Insecticide With Active Methomyl Ingredients Against Hematological Parameters As Information on Stress Conditions of Tilapia Strain Jatimbulan (Oreochromis niloticus)

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Abstract

Methomyl is used to control and eliminate pests (insects) in agriculture. Methomyl residues have been detected in agricultural products and natural waters. The purpose of this study was to analyze the effect of exposure to insecticides with the active ingredient methomyl with different concentrations on hematology (erythrocytes, hemoglobin, hematocrit, leukocytes) as information on stress conditions for Tilapia Jatimbulan which were observed on the 4th day after the first exposure and the 4th day after the exposure 7th (end of study 28 days). The study used a completely randomized design with 1 control without exposure and 3 exposure treatments namely concentrations of 1.8 mg/L (Treatment A), 4.015 mg/L (Treatment B), 6.2 mg/L (Treatment C), with each 3 repetitions. Six tilapia fish measuring 9-12 cm and weighing an average of 30.54 ± 1.17 g were put into an aquarium (30 L) containing fresh water and insecticide with the appropriate concentration of treatment. Four days after the first exposure, one fish was taken from each aquarium to be analyzed according to the observed parameters. Then the fish were temporarily moved (± 30 minutes) to another aquarium, the treatment aquarium was cleaned and filled with insecticide again with the concentration according to the treatment then the fish were put back in for (second) exposure for 4 days. The treatment was repeated every 4 days until the 7th exposure (28th day). Insecticide Lannate® 25 WP which was used for detection by FTIR and GCMS, is known to contain 32.55% methomyl content. Tilapia experienced stress due to hypoxia, after the first exposure (day 4), marked by increased hematology according to the increase in insecticide concentration. After 7 exposures, hypoxic stress still occurred in treatment C, marked by an increase in hematology. It is know

that the concentration of insecticide exposure of 6.2 mg/L resulted in fish experiencing severe stress up to 7 times the exposure (28 days at the end of the study).

Keywords: *Erythrocytes, Hematocrit, Hemoglobin, Insecticides, Leukocytes, Methomyl, Stress.*

INTRODUCTION

Methomyl (C5H10N2O2S) active ingredient insecticide is a pesticide used to control and eliminate pests (insects). Its use has contributed greatly to increasing agricultural yields. However, its strong solubility in water, use not based on ecological aspects and industrial and agricultural discharges into the environment have resulted in residues and are detected in agricultural products and natural waters (Kongphonprom and Burakham 2016; Haider and 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 (Wismaningsih and Oktaviasari, 2016; Wartono et al. 2018).

The application of an insecticide with the active ingredient methomyl is by spraying it on the plants. This method leaves residue on the surface of the soil which can be carried away by runoff water so that it enters the waters and causes pollution (Lin et al. 2020). The mechanism of methomyl degradation in the aquatic environment is the hydrolysis reaction. Starting with the reaction of water (H2O) with methomyl so that it will produce the product methomyl oxime. Furthermore, the oxime product (RRC=NOH) will produce acetonitrile through Beckmann rearrangement and elimination (Murti and Matsumura, 2012; Chau and Afghan, 2020).

The process of entering insecticide residues into the waters through river flow (Suryono et al. 2016). Watersheds (DAS) can carry insecticide residues to the waters (Prabowo and Subantoro 2012) such as lakes and reservoirs. One of the causes of the deteriorating water quality of lakes and reservoirs is the influx of contaminants from upstream (land) areas carried by rivers (Sulaiman et al., 2020). This can have a negative impact on fish as aquatic organisms (Ihsan et al. 2018).

Insecticide residues can enter the body through the gills. This is because the gills are located on the outside and have a wide and open surface so that there is direct contact with water. In addition, in the gills there are many capillaries or blood vessels (Authman et al. 2013). Water enters through the gill lamellae, so that insecticide residues suspended in water very easily stick to the gill mucus. The more material that sticks, the mucus cells in the gills will produce more mucus and cover the lamellae. This occurs as a result of the entry of toxic materials into the gill tissue. The amount of mucus in the lamellae causes oxygen diffusion to be disrupted resulting in the body experiencing hypoxia. Hypoxia is a condition in which cells and even tissues experience a lack of oxygen. This situation can trigger stress conditions (Lestari et al. 2018), such as hematological changes (Chinnamani et al. 2018). In addition, insecticide residues will be carried into the body by the blood stream in the gills. One of the organs that will receive blood that carries toxic materials is the kidney which affects hematology (Irene et al. 2021).

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 and 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), The selection of tilapia as a test biota to see the effects of toxic substances

because it represents the actual environmental conditions, meaning that 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.

Based on the above background, this study aims to analyze the effect of 1x and 7x exposure to insecticides with the active ingredient methomyl on hematology (erythrocytes, hemoglobin, hematocrit and leukocytes) as information on stress conditions in fish.

Materials and Methods

Location and Time of Research

The research was conducted from November 2022 to January 2023. The stages of maintenance, observation of hematology, acclimatization and water quality were carried out at the Fish Cultivation Laboratory, Fish Reproduction Division, Faculty of Fisheries and Marine Sciences (FPIK), Brawijaya University, Malang. FTIR Test at the Organic Chemistry Laboratory, Chemistry Study Program, Faculty of Science and Technology, State Islamic University of Maulana Malik Ibrahim, Malang. GCMS test at the Integrated Research and Testing Laboratory (LPPT) Unit 2, part of the Central Chemical and Physical Analysis Laboratory (LAKFIP), Gadjah Mada University, Yogyakarta.

