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TOBACCO POWDER (*Nicotiana tobacum*) AS BIO-PESTICIDE: ITS IMPACT ON TILAPIA (*Oreochromis niloticus*) MORTALITY AT DIFFERENT DOSES

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ABSTRACT Tilapia is a pest in shrimp ponds that needs to be eradicated because it is a competitor that disrupts the life of cultivated shrimp. Tobacco leaves (*Nicotiana tobacum*) contain nicotine which can be used as a botanical pesticide. This study aims to determine the effect of tobacco powder on the mortality of tilapia as a pest in ponds. The materials used are low-quality tobacco powder and tilapia measuring 7-9 cm. The study used an experimental method with a Completely Randomized Design with treatments A (0 ppm or control), B (10 ppm), C (20 ppm) and D (30 ppm) with three replications. Data analysis used analysis of variance (One Way Anova). The results of statistical tests showed significant differences between treatments and controls. The best treatment for tilapia mortality (*Oreochromis niloticus*) was treatment C (20 ppm) and D (30 ppm) followed by B (10 ppm) while treatment A (0 ppm) did not affect tilapia mortality (*Oreochromis niloticus*).

Keywords: *Pests, Tilapia (Oreochromis niloticus), Pesticides, Tobacco (Nicotiana tobacum)*

INTRODUCTION

The most widely cultivated shrimp commodities in ponds consist of white shrimp (*Litopenaeus vannamei*) and tiger shrimp/*Penaeus monodon* (Fahmi, 2000). According to Fahmi (2000), shrimp or milkfish that are raised in traditional ponds are not given artificial feed and get their food from natural feed from fertilization. Traditional pond management carried out by farmers only involves putting in seawater when the tide is high and removing it when the water starts to recede. This activity has the potential to bring unwanted wild aquatic animals into the pond, either in the form of eggs, seeds or those that are already adults (Fahmi, 2000). Unwanted wild animals that enter the pond can be categorized as pests (Rahmaningsih, 2018).

The characteristics of pests that cause losses to cultivation efforts are categorized into three types, namely nuisance characteristics, competitive/competitor characteristics and predator/predator characteristics. Pond pests with nuisance characteristics can cause losses in the form of embankments. Ponds leak because they make holes. Examples of pests such as crabs. Animals such as snapper, snakehead, snakes and others are pests that are categorized as predators because they can prey on cultivated organisms. While as competitors, pests such as tilapia can compete with cultivated organisms in getting oxygen and food (Soeseno 1993).

Tilapia (*Oreochromis niloticus*) is one of the fish that is classified as a pest in the shrimp farming process in ponds. According to Effendie (2003), the presence of tilapia in traditional vannamei shrimp ponds can be categorized as a pest. Tilapia that enters the pond will compete for food and living space with the shrimp being farmed. According to Dailami *et al.*, (2021) tilapia has the characteristic of easily reproducing wildly with rapid growth. This This can cause the tilapia population uncontrolled so it can interfere development of cultured organisms. Pest control in vannamei shrimp ponds can be done using chemicals or natural materials. According to Fahmi (2000), pesticides used to control pests in Shrimp ponds aim to kill pests without disrupting the life of the

shrimp and can be in the form of factory chemicals or traditional materials. Furthermore, Fahmi (2000) stated that traditional pest control materials can be in the form of natural materials that are toxic to pond pests and easy to obtain. Examples of natural pesticide materials that can be used are tuba roots, tea seeds and tobacco.

Natural pesticides have organic properties that are easily decomposed in nature so that their toxicity can be lost in a few days. This is one of the efforts to reduce chemicals that aims to support food safety for aquaculture products (Prariska *et al.,* 2017). According to Kardinan (2005), the impact of chemical pesticides on the environment can be reduced by using natural pesticides. Some of the advantages of natural pesticides are that they are easily decomposed and relatively inexpensive. Natural pesticides are also safe for humans and animals because their toxic residues are easier to remove. Said *et al.,* (2015) stated that tuba roots (*Derris elliptica*) as one of the natural pesticides can kill fish after 30 minutes of treatment. The results of research by Lukman *et al.,* (2014) stated that the concentration of fresh tuba roots of 2 ppm is a concentration that can kill tilapia effectively.

