Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 5, No. 1, January 2020

Research Paper

Acute Toxicity Test of *Jatropha curcas L.* on Nile Tilapia Seeds (*Oreochromis niloticus L.*)

Nurhidayanti[1]*

[1]*Lecturer of Medical Laboratory Technology, STIKes Muhammadiyah Palembang*
***Corresponding author**: nuri89_yanti@gmail.com

Abstract
This study is investigating acute toxicity test of *Jatropha curcas L.* on Nile tilapia seeds (*Oreochromis niloticus L.*). This study had been conducted in May 2017, in Balai Riset Perikanan dan Perairan Umum (BRPPU) Mariana, Banyuasin. The purpose of this study is to determine the concentration of Jatropha curcas seed powder 50% death of Nile tilapia seeds (LT_{50}). This acute toxicity test was used by biological test with exposure time of 96 hours. The concentrations of Jatropha curcas tested were 0 (control), 240, 288, 346, 415, 498, 597, and 716 ppm, then every concentrations was applied for 96 hours. We analyze data using Spearman-Karber method with SPSS. It showed LC_{50} of 24 hours was 551,271 ppm, LC_{50} of 48 hours was 466,513 ppm, and LC_{50} of 96 hours was 393,892 ppm. Meanwhile, LT_{50} for 597 ppm of *Jatropha curcas* was 1285, 166 minutes, and LT_{50} for 761 ppm was 784,121 minutes. The higher the concentration of *Jatropha curcas* seeds is the fastest time that causes the death of Nile tilapia seeds, *Jatropha curcas* seeds have moderate toxicity.

Keywords
Acute toxicity, *Jatropha curcas L.*, biological test, *Oreochromis niloticus L*

Received: 12 December 2019, Accepted: 15 January 2020
https://doi.org/10.26554/sti.2020.5.1.18-22

1. INTRODUCTION

Jatropha curcas L. is being fostered as a sustainable source of bioenergy and food. The most valuable parts of the plant are the kernels containing high amounts of oil and protein suitable for creating a range of beneficial products (Montes and Melchinger, 2016). In addition, drought-resistant annual plants can thrive in marginal areas of Eastern Indonesia, such as NTT, NTB, Sulawesi, Maluku, and Papua.

Jatropha plants are poisonous plants. The *Jatropha curcas* L is a plant of the family *Euphorbiaceae.* The whole parts of Jatropha plant are poisonous, especially the seeds. However, the phorbol esters in the toxic varieties of *Jatropha render* the most important byproduct of the biofuel extraction, the seed kernel meal, unsuitable for consumption. The adverse effects of toxicity have been firmly established on microorganisms to higher animals using extracts from fruit, seed, oil, roots, latex, bark, and leaves (Devappa et al., 2010). This affects the benefits of the plant negatively.*Jatropha curcas* are very dangerous for small children, because the seeds are delicious and tasty taste but contain toxins.

Jatropha leaves and seeds fall to some part of the and they would be endanger for life of aquatic biota. The reason is the leaves or seeds of jatropha contains toxins and causes negative effects in the form of aquatic biota death. Water is a primary need for life on earth, especially for humans. Therefore, if the used water contains toxins, it will disturb human health. Toxins that accumulated in the body's tissues could be poison to body's organs, so that the body's organs could not be functioned anymore and disrupted health and death.

Tilapia (*Oreochromis niloticus L.*) deserves to be used as a biological indicator because when it meets the requirements, namely this organism is sensitive to toxic materials and environmental changes, widespread distribution and easily obtained in large quantities, has economic value, reaction and ecological importance both regionally and nationally, easily maintained in a laboratory (Silva et al., 2017).

Biological testing is testing a toxic compound using living organisms. The purpose of the biological test is to determine the organism's response to the magnitude of the concentration of the toxic compound. Toxicity tests are using to evaluate the amount of toxin concentration and the duration of exposure which can have toxic effects on biological tissues. Toxicity is a relative toxicity associated with its potential which results in negative effects for living things.

According toAli et al. (2019), toxicity was influenced by several factors including the composition and type of toxin, the concentration of the toxicant, the duration of the frequency of

exposure to environmental characteristics and the species of recipient biota. Toxic can cause negative effects for biota in the form of structural and functional changes, both acutely and chronically. Acute toxicity testing can be done by determining Lethal Concentration 50% (LC_{50}) and Lethal Time 50% (LT_{50}) which aims to determine the level of jatropha toxicity to test animals. Based on the description above, it is necessary to do a jatropha toxicity test so that it can be known how much concentration can kill fish using biological tests.

