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TOLERANCE OF EARTHWORMS (*Eudrilus eugeniae*) TO 2,4-D DIMETHYL AMINE HERBICIDE

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ABSTRACT

The purpose of this study was to determine (1) the tolerance of earthworms (*Eudrilus eugeniae*) to the herbicide 2,4-D dimethyl amine, and (2) the response of increased biomass, behavior, and morphology of earthworms (*Eudrilus eugeniae*) when exposed to subletal doses of the herbicide 2,4-D dimethyl amine. This research is an experimental research conducted in two stages of research. The first stage to look at herbicide toxicity, includes LD50, upper threshold, and lower threshold. The second stage is a follow-up test study using a one-factor Complete Random Design (RAL) to determine the effect of subletal dose of LD50-48 hours on the increase in biomass, behavior, and morphology of earthworms (*Eudrilus eugeniae*). The object of the study is the earthworm (*Eudrilus eugeniae*) which already has a clitelum with a biomass of 25 grams for each treatment tank. Data analysis was carried out with Proit Analysis to determine the upper threshold, lower threshold, and LD50 value while the analysis of the difference in earthworm biomass from the treatment group was carried out using the One-Way ANOVA test at a significance level of 95%. The average treatment was further tested using the DMRT test in the SPSS version 24.0 program. The results showed that: (1) earthworm tolerance to the herbicide 2,4-D dimethyl amine, at the upper threshold value of 9996.014 ppm, the lower threshold of 3409.972 ppm, the LD50-48 hours of 9015.858 ppm which is practically non-toxic, (2) the response of earthworms (*Eudrilus eugeniae*) when exposed to subletal doses of the herbicide 2,4-D dimethyl amine herbicideIt is characterized by a reduced earthworm appetite, a shrinking body shape, and a decrease in biomass.

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1. INTRODUCTION

Weeds are plants that grow where they are not needed (Nasution, 1986). Most weeds are fast-growing plants that can produce large numbers of seeds in a short period of time. Weeds are highly undesirable on farmland because they reduce productive crop yields. Weed restrictions are technical and plastic. Technical because it is related to the plant production process. The presence of weeds in agriculture will be very detrimental to cultivated crops and interfere with the growth of cultivated plants. Therefore, it is necessary to control weeds on cultivated plants to reduce or inhibit weed growth. Some weed control methods include mechanical, physical, technical culture, and chemical precautions.

Chemical weed control is weed control by applying certain chemicals that are toxic/toxic and damage plant tissues/weeds. The chemicals used to control weeds are called herbicides. Herbicides are still considered the best solution for farmers to control weeds that interfere with plants.

Herbicides are compounds that are commonly used to eradicate weeds on plants. The advantages of herbicides are that they can control growing weeds, are able to control weeds from the start, and reduce root damage compared to mechanical weeding of the soil. In addition to its advantages, herbicides also have disadvantages, namely adverse effects on human health, resistant weed species, pollution, and residues that can poison non-target plants and animals (Wawan, 2013). The use of herbicides such as herbicide DMA 6 which contains the active ingredient 2,4-D dimethyl amine to eradicate weeds in the soil, is used for broadleaf weeds (*Sphenoclea zeylanica* and *Ludwigia octovalvis)* and grass groups (*Cyperus irria* and *Fimbristylis littoralis*) in rice plants. The active ingredient 2,4-D dimethyl amine is still considered one of the ultimate weapons against weed attacks on rice plants (Moenandir, 1990).

The use of herbicides by farmers can leave residues scattered in the surrounding environment such as surface water, groundwater, soil and plants. Herbicides can affect the lives of non-target organisms. Measuring the dose of herbicides, farmers generally only use a packing cap or tablespoon to estimate, rather than using the correct measuring tool. The methods used by farmers when applying herbicides have the opportunity to exceed the dosage and frequency of administration.