According to Munajat and Budiana (2003), one type of plant that can be used as natural esticide is tobacco (*Nicotiana tobacum*). The high nicotine content in the leaves and stems of tobacco is the main factor in choosing tobacco as a natural poison. Aquatic pests such as trisipan (Cerithidae chingulata) can be effectively eradicated by tobacco plants. The use of tobacco with the active ingredient nicotine to eradicate pond pests was reported by Darmono (1991). The dosage of tobacco used as a pest control is 200 - 400 kg / Ha.

Rudiyanti (2010) stated that tobacco plants (*Nicotiana tobacum*) have a fairly powerful killing ability against pests and fish diseases because of the high nicotine content. This is the main factor in using tobacco plants as natural pesticides. Nicotine can be used as a poison contact, stomach poison and fumigant namely nicotine alkaloid, nicotine sulfate and other nicotine content. Nicotine is a poison that reacts very quickly attacking the nerves.

The use of tobacco plants as natural pesticides to control tilapia pests in shrimp ponds empirically has not been widely carried out compared to tea seeds and tuba roots. Therefore, research is needed related to the toxicity of tobacco to tilapia.

MATERIALS AND METHODS

This study used an experimental method conducted in Grinting Village, Bulakamba District, Brebes Regency, Central Java. This study used three treatments of tobacco powder doses with one control treatment. Each treatment was repeated 3 times. The treatment of tobacco powder doses used were:

1) Treatment A (Tobacco Powder Dose 0 mg/liter) or control.
2) Treatment B (Tobacco Powder Dose 10 mg/liter).
3) Treatment C (Tobacco Powder Dose 20 mg/liter).
4) Treatment D (Tobacco Powder Dose 30 mg/liter).

The number of tilapia used as test animals was 10 per container following the experiment conducted by Abdulah *et al.,* (2015). The determination of the test container for each treatment was carried out randomly.

The research preparation stage includes the preparation of 12 30-liter buckets. The buckets were cleaned and arranged according to a random arrangement scheme. Each bucket container was filled with 20 liters of water with a salinity of 20 ppt. Each bucket container was supplied with oxygen through an aerator hose. Measurement of water quality parameters was carried out before the fish were put into the aquarium including temperature, pH, DO, and salinity. The test animals used were tilapia measuring 7-9 cm from the Tegal Regency Fish Seed Center. Before placed to in receptacle treatment, the tilapia fish to be tested are first kept in a stock container measuring 60 x 40 x 40 cm for

one week.

The tobacco powder used is dried tobacco powder from post-harvest processing of tobacco leaves. The powder is then weighed with each weight according to the treatment dose, namely treatment B 200 mg, treatment C 400 mg, and treatment D 600 mg. The weighed tobacco leaf powder is then put into a glass container. Furthermore, the tobacco powder is soaked in fresh water so that all the tobacco powder is submerged so that the suspension dissolves for 24 hours. The tobacco leaf powder extract is directly applied to each treatment container.

Observations were made after tobacco powder was applied to each treatment container. The research parameters observed were fish behavior, the number of dead fish, the duration of death and other conditions that occurred during the study. Analysis of research data using, Normality Test, Homogeneity Test, Anova Test, Completely Randomized Design (CRD) Test, Additivity Test, Tukey Test, and Duncan Test. The water quality parameters measured were temperature, dissolved oxygen, pH, and salt content (salinity).

Data collection on tilapia mortality was conducted by observing the number of fish that died and those that were still alive 24 and 48 hours after application. After the first 15 minutes of tobacco powder application, tilapia behavior was observed. exposed to tobacco powder suspension. According to Shahabuddin *et al.,* (2005), the mortality rate of tilapia can be calculated using the formula:

$$M = \frac{\text{Number of dead fish}}{\text{Total amount of fish}} \times 100\%$$

Information:
M = Mortality (%)

RESULTS AND DISCUSSION

Results of Visual Observation of Fish, Fish Mortality and Water Quality During Maintenance

The study of the effect of giving tobacco powder with different doses on tilapia mortality was carried out by contact poisoning, namely by mixing tobacco extract into the tilapia maintenance container. The tobacco poison suspension began to appear after five minutes of soaking the tobacco powder in a glass container before being spread into the tilapia maintenance container. This is indicated by the increasingly concentrated color of the soaking water. The higher the dose of tobacco soaked, the thicker the resulting soaking water (Figure 1).

