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The Effect of Synthetic Pesticides and Botanical Pesticides From Paitan Leaves (*Thitonia diversifolia*) on Death, Behavior of Earthworms, Hatchability of Coccon and Histology of Earthworms

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ABSTRACT
The use of synthetic pesticides in pest control is increasing from year to year. The effects of using synthetic pesticides can affect non-target organisms that live in the soil, such as earthworms. Therefore, it is necessary to develop botanical pesticides based on paitan leaf extract (*Thitonia diversifolia*) which are environmentally friendly. The method of research used is laboratory experimental research with a *Completely Randomized Design* (CRD) research design. The aim of this research was to analyze the effect of exposure to synthetic pesticides and botanical pesticides from paitan leaf extract on the growth, behavior of earthworms, cocoon hatchability and histology. Making paitan leaf extract using the maceration method with ethanol solvent. The pesticide concentration in the study consisted of negative control (K-), synthetic pesticide (K+), and 5% paitan leaf extract. Based on the research results, it shows that earthworms exposed to synthetic pesticides died more (79 %) compared to treatment with paitan leaf extract (39 %), and K(-) 15%. The most cocoons that successfully hatched were found in the treatment K(-) Based on research results, botanical pesticides, paitan leaves are safe for the life of earthworms.

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1. INTRODUCTION

Indonesia is an agricultural country with the majority of its population being farmers (Maulana *et al*., 2020). This causes farmers to rely heavily on the presence of pesticides. The use of synthetic pesticides has increased from year to year (Ministry of Agriculture, Directorate General of Food Crops, 2020) because they play an important role in controlling pests. The application of pesticides is still carried out by spraying and spreading, allowing most of the synthetic pesticides to fall to the surface of the soil and possibly affecting the organisms in it.

Synthetic pesticides are also commonly known to be unable to be broken down by natural processes, which means they remain in the environment for long periods of time. This poses a significant risk to the environment and non-target organisms. One of the efforts to maintain environmental health and soil quality is to use alternatives, namely by switching to using botanical pesticides. In this research, paitan leaves will be used considering that this type of leaf is easy to obtain and is rarely used by the public. The paitan leaf plant (*Tithonia diversifolia*) contains active ingredients in the form of alkaloids, flavonoids, saponins, tannins, terpenoids and phenolics (Jayati & Nopiyanti, 2020).

With the development of a plant-based pesticide made from paitan leaf extract (*Thitonia diversifolia*), it is hoped that farmers will be able to use plant-based pesticides to control pests that are safe, environmentally friendly and non-toxic to non-target organisms (Djukri *et al*., 2018). This research used experimental animals in the form of earthworms. The role of earthworms in the ecosystem is very important for agricultural sustainability. It is necessary to address the use of both types of pesticides and their effects on behavioral responses, hatchability and histology of earthworm cocoons. Regarding synthetic pesticides, it has an impact on the extinction of certain species, this is proven by research (Jeyaprakasam *et al*., 2021) which causes changes in the behavior of earthworms in the ecosystem. The research carried out showed that the pesticide toxicity test

with the malathin type caused morphological abnormalities and a decrease in population and shrinkage and swelling of the clitellum. Research regarding the use of botanical pesticides made from paitan leaf extract (*Tithonia diversifolia*) against non-target organisms, especially earthworms, has not yet been carried out. For this reason, this research aims to analyze the differences in the effects between exposure to synthetic pesticides and botanical pesticides from paitan leaf extract (*T. diversifolia*) on the growth and behavior of earthworms, hatchability of cocoons and histology of earthworms.

2. RESEARCH METHOD

The type of research used is laboratory experimental research with a *Completely Randomized Design* (CRD) research design. The toxicity test method used is based on the OECD (1984) guidelines for testing chemicals. The aim is to identify the toxic potential of chemicals against earthworms using petri dishes and filter paper (Jeyaprakasam *et al*., 2021). The stages in this research were divided into 5, including: the simplicia making stage, the extract making stage, the earthworm media preparation stage, the earthworm acclimatization stage, the treatment stage in the form of preparing botanical and synthetic pesticide solutions and applying pesticides to earthworms and cocoons, and the stage of making earthworm intestinal preparations. The steps that will be taken in this research are as follows:

a) Making Simplicia

The paitan leaves used in this research were obtained around Karangploso land, Malang Regency. The leaves selected are old leaves and dark green in color. The paitan leaves obtained were then cleaned of adhering dirt and air-dried at room temperature for 2 days. After that, dry the leaves in the oven at 50°C for 2 days. The dried leaves are then ground with a blender until they become powder (Hartini *et al*., 2022)

b) Making Extracts

Extract preparation, the maceration method used to obtain paitan leaf extract, was carried out 2 times with a ratio of 1: 10 (Rahayu *et al.,* 2020), 100 g of leaf powder was weighed, put into an Erlenmeyer glass and 500 ml of 96% ethanol was added. The next stage is stirring and shaking at a speed of 100 rpm for 24 hours. The maceration results were filtered and the residue was added with 500 ml of 96% ethanol then shaken at a speed of 100 rpm for 24 hours. The results of the maceration were filtered using Whatman filter paper and then mixed with the results of the first filter. The filtered results that have been mixed are then evaporated using a rotary evaporator at a temperature of 50 °C to separate the solvent to obtain a thick extract. The paitan leaf extract obtained was stored in a refrigerator at 4°C. The extraction method used in this research is maceration, because this method does not use heat in the process so it will not damage the flavonoid compounds contained in the leaves and the use of ethanol with a concentration of 96% is because the higher the ethanol concentration used, it is assumed that the bioactive components contained in the 96% ethanol extract are more than the 50% and 70% ethanol extracts (Riwanti *et al.,* 2020).

c) Preparation of earthworm test media

Preparation of earthworm media. The work procedure carried out in this process is to prepare the test medium in the form of filter paper which is shaped round according to the size of the cup with a diameter of 90 mm. Then the filter paper is put into a petri dish. Each replication contained 3 earthworms and each cup would be filled with 1 worm. The total number of earthworms used in this research was 100 earthworms and 90 earthworm cocoons. The type of sampling uses simple random sampling because the samples observed are taken randomly from the research site without paying attention to the levels in the population (Apriliani *et al*., 2019).

d) Preparation of Earthworm Cocoon Test Media

Prepare the earthworm rearing containers used in this research, namely 180 rectangular plastic boxes measuring 16.5 cm x 11 cm x 7.5 cm. The next stage is the media in the form of soil mixed with organic material, the ratio of soil to organic material is 1:1, placed in a plastic box each containing a height of 5-7 cm and weighing approximately 500 grams, then moistened with water to maintain the humidity of the media. Cultivate these worms because earthworms prefer moist places.

e) Preparation of solutions of 2 types of pesticides

The pesticides used include a 3% botanical pesticide solution, obtained by making a 3% suspension obtained from a paste-shaped extract of 5 ml of paitan leaves added with distilled water until it reaches a weight of 100 ml and stirred until smooth. Then fill or spray the filter paper with 1 ml of pesticide. Earthworms were put into the test container, 1 each. These earthworms are kept in a place that is not exposed to direct sunlight, dark and must be kept in humid conditions (Mufti, 2019)

f) **f) Toxicity Test Application**

Carried out following the methods described in the OECD (1984) guideline for testing chemicals no. 207. This is a simple screening test to identify the toxic potential of chemicals to earthworms. The petri dish used was 90 mm in diameter, the filter paper was shaped round according to the size, synthetic and botanical pesticide solutions and 1 ml of ordinary tap water were used. The application starts with washing the earthworms with deionized water or distilled water until the dirt was clean, then the earthworms are drained and

placed on filter paper for 1 hour to remove their stomach contents. After that, they are rinsed again with deionized water (Aquadest), drained, and prepared a solution. The pesticide was then dripped or sprayed into filter paper that has been prepared previously with 1 ml given to each cup, put the drained earthworms into the cup that has been given to each treatment and the test was carried out in the dark at a temperature of 28 ± 2^0 C for 48 hours. After 48 hours, earthworms were monitored for death by gentle mechanical stimulation of the frontal region.

Data analysis

Data on the percentage of earthworm death and cocoon hatchability using Microsoft Excel 2016 and descriptive analysis.