Weeds are controlled by spraying using 2,4-D dimethyl amine, the active ingredient in DMA6 that can be accumulated by non-target animals such as earthworms. It is important to understand the accumulation of 2,4-D-dimethyl amine by non-target animals. Some organisms can be used as indicators of environmental pollution. Among such organisms are earthworms. This is very detrimental if the impact of .4-D dimethyl amine can kill earthworms, considering the role of earthworms can help the decomposition process of organic matter.

Earthworms are animals that are usually the main component of animal biomass in the soil, and play an important role as decomposers and their sensitivity due to environmental impacts (Edwards & Bohlen, 1996; Hammond, 2000). Earthworms are one of the ecosystem engineers that can change habitat conditions by carrying out a series of activities, especially eating activities (Jones, Lawton and Shachak, 1994). The role of earthworms is very important in the process of decomposition of soil organic matter. Along with other soil microbes, especially bacteria, earthworms play a role in the biochemical cycle. Earthworms feed on leaf litter and other dead plant matter, thus decomposing and disintegrating (Schwert, et al., 1990; Scheu, 2003; van Groenigen, Lubbers, Vos, Brown, De Deyn dan van Groenigen, 2014). If the soil has been exposed to herbicides, the organic matter that has been exposed to herbicides is eaten into the body of the earthworm. Herbicides in soil can reduce the development and affect the morphology of earthworms (Palungkun, 2010).

Based on the description above, there are problems related to the influence of herbicides on non-target animals such as earthworms that need to be known. There is a need for research to determine the toxicity of the herbicide 2,4-D dimethyl amine in earthworms (*Eudrilus eugeniae*) and the response of earthworms (*Eudrilus eugeniae*) when exposed to sublethal doses of the herbicide 2,4-D dimethyl amine. High doses can have an effect on reducing the growth of earthworms .

2. RESEARCH METHOD (11 Pt)

2.1. Types of Research

This research is an experimental research. This research was conducted in two stages of research, the first stage to look at herbicide toxicity, including LD50, upper threshold and lower threshold. The second phase was a follow-up test study using a one-factor Complete Random Design (RAL) to determine the effect of the LD50-48 h subletal dose on the growth of earthworms (*Eudrilus eugeniae*). In the second phase of the test, 5 subletal doses of LD50-48 hours were performed.

2.2. Research Time and Place

This research was conducted at the Biology Garden, Faculty of Mathematics and Natural Sciences, Yogyakarta State University from February to May 2022.

2.3. Research Object

The object in this study is an earthworm (*Eudrilus eugeniae*) which already has a clitelum with a biomass of 25 grams for each treatment tank.

2.4. Research Procedure

1. **Test Animal Preparation**

   In this study, earthworms derived from species *Eudrilus eugeniae.* Earthworm culture was obtained from a earthworm cultivation group in Godean, Sleman. The earthworm used is an adult earthworm (the clitum has formed).

2. **Media Preparation**

   Palm oil is obtained from palm oil sellers in Bendo, Tulung, Klaten. The container used for the experiment was in the form of a plastic tub measuring 17x11x4.5 cm which was used for the preliminary test and a size of 40x31x13 cm which was used for the follow-up test.

3. **Preliminary Test**

   This test was carried out with the aim of determining the dosage range of the herbicide that kills the earthworm *Eudrilus eugeniae*. in the range of 0% < < 100% mortality will be used for subsequent tests. This test used a plastic tub, each plastic tub measuring 17x11x4.5 cm containing 250 grams of palm oil media and then filled with 10 earthworms and adapted for 24 hours before being treated. The doses in this treatment are 0 ppm, 1650 ppm, 3300 ppm, 4950 ppm, 6600 ppm, 8250 ppm, and 9900 ppm. For each dose to be tested, the herbicide is dissolved in a liter of water. To determine the dose, dilution is carried out with the following formula:

   $$M1 \times V1 = M2 \times V2$$

   After that, the herbicide is sprayed onto each medium containing earthworms according to the predetermined dose. Observations were made every 24 hours for 48 hours to see the mortality percentage of the *Eudrilus eugeniae* earthworm. The data obtained was then analyzed by probit analysis

4. **Advanced Test**

   Before being treated with various doses of herbicides, earthworms are first weighed at 25 grams to determine their initial weight before being treated and then put into a plastic tub measuring 40x31x13 cm containing 1 kg of palm oil and adapted for 24 hours before being treated.