A B

Figure 1. Density of Tobacco Toxic Suspension:
A : Immediately after Immersion;
B : 5 minutes after Immersion

Visual observation of fish in the treatment of tobacco powder administration after 10 minutes of application has shown changes in behavior. The movement of fish in each treatment looks different, it is suspected that the difference in the administration of tobacco powder has an effect on fish movement. The swimming movements of the fish become irregular, floating and less responsive

(Figure 2). While in the treatment of 0 ppm tobacco dose (control) the activity of tilapia fish still looks normal.

Figure 2. Changes in Fish Movement after Tobacco Powder Application

Fish mortality data after application (HAA) for each treatment is presented in table 1 below:

Table 1. Fish Condition at 1 Hour After Application (HAA)

No	Treatment	10 Minutes	30 Minutes	60 Minutes
1.	A	Fish swimming at bottom of the tank (normal)	Fish swimming at bottom of the tank normal)	Fish swimming at bottom of the tank (normal)
2.	B	Fish swimming irregularly (floating)	50 % The fish is inactive (dead)	90 % The fish is inactive (dead)
3.	C	Fish swimming irregularly (floating)	80 % The fish is inactive (dead)	100 % The fish is inactive (dead)
4.	D	Fish swimming irregularly (floating)	80 % The fish is inactive (dead)	100 % The fish is inactive (dead)

Observations of tilapia mortality after one hour of administering tobacco powder in the dose treatments B (10 mg/liter, C (20 mg/liter) and D (30 mg/liter) respectively 90%, 100% and 100%. Mortality of 0% occurred in treatment A (0 ppm) or control. The results of statistical tests showed a significant difference between treatment and control. The best treatment for mortality of tilapia (*Oreochromis niloticus*) was treatment C (20 mg/liter) followed by D (30 mg/liter) and B (10 mg/liter) while treatment A (0 mg/liter) did not affect mortality of tilapia (*Oreochromis niloticus*).

Observation of tilapia mortality was continued at 24 Hours After Application (JSA). The observation data in table 2 shows that in all treatments of tobacco powder administration resulted in a 100% mortality rate. While in the control treatment, there was no tilapia mortality. Visually, dead tilapia carcasses were seen floating in treatment containers B (10 mg/liter), C (20 mg/liter, and D (30 mg/liter). While in treatment A (0 mg/liter) or control, all the fish were still alive.

Table 2. Fish Mortality Data (%) 24 Hour After Application (HAA)

	Tobacco Powder Dosage Treatment (ppm)			
Reply	A	B	C	D
1	0	100,00	100,00	100,00
2	0	100,00	100,00	100,00
3	0	100,00	100,00	100,00
Average	**0**	**100,00**	**100,00**	**100,00**

The data of water quality parameter measurements during the experiment are presented in Table 3. Water quality parameter measurements were carried out at the beginning before the distribution of tilapia and at the end of the trial period which was carried out for 24 hours of maintenance. The results of water quality parameter measurements, both physical and chemical, during the study listed in Table 3 are still suitable for tilapia life. The water quality parameters observed include temperature, pH, dissolved oxygen (DO), and salinity.