2. EXPERIMENTAL SECTION

This type of experimental research is using a biological test method with a static medium which is carried out in two stages. The two stages are preliminary test with the aim of getting the concentration range to be used in the toxicity test, and the second one is toxicity test with the aim to find out the number and time of death of fish seeds due concentration of jatropha seeds to be tested for 96 hours, so that the LC_{50} and LT_{50} values can be known.

This research was conducted on May 2017, located in the Laboratory of the Balai Riset Perikanan dan Perairan Umum (BRPPU) Mariana Palembang. The sample in this study was tilapia seed with an average length of 3-4 cm. The steps in concentration for the toxicity test are as follows: Prepared 8 glass aquariums containing 10 liters of water mixed with Jatropha seed powder based on concentration and control. Then put healthy test animals and have been selected each of 10 tails each aquarium and recorded when entering the test animals. Then the observation of the death of tilapia seedlings for 24 hours, 48 hours and 96 hours was carried out to obtain the LC_{50} value. Observations were made on the death of tilapia fish seeds, namely for 96 hours every 10 minutes in the first 6 hours, 30 minutes for the second 6 hours, 1 hour in the next 12 hours, every 6 hours for 72 hours later to determine the LT_{50} value. The number of dead fish recorded at each observation. Data were analyzed by the Spearman-Karber method.

3. RESULTS AND DISCUSSION

3.1 Introduction Test

The preliminary test was carried out for 24 hours with various concentrations of jatropha seeds, they are 0 ppm (control), 10 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, 1000 ppm, obtained critical range values, the upper threshold concentration (n) of 800 ppm caused 100% mortality of tilapia seeds and a lower threshold concentration (n) of 200 ppm did not cause death effects on tilapia seed. This can be seen in Table 1.

From the preliminary test that has been carried out for 24 hours, weget the critical range value of 800 ppm for the maximum range and 200 ppm for the minimum range. So that the range obtained for acute toxicity tests are 0 ppm (control), 240 ppm, 288 ppm, 346 ppm, 415 ppm, 498 ppm, 597 ppm, 716 ppm. The results of acute toxicity tests at various levels of jatropha seed concentration on tilapia seed yield LC_{50} values for 24 hours, 48 hours, 96 hours. This can be seen in Table 2.

Table 1. The number of fish that died at various concentrations of jatropha seeds in the preliminary test for 24 hours

Concentration (ppm)	lots of fish tested	Many fish die	Critical Range
0	10	0	
10	10	0	
100	10	0	
200	10	0	Min
400	10	3	
600	10	8	
800	10	10	Max
1000	10	10	

Table 2. LC_{50} values of jatropha seeds to tilapia (*Oreochromis niloticus L.*) seeds with a variation of time of 24 hours, 48 hours, and 96 hours

Treatment time (hour)	LC50 (ppm)	Confidence interval 95% Upper limit (ppm)	Lower limit (ppm)
24	551,271	605,808	507,430
48	466,513	511,696	430,080
96	393,892	437,718	356,252

The above results show that the higher the concentration of Jatropha seeds given to the static medium, the higher the number of dead tilapia seeds, and if the concentration of Jatropha seeds is low then the time needed to kill tilapia seeds is getting longer.

3.2 Regression Equation

Regression equations obtained based on probit values for LC_{50} for 24 hours, 48 hours, 96 hours can be seen in Figures 1, 2 and 3.

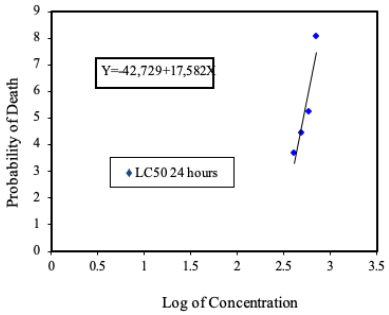

Figure 1. LC_{50} values for 24 hours with a regression equation are: Y = -42,729 + 17,582X

LC_{50} value for 24 hours was 551,271 ppm, with an upper limit at a 95% confidence level of 605,808 ppm and a lower limit of 507,430 ppm. The results of this test indicate that the administration of Jatropha seeds concentration of 551,271 ppm can cause

the death of 50% of test fish within 24 hours.

These results indicate that jatropha seeds were toxic and have the ability to kill tilapia. Disposing of Jatropha seeds waste from the use as biodiesel material contains several chemicals including curcin, phorbol ester and other chemicals that can cause toxic effects if it falls on water areas.