Research Design

The study used a completely randomized design (CRD) with 3 treatments of exposure to insecticide concentrations with the active ingredient methomyl, namely 1.8 mg/L (Treatment A), 4.015 mg/L (Treatment B), 6.2 mg/L (Treatment C), and without exposure to insecticides with the active ingredient methomyl (Control) with 3 replications each.

FTIR Spectrophotometer

The FTIR test is used to analyze the presence of functional groups which are characteristic of methomyl whose results are displayed as wave numbers or wavelengths (peaks). This is appropriate (Lee et al. 2017). Analysis was carried out using the Potassium Bromide (KBr) pellet method, namely by taking a sample of the insecticide with the active ingredient methomyl as much as 1 mg and then grinding it until smooth using a mortar then mixing it with 100 mg KBr or with a mass ratio of 1: 100. Then the mixture was again crushed until smooth using mortars.

The sample size requirement which is dry powder is 100 mesh. After that, pellets were made using a hand press with a pressure weight of 8 tons, resulting in transparent pellets. Transparent pellets were tested and the IR spectrum was interpreted using Varian Resolutions 4.0.5 software with a wavelength range of 4000-400 cm-1. Refers to Subayu et al. (2021).

Gas Chromatography–Mass Spectrometry (GCMS)

As much as 1 g of the insecticide sample with the active ingredient methomyl was dissolved in a mixture of Acetone : Diethylether : Petrolium Benzene with a ratio of 1 ml: 1 ml: 1 ml, in a microtube and then vortexed. Then the resulting sample supernatant is transferred to the GC Vial and then ready to be injected into the GCMS tool through the injector, in the sample injector it is heated (Table 1) so that later the sample enters the system in the form of steam. Then the sample that has turned into steam will be carried by the carrier gas into the column.

Table 1. Column and tool conditions

A. Column :	
Type	HP-5MS UI
Length	30 m
I. D.	0.25 mm
Movie	0.25 µm
Max Temp	325/350ºC
B. Tool condition:	
Carrier Gas	Helium UHP (He)
Injector Temperature	250ºC
Split flow	30 ml/min
Split ratio	Splitless
Front Inlet Flow	1.50 ml/min
MS transfer line temp	280ºC
Ion Source temp	250ºC
Mass Range	70-400 (amu)
Purge Flow	3 ml/min
Gas Saver Flow	10 ml/min
Gas Saver Time	2.5 min

The column is placed in a heated room at a temperature (Table 2) called the column oven, in which the sample will be separated into single compounds based on chemical and physical interactions between the sample, the mobile phase (carrier gas), and the stationary phase (composing material). column). After the sample is separated it will go to the detector (Mass Spectrometry (MS)), in MS the sample which has been separated into single compounds will be identified based on its m/z. Furthermore, the identification process using the GCMS tool will produce active compounds which can be seen from the peaks of the chromatogram as identification of data from chromatography and mass spectrometry (MS) results seen from the mass spectrum with the molecular weight of each compound. It should be noted that the GCMS is equipped with a library, so that after the detection of compounds in the form of peaks it can be compared with the library, so that compounds that have these peaks are identified. This is appropriate Hotmian et al. (2021).

Table 2. Column temp

No	Retention Time (min)	Rate (ºC/min)	Target value (ºC)	Hold time (min)
1	1	0	50	1
2	4	25	125	0
3	25	10	300	3.5

Maintenance and Acclimatization of Test Fish

The Jatimbulan tilapia strain was obtained from the Umbulan Pasuruan Freshwater Cultivation Development UPT (PBAT), which was reared 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 ingredient methomyl (test treatment)

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.

Exposure is done first before the fish are put in the aquarium. The concentration of the treatment refers to the LC50 results of tilapia size 9-12 cm in the study Islamy et al. (2017), that is 4.015 mg/L (Perlakuan 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% ie 1.8 mg/L (Treatment A).

Before exposure the fish were temporarily moved (± 30 minutes) to another aquarium, the treatment aquarium was cleaned and filled with insecticide again with the concentration according to the treatment then the fish were put back in for (second) exposure for 4 days. The treatment was repeated every 4 days until the 7th exposure (28th day). This refers to conditions in nature where fish that live freely in nature and in reservoirs or lakes will be exposed to toxic substances from flowing water or new incoming water from the DAS (River Basin), in this case fish may be exposed to toxic substances more than 1 time.

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.

Blood Sampling

Fish blood was collected 2 times, namely on the 4th day after the first exposure and the 4th day after the 7th exposure (end of the study on the 28th day). Each aquarium was taken one fish to take blood. This is appropriate Benning (1978), that acetonitrile began to be detected on day 3 after the introduction of pesticides with the active ingredient methomyl in the environment. In addition, in the exposure stage (treatment test) repeated exposure was carried out on day 5 starting from the previous exposure, so that acetonitrile (methomyl degradation product) resulted in changes in hematological levels.