Table 3. Average Data of Tilapia Water Quality Parameter Measurement Results During The Study

Parameter	Treatment	Research Results	Quality Standard
Temperature (^{O}C)	A	30	25 – 30 (Amri, 2013)
	B	30	
	C	30	
	D	30	
pH	A	7,00 – 7,18	6,0 – 9,0 (Setyo, 2006)
	B	6,96 – 7,17	
	C	6,97 – 7,12	
	D	7,03 – 7,19	
DO (ppm)	A	6,17 – 6,63	4,0 – 8,5 (Amri, 2013)
	B	6,20 – 6,67	
	C	6,20 – 6,70	
	D	6,23 – 6,63	
Salinity (ppt)	A	20	20 – 25 (Setyo, 2006)
	B	20	
	C	20	
	D	20	

DISCUSSION

Fish Visuals

Visual observation of fish in the tobacco powder treatment after 10 minutes of application has shown changes in behavior. The swimming movements of the fish began to look uncontrolled, such as weak and tended to float following the water current due to oxygen aeration. The fish also looked stressed, gasping with a weak response. The activity of tilapia fish that experienced this disorder is thought to be due to exposure to tobacco poison. The symptoms of tilapia fish above are in accordance with the results of research by Yosmaniar *et al*, (2009) which stated that clinical symptoms due to pesticide poisoning that arise after application are in the form of irregular swimming movements and gill covers, followed by fish conditions that begin to weaken and then die. The abnormal behavior of the fish above is in accordance with the statement of Sudarmo (1991). Fish movements become hyperactive, thrashing, paralyzed and then die after being exposed to pesticide poison. Rudiyanti (2010) stated that fish exposed to tobacco poison experience changes in behavior. Clinical symptoms of animals contaminated with poison show signs of stress in the form of less stable movements until finally the fish tend to be at the bottom of the treatment container.

The changes in the fish are one way to minimize the biochemical process in the poisoned body so that the lethal effect becomes slower. Lukman *et al.*, (2014) stated that tilapia exposed to tuba root poison (*Derris elliptica*) showed signs such as swimming to the surface of the water (gasping). The fish will jump as if they are going to get out of the treatment container. Then the tilapia slowly descends to the bottom of the water and faints. Furthermore, marked by the immobilization of the gill

cover, the fish dies.

The nicotine poison content in tobacco powder works through contact, stomach and respiration and is systemic which causes death in fish (Munajat and Budiana, 2003). Nicotine poison in tobacco powder extract works in the fish's body by stimulating the ganglion in the nervous system through post-synaptic membrane deposarization. At higher doses with longer effects, it can inhibit the stimulus in the ganglion so that the coordination system of stimuli from body organs to the central nervous system is disrupted which results in loss of body balance, uncontrolled movements and numbness (Wattimena and Soebito, 1990).

Fish Mortality

Based on the results of the experiment of giving tobacco powder to tilapia mortality, it showed that treatments B (10 ppm), C (20 ppm) and D (30 ppm) were significantly different in killing tilapia within 1 to 24 hours after application compared to the control. However, there was no significant difference between the doses of tobacco powder on tilapia mortality. Rudiyanti (2010) stated that a dose of 50 ppm tobacco extract was able to provide a mortality rate of 100% for fish seeds.

The death of tilapia due to exposure to tobacco is caused by tobacco containing poison. Direct exposure to tobacco poison through water intake through the gill membrane, ingestion of food and direct absorption from sediment is the cause of the fish death process. As is known, tobacco contains nicotine which is categorized as one of the natural pesticides. In addition, tobacco also contains saponins which are contact poisons and stomach poisons (Djojosumarto, 2008).

Nicotine compound has a molecular weight of 162.23 grams/mol and is a type of alkaloid with the compound formula $C_{10}H_{14}N_2$. The nitrogen element is the largest alkaloid content with basic to neutral properties. Except for the seeds, all parts of the tobacco plant contain nicotine. As much as 2-8% nicotine content is found in dry tobacco. Nicotine content of 0.6 - 5.5% is found in tobacco materials for making cigarettes. The stimulant properties of nicotine affect the work of the nerves in mammals which makes them calm and relaxed. In addition to being a stimulant that provides a calm and relaxing effect, nicotine can also act as a poison that causes the death of organisms (Glio, 2017; Novizan, 2002; Tirtosastro and Murdiyati, 2017). As an organic chemical compound, the toxic properties of nicotine can be used to kill pests. Nicotine is also a neurotoxin with a very fast reaction. The nicotine content as a contact poison, fumigant and stomach poison is nicotine alkaloid, nicotine sulfate and other nicotine content (Cahyono, 1998).