3. RESULT AND DISCUSSION

3.1 Results of 24 Hour and 48 Hour Contact Toxicity Tests on Earthworm Mortality

This research used 2 different types of pesticides, namely synthetic pesticides containing the active ingredients lambda chyhalotrin and Thiametoxam and botanical pesticides made from paitan leaf extract (*T. diversifolia*) and a negative control treatment which was only given water. Observation results show that the highest mortality rate for earthworms occurs in the K+ (synthetic pesticide) treatment, with a death percentage of 79% shown in table 1 and figure 1.

Table 1.Death or Mortality Percentage Values based on toxicity tests for 3 treatments

Treatment	Time of Observation (hours)	Observation Initial	Number of Dead	Number of Lives	Percentage (%) of deaths
P1	24	33	6	27	18
	48	33	13	20	39
K+	24	33	19	14	58
	48	33	26	7	79
K-	24	33	3	30	9
	48	33	5	28	15

The synthetic pesticides in the research used pesticides with the active ingredients lambda cyhalothrin and thiamethoxam. The active ingredient Lambda-cyhalothrin is a pyrethroid insecticide which works on the insect nervous system and regulates nerve function by interfering with sodium channels, while the active ingredient thiamethoxam is classified as a second generation neonicotinoid insecticide which is widely applied throughout the world to control various insects. This active ingredient contains Se nanoparticles which have significant residual effects over a period of 14 days in the environment (Kang *et al*., 2021).

Pesticide residues in the environment have toxic effects on ecosystems and living creatures. Recent research by Foucault *et al.* (2021) stated that exposure to low doses of pesticides on human bone marrow mesenchymal stem cells/stromal cells caused a decrease in aldehyde dehydrogenase-2 (ALDH2) activity, inhibition of cell proliferation, DNA damage to senescence. Exposure to pesticides containing organophosphates can have detrimental toxic effects on health including histopathological damage, neurotoxicity, hepatotoxic disorders, oxidative stress, apoptosis, disruption of signaling, decreased cell proliferation, and senescence (Adaninggar *et al*., 2024). The activity of synthetic pesticides also acts as a potential neurotoxicant in non-target species because it inhibits an important enzyme, namely acetylcholinesterase (AChE) in the nervous system of insects and other animal species. Pesticides will also affect and change the eco-physiology of earthworms (Singh & Bisht, 2019).

Exposure to plant-based pesticides made from paitan leaf extract caused mortality of 39% or 61% of earthworms were still able to survive exposure to the plant-based pesticides developed. In general, paitan leaves contain compounds including alkaloids, tannins, flavonoids, terpenoids and saponins and show activity as antibacterial, antiprotozoal, and have been traditionally tested as a botanical pesticide to repelent pests, grasshoppers and fleas with quite effective results (Baideng *et al.* , 2020). The lowest percentage of earthworm deaths occurred in the kontrol (-) treatment shown in figure 2.

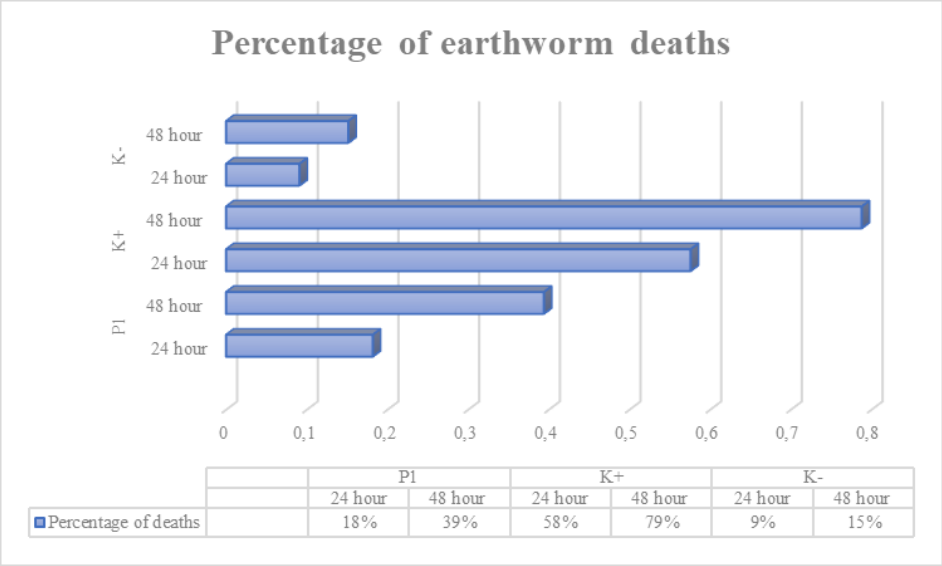


Figure 1. Percentage of Earthworm Deaths

3.2 Toxicity Test Results on Earthworm Behavioral Responses

Exposure to pesticides or treatments carried out in research shows that each treatment produces different responses (Table 2) as follows:

Table 2. the effect of pesticide treatment on the behavioral response and morphological condition of earthworms

Treatment	Behavioral Response	Morphological Condition
K-	Active response and movement around the cup or medium	Normal and no swelling in the clithelium
K+	Slow response, moving in circles and dodging	The clithelium swells and coelom fluid comes out of the body
P1	Respond slowly and move around the cup	Part of the worm's body has swelling in the posterior part

Synthetic Pesticide Contact Toxicity Test

Contact toxicity of the pesticides lambda cyhalothrin and thiametoxam was carried out on the earthworm *Eudrillus euginae* and used the filter paper contact method. The results of observations of behavioral responses obtained included moving to avoid, circle, roll and surround the cup in all individuals exposed to botanical pesticide a concentration of 3%. The conditions of the individuals exposed are shown in the figure

24 hours 48 hours

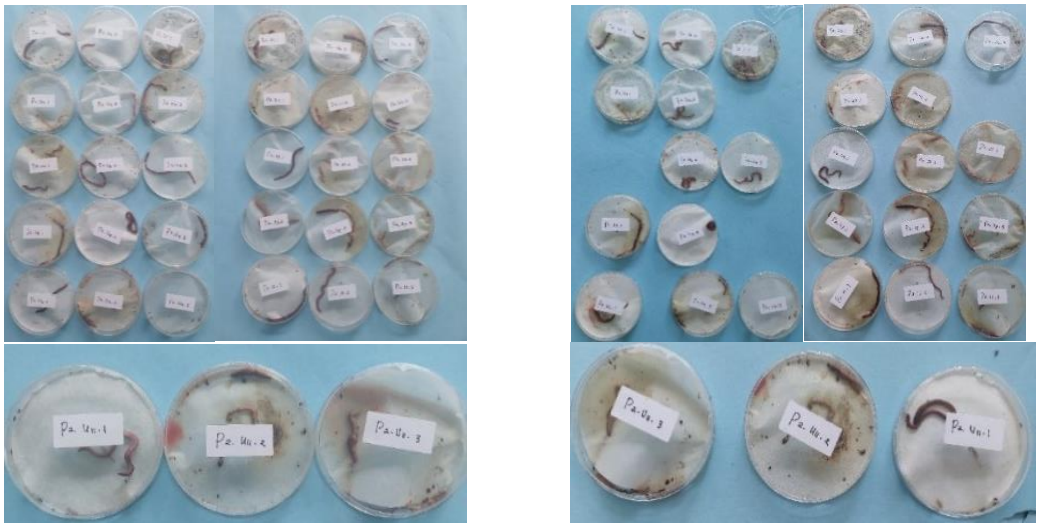


Figure 2. Behavioral response of worms due to exposure to synthetic pesticides

Botanical Pesticide Contact Toxicity Test

The visible morphological characteristics are the discharge of fluid or coelom from the worm's body which is red or yellow, and the body is split into several parts but the anterior part still moves by avoiding or surrounding the cup as shown in Figure 4.