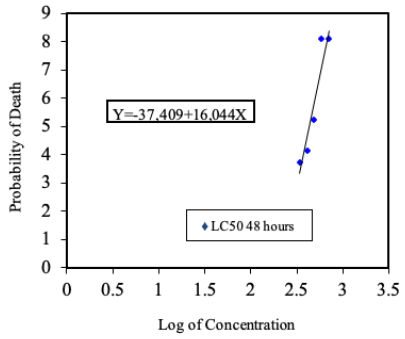

Figure 2. LC_{50} values for 48 hours with a regression equation are: Y = -37,402 + 16,044X

LC_{50} at 48 hours is 466,513 ppm, with the upper limit at the 95% confidence level of 511,696 ppm and the lower limit were 430,080 ppm. The results of this test indicate that the administration of Jatropha seeds concentration of 466.513 ppm has caused the death of 50% of test fish within 48 hours.

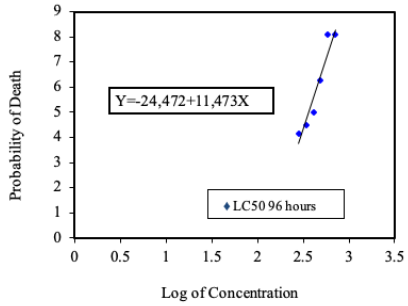

Figure 3. LC_{50} values for 96 hours with a regression equation are: Y = -24,472 + 11,473X

At 96 hours exposure, the LC_{50} value was 393,892 ppm, with an upper limit of 95% confidence level, 437,718 ppm and the lower limit was 356,252 ppm. The results of this test indicate that the administration of Jatropha seeds concentration of 393,892 ppm can cause the death of 50% of test fish within 96 hours.

The results if this study showed that the higher the concentration of Jatropha seeds given to the static medium, the higher the number of dead tilapia seeds, and if the concentration of Jatropha seeds is low then the time needed to kill tilapia seeds is getting longer. The results of this study were confirmed by the opinion of He et al. (2011), that the more or more concentrated the concentration of toxic substances given, the greater the impact on the death of the target organism caused by the accumulation of toxins caused by the toxic substances.

LC_{50} values for 24, 48, and 96 hours from the range of concentration of Jatropha seeds that have been determined that the concentration of Jatropha seeds that have been applied with test animals have the ability to kill tilapia seeds. This is presumably because in Jatropha seeds there are chemicals that can kill tilapia seeds.

The death of the test fish was caused by a toxic substance (jatropha seeds) absorbed into the body of the fish interacting with cell membranes and enzymes so that the enzyme was immobilized. Thus, the work of the enzyme is inhibited or selective transmission of ions occurs through the cell membrane. This is in line with the statement of Tchounwou et al. (2012) that toxic substances can inhibit the work of enzymes in the body of fish. Another cause is related to the availability of dissolved oxygen, where jatropha seeds with high concentrations will inhibit the entry of oxygen from the air into the test solution (jatropha seeds water) so that the fish eventually run out of oxygen. Tanya et al. (2011), said that the concentration of dissolved oxygen depends on the level of water saturation itself, water saturation can be caused by colloids that float in water or the number of jatropha seeds dissolved in water.

From the results obtained indicate that jatropha seeds have a relatively high level of toxicity against tilapia seeds. According to Ohtani et al. (2017), LC_{50} for 96 hours which included moderate toxic toxic power that is 100-1000 ppm. Jatropha seeds are stated as being toxic in fish and invertebrates in the water.

This can be seen from the behavior of the fish in response to toxic substances given, such as fish gliding quickly to and fro until some fish that jump out of the aquarium, the fish's breath became wheezing, lethargic and weak, then lying at the bottom of the aquarium. Over time the fish's body color became pale, and in the ventral part is greenish-white, then produces excessive mucus, and before death the fish moves irregularly.

Allegedly the main cause of death in tilapia seeds, because the concentration of phorbol esters contained in jatropha seeds is still quite high. This is because phorbol ester is stable and not easily degraded quickly, this condition causes long-term physiological responses such as uncontrolled cell proliferation and differentiation. Based on this fact it was found that jatropha seeds are toxic substances that have an acute effect on a biota that lives in waters, according to He et al. (2011), said that a toxicant effect on organisms was acute if the substance is able to kill within a period of no more of 14 days.