The process requires the use of a syringe rinsed with 3.8% Na-citrate anticoagulant. Fish blood was taken from the linea lateralis then the blood was put into the eppendorf for observation. Fish blood samples were taken for each treatment group by taking 1 test animal (replication) from each aquarium. Calculation of the number of leukocytes and erythrocytes was carried out according to Blaxhall dan Daisley (1973), while the calculation of hematocrit levels follows Idzni et al. (2018), and hemoglobin levels follow Docan et al. (2010). Measurements were made using a hematocrit scale. Erythrocytes tube height is the hematocrit value expressed in %. The observed water quality, namely temperature and pH using a pH meter and DO using a DO meter, was measured twice a day during the study, namely at 08.00 (morning) and 16.00 (afternoon).

Data Analysis

Blood profile data (erythrocytes count, hemoglobin level, hematocrit and leukocytes count) were tested by One Way ANOVA at 95% confidence level to determine the effect of treatment between groups. If the results of the ANOVA test show that there are significant differences between treatments, then continue with the Duncan's Multiple

Range Test (DMRT) to determine differences between treatments and choose the best treatment. Statistical analysis was performed with SPSS version 26.0. Water quality data were analyzed descriptively.

Results and Discussion

Fourier Transform Infrared Spectroscopy (FTIR) Test

The FTIR test results show several wave numbers which can be seen in Figure 1 and Table 3.

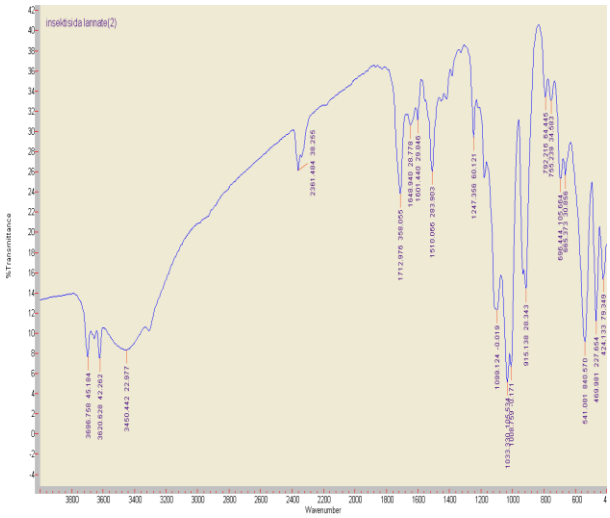


Figure 1. Lannate® 25 WP insecticide spectrum from FTIR test results (Organic Chemistry Laboratory, Chemistry Study Program, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University, Malang, 2023)

The wavenumbers generated from this FTIR test indicate the presence of methomyl. Powered by Suramana et al. (2001), Methomyl detection using FTIR found the presence of amide I (1653, 1654 cm-1) and amide II (1543, 1544 cm-1) groups. The amide I region comprises vibrations mainly due to the C=O strain mode with some contribution from N–H bending and C–N stretching and the amide II region is mainly associated with N–H bending with a large contribution from the C–N stretching mode. The results of this study are also supported by Srikhaow et al. (2022), that the detection of methomyl in an aqueous solution on Biochar using FTIR found the presence of a carbonyl functional group with a wave number of 1634 cm-1.

Table 3. Data on wavenumbers and functional groups of the insecticide Lannate® 25 WP

Wavenumber (cm^{-1})		Ribbon Shape	Compound Type	Functional Groups	Literature
Lannate® 25 WP	Wave Range				
3696	3000-3700	Sharp	Amine	N-H (Stretch)	Parihar *et al*. (2018)
3620	3000-3700	Sharp	Amine	N-H (Stretch)	Parihar *et al*. (2018)
3450	3300-3500	Widened	Amine	N-H (Stretch)	Kommuri *et al*. (2018)
2361	2361	Sharp	Amine	N-H (Ulur)	Senthilkumar and Rajendran (2017)
1712	1640-1800	Sharp	Carbonyl/ Carboxyl group	C=O (Stretch)	Samanta *et al*. (2011)
1648	1640-1800	Sharp	Carbonyl/ Carboxyl group	C=O (Stretch)	Samanta *et al*. (2011)

1601	1550-1640	Sharp	Primary, Secondary Amine and Amida	N-H (Bend)	Shoukat and Yoo (2018)
1510	1510	Sharp	Methyl	C-H (CH_3) (Bend)	Hamdani and Haryanto (2021)
1247	1150-1270	Sharp	Carbonyl esters	C=O	Lingegowda *et al*. (2013)
1099	1050-1150	Sharp	Amine	C-N (Stretch)	Kommuri *et al*. (2018)
1033	1030-1050	Sharp	Amine	C-N (Stretch)	Zhang *et al*. (2013)

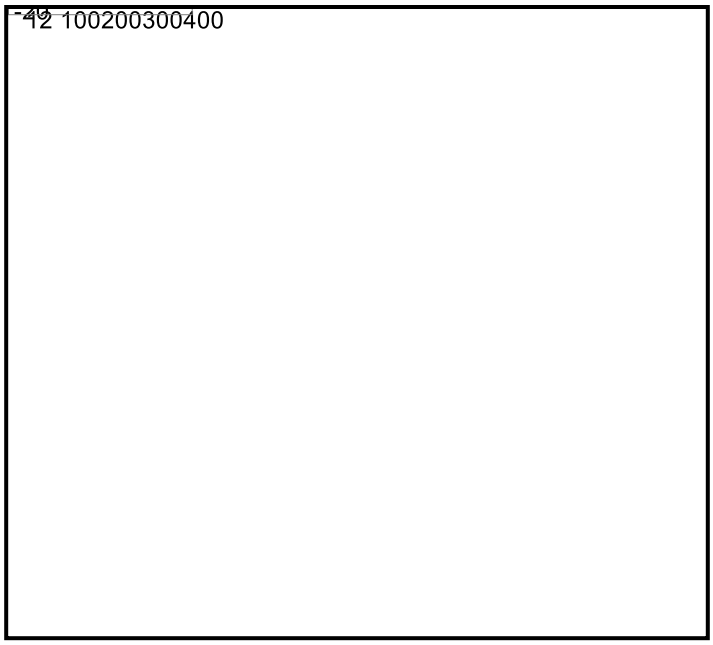
Gas Chromatography–Mass Spectrometry (GCMS) Test