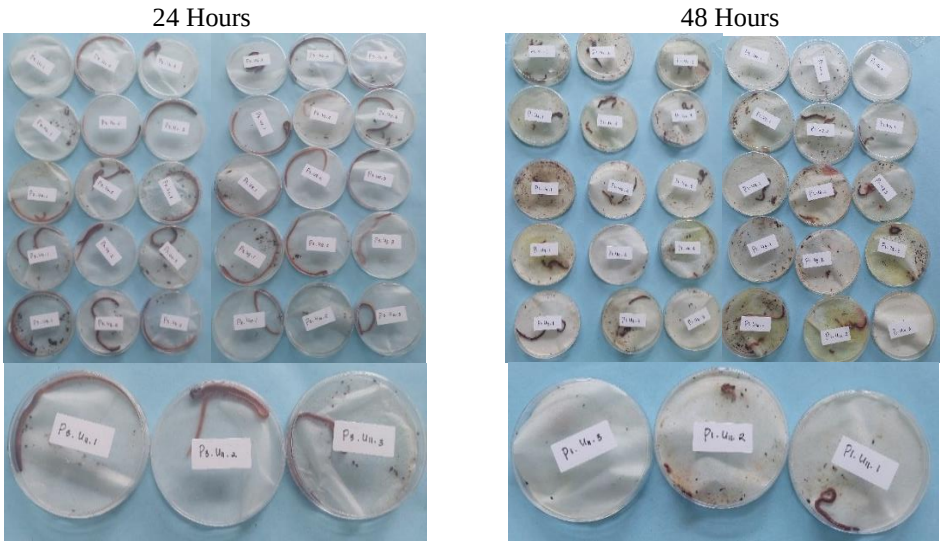


Figure 3. Behavioral response of worms due to exposure to botanical pesticides

Water Only Contact Toxicity Test

The behavioral response of earthworms from exposure to water alone or control, the water used was ordinary tap water originating from the Greenhouse, State University of Malang. Tap water generally has the characteristics of ideal clean water, which does not have to be clear, colorless, tasteless and odorless, and does not contain pathogenic germs and all creatures that endanger human health. If viewed based on chemical parameters, the water does not contain toxic chemicals, or metal content that exceeds clean water quality standards (Singkam *et al*., 2021). This statement is in accordance with research data obtained where the negative control treatment had a lower mortality percentage than other treatments. The behavioral responses produced by this treatment can be seen in Figure 5 with responses including moving in circles and avoiding and also surrounding the cup, earthworms that experience mortality and death, namely fluid comes out of the body and is red or yellow in color.

This treatment does not use pesticides but there are still worms that experience mortality, this may be influenced by factors including light, pH around neutral and good air and water circulation. The media temperature should be maintained in the range of 18-27ºC, while the humidity required is between 50-80%. However, the temperature and humidity requirements of earthworm media vary between species and the adaptability of each species (Brata, 2006).

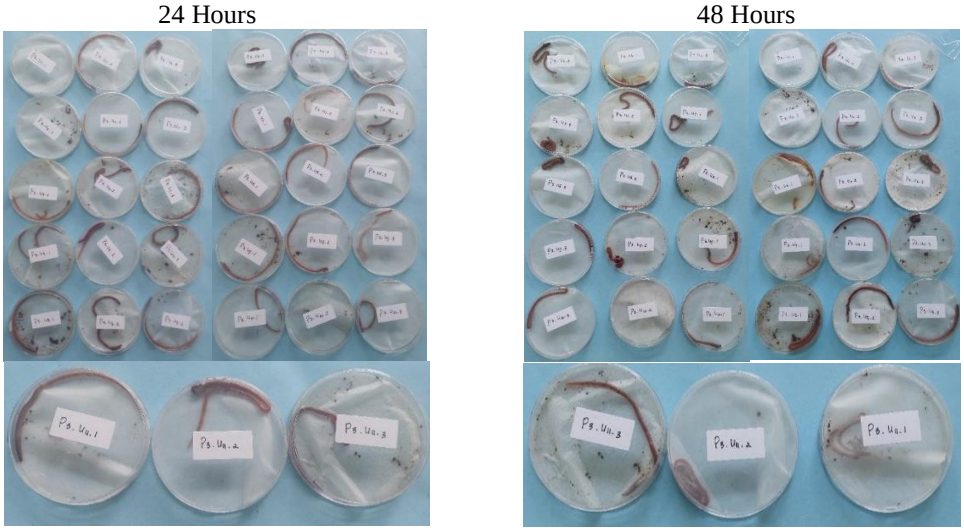


Figure 4. Behavioral response of worms due to exposure to water only

3.3 Toxicity Test Results on Cocoon Hatchability

Observation data on the hatchability of earthworm (*Eudrillus euginae*) cocoons under the influence of the insecticide lambda cyhalothrin and thiamethoxam, in the form of the number of cocoons that hatch. In this study, cocoons were reared for 15 days until all the cocoons hatched and became worms. Earthworm cocoons are placed on the surface of the soil, so when insecticide is applied, the residue from this insecticide can directly contact the earthworm's body and can also contact the cocoon. Figure 6 shows the percentage of cocoon hatchability. The K- treatment or giving water alone had the highest percentage of hatching compared to other treatments, namely 57%. The P1 or botanical pesticide treatment had a cocoon hatchability percentage of 40%, and the lowest cocoon hatchability percentage occurred in the K+ or synthetic pesticide treatment with a hatchability percentage of 17%.