Water Quality

The results of water quality parameter observations during the experiment showed that it was still in the range that was suitable for tilapia cultivation in all treatments. The water temperature before and after the experiment was 30 0C. According to Amri (2013), a good temperature for tilapia is between 25 - 30 0C. The results of pH measurements before the experiment were 6.96 - 7.03 and after the experiment 7.12 - 7.18, still in the range that was suitable. According to Setyo (2006), the range of pH values that were suitable for tilapia was 6.0 - 9.0. The dissolved oxygen value ranged from 6.17 - 6.63 both before and after the experiment and was still in the range that was good for tilapia according to Amri (2013). The results of water salinity measurements in the treatment and control containers were 20 ppt both before and after the tobacco powder treatment. According to Arifin (2016), tilapia can live and reproduce in waters with a salinity of 0 to 28 ppt.

CONCLUSION

The results of the research that has been conducted show that the administration of tobacco powder doses C (20 ppm), D (30 ppm) and B (10 ppm) has a significant effect on tilapia mortality after 1 hour of treatment (JSA) compared to the control. The best treatment for tilapia mortality is treatment C (20 ppm) and D (30 ppm) followed by treatment B (10 ppm), while treatment A (0 ppm) has no effect on tilapia mortality. Water quality during the research was in the range that is suitable for tilapia cultivation. It is recommended that there be research on the use of tobacco powder in controlling pests in shrimp ponds.

REFERENCES

Abdullah, Said., Sulistyawati and Sumoharjo. 2015. Acute Toxicity Test of Tuba Root Suspension (*Derris elliptica*) on Fish Nile (*Oreochromis niloticus*).Aquawarman Journal 1(1) : 1-6.

Amri, K. and Khairuman. 2003. Intensive Tilapia Cultivation. Agromedia Pustaka. Jakarta.

Amri, K. and Khairuman. 2013. Fish Cultivation. Agromedia. Jakarta.

Andriani, Yuli. 2018. Tilapia Fish Cultivation. Deepublish. Yogyakarta. 78 pp.

Arifin, MY 2017. Growth and Survival Rate of Red Strain and Black Strain Tilapia (*Oreochromis sp*) Maintained in Saline Media. Scientific Journal of Batanghari University, Jambi 16 (1): 159 – 166.

Boyd, CE, 1990. Water Quality in Ponds for Aquaculture. Birmingham Publishing Co. Birmingham, Ala

Cahyono, B. 1998. Tobacco, Cultivation and Farming Business Analysis. Kanisius. Yogyakarta. 126 pp.

Dailami, M., Rahmati, A., Saleki, D., Hamid, A. 2021. Tilapia Fish. Brainy Bee. Poor. 128 pp.

Darmono. 1991. Cultivation of Penaues Shrimp. Kanisius Publisher. Yogyakarta. 104 pp.

Dewi, E., R., R. Agustina and Eridani. 2020. The Effect of Tobacco Leaf Powder (*Nicotiana tabaccum* Linn.) on the Mortality of Golden Snails (*Pomacea canaliculata*). Agroristek Journal 3 (1): 35 – 43.

Djojosumarto, P. 2008. Complete Guide to Pesticides and Their Applications. Agromedia. 1, 13-31.

Effendie, MI 2002. Fisheries Biology. Nusantara Library Foundation. Yogyakarta. 5 pages.

Effendie, MI. 2003. Water Quality Review. Kanisius, Yogyakarta.

Emiliani, N,. Djufri,. M. Ali S. 2017. Utilization of Tobacco Plant Extract (*Nicotiana tobacum*) as Organic Pesticide for Controlling Golden Snail Pests (*Pomaceace canaliculara* L.) in the Rice Field Area of Gampong Tungkop, Aceh Besar. Scientific Journal of Students of the Faculty of Teacher Training and Education, Unsyiah 2 (2): 59 – 70.

Ervina Dewi, Rahmi Agustina and Eridani. 2020. The Effect of Tobacco Leaf Powder (*Nicotiana tabaccum* Linn.) on the Mortality of Golden Snails (*Pomacea canaliculata*). Journal of Agroristek 3 (1): 35 – 43.