Another research using castor beans was also carried out by several researchers, including Devappa et al. (2010), who tested the toxicity of castor beans for sheep and goats. The results of his study explained that at concentrations of 0.5 and 1 g / kg of jatropha seeds can cause major pathological changes including bleeding in the rumen, reticulum, lungs, kidneys and heart, a decrease in the amount of protein and calcium in serum in the sheep and goats.

The results of this study are supported by the opinion of Mastan et al. (2012), stating that at higher concentrations of

5.5-14 g / kg of jatropha seeds can cause death in goats, sheep and pigs within a few hours or up to three days later. At lower concentrations of 0.25 g / kg of jatropha seeds can cause death after 7-21 days The results of this study indicate that tilapia is more sensitive to jatropha seeds compared to ruminant animals such as sheep and goats.

Toxicity research on tilapia have been conducted by Parliza (2010), he was using cigarette tobacco with an LC_{50} value of 96 hours of 211.552 ppm. Based on these results it could be seen that the waste water with lower toxicity (toxicity) than jatropha seed powder, while cigarette tobacco is more toxic than jatropha seed powder.

There is a relationship between the concentration of toxic substances with exposure time which means that the toxic activity in killing test fish depends on the time or duration of exposure of the poison to the test fish (Silva et al., 2017).

3.3 Middle Death Time

To saw the length of time required by jatropha seeds can cause 50% (LT_{50}) of tilapia (*Oreochromis niloticus L.*) seed death. This can be seen in Table 3.

Table 3. Time of middle death (LT_{50}) of tilapia seed given concentration of jatropha seeds on static medium

Jatropha Seed Concentration (ppm)	LT50 (minute)	Confidence interval 95% Upper limit (sec)	Lower limit (sec)
597	1,258,166	1,381,378	1,159,394
716	784,121	837,450	737,331

From Table 3 above, at a concentration of 716 ppm, 50% of the test fish died within 784,121 minutes is faster than at a concentration of 597 ppm where LT_{50} was 1258,166 minutes. It means that the higher concentration of jatropha seeds, the faster it will cause the death of test animals.

3.4 Probit Value Regression Equation

The regression equation obtained based on probit values for LT_{50} with concentrations of 597 and 716 ppm, this can be seen in Figures 4 and 5 below.

In pictures 4 and 5 it can be seen that at higher concentrations results in lower time (LT_{50}), whereas at lower concentrations, the time needed to kill the test fish is getting longer. This explains that the lower the concentration of Jatropha seeds given to the test fish, the longer the time needed to kill 50% (LT_{50}) of the test fish.

At a concentration of 716 ppm, 50% of test fish died within 784,121 minutes far faster than at a concentration of 597 ppm where LT_{50} was 1258,166 minutes. At a concentration of 716 ppm, 50% of the test fish died within 784,121 minutes faster than the concentration of 597 ppm where LT_{50} was 1258,166 minutes. Higher concentration lower time (LT_{50}), whereas at lower concentrations, the time needed to kill the test fish is

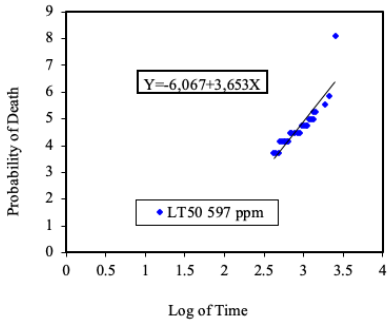

Figure 4. LT_{50} values at a concentration of 597 ppm with a regression equation are: Y = -6,067 + 3,653X

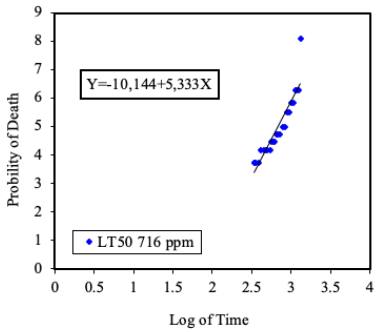

Figure 5. LT_{50} values at a concentration of 716 ppm with the regression equation are: Y = -10,144 + 5,333X

getting longer. This explains that the lower the concentration of Jatropha seeds given to the test fish, the longer the time needed to kill 50% (LT_{50}) of the test fish. If the concentration of Jatropha seeds is given the higher the shorter the time needed to kill 50% (LT_{50}) of test fish because the higher the concentration, the more active substances that enter the body of tilapia seed (Silva et al., 2017).

