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Insecticide with the Active Ingredient Methomyl Interferes with the Growth and Survival of the Jatiumbulan Tilapia Strain (*Oreochromis niloticus*)

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Abstract: Insecticides with the active ingredient methomyl are a class of pesticides used to protect agricultural crops from attack by pests such as insects. The area that used this insecticide was the result of previous research, namely the Musi Rawas Sawah Center, South Sumatra and Ngantru District, Tulungagung Regency. However, do not rule out many other areas that use it. This study aims to prove that the insecticide with the active ingredient methomyl interferes with the survival and growth of tilapia (*O. niloticus*) strain Jatimbulan. The treatment consisted of a control and three exposure concentrations namely 1.8, 4.015 and 6.2 mg/L with 3 repetitions each. Exposure was carried out artificially once in rearing water and observations were made for 4 days (96 hours). In conclusion, the results showed that with increasing concentrations of insecticide exposure to the active ingredient methomyl (Lannate with 25% methomyl), stress occurred more quickly which resulted in decreased survival, time to death, growth, and clinical symptoms. However, the concentration of 6.2 mg/L (treatment C) gave significantly different results in survival parameters compared to the 1.8 mg/L (A) and 4.015 mg/L (B) treatments. This is because in treatment C there was stress to death and this condition did not occur in treatments A and B.

Keywords: Clinical symptoms; Growth; Insecticide; Methomyl; Survival rate

Introduction

Methomyl is a pest that is widely used around the world. Its application has made a great contribution to pest control, thereby increasing agricultural yields and agricultural profits. However, its strong solubility in water, popular use, and industrial and agricultural discharges to the environment have resulted in detectable levels of methomyl residues in some bodies of water and food (Kongphonprom & Burakham, 2016). Pesticides consist of several groups, one of which is insecticides. Metomil ($C_5H_{10}N_2O_2S$) is one of the most easily found insecticides on the market and is the most widely used commercially in agriculture worldwide (Malhat et al., 2015; Wartono et al., 2021). Methomyl is used to control and eliminate many agricultural pests such as insects and so on. This is because methomyl has a wide range of biological activities, so it is not uncommon for methomyl residues to be detected in agricultural products and natural waters (Haider & Kata, 2020). Insecticides with the active ingredient methomyl are a class of carbamate pesticides that are often used by farmers in the Musi Rawas Paddy Field Sentra, South Sumatra and Ngantru District, Tulungagung Regency in eradicating pests (Wismaningsih & Oktaviasari, 2016; Wartono et al., 2018).

The process of entering pesticide residues into waters, one of which is through waterways or river flows (Suryono et al., 2016). Watersheds (DAS) can carry insecticide residues to the waters (Prabowo & Subantoro, 2012) such as lakes and reservoirs. One of the causes of the often deteriorating water quality of lakes and reservoirs is the influx of contaminants from

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upstream (land) areas carried by rivers (Sulaiman et al., 2020). This can have a negative impact on fish as aquatic organisms (Sumantri et al., 2017; Ihsan et al., 2018).

Tilapia (O.niloticus) is a type of fish that is very popular with the community, because of its fast growth, easy to obtain feed, and can be kept in all places (Iskandar & Elrifadah, 2015). Therefore tilapia is a freshwater fish that has large enough consumers so that tilapia cultivation is highly developed, such as the jatimbulan tilapia strain which is widely cultivated in floating net cages (KJA) in the Pasuruan area, East Java. According to Tyas et al. (2016), tilapia was selected as a test biota to see the effects of toxic substances because it represents the actual environmental conditions, meaning that it has a wide distribution, namely euryhaline. Tilapia inhabit a variety of freshwater habitats, including rivers, reservoirs and lakes. Based on this, the potential for tilapia to encounter toxic materials in the environment is very large.

This research was conducted to provide information related to exposure to insecticides with the active ingredient methomyl which can interfere with the growth and survival of the Jatimbulan tilapia strain (*Oreochromis niloticus*).

Method

Location and Time of Research

The research was conducted at the Fish Cultivation Laboratory, Fish Reproduction Division, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, in December 2022.

Research Design

The research used a completely randomized design (CRD) with 4 treatments and 3 replications. The treatments used were the concentration of the insecticide with the active ingredient metomyl 1.8 mg/L (Treatment A), the concentration of the insecticide with the active ingredient metomyl 4.015 mg/L (Treatment B), the concentration of the insecticide with the active ingredient metomyl 6.2 mg/L (Treatment C), and without administration of an insecticide with the active ingredient methomyl (Control).

