn metal, it was observed that the protein content in the bodies of these worms decreased, the lowest protein content recorded at the concentration was 1500 mg.kg<sup>-1</sup>, which amounted to 0.045 mg g<sup>-1</sup>, The decrease in the protein content in the bodies of worms is due to an inverse relationship between the concentration of contamination and the protein content. The results of the current study contrasted with those of (20,21) indicated that when earthworms *L. mauritii* and *D. Will* were treated with phosphate pesticides, there was variation in the protein level, so the protein concentration was high when exposed to low and medium levels, and the protein level was lower. At higher concentration however, the protein level in earthworms in all treatments was higher than in the control group.

**Table 4. The effect of treating earthworms with concentrations of the fungicide**

Concentration	0	1000 mg.kg <sup>-1</sup>	200 mg.kg <sup>-1</sup>	40 mg.kg <sup>-1</sup>
Amount of protein mg.g <sup>-1</sup>	0.1265 a	0.101 b	0.0915 b	0.084 b

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