   Based on the preliminary test, an LDvalue of 50-48 hours from the herbicide used was 9015.858 ppm with a range of 3409.972 ppm to 9996.014 ppm. Based on this value, five doses of herbicide were determined, with concentration 0 ppm, 825 ppm, 1650 ppm, 2475 ppm, and 3300 ppm. After that, it is sprayed onto earthworms according to the predetermined dose. Then the tub is covered with newspaper and then sprayed with water to maintain moisture and prevent the entry of animals or fleas. Feeding in the form of tofu pulp is done every two days. Observation of worm biomass was carried out every two weeks for 1.5 months and observation of feeding behavior was carried out daily for 1.5 months.

2.5. Data Collection Techniques

Observation of the percentage of earthworm mortality is carried out once every two weeks by sorting and considered dead if the worms do not respond to stimuli and then calculated using a counter. Monitoring of the behavior and morphology of earthworms is carried out every two days by directly observing the response of earthworms. Observation of the increase in earthworm biomass is carried out every two weeks by means of earthworms being weighed using analytical scales.

2.6. Data Analysis Techniques

The data in the study will be processed and presented in the form of tables and graphs. The data from the toxicity test was analyzed with Probit Analysis to determine the values of the upper threshold, lower threshold, and LD50 while the analysis of the difference in earthworm biomass from

the treatment group was carried out using the One-Way ANOVA test at a significance level of 95%. The average treatment was further tested using the DMRT test in the SPSS version 24.0 program

3. RESULTS AND ANALYSIS

3.1. Result

Upper Threshold, Lower Threshold, LD50 Obtained from Earthworm (*Eudrilus eugeniae*) Tolerance to 2,4-D Dimethyl Amine Herbicide

In this study, a preliminary test was conducted to obtain an upper threshold, lower threshold, and LD50-48 hours using 7 treatment doses, namely 0 ppm, 1625 ppm, 3300 ppm, 4950 ppm, 6600 ppm, 8250 ppm, and 9900 ppm. The mortality percentage of earthworms (*Eudrilus eugeniae*) can be seen in Table 1 as follows:

Table 1. Data on the Percentage of Mortality of Earthworms (*Eudrilus eugeniae*) in the Preliminary Test of Herbicide *2,4-D Dimethyl Amine* for 48 Hours

Dosage (ppm)	Earthworm Mortality (%)	
	24 hours	48 hours
0	0	0
1650	0	10
3300	0	10
4950	10	20
6600	10	20
8250	10	40
9900	60	80

Based on the data in Table 1, it can be seen that the higher the dose of treatment given, the higher the mortality rate in earthworms. And vice versa, the lower the dose of treatment given, the smaller the percentage of earthworm deaths.

The upper threshold value, the lower threshold value, and the LD50-48 hr value of the herbicide 2,4-D dimethyl amine against earthworms were probit analyzed using SPSS software, the results of the analysis can be seen in Table 2 as follows:

Table 2. LD50-48 h value of Herbicide Toxicity of 2,4-D Dimethyl Amine Herbicide to Earthworm (*Eudrilus eugeniae*)

Probit	95% Confidence Interval for Concentration			95% Confidence Interval for Logs (Concentration)		
	Estimation	Lower Threshold	Upper Threshold	Estimation	Lower Threshold	Upper Threshold
.500	9015.858	3409.972	9996.014	3.955	3.533	4.000

Based on the results of probite analysis in Table 3, it is known that the upper threshold value of 2,4-D dimethyl amine herbicide in earthworms is 9996.014 ppm, while the lower threshold value of 2,4-D dimethyl amine herbicide in earthworms is 3409.972 ppm. The upper threshold value (LD100-24 hours) is the level of a toxic substance that causes 100% mortality in a number of test biota (animals/plants/microorganisms), while the lower threshold value (LD0-48 hours) is the level of a toxic substance that causes 0% mortality (100% life) in a number of test biota (animals/plants/microorganisms) (Sukiya & Putri, 2014).