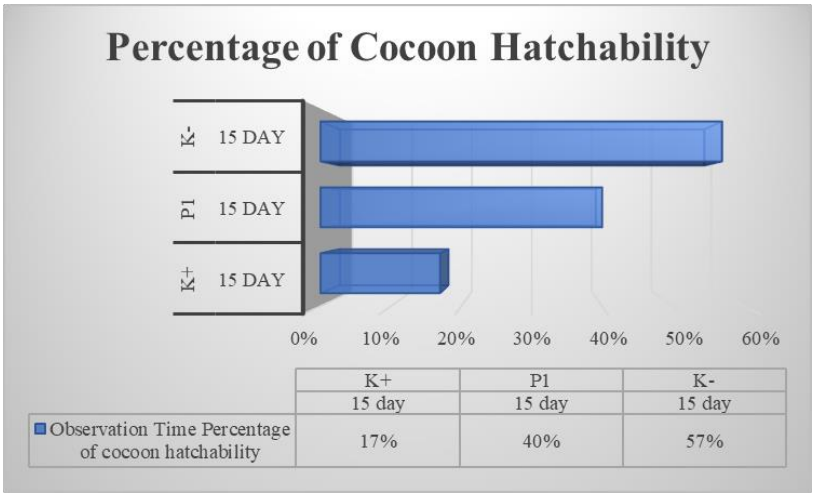


Figure 5. Behavioral response of worms due to exposure to synthetic pesticides

Earthworm cocoons have a hard and strong structure, this kind of structure means that when the cocoon is treated with insecticide the embryo inside the cocoon is not affected. The observation data on cocoon hatchability obtained was appropriate in that pesticides made from lambda cyhalothrin and thiamethoxam affected cocoon hatchability and were only able to hatch by 17%. Generally, pesticides have an effect on the target by inhibiting acetylcholinesterase in nerves, this inhibition is irreversible. Under normal circumstances acetylcholinesterase will degrade acetylcholine into choline and acetic acid. However, with the presence of organophosphates which block the action of acetylcholinesterase, the process of acetylcholine degradation will be hampered and cause a buildup of acetylcholine in the post synapse, if this event continues it will cause death. Apart from that, the active ingredients contained can reduce the hatchability of earthworms, disrupt the development of earthworms and also affect the morphology of earthworms (Santoso *et al*., 2016).

The development of the embryo will continue to develop as usual, but some cocoons do not develop and cannot hatch new individuals. The difference in the number of cocoons that hatch is also caused by changes in temperature and limited sources of food reserves in the cocoon and the ability to detoxify or excrete toxicants is related to the insecticide concentration. Generally, the development of the cocoon starts from being cream colored, then over time it will change color to yellowish white and then when the young worm is ready to come out, the color of the cocoon will change to brownish with visible red movement in it from the blood flow of the young earthworm. Then, when the young worms come out, they will appear transparently empty (Santosa *et al.*, 2016).

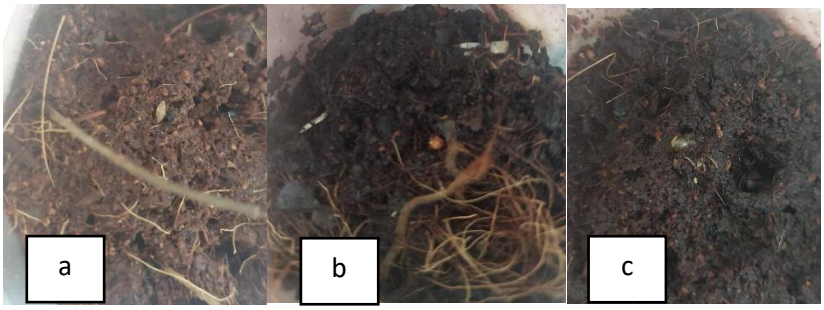


Figure 6. Condition of earthworm cocoons (a. the cocoon is damaged , b. indications that there are earthworm embryos, c. indications that the earthworms have come out)

Figure 6 shows the condition of cocoons that have been exposed to pesticides, which can be seen in picture a. that the pesticide applied caused damage to the cocoon. It can be seen that the cocoon is dark yellow and its shape is no longer intact, the picture shows the cocoon is brownish yellow which indicates the development of an earthworm embryo inside it, and picture c shows that the cocoon is transparent brown with an intact egg-shaped shape and indicates that the worms come out of the cocoon and the young worms come out into the media. In general, earthworms can live well if the environmental conditions in which they live can support their needs. These environmental conditions have criteria in the form of temperatures that are not too high or too low, abundant organic matter, appropriate humidity, appropriate soil pH and sufficient oxygen availability (Santosa *et al*., 2016).