Death of tilapia seed due to exposure to jatropha seeds suspected nerve poisoning. In addition, the results of research conducted by King et al. (2013) explained that curcin contained in jatropha seeds can function as a binding of glycoprotein (biomolecules which are a combination of protein and carbohydrates) on the cell surface. The curcin mechanism is related to N-glycosidase activity which can then affect metabolism. In addition, curcin is a powerful anti-inhibitor action against protein synthesis. Curcin from jatropha seeds is not seen as a cause in short-term toxicity, but the toxic effect will increase if it is combined with other toxins such as phorbol ester (He et al., 2011) Phorbol esters could be cause extraordinary biological effects even in low concentrations. Its influence causes skin irritation and triggers tumors because it stimulates PKC (Protein Kinase C), which affects the distribution of signals and cell and tissue development as well as a variety of strong biological effects on test animals, thus disrupting the digestive process and damaging

the intestinal membrane, damaging the liver, lung lungs, blood vessels and spinal cord and eventually cause death (King et al., 2011).

4. CONCLUSIONS

Jatropha seed powder is a medium toxicant to tilapia seeds. The concentration of jatropha seeds that can cause 50% death (LC_{50}) in tilapia seeds, namely 24 hour LC_{50} of 551,271 ppm, 48 hour LC_{50} of 466,513 ppm, and LC_{50} 96 hours of 393,892 ppm. The time needed for Jatropha seeds that can cause death of 50% (LT_{50}) of tilapia seeds is at a concentration of 716 ppm for 784.121 minutes and 597 ppm for 1258,166 minutes. The higher the concentration of jatropha seeds applied to test animals, the faster the time needed to kill tilapia seeds.

REFERENCES

Ali, H., E. Khan, and I. Ilahi (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry*, **2019**

Devappa, R. K., H. P. Makkar, and K. Becker (2010). Jatropha toxicity—a review. *Journal of toxicology and environmental health, Part B*, **13**(6); 476–507

He, W., A. J. King, M. A. Khan, J. A. Cuevas, D. Ramiaramanana, and I. A. Graham (2011). Analysis of seed phorbol-ester and curcin content together with genetic diversity in multiple provenances of Jatropha curcas L. from Madagascar and Mexico. *Plant physiology and biochemistry*, **49**(10); 1183–1190

King, A. J., Y. Li, and I. A. Graham (2011). Profiling the developing Jatropha curcas L. seed transcriptome by pyrosequencing. *BioEnergy Research*, **4**(3); 211–221

King, A. J., L. R. Montes, J. G. Clarke, J. Affleck, Y. Li, H. Witsenboer, E. van der Vossen, P. van der Linde, Y. Tripathi, E. Tavares, et al. (2013). Linkage mapping in the oilseed crop J atropha curcas L. reveals a locus controlling the biosynthesis of phorbol esters which cause seed toxicity. *Plant biotechnology journal*, **11**(8); 986–996

Mastan, S. G., P. D. Sudheer, H. Rahman, M. P. Reddy, and J. Chikara (2012). Development of SCAR marker specific to non-toxic Jatropha curcas L. and designing a novel multiplexing PCR along with nrDNA ITS primers to circumvent the false negative detection. *Molecular biotechnology*, **50**(1); 57–61

Montes, J. M. and A. E. Melchinger (2016). Domestication and Breeding of Jatropha curcas L. *Trends in plant science*, **21**(12); 1045–1057

Ohtani, M., Y. Nakano, R. Sano, T. Kurata, and T. Demura (2017). Toxic Substances in Jatropha Seeds: Biosynthesis of the Most Problematic Compounds, Phorbol Esters. In *The Jatropha Genome*. Springer, pages 97–111

Parliza, E. (2010). *Uji Toksisitas Tembakau Rokok terhadap Ikan Nila (Oreochromis niloticus L.)*. Master's thesis, Jurusan Biologi. Fakultas MIPA. Universitas PGRI Palembang.

Silva, M. A., T. Motta, D. B. Tintor, T. A. Dourado, A. L. Alcântara, A. A. Menegário, and J. R. Ferreira (2017). Tilapia (Oreochromis niloticus) as a biondicator of copper and cadmium toxicity. A bioavailability approach. *Journal of the Brazilian Chemical Society*, **28**(1); 143–151

Tanya, P., S. Dachapak, M. M. Tar, and P. Srinives (2011). New microsatellite markers classifying nontoxic and toxic Jatropha curcas. *Journal of genetics*; 1–3

Tchounwou, P. B., C. G. Yedjou, A. K. Patlolla, and D. J. Sutton (2012). Heavy metal toxicity and the environment. In *Molecular, clinical and environmental toxicology*. Springer, pages 133–164