The LD50-48 hr value of herbicide *2,4-D dimethyl amine* in earthworms was 9015.858 ppm. LD50 shows the number of toxins per unit weight of the organism that can kill 50% of the population of the animal types that are tested (Tarumingkeng, 1992). The data showed

subletal dose data of LD50-48 hours which would later be tested in follow-up tests by taking doses below the lower threshold value of 3409.972 ppm.

The effect test of the subletal dose of LD50-48 h herbicide *2,4-D dimethyl amine* on earthworms was carried out with 5 treatment doses with 5 replicates each. The doses used to test the effect of subletal dose LD50-48 hours of herbicide *2,4-D dimethyl amine* on earthworms were 0 ppm, 825 ppm, 1650 ppm, 2475 ppm, and 3300 ppm.

Effect of Subletal Dose of Herbicide *2,4-D Dimethyl Amine* on the Behavior and Morphology of Earthworms (*Eudrilus eugeniae*)

The results the observation of the effect of subletal dose of the herbicide *2,4-D dimethyl amine* on the behavior and morphology of earthworms (*Eudrilus eugeniae*) in Table 3.

Table 3. Effect of Herbicide *2,4-D Dimethyl Amine* on the Behavior and Morphology of Earthworms (*Eudrilus eugeniae*)

Dosage (ppm)	Things observed		
	Appetite	Behaviour	Morfologi
0 ppm	Earthworms feed normally by finishing their feed within 1 day.	Earthworms move normally.	The body of earthworms is purplish-red.
825 ppm and 1650 ppm	Earthworms have no appetite in the first week, earthworms begin to eat on the 4th day after treatment and for the following days the feed is exhausted within 1 day.	Earthworms move slightly irregularly to the touch and recover by the second week.	The earthworm's body is slightly pale in color in the first week and returns to normal in the second week.
2475 ppm and 3300 ppm	Earthworms have no appetite in the first week, earthworms begin to eat on the 6th day after treatment and for the following days the feed is exhausted within 1-2 days.	Earthworms move very, very irregularly when touched in the first to third week and return to normal in the fourth week.	The earthworm's body is very pale in color in the first week. In the sixth week, it was seen that earthworms had small and short bodies.

Based on Table 3, there is an effect of the herbicide *2,4-D dimethyl amine* in earthworms (*Eudrilus eugeniae*) on the behavior, morphology, and appetite of earthworms (*Eudrilus eugeniae*). The higher the dose given, the lower the appetite of earthworms (*Eudrilus eugeniae*). Feeding is carried out every morning. Shortly after being treated with *2,4-D herbicide dimethyl amine* and fed tofu pulp which has reduced its moisture content to approximately 75%, earthworms do not eat immediately and are only exhausted after 4 days for treatment of 825 ppm and 1650 ppm. In the control treatment, the appetite of earthworms (*Eudrilus eugeniae*) was normal and depleted feed within one day. Earthworms treated with *2,4-D dimethyl amine* herbicide, the fastest feeding speed recovered was at 825 ppm and 1650 ppm treatment. Within one week, earthworms in these two treatments have eaten once a day marked as fed out. At 2475 ppm and 3300 ppm treatments, feeding behavior only recovered after 6 days and feed was only exhausted one to two days. This happens because earthworms are still in the adjustment period after being treated with *a 2,4-D dimethyl amine* herbicide.