The GCMS test results in Figure 2 and Table 4 show that the insecticide Lannate® 25 WP contains methomyl. It is known from the resulting peaks of 88.0 m/z and 105 m/z which are characteristic of methomyl. This result is supported by Tsatsakis et al. (1996), Methomyl has peaks 58.0 m/z, 88.0 m/z, 105.0 m/z.

Figure 2. GCMS test result of insecticide Lannate® 25 WP. A) Mass spectrum of the sample; B) Ethanimidothioic acid, N-hydroxy-, methyl ester; Methomyl; and a-(Methylthio) acetamide from GCMS Database Library (LPPT Unit 2, LAKFIP, UGM, Yogyakarta, 2023)

A

B



Based on Table 4, the dominant compound is seen from the area (%) it has so it is known that the methomyl content contained in Lannate® 25 WP is 32.55%. This result is supported by Kartika et al. (2018), that the area shows the abundance of a component. This is also appropriate Satioko et al. (2013), GCMS results for a yeast dose sample of 75 g showed an ethanol peak at 2.199 minutes with an area of 85.22%. From the GCMS test, bagasse contains the best ethanol content of 85.22%.

Table 4. The dominant compound content in Lannate® 25 WP insecticide GCMS test results

No	Retention Time (min)	Hit #	Chemical Formula	Mol. Weight	Rel. Area (%)
1.	9,35	1: Ethanimidothioic acid, N-hydroxy-, methyl ester	C_3H_7NOS	105	32.55
		2: Methomyl	$C_5H_{10}N\ 2\ O_2S$	162	
		3:a-(methylthio)acetamide	C_3H_7NOS	105	

All compounds detected at the same retention time constitute a single compound. This is because the GCMS tool consists of GC and MS tools. The GC tool will separate compounds using high temperatures so that the compounds will experience the release of several bonds from their initial chemical structure followed by the release of electrons and a decrease in

molecular weight. Furthermore, the MS tool will detect these compounds based on their molecular weight.

Blood profile

Erythrocytes

Tilapia experienced stress due to hypoxia, after the first exposure (day 4), characterized by increased erythrocytes as the concentration of insecticide increased. After 7 exposures, hypoxic stress still occurred in treatment C (6.2 mg/L), marked by an increase in hematology (Table 5). However, the erythrocytes values obtained in all treatments were still in the normal category. This is appropriate Dianti et al. (2013), The number of erythrocytes in tilapia generally ranges from 1.05 - 3.0 × 106 cells/mm-3.

Table 5. Erythrocytes measurement data

Treatment (mg/L)	Erythrocytes (× 10^6 cells/mm^{-3})	
	Day-4	Day-28
A (1.8)	1.30±0.04[b]	1.20±0.02[b]
B (4.015)	2.12±0.02[c]	1.62±0.04[c]
C (6.2)	2.65±0.03[d]	2.68±0.02[d]
K (Control)	1.14±0.04[a]	1.15±0.03[a]

Remarks: Different letter symbols in columns and rows show a noticeable difference (P<0.05)

The results of this study are supported by Bangsa et al. (2015), that fish seek to increase the number of erythrocytes back to normal conditions to reduce the level of stress experienced and to adjust their physiological conditions. Erythrocytes are the most numerous cells. Saparuddin (2019), stated that as the number of erythrocytes increased, the oxygen absorption activity by the erythrocytes increased. The body of the fish compensates if there is a change that causes the fish to experience a lack of oxygen and to overcome this the fish will increase the number of erythrocytes. The increase in red blood cells is an effort of fish body homeostasis in an effort to increase hemoglobin to bind oxygen. Stressed fish will experience hematopoiesis (an increase in blood cells) so that their erythrocytes increase as an effort to adjust to the addition of oxygen (Samsisko et al., 2014).

Hemoglobin

Tilapia experienced stress due to hypoxia, after the first exposure (day 4), characterized by an increase in hemoglobin as the concentration of insecticide increased. After 7 exposures, hypoxic stress still occurred in treatment C (6.2 mg/L), marked by an increase in hemoglobin and not in the normal category (Table 6). This is appropriate Salasia et al. (2001), Normal hemoglobin levels in tilapia range from 5.05 - 8.33 G%.

Table 6. Data on hemoglobin measurements

Treatment (mg/L)	Hemoglobin (G %)	
	Day-4	Day-28
A (1.8)	6.60±0.36[a]	5.57±0.40[a]
B (4.015)	7.53±0.45[b]	6.60±0.36[b]
C (6.2)	8.63±0.32[c]	9.40±0.53[c]
K (Control)	5.93±0.40[a]	6.00±0.50[ab]

Remarks: Different letter symbols in columns and rows show a noticeable difference (P<0.05)

The results of this study are supported by Uyun and Indriawati, (2013) and Lavabetha et al. (2015), that hypoxia can stimulate the formation of new red blood cells into the blood and cause an increase in Hemoglobin (Hb) levels to meet tissue oxygen needs in order to maintain its life. This is because Hb is contained in the erythrocytes.