Maintenance and Acclimatization of Test Fish

Tilapia strain Jatiumbulan was obtained from UPT Umbulan Pasuruan Freshwater Cultivation Development (PBAT), which is kept in floating net cages (KJA). Maintenance and acclimatization of 72 test fish with a length of 9-12 cm and an average weight of 30.54 ± 1.17 g were reared separately in 12 aquarium units with a size of 30 x 30 x 50 cm.

Maintenance (holding) was carried out in tanks for 14 days and acclimatization for 1 week in an aquarium. During this stage the test fish were given feed 3 times a day in the form of PF 1000 pellets with a protein content of 39-41%. Water replacement is carried out every 2 days as much as 20% of the total 30 liters of water used so that the water conditions remain clean. Test fish that are dead or abnormal are immediately discarded. Only healthy fish were used in this study.

Exposure to Insecticides with the Active Ingredients of Mehtomyl (Test Treatment)

The active ingredient insecticide used is Lannate® 25 WP containing 25 % methomyl as the active substance. Each aquarium was filled with 6 test fish that had been reared and acclimatized beforehand, with a reference of 5 liters per 1 fish so as not to disturb the survival of the test fish. The day before use the fish is fasted (Fadly et al., 2021).

Exposure is done first before the fish are put in the aquarium. The concentration of the treatment refers to the LC_{50} results of tilapia size 9-12 cm in the study Islamy et al. (2017), that is 4.015 mg/L (Treatment B). The concentration of carbamate pesticide residue which is a class of methomyl pesticides was detected in fish meat from aquaculture ponds in Sukabumi, West Java, reaching 1.88 mg/L (Taufik, 2011). The range of increase and decrease in exposure concentration is 55%. 4.015 mg/L + 55% = 6.2 mg/L (Treatment C) and 4.015 mg/L - 55% = 1.8 mg/L (Treatment A). The method of determining the exposure concentration in the treatment refers to Mulyani et al. (2014).

A total water change is carried out every 5 days followed by re-exposure of the insecticide in the rearing container (Rahayu et al., 2017). In addition, this refers to conditions in nature where fish that live freely in nature and in reservoirs or lakes will be exposed to toxic materials from running water or new incoming water from the DAS (River Basin). This is because reservoirs and lakes have little or no outlet channels.

Exposure was carried out artificially (artificial), namely deliberately exposed to the media of test fish rearing water. Siphoning is done so that the water conditions remain clean so as not to disturb the life of the test fish. Siphoning is done by filtering the water using a cloth so that the feces and water are separated. Then the water is put back into the aquarium. One of the focus points in this research is water so it cannot use a circulation system.

Parameters and Observation Time

Parameters observed were clinical symptoms, absolute growth, survival and time of death, and water quality in the form of temperature, pH and DO. The observation time was before giving treatment and the 4th day (96 hours) after exposure. Water quality measurements were carried out 2 times a day, namely at 08.00 (morning) and 16.00 (afternoon). Observation of clinical symptoms was carried out after the treatment test (post-exposure) was carried out by observing physical (external) changes, fish movements, position or distribution of fish, and appetite. Fish growth is calculated using Formula 1:

G (growth) = Wt (final mass) – W0 (initial mass) (1)

Fish survival is calculated using the following formula: SR (*survival rate* %) = N_t (final amount) – N_0 (initial amount)

Measurement of water quality parameters, namely pH and temperature, used a pH meter while DO used a DO meter. The research schedule or research flow can be seen in Figure 1.

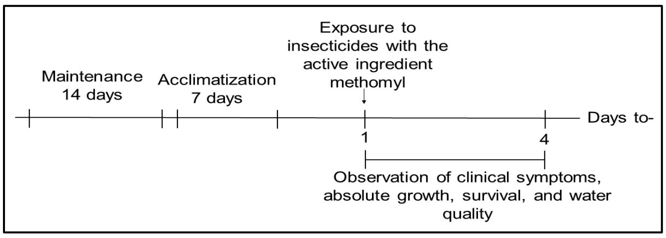

Figure 1. Schedule research or research flow

Data Analysis

Growth and survival data were tested with One Way ANOVA at the 95 % confidence level to determine the effect of treatment between groups. If the results of the ANOVA test show that there is a significant difference between treatments, then continue with the Duncan's Multiple Range Test (DMRT) to determine which treatment gives a significantly different response. Statistical analysis was performed with SPSS version 26.0. While data on clinical symptoms and water quality were analyzed descriptively.