The behavior and morphology of earthworms after being given the herbicide *2,4-D dimethyl amine* , namely the movement of earthworms was slightly irregular and the body was slightly pale in the first to second week for treatment at 825 ppm and 1650 ppm and only recovered in the second week. As for the treatment of 2475 ppm and 3300 ppm, the movement of earthworms is very irregular and its body is very pale in the first to third week and will recover in the fourth week.

Effect of Subletal Herbicide Dose *of 2,4-D Dimethyl Amine* on Biomass Increase of Earthworm (*Eudrilus eugeniae*)

Earthworm biomass is observed every 2 weeks in 1.5 months. From this data, it will be possible to determine the average increase in earthworm biomass and the speed of increase in earthworm biomass per dose. In Figure 1, there is an average value of biomass increase of earthworms *E. eugeniae* presented in the form of a graph.

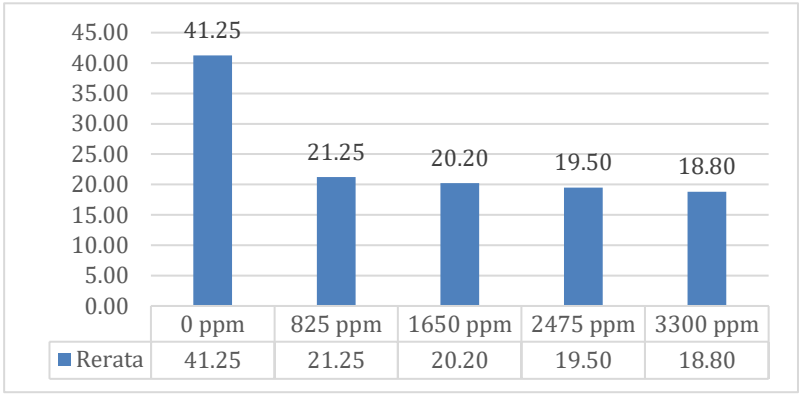
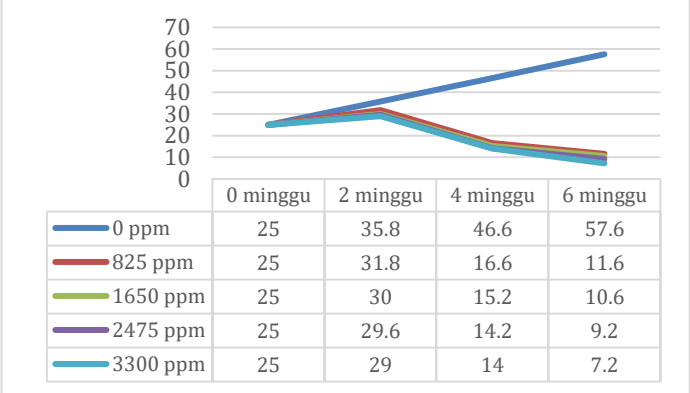


Figure 1. Graph of the Average Biomass Increase of Earthworm (*Eudrilus eugeniae*) After Receiving Herbicide Treatment *with 2,4-D Dimethyl Amine Herbicide*

Based on the graph above, it is known that the highest average increase in earthworm biomass is found in the control treatment. Meanwhile, the herbicide treatment with a dose of 3300 ppm has an average biomass that tends to be lower among others. On the graph, it can be seen that the higher the dose of treatment given, the lower the earthworm biomass.

In Figure 3, there is a value of the change pattern of earthworm biomass (*Eudrilus eugeniae*) presented in the form of a graph.



	0 minggu	2 minggu	4 minggu	6 minggu
0 ppm	25	35.8	46.6	57.6
825 ppm	25	31.8	16.6	11.6
1650 ppm	25	30	15.2	10.6
2475 ppm	25	29.6	14.2	9.2
3300 ppm	25	29	14	7.2

Figure 2. Graph of Biomass Increase Pattern of Earthworm (*Eudrilus eugeniae*) After Receiving Herbicide Treatment *of 2,4-D Dimethyl Amine*

Based on Figure 2, it is known that the fastest pattern of earthworm biomass increase occurs in earthworms that are given control treatment in week 6 of observation. The graph shows the average increase in earthworm biomass in each different treatment every week. In the control treatment, the average increase in biomass was highest in week 6, while for the treatment of 825 ppm, 1650 ppm, 2475 ppm, and 3300 ppm, the average increase in biomass was highest in week 2.