Hematocrit

Tilapia experienced stress due to hypoxia, after the first exposure (day 4), marked by an increase in hematocrit as the concentration of insecticide increased. After 7 exposures, hypoxic stress still occurred in treatment C

(6.2 mg/L), marked by an increase in hematocrit (Table 7). However, the hematocrit values obtained in all treatments were still in the normal category. This is appropriate Nur et al. (2018), If the hematocrit value of fish is less than 22%, anemia is indicated, whereas if the hematocrit value is greater than 60%, it indicates that the fish is in a state of stress.

Table 7. Data on hematocrit measurements

Treatment (mg/L)	Hematocrit (%)	
	Day-4	Day-28
A (1.8)	22.67±1.53^{a}	22.33±1.53^{a}
B (4.015)	23.00±1.00^{a}	22.67±1.53^{a}
C (6.2)	35.00±1.00^{b}	37.67±1.53^{b}
K (Control)	22.33±1.53^{a}	23.00±1.00^{a}

Remarks: Different letter symbols in columns and rows show a noticeable difference (P<0.05)

The results of this study are in accordance with Uyun dan Indriawati (2013) and Lavabetha et al. (2015), the onset of hypoxia causes stimulation of the formation of new red blood cells into the blood and causes an increase in Hb levels followed by hematocrit to meet tissue oxygen needs in order to maintain its life. According to Royan et al. (2014), a hematocrit value of 20% means that the blood contains 20% red blood cells. The results of this study can be strengthened by Benfey dan Biron (2000), that fish seek to increase the number of erythrocytes back to normal conditions to reduce the level of stress experienced and to adjust their physiological conditions.

Leukocytes

Tilapia experienced stress due to hypoxia, after the first exposure (day 4), characterized by an increase in leukocytes as the concentration of insecticide increased. After 7 exposures, hypoxic stress still occurred in treatment C (6.2 mg/L), marked by an increase in leukocytes (Table 8). However, the leukocytes values obtained in all treatments were still in the normal category. This is appropriate Dianti et al. (2013), The number of leukocytes in tilapia generally ranges from 2×104-15×104 cells/mm-3.

Table 8. Leukocytes measurement data

Treatment (mg/L)	Leukocytes (× 10^4 cells/mm^{-3})	
	Day-4	Day-28
A (1,8)	2,34±0,26^{ab}	2,27±0,15^{a}
B (4,015)	3,00±0,23^{b}	2,66±0,26^{a}
C (6,2)	4,06±0,19^{c}	4,36±0,38^{b}
K (Control)	2,21±0,57^{a}	2,23±0,26^{a}

Remarks: Different letter symbols in columns and rows show a noticeable difference (P<0.05)

It is supported by Abdel-Aziz et al. (2010) and Chouw et al. (2019), Fish kidney consists of two parts, namely the head kidney and vertebrae kidney. Head kidney functions as a hematopoietic tissue, which plays a role in the formation of blood such as erythrocytes, leukocytes, platelets and so on. Stress conditions can reduce the health of fish as happened in this study. This is reinforced by Ni et al. (2016), more leukocytes are produced if the body's condition is sick. Leukocytes play a key role in the immune system and are especially important under stressful conditions. It can also be strengthened by Dianti et al. (2013), The increase in total leukocytes in fish is caused by the entry of foreign compounds into the body which causes the fish to experience stress, so that the total leukocytes in the fish's body increases. Examples of foreign compounds are pesticides or insecticides. It is supported by Samsisko et al. (2014), The increase in the number of leukocytes is called leukocytosis which is an "ephinephrine" reaction in which neutrophils and lymphocytes are mobilized into the general circulation thereby increasing the total number of leukocytes.

Water quality

The results of water quality measurements including temperature, pH and dissolved oxygen (DO) in each rearing medium were within the range of values appropriate for tilapia life. The results of observations of water quality during the study can be seen in Table 9.

Table 9. Water quality during the study

No	Parameters	Range of parameters during the study	Range of research results	Reference
1	Temperature (°C)	24-26	22-30	(Muarif, 2016)
2	Ph	6.50-8.00	6-9	(SNI, 2009)
3	Dissolved oxygen (ppm)	4-5	≥ 3 mg/L	(SNI, 2009; Pramleonita *et al*., 2018)

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. In addition, the range of temperature and pH observations in this study is included in the solubility range of methomyl in aquatic environments. Solubility in water means that there is a mechanism for decreasing the status and concentration of a core (active) compound as a result of reacting with water resulting in a decomposition process into other compounds or another compound is formed, namely methomyl to acetonitrile. According to Benning (1978) and World Health Organization (2002), Acetonitrile as a decomposition product of the active ingredient methomyl was detected on the 3rd day after the pesticide with the active ingredient methomyl entered the waters. Therefore, there is a role for acetonitrile in the degradation of gill tissue and the effects of stress.