Result and Discussion

Clinical Symptoms

The results of post-exposure clinical symptoms observations can be seen in Figure 2 and Table 1. Clinical symptoms are one of the visible signs that fish are experiencing stress due to environmental changes caused by exposure to insecticides with the active ingredient metomyl. The results of observations of clinical symptoms (Figure 2 and Table 1) are supported by Firdaus et al. (2018), that there is a change in the color of the fish's body, in this case exposure affects the color pigment on the fish's scales, unbalanced swimming movements, peeling scales, damaged fins and tail, separating to the bottom or surface, decreased appetite, which ends in death. It is also compatible Barton (2002) and Tang et al. (2018), tertiary stress physiological response, refers to aspects of the animal's overall appearance such as changes in growth, condition, overall resistance to disease, metabolic scope for activity, behavior, and ultimately leads to survival. The tertiary response is a decrease in fish appetite which causes a decrease in the body's defense system resulting in death.

Figure 2. Post-exposure test tilapia clinical symptoms (96 hours). A) Control; B) Treatment A; C) Treatment B; D) Treatment C; E) The condition of fish that are attacked by fungi in the mouth area due to decreased resistance to pathogen attack; F) Condition of dead fish; G) The body shape of the fish has decreased appetite.

Table 1. Comparison of Clinical Symptoms After Exposure (96 Hours)

Treatments	Clinical Symptoms
A (1.8 mg/L)	– There are fish with dark colors but there are still fish with clear colors
	– No deaths
	– Appetite is still normal and there is no leftover feed
	– There are fish that are responsive and unresponsive to hand movements
	– Active and passive movement as well as moving spread on the surface to the bottom of the waters
B (4.015 mg/L)	– The color of the fish is all black
	– No deaths
	– Appetite decreased but the remaining feed is small
	– In some fish the swimming movements are unbalanced because the pectoral and tail fins are damaged
	– There are fish that are responsive and unresponsive to hand movements
	– Passive movement and spread on the surface to the bottom of the waters
	– The fish gasped at the surface
C (6.2 mg/L)	– The color of the fish is all black
	– There's a death
	– The characteristics of fish that have died are peeling scales and damaged fins
	– Decreased appetite and a lot of leftover feed

Treatments	Clinical Symptoms
	– Some of the test fish had unbalanced swimming movements due to damage to the pectoral and tail fins – There are fish that are responsive to hand movements and unresponsive – Slow swimming movements and separate themselves from the group – Move spread on the surface to the bottom of the waters – The fish gasped at the surface – Some in the mouth of the fish affected by the fungus
K (Control)	– Clear fish color (normal) – No deaths – Normal appetite and no leftover feed – Fish swimming movements are normal and responsive to hand movements – Active movement

Survival Rate and Time of Death

The results of observations of survival and time of death during the study can be seen in Figures 3 and Table 2.

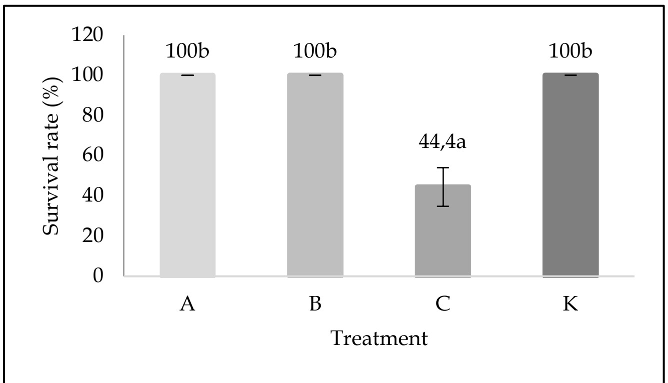

Figure 3. Survival (%) throughout the study. Treatment A (1.8 mg/L); B (4.015 mg/L); C (6.2 mg/L); K (Control)

Table 2. Time and number of deaths (hours) during the study. Treatment A (1.8 mg/L); B (4.015 mg/L); C (6.2 mg/L); K (Control)