Ezraneti, R and Fajri, N. 2016. Toxicity Test of Mahkota Dewa Leaf Powder (Phaleria macrocarpa) on

Tilapia Fish Seeds (*Oreochromis niloticus*). Acta Aquatica Journal 3 (2): 62 – 65.

Fahmi. 2000. Several Types of Predatory Fish in Traditional Ponds and How to Handle Them. Jurnal Oseana XXV (1) : 21 – 30.

Ghozali, Imam. 2012. Multivariate Analysis Application with IBM SPSS Program. Yogyakarta: Diponegoro University.

Ghozali, Imam. 2019. Multivariate Analysis Application. Diponegoro University Semarang

Glio, MT 2017. Making Botanical Pesticides for Hydroponics, Aquaponics, Vertical Cultivation, and Organic Vegetables. Jakarta: PT. Agromedia Pustaka.

Gomez, KA and Gomez, AA 1995. Statistical Procedures for Agricultural Research. Translation of Statistical Procedures for Agriculture Research. Translator: Endang Sjamsuddin and Justika S, Baharsjah, Jakarta: UI Press. 698 pp.

Hadi Pranggono, Tri Yusufi Mardiana and Nurul Afifah. 2019. The Effect of Giving Saponin with Different Doses on the Mortality of White Snapper (*Lates calcalifer*). PENA Aquatics Journal 18 (1) : 41 – 45.

Husna, U,. Sri Fitri, S,. and Nazlia, S,. 2023. Comparison Superiority Income from Intensive and Semi-Intensive Pond Cultivation Systems in Vannamei Shrimp Cultivation in Baitussalam District. MAHSEER Journal 5 (1) : 32 – 42.

Isnawan, H, B,. Trisnawati, W, D., Hidayat, T,. and Nurkomar, I. 2019. Agricultural Statistics Guide. Agrotechnology Study Program, Faculty of Agriculture, University of Muhammadiyah Yogyakarta. Yogyakarta. 59 pp.

Kardinan, A. 2005. Botanical Pesticides, Recipes and Applications. Penebar Swadaya, Jakarta, 88 pp.

Khairuman and Amri, K. 2013. Tilapia Fish Cultivation. Agromedia Pustaka. Jakarta. Khairul, Amri and

Kanna, I. 2008. Intensive, Semi-Intensive and Traditional Vaname Shrimp Cultivation. Gramedia Pustaka, Jakarta. 168 pp.

Kordi, G and Tanjung, AB 2005. Water Quality Management. Rineka Cipta. Jakarta.

Kordi, G. (2009). Aquaculture. PT. Citra Aditya Bakti. Bandung.

Lukman, Mulyana and Mumpuni, FS. 2014. Effectiveness of Giving Tuba Root (*Derris elliptica*) on the Duration of Death of Tilapia Fish (*Oreochromis niloticus*). Journal of Agriculture 5 (1) : 22 – 31.

Matsumura, F. 1975. Toxicology of Insecticides. Plenum Press, New York and London. pp. 105 – 114.

Meikawati, W,. 2013. Utilization of Tobacco Plant Extract (*Nicotianae tobacum*) as Pesticide for Controlling Armyworm Pests on Chili Plants. Proceedings of the National Seminar, ISBN: 978-979- 98438-8- 3: 455-460.

Monalisa, S,. and Minggawati, Infa. 2010. Water Quality Affecting the Growth of Tilapia (*Oreochromis sp*.) in Concrete and Tarpaulin Ponds. Journal of Tropical Fisheries 5 (2) : 526 – 530.

Muchlisin ZA., Arisa AA., Muhammadar AA., Fadli N., Arisa II. and Siti Azizah MN. 2016. Growth Performance And Feed Utilization Of Keureling (*Tor tambra*) Fingerlings Fed A Formulated Diet With Different Doses of Vitamin E (*alpha-tocopherol*). Archives of Polish Fisheries 23 : 47-52.

Munajat, A and Budiana, NS 2003. Botanical Pesticides for Fish Diseases. Penebar Swadaya, Jakarta, 87 pp.