The results of this study are in accordance with Fang et al. (2019), related to the solubility of methomyl, which can be dissolved at a water temperature of 20-30 ºC. According to Srikhaow et al. (2022), that methomyl has solubility at the pH of the aquatic environment, namely 6, 7 and 8. Based on these data, stress on O. niloticus is caused by exposure to the insecticide with the active ingredient methomyl in rearing water.

Conclusion

Based on FTIR and GCMS test results Lannate® 25 WP contains 32.55% methomyl. After the first exposure (day 4), tilapia experienced hypoxic stress, which was characterized by an increase in blood hematology according to the increase in insecticide concentration. After 7 exposures, hypoxic stress still occurred in treatment C, marked by an increase in hematology. It is know that the concentration of insecticide exposure of 6.2 mg/L resulted in fish experiencing severe stress up to 7 times the exposure (28 days at the end of the study).

Acknowledgement

This research is the first part of a research conducted with Sri Andayani, Nurhalisa and Afella Osa on “The Effect of Insecticides With Active Methomyl on Tilapia Strain Jatimbulan (Oreochromis niloticus)”.

Reference

Abdel-Aziz, E. S. H., Abdu, S. B. S., Ali, T. E. S., & Fouad, H. F. (2010). Haemopoiesis in the Head Kidney of Tilapia, Oreochromis niloticus (Teleostei: Cichlidae): A Morphological (Optical and

Ultrastructural) Study. Fish Physiology and Biochemistry, 36(3), 323–336. https://doi.org/10.1007/s10695-008-9297-z

Authman, M. M., Ibrahim, S. A., El-Kasheif, M. A., & Gaber, H. S. (2013). Heavy metals pollution and their effects on gills and liver of the Nile Catfish inhabiting El-Rahawy Drain, Egypt. Glob. Vet., 10, 103–115.

Bangsa, P. C., Sugito, Zuhrawati., R., Daud., N., Asmilia, & Azhar. (2015). Pengaruh peningkatan suhu terhadap jumlah eritrosit ikan Nila (Oreochromis niloticus). Jurnal Medika Veterinaria, 9(1), 9–11.

Benfey, T. J., & Biron., M. (2000). Acute stress response in triploid Rainbow Trout (Oncorhynchus Mykiss) and Brook Trout (Saloelinus Fontinalis). Aquaculture, 184, 167–176.

Benning, D. S. (1978). Special Scientific Report. U.S. Fish and Wildlife Service.

Blaxhall, P. C., & Daisley, K. W. (1973). Routine haematological methods for use with fish blood. Journal of Fish Biology, 5(6), 771–781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Chau, A. S. Y., & Afghan, B. K. (2020). Anal of Pest in Water Anal Nitrogen Cont Pest (A. S. Y. Chau & B. K. Afghan (eds.)). CRC Press.

Chinnamani, S., Mageswari, M., Murugaian, P., & Sivasuriyan, S. (2018). Hamatological Response in a Freshwater Fish Channa striatus Exposed to Endosulfan Pesticide. International Journal of Zoology and Applied Biosciences, 3(4), 344–348.

Chouw, A., Putera, B. W., Sartika, C. R., Diantini, A., Djuwantono, T., Faried, A., Dewi, D. A. P., & Riswandani, J. (2019). Pengaruh Usia Ibu Hamil terhadap Jumlah Sel Punca Hematopoietik dan Very Small Embryonic-like Stem Cell pada Darah Tali Pusat. Indonesian Journal of Clinical Pharmacy, 8(2), 114–120. https://doi.org/10.15416/ijcp.2019.8.2.114

Dianti, L., Prayitno, S. B., & Ariyati, R. W. (2013). Ketahanan nonspesifik ikan mas (Cyprinus carpio) yang direndam ekstrak daun jeruju (Acanthus ilicifolius) terhadap infeksi bakteri Aeromonas hydrophila. Aquaculture Management and Technology, 2(4), 63–71. http://ejournal-s1.undip.ac.id/index.php/jfpik

Docan, A., Cristea, V., Grecu, L., & Dediu, L. (2010). Haematological response of the European catfish, Silurus glanis reared at different densities in “flowthrough” Production system. Archiva Zootechnica, 13(2), 63-70.

Fang, L., Song, C., Zhang, J., Zhang, S., Zhang, C., Chen, J., & Meng, S. (2019). Effects of Multiple Environmental Factors on the Elimination of Methomyl in Aquaculture Water. Aquaculture Environment Interactions, 11, 213–220. https://doi.org/10.3354/aei00304

Haider, H. H., & Kata, F. S. (2020). The Protective Role of Ascorbic Acid in the Reproductive Functions of Female Laboratory Mice Treated with Methomyl Pesticide. Journal of Basrah Researches, 46(2), 89–102.