The effect of the herbicide *2,4-D dimethyl amine* on the biomass of earthworms (*E. eugeniae*) was carried out by *the One Way Anova* analysis test which can be seen in Table 4 as follows:

Table 4. Results of *the One Way Anova* Analysis Test on the Effect of Subletal Herbicide Dose *of 2,4-D Dimethyl Amine* on the Increase in Biomass of Earthworms (*Eudrilus eugeniae*)

	Sum of Squares	Df	Mean Square	F	Sig.
Herbisida	1466.620	4	366.655	3.369	.037
Trial Error	1632.580	15	108.839		
Total	3099.200	19			

Remarks: Significance at P < 0.05

The test results found a significance value of 0.037. This shows that there is an effect of treatment on earthworm biomass. This is because the sig value < 0.05. Because of the significant results, a follow-up test was carried out by Duncan at a level of 5% to compare the average difference between treatments. The results of the Duncan test are as follows:

Table 5. Duncan Advanced Test (DMRT) with a Level of 5% on the Effect of Subletal Herbicide *Dose 2,4-D Dimethyl Amine* on Earthworm (*Eudrilus eugeniae*) Biomass

Dose	N	Alpha value = 0.05	
		1	2
3300.00	4	18.8000	
2475.00	4	19.5000	
1650.00	4	20.2000	
825.00	4	21.2500	
.00	4		41.2500
Itself.		.764	1.000

The results of the Duncan test showed that the control dose treatment was different for the doses of 825 ppm, 1650 ppm, 2475 ppm, and 3300 ppm.

Media Climatic Data during the Study of Earthworm Tolerance (*Eudrilus eugeniae*) to Herbicide *2,4-D Dimethyl Amine*

The results of climate data measurement of the effect of the herbicide *2,4-D dimethyl amine* on earthworms (*Eudrilus eugeniae*) in this study can be seen in Table 6 as follows:

Table 6. Media Climatic Data during the Study of Earthworm Tolerance (*Eudrilus eugeniae*) to Herbicide *2,4-D Dimethyl Amine*

Dosage (ppm)	Suhu (^{oC})	pH	Humidity (%)
0	25,30	6,87	19.70
825	26,70	6,72	18,90
1650	26,95	6,675	18,65
2475	27,25	6,645	18,25
3300	27,70	6,6	17,75

Climate data on the media was calculated every two weeks for 1.5 months. The measured data are temperature, pH, and humidity. The average temperature obtained from the lowest measurement results is 25.30oC, while the highest is 27.70oC. Then the lowest average pH is 6.6 and the highest is 6.87. Then the lowest average humidity is 17.75% and the highest is 19.70%.

The average temperature, pH, and humidity that have been measured according to the criteria necessary for earthworm life. Earthworms live in an ideal temperature range between 24-30oC and humidity of about 15-50% (Rukmana, 2008: 28) and according to Sugiantoro (2012: 61), the ideal pH is around 6-7.2.

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3.2. Discussion

Upper Threshold, Lower Threshold, LD50 Obtained from Earthworm (*Eudrilus eugeniae) Tolerance to* 2,4-D Dimethyl Amine *Herbicide*

The application of the herbicide type 2,4-D with the active ingredient *2,4-D dimethyl amine* 825 g/l has an effect on earthworms *E. eugeniae* can be seen in Table 1. After 48 hours of application, *E. eugeniae* earthworms died at doses of 1650 ppm, 3300 ppm, 4950 ppm, 6600 ppm, 8250 ppm and 9900 ppm, while there were no deaths in control.