3.4 Toxicity Test Results On Histology Of Earthworm

The results of the toxicity test on the histology of earthworms are shown in table 3 and figure 7, this is in accordance with research conducted by Duo *et al.*, (2022) that in the control treatment the structure of the epidermis and muscles did not show clear changes, exposure to pesticides with moderate concentrations resulted in damage to the epidermis, such as enlarged vacuoles, deformed epithelial cells, muscle degradation, and the spaces between cells getting bigger. Exposure to the highest concentrations of pesticides causes severe damage to the body walls of earthworms, the epithelial tissue is loosely arranged, some cells are degraded, muscles experience shrinkage or thinning and their structure is irregular.

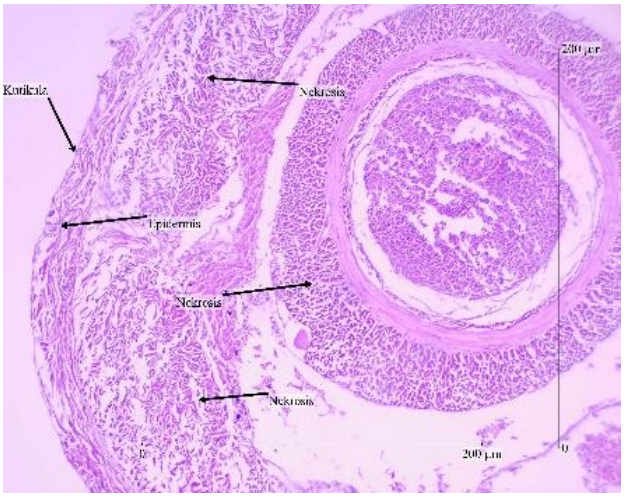
Table 3. Effect of pesticide treatment on earthworm gut histology

Treatment	Cuticle	Epidermal tissue	Muscle tissue
K-	Normal	Epidermis tissue shows a structure that is still intact and clearly arranged	Setae segmentation is clearly visible
K+	Exfoliate	damage to the epidermal tissue and necrosis occurs	the structure of the worm from the outside to the inside appears unclear
P1	Normal	Epidermal tissue shows a structure that is still intact and clearly arranged	Setae segmentation is clearly visible

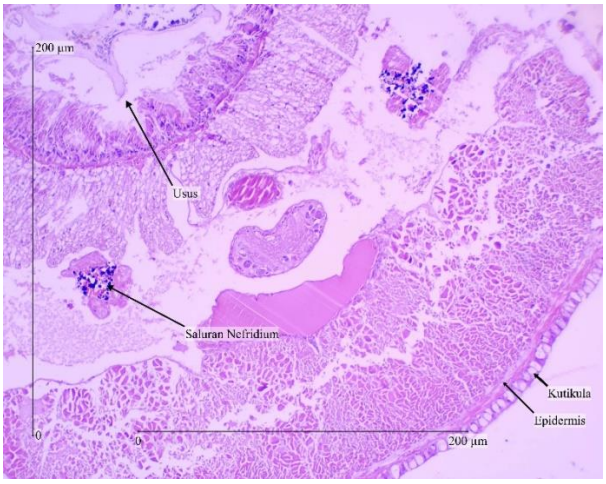
Generally, earthworms have thin cuticles and are composed of cells that are sensitive to chemical stimuli. This research used 3 types of treatment, namely synthetic pesticide treatment, botanical pesticide treatment and control. The contact toxicity test uses filter paper, the liquid is sprayed on the filter paper, and new earthworms are put into a petri dish. The findings in this study show that chemicals can stick to and reach the coelomic fluid because earthworm skin contains secretory cells and produces mucus. This is through the skin of earthworms, the active ingredients that stick to the worm's body from the treatment given will be transported throughout the body, causing toxic symptoms. In addition, epidermal tissue is the initial barrier for earthworms to survive exposure to pollutants so that if damaged, contaminants can enter the organism's body (Duo *et al*., 2021).