Treatment	24 hours	48 hours	72 hours	96 hours
K1	0	0	0	0
K2	0	0	0	0
K3	0	0	0	0
A1	0	0	0	0
A2	0	0	0	0
A3	0	0	0	0
B1	0	0	0	0
B2	0	0	0	0
B3	0	0	0	0
C1	0	2	0	2
C2	2	1	0	0
C3	0	0	3	0

Note : numbers 1, 2, 3 are treatment repetitions

The results of the study in terms of the survival of *O. niloticus* showed that exposure to the insecticide with the active ingredient methomyl in rearing water had a significantly different effect between treatment C and treatments A, B, and K (Figure 3). Based on these data, it can be indicated that *O. niloticus* was under stress which resulted in death as happened in treatment C while in treatments A and B it did not cause death. This could be due to the inability of the fish immune system to deal with exposure to insecticides with the active ingredient methomyl, especially at a concentration of 6.2 mg/L (Treatment C) which caused the highest mortality within 24 to 96 hours (Table 2). It is supported by Nurhalisa, Nur, & Suryani (2022), that low survival is caused by stressful conditions. This statement is also supported by Barton (2002) dan Tang *et al*. (2018), that the tertiary response, refers to changes in growth, condition, overall resistance to disease decreases, metabolic scope for activity, behavior, and ultimately leads to survival.

Growth

The results of absolute growth observations during the study can be seen in Figure 4 below :

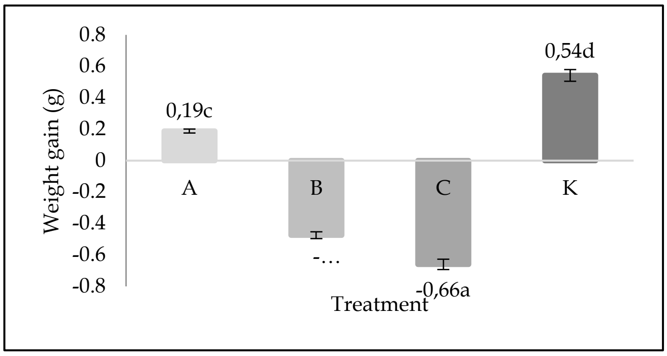

Figure 4. Absolute growth (G) during the study. Treatment A (1.8 mg/L); B (4.015 mg/L); C (6.2 mg/L); K (Control)

The results of the research in terms of the absolute growth of *O. niloticus* showed that exposure to the insecticide with the active ingredient methomyl in rearing water had a significantly different effect between treatments (Figure 4). Based on these data, it can be indicated that *O. niloticus* is under stress so that the energy for its growth cannot be used properly. Stressful conditions can cause a decrease in appetite which results in weight loss. This is supported by statements Bastien & Benjamin (2019), that stress is related to growth. Deane & Woo (2009), explained that fish experiencing stress causes growth hormone to decrease. Barton (2002) and Tang *et al*. (2018), that in the tertiary stress response there is a decrease in fish appetite which causes a decrease in growth.

Water quality

Water quality during the study can be seen in Table 3 below:

Table 3. Water quality during the study

Parameter	Parameter range during the study
Temperature (°C)	24-26
pH	6.50-8.00
Dissolved oxygen (ppm)	4-5

The results of observations of water quality during the research showed that the water in the rearing tanks supported the life of *O. niloticus* so that it did not cause stress. This is supported by research results Muarif (2016), the optimum temperature for tilapia is 22-30 °C, SNI (2009), the optimum pH for tilapia is 6-9 and according to dissolved oxygen to support the survival of tilapia that is ≥ 3 mg/L (SNI 2009; Darwisito, Sinjal, & Wahyuni, 2015; Mujalifah, Santoso, & Laili, 2018; Pramleonita, Yuliani, Arizal, & Wardoyo, 2018). Based on these data, stress on O. niloticus was caused by exposure to the insecticide with the active ingredient methomyl in rearing water.

Conclusion

The results of this study can provide information about insecticides with the active ingredient methomyl which can interfere with survival and growth. Along with an increase in the concentration of exposure given resulted in a decrease in survival which has an impact on decreased growth. This is because energy will be used first for survival and then growth. In addition to causing changes in appearance or clinical symptoms. Based on the results of this study it is proven that the insecticide with the active ingredient methomyl can cause stress in fish. In conclusion, our results show that when the exposure concentration of the insecticide with the active ingredient methomyl (Lannate with 25% methomyl) is increased, stress occurs more rapidly. Therefore, it is necessary to increase awareness about environmental problems because of the bad impacts that can be caused.

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