From Table 1, it can be seen that in the herbicide *treatment of 2,4-D dimethyl amine*, the highest mortality percentage of earthworms *E. eugeniae* was found in the treatment with a dose of 9900 ppm, which was 80%. In this study, the higher the dose of herbicide given to earthworms *E. eugeniae* , the higher the mortality rate.

The results of probit analysis showed that the upper threshold value *of 2,4-D dimethyl amine* herbicide was 9996.014 ppm, the lower threshold value was 3409.972 ppm, and the LD50 value was 9015.858 ppm. The high toxic levels of *2,4-D dimethyl amine* against earthworms are due to the nature of the active compound *2,4-D dimethyl amine* which can cause narrowing and swelling in the clitellar region thereby interfering with the enzymatic processes and life of earthworms (Correira & Moreira, 2010).

Effect of Subletal Dose of Herbicide *2,4-D Dimethyl Amine* on the Behavior and Morphology of Earthworms (*Eudrilus eugeniae*)

The herbicide active ingredient *2,4-D dimethyl amine* is a chemical intended to control plant pests. But it can also cause effects on soil organisms because this substance has systemic toxic properties. This property causes residues that can enter through the body surface of soil organisms such as earthworms and enter their digestive system and nervous system. Earthworms exposed to the active ingredient *2,4-D dimethyl amine* undergo several changes, namely body color, body shape, behavior, and appetite speed. Earthworms that receive treatment for herbicide exposure have a pale color. The higher the dose given, the paler the earthworm's body color. The body color of earthworms *E. eugeniae* in the control treatment tended to be purplish-red. This suggests that the herbicide *2,4-D dimethyl amine* may affect the pigmentation of earthworms' body color.

The shape of earthworms exposed to the herbicide *2,4-D dimethyl amine* is seen shrinking and shortening and its body looks cut off. Dead earthworms have similar characteristics, namely the body is cut and dried. This is likely due to cell lysis due to herbicide exposure. This lysis causes the earthworm's body to be torn and cut into pieces.

The movement behavior of earthworms exposed to the herbicide *2,4-D dimethyl amine* at the beginning of the herbicide treatment was that it moved uncontrollably and then over time became more passive. However, when touched, his movements become aggressive. The eating behavior of earthworms exposed to the herbicide *2,4-D dimethyl amine* is different compared to earthworms that are not exposed to herbicides. Shortly after being treated with *2,4-D dimethyl amine* herbicide and fed tofu pulp, earthworms did not eat immediately and were only exhausted after 4 days for treatment of 825 ppm and 1650 ppm.

In the control treatment, appetite is normal and feed is depleted within one day. Earthworms treated with *2,4-D dimethyl amine* herbicide, the fastest feeding speed was at 825 ppm and 1650 ppm which was marked after 4 days of regular feeding earthworms once a day. While the treatment was 2475 ppm and 9025 ppm, earthworms only ate after 6 days and the feed ran out in 1-2 days.

The possibility of herbicide accumulation causes *the organs of E. eugeniae* to experience disturbances so as to reduce appetite which results in a decrease in the rate of feed consumption and the use of energy from food is more widely used to defend itself from environmental pressure and replace parts of the body cells damaged by foreign substances so

that the excess energy from the use of this process is very little used to increase body weight (Heath, 1987). This suggests that growth can be said to be a sensitive parameter in determining the effect of the herbicide *2,4-D dimethyl amine* on *E. eugeniae*.

Pollutants (*2,4-D dimethyl amine*) can directly or indirectly affect eating behavior, way of eating, absorption, digestion, assimilation, excretion and changes in hormonal levels that ultimately affect growth. The power of fluctuations and availability of food, soil conditions and earthworm conditions affect the amount of energy consumed by an earthworm, so that the energy consumed can be greater or smaller than the energy it uses. This results in an increase or decrease in growth energy (Tang and Affandi 2002). Normally, according to Waren (1971), about 70% of the energy value from food is prioritized and used for the maintenance of body tissues, but if earthworms are sick or experience environmental disturbances, it will affect earthworms to use energy to maintain their lives more than usual.