Hamdani, A. H., & Haryanto, A. D. (2021). The Face Mask Waste Recycling Generated During Covid-19 Pandemic In Indonesia. Journal Of Geological Sciences And Applied Geology, 5(2), 31–34. http://journal.unpad.ac.id/gsag/article/view/34996

Hotmian, E., Suoth, E., Fatimawali, F., & Tallei, T. (2021). Analisis GC-MS (Gas

Chromatography - Mass Spectrometry) Ekstrak Metanol Dari Umbi Rumput Teki (Cyperus rotundus L.). Pharmacon, 10(2), 849–856. https://doi.org/10.35799/pha.10.2021.34034

Idzni, S. A., Junardi, & Rousdy, W. (2018). Kadar Hematokrit dan Hemoglobin Ikan Sapu-sapu (Pterygoplichthys pardalis) Terpapar Logam Berat Merkuri Klorida. Jurnal Protobiont, 7(3), 68–71. https://doi.org/10.26418/protobiont.v7i3.29085

Ihsan, T., Edwin, T., & Anggraeni, W. (2018). Behavioral responses of Nile tilapia (Oreochromis niloticus) by sublethal exposure to chlorpyrifos: a case study in Twin Lakes of West Sumatra. Environmental Health Engineering and Management, 5(4), 205–210. https://doi.org/10.15171/ehem.2018.28

Irene, C., Eddiwan, E., & Windarti, W. (2021). Study Of Gill, Kidney And Liver Structure Of Pangasius Hypophthalmus In The Tanjung Kudu Lake And Sail Rivers, Riau Province. Asian Journal of Aquatic Sciences, 4(2), 134–143. https://doi.org/10.31258/ajoas.4.2.134-143

Iskandar, R., & Elrifadah. (2015). Pertumbuhan dan Efisiensi Pakan Ikan Nila (Oreochromis niloticus) yang diberi pakan buatan berbasis kiambang. ZIRAA'AH, 40(1), 18–24.

Islamy, R. A., Yanuhar, U., & Hertika, A. M. S. (2017). Assessing the Genotoxic Potentials of Methomyl-based Pesticide in Tilapia (Oreochromis niloticus) Using Micronucleus Assay. The Journal of Experimental Life Sciences, 7(2), 88–93. https://doi.org/10.21776/ub.jels.2017.007.02.05

Kartika, F. A. C., & Proborini, W. D. (2018). Analisa Komposisi Minyak Atsiri Kulit Jeruk Manis Hasil Ekstraksi Metode Microwave Hydrodiffusion and Gravity Dengan Gc-Ms. Reka Buana, 3(1), 53–58. https://doi.org/10.33366/rekabuana.v3i1.918

Kommuri, P. K., Mugada, N., & Kondamudi, R. B. (2018). Fourier Transform Infrared (Ftir) Spectroscopy Study of Spiny Lobsters From Visakhapatnam Coast, Andhra Pradesh, India. International Research Journal Of Pharmacy, 9(12), 96–99. https://doi.org/10.7897/2230-8407.0912300

Kongphonprom, K., & Burakham, R. (2016). Determination of Carbamate Insecticides in Water, Fruit, and Vegetables by Ultrasound-Assisted Dispersive Liquid-Liquid Micro Extraction and High-Performance Liquid Chromatography. Anal. Lett., 49(6), 753–767.

Lavabetha, A. R. R. R., Hidayaturrahmah, Muhamat, & Budi, S. H. (2015). Profil Darah Ikan Timpakul. Journal of Bioscientiae, 12(1), 78–89. http:/fmipa.unlam.ac.id/bioscientiae

Lee, L. C., Liong, C. Y., & Jemain, A. A. (2017). A Con- temporary Review on Data Preprocessing (DP) Practice Strategy in ATR-FTIR Spectrum. Chemometrics and Intelli- Gent Laboratory Systems, 163, 64–75. https://doi.org/doi:https://doi.org/10.1016/j.chemolab.2017.02.008

Lestari, W. P., Wiratmini, N. I., & Dalem, R. A. A. G. (2018). Struktur Histologi Insang Ikan Mujair (Oreochromis mossambicus L.) Sebagai Indikator Kualitas Air Lagoon Nusa Dua, Bali. Simbiosis, 6(2), 45–49. https://doi.org/10.24843/jsimbiosis.2018.v06.i02.p03

Lin, Z., Zhang, W., Pang, S., Huang, Y., Mishra, S., Bhatt, P., & Chen, S. (2020).

Current Approaches to and Future Perspectives on Methomyl Degradation in Contaminated Soil/Water Environments. Molecules, 25(3), 1–16. https://doi.org/10.3390/molecules25030738

Lingegowda, D. C., Kumar, J. K., Prasad, A. G. D., Zarei, M., & Gopal, S. (2013). Ftir Spectroscopic Studies On Cleome Gynandra – Comparative Analysis Of Functional Group Before And After Extraction. Romanian Journal of Biophysics, 22(3–4), 137–143.

Muarif, M. (2016). Karakteristik Suhu Perairan Di Kolam Budidaya Perikanan. Jurnal Mina Sains, 2(2), 96–101. https://doi.org/10.30997/jms.v2i2.444

Murti, C. R. K., & Matsumura, F. (2012). Biodegradation of Pesticides (C. R. K. Murti & F. Matsumura (eds.); I). Springer US. https://doi.org/10.1007/978-1-4684-4088-1

Ni, M., Wen, H. S., Li, J., Chi, M., Bu, Y., Ren, Y., Zhang, M., Song, Z., & Ding, H. (2016). Effects of stocking density on mortality, growth and physiology of juvenile Amur sturgeon (Acipenser schrenckii). Aquaculture Research, 47(5), 1596–1604. https://doi.org/10.1111/are.12620

Nur, I., Asnani, A., & Ma'ruf, K. (2018). Pengaruh Suplementasi Kitin Dari Kulit Udang terhadap Profil Darah Ikan Mas (Cyprinus carpio). JSIPi (Jurnal Sains Dan Inovasi Perikanan) (Journal of Fishery Science and Innovation), 2(2), 69–75. https://doi.org/10.33772/jsipi.v2i2.7576

Parihar, D., Kumar, S., Kumar, S., & Bhati, V. (2018). FTIR analysis by application of organic nutrients in sativa (Stevia rebaudiana Bert.). International Journal of Chemical Studies, 6(3), 1927–1930.