Ningsih, Setia Y. 2019. Effectiveness Test of Tobacco Waste Extract as Botanical Pesticide Against Helicoverpa armigera Caterpillars (*Lepidoptera: noctuidae*) on Corn. Brawijaya University Thesis.

Nisrinah, Subandiyono, T. Elfitasari. 2013. The Effect of Bromelain Use on the Level of Protein Utilization of Feed and Growth of Dumbo Catfish (*Clarias gariepinus*). Journal of Aquaculture Management and Technology

Novizan. 2002. Making and Utilizing Environmentally Friendly Pesticides. Depok. PT. AgroMedia Pustaka. pp 30-31.

Paiman. 2015. Experimental Design for Agriculture. UPY Press. Yogyakarta. Pranggono, H., Mardiana and Afifah. 2019. The Effect of Giving Saponin with Different Doses on Mortality of White Snapper (*Lates calcalifer*). PENA Aquatics Journal 18(1): 41 – 45.

Prariska, Dony., Tanbiyaskur, and Azhar M. Hanif. 2017. Toxicity Test of Tuba Root Extract (*Derris elleptica*) on Red Tilapia (*Oreochromis sp*). Journal of Fisheries Sciences and Aquaculture 12 (1): 41 – 48.

Rahmaningsih, S. 2018. Fish Pests and Diseases. Deepublish. Yogyakarta. 356 pp.

Riri Ezraneti and Nurul Fajri. 2016. Toxicity Test of Mahkota Dewa Leaf Powder (*Phaleria macrocarpa*) on Tilapia Fish Seeds (*Oreochromis niloticus*). Journal of Acta Aquatica, 3:2, 62-65.

Rohman, M. 1986. Effectiveness of Tea Seed Cake (*Saponin*) as a Wild Fish Eradicator in Ponds. Thesis. Bogor Agricultural University.

Rudiyanti, S. 2010. Toxicity of Tobacco Leaf Extract (*Nicotiana tobacum*) on the Growth of Tilapia Fish. Journal of Fisheries Science and Technology 6 (1) : 56 – 61.

Said, A., Sulistyawati and Sumoharjo. 2015. Acute Toxicity Test of Tuba Root Suspension (*Derris eliptica*) Against Tilapia (*Oreochromis niloticus*). Aquawarman Journal 1(1) : 1-6.

Santoso, S. 2001. SPSS Processes Statistical Data Professionally. PT Gramedia Jakarta.

Setyo, BP, 2006. Effect of Different Chromium (Cr+3) and Salinity Concentrations on Feed Utilization Efficiency for the Growth of Tilapia (*Oreochromis niloticus*). Thesis. Postgraduate Program, Dipanegoro University, Semarang. (Unpublished).

Soeseno. 1993. General Fisheries Basics. Yasagama, Jakarta.

Starkweather, J. 2010. Homogeneity of Variances. Research and Statistical Support Consultant. Retrieved May 10, 2019, from:https://it.unt.edu/sites/default/files/levene_jds_mar2010.pdf

Sucipto, Adi and Prihartono, R. Eko. 2005. Bangkok Red Tilapia Breeding. Penebar Swadaya. Jakarta. 90 pp.

Sudarmo, S. 1991. Pesticides. Kanisius Publisher, Yogyakarta, 130 pp.

Supono, S. 2017. Shrimp Cultivation Technology. University of Lampung. Lampung. 129 pp.

Tirtosastro, S., Murdiyati, AS 2017. Chemical Content of Tobacco and Cigarettes. Tobacco Plant Bulletin, Industrial Oil Fiber 2 (1) : 33 – 44.

Tobing, R. 1989. Chemistry of Natural Materials. Department of Education and Culture. Directorate General of Higher Education. Development Project for Educational Personnel Education Institutions. Jakarta.

Wattimena, JR and Soebito, S. 1990. Drug Compounds, Textbook of Pharmaceutical Chemistry. (Second Edition). Yogyakarta: Gadjah Mada University Press.

Yosmaniar, Supriyono, Edy and Sutrisno. 2009. Lethal Toxicity of Molluscicides Niclosamide on Goldfish (*Cyprinus carpio*) Seeds. Journal of Aquaculture Research 4 (1): 85 – 93.