Effect of Subletal Herbicide Dose *of 2,4-D Dimethyl Amine* on Biomass Increase of Earthworm (*Eudrilus eugeniae*)

The results showed that the administration of *2,4-D dimethyl amine* herbicide treatment on earthworms *E. eugeniae* with different dose levels had an effect on the increase in body biomass. Earthworm biomass treated *with 2,4-D dimethyl amine* herbicide showed a decrease as seen in Figure 1.

Figure 1 shows that the higher the dose of treatment given, the lower the increase in earthworm biomass. In the graph, it can be seen that earthworms with the highest dose treatment of 3300 ppm have a low average biomass increase, this is likely due to the high dose given so that appetite decreases and some body tissues do not function properly. At 825 ppm and 1650 ppm treatment, earthworms took about three days to recover appetite, while at 2475 ppm and 3300 ppm, earthworms took about five days to recover appetite. The higher the dose of treatment given, the higher the rate of tissue damage to the body, resulting in the recovery of the earthworm's appetite for longer.

Based on Figure 2, earthworms in all treatments experienced an increase in biomass. Earthworms in each treatment experienced a different biomass rate of increase. Earthworms in the control treatment had an increase in biomass in week 6, while in the dose treatment of 825 ppm, 1650 ppm, 2475 ppm, and 3300 ppm had an increase in biomass rate in week 2. This is likely because the higher the dose given, it will cause the body tissues to not function properly. When earthworms have passed the recovery period, their appetite will return to normal and their eating speed is high due to the recovery of body tissues damaged by herbicide exposure. After the body tissue improves, earthworms will eat feed as necessary because the body condition has recovered.

The decrease in the body biomass of *E. eugeniae* earthworms can be clearly seen in the observation of the 4th MST at the treatment doses of 825 ppm, 1650 ppm, 2475 ppm, and 3300 ppm. The weight loss is suspected to occur due to disruption of physiological processes and body metabolism due to treatment. The effect of *the dose of 2,4-D dimethyl amine* is an environmental pressure for *the earthworm E. eugeniae* so that the animal will reduce its growth.

Climatic Data Media

Climatic data on the media was calculated every two weeks for 6 weeks. The temperature and pH measured during the study increased. The average temperature obtained from the lowest measurement results is 25.30ºC while the highest is 27.70ºC · The temperature that has been measured is in accordance with the criteria that earthworms need to thrive. In accordance with the statement of Rukmana (2008: 28) earthworms live in the ideal temperature range between 24-30oC. Too high a temperature causes earthworms to be unable to survive.

The average pH in this study was the lowest was 6.6 and the highest was 6.87. The results of this pH measurement are in line with the statement of Sugiantoro (2012: 61), the ideal pH is around 6-7.2. The condition of the media that is too acidic causes the earthworm cache to break due to protein poisoning and the earthworm skin also suffers serious injuries.

The average humidity in this study was the lowest was 17.75% and the highest was 19.70%. The results of this humidity measurement are in line with the statement of Rukmana (2008: 28) earthworms live in the humidity range of around 15-50%. Soil moisture that is too high or wet can cause earthworms to turn pale and then die. On the other hand, if the soil moisture is too dry, the earthworm will immediately enter the soil and stop feeding and eventually die.

CONCLUSION (11 Pt)

Based on the results of the study entitled "Tolerance of Earthworms (*Eudrilus eugeniae*) to Herbicide *2,4-D Dimethyl Amine*" it can be concluded that:

1. Earthworm tolerance to the herbicide *2,4-D Dimethyl Amina*, at an upper threshold value of 9996,014, ppm, lower threshold of 3409,972 ppm, LD50-48 hours of 9015,858 ppm which is practically non-toxic.
2. The response of earthworms (*Eudrilus eugeniae*) when exposed to subletal doses of the herbicide *2,4-D dimethyl amine* is characterized by reduced earthworm appetite, shrinking body shape and decreased biomass increase.

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