The treatment in this research also used paitan leaves (*Tithonia diversifolia*) as a botanical pesticide. Paitan leaves (*Tithonia diversifolia*) are plants that contain alkaloids, tannins, flavonoids, terpenoids and saponins. This content makes paitan leaves neurotoxin and stomach poison for invading organisms (Kawura *et al*., 2022). However, in this study, P1 treatment did not cause severe toxicity as shown in Figure 8, so it can be concluded that the botanical pesticide made from paitan leaves (*Thitonia diversifolia*) is safe against non-target organisms, especially earthorms.

K+



K-



P1

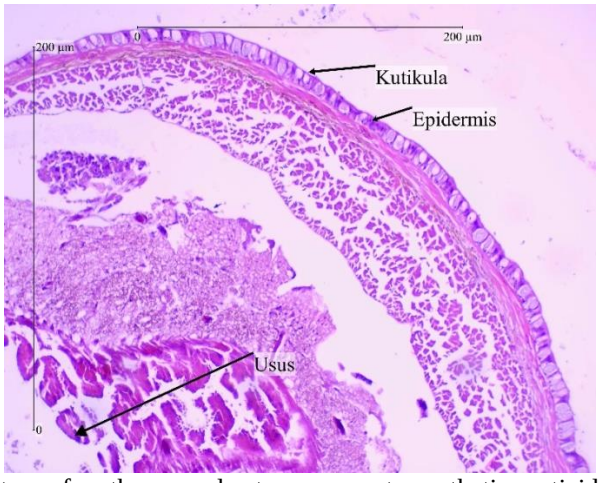


Figure 7. Histological picture of earthworms due to exposure to synthetic pesticides, controls and botanical pesticides

Figure 7 is the histology result of exposure to synthetic pesticides and vegetable pesticides as well as the control treatment. The K+ image is the result of synthetic pesticide treatment made from lambda chyhalothrin and Thiametoxam, the layers of the worm structure from the outside to the inside appear unclear. Organs in the coelom are difficult to identify. Longitudinal sections of the worm show damage to the epidermis and loss of its cuticle structure and glandular cells. The circular and longitudinal muscle layers appear fragmented. There is visible destruction and loss of parenchyma in the central area of the worm which should show several structures such as the intestines, nephridia and typhlosol. Image K- is the result of control treatment, the cuticle layer shows normal results, the layers of the worm structure from the outside to the inside appear complete. The epidermis shows an intact structure of the cuticle, epidermal gland cells, and basement membrane bordering the circular muscle. The internal organs of the coelom appear clear and relatively intact. The nephridia and intestines appeared intact. Setae segmentation is clearly visible in longitudinal section.

Figure P1 is the result of treatment with botanical pesticides made from paitan leaf extract, showing normal cuticles, the layers of the worm structure from the outside to the inside appear complete. The epidermis shows an intact structure of the cuticle, epidermal gland cells, and basement membrane bordering the circular muscle. The internal organs of the coelom appear clear and relatively intact. The nephridia and intestines appeared intact. Setae segmentation is clearly visible in longitudinal section. Debris in the middle of the worm's body in longitudinal section is thought to be ingesta in the intestine. Pigment deposits suspected to be lipofuscin or hematin were seen in the circular muscle layer. It can be concluded that the most significant changes in histological conditions occurred in the K+ or synthetic pesticide treatment.

4. CONCLUSION

Based on the results of the research and discussion, it can be concluded that insecticides containing the active ingredients lambda cyhalothrin and thiamethoxam caused 79% mortality within 48 hours and 58% within 24 hours after application. Meanwhile, the use of botanical pesticides made from paitan leaves resulted in

mortality percentages of 18% and 39% during 48 hours of observation. These results show that the vegetable pesticides exposed to earthworms are still able to survive in these environmental conditions, and treatments that use water alone have higher mortality rates. lower than other treatments. The highest cocoon hatchability occurred in the K+ treatment and the vegetable pesticide treatment made from paitan leaves could hatch cocoons with a percentage of 40%

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