Prabowo, R., & Subantoro, R. (2012). Kualitas Air dan Beban Pencemaran Pestisida di Sungai Babon Kota Semarang. Mediagro, 8(1), 9–17.

Pramleonita, M., Yuliani, N., Arizal, R., & Wardoyo, S. E. (2018). Parameter Fisika Dan Kimia Air Kolam Ikan Nila Hitam (Oreochromis niloticus). Jurnal Sains Natural Universitas Nusa Bangsa, 8(1), 24–34.

Royan, F., Rejeki, S., & Haditomo, A. H. C. (2014). Pengaruh Salinitas Yang Berbeda Terhadap Profil Darah Ikan Nila (Oreochromis niloticus). Journal of Aquaculture Management and Technology, 3(2), 109–117. http://ejournal-s1.undip.ac.id/index.php/jam

Salasia, S. I. O., Sulanjari, D., & Ratnawati, A. (2001). Studi Hematologi Ikan Air Tawar. Biologi, 2(12), 710–723.

Samanta, A., Ojha, K., & Mandal, A. (2011). Interactions between acidic crude oil and alkali and their effects on enhanced oil recovery. Energy and Fuels, 25(4), 1642–1649. https://doi.org/10.1021/ef101729f

Samsisko, R. L. W., Suprapto, H., & Sigit, S. (2014). Respon hematologis ikan kerapu Tikus (Cromileptes Altivelis) pada suhu media pemeliharaan yang berbeda. Journal Of Aquaculture And Fish Health, 3(1), 36–43.

Saparuddin. (2019). Respon hematologi ikan Nila (Oreochromis niloticus) pada suhu pemeliharaan yang berbeda. Jurnal Matematika, Sains, Dan Pembelajaannya, 5(2), 121–126.

Satioko, T. R., Wahyuni, S., & Santoso, N. B. (2013). Pemanfaatan Bagas Limbah Pabrikgula Jatibarang Brebes Menjadi Bioetanol. Indonesian Journal of Chemical Science, 2(3), 207–211.

http://journal.unnes.ac.id/sju/index.php/edaj

Senthilkumar, S., & Rajendran, A. (2017). Synthesis, Characterization and Electrical Properties of Nano Metal and Metal-Oxide Doped with Conducting Polymer Composites by in-Situ Chemical Polymerization. MOJ Polymer Science, 1(6), 1–4. https://doi.org/10.15406/mojps.2017.01.00031

Shoukat, T., & Yoo, P. J. (2018). Rheology of asphalt binder modified with 5W30 viscosity grade waste engine oil. Applied Sciences (Switzerland), 8(7), 1–18. https://doi.org/10.3390/app8071194

SNI. (2009). Produksi Ikan Nila (Oreochromis nilotocus bleeker) Kelas Pembesaran Di Kolam Air Tenang.

Srikhaow, A., Chaengsawang, W., Kiatsiriroat, T., Kajitvichyanukul, P., & Smith, S. M. (2022). Adsorption Kinetics of Imidacloprid, Acetamiprid and Methomyl Pesticides in Aqueous Solution onto Eucalyptus Woodchip Derived Biochar. Minerals, 12(5). https://doi.org/10.3390/min12050528

Subayu, N., Andayani, S., Fadjar, M., & Fahrurrozi, A. (2021). Analysis of the content of secondary metabolites using Uv-vis and Ftir Spectrophotometry from the Methanol extract of Rhizophora mucronata Leaves. Eco. Env. & Cons., 27, 76–78.

Sulaiman, P. S., Rachmawati, P. F., Puspasari, R., & Wiadnyana, N. N. (2020). Upaya Pencegahan Dan Penanggulangan Kematian Massal Ikan Di Danau Dan Waduk. Jurnal Kebijakan Perikanan Indonesia, 12(2), 59–73. https://doi.org/10.15578/jkpi.12.2.2020.59-73

Suramana, T., Sindhuphak, R., Dusitsin, N., Posayanonda, T., & Sinhaseni, P. (2001). Shift in FTIR spectrum patterns in methomyl-exposed rat spleen cells. Science of the Total Environment, 270(1–3), 103–108. https://doi.org/10.1016/S0048-9697(00)00790-7

Suryono, C. A., Rochaddi, B., & Irwani, I. (2016). Kajian Awal Kontaminasi Pestisida Organoklorin dalam Air Laut di Wilayah Perairan Paling Barat Semarang. Buletin Oseanografi Marina, 5(2), 101–106. https://doi.org/10.14710/buloma.v5i2.15728

Taufik, I. (2011). Pencemaran Pestisida Pada Perairan Perikanan. Media Akuakultur, 6(1), 69–75.

Tsatsakis, A. M., Tsakalof, A. K., & Michalodimitrakis, E. N. (1996). The analysis of methomyl, a carbamate pesticide, in post-mortem samples. Science and Justice - Journal of the Forensic Science Society, 36(1), 41–45. https://doi.org/10.1016/S1355-0306(96)72553-4

Tyas, N. M., Batu, D. T. F. L., & Affandi, R. (2016). Uji Toksisitas Letal Cr6+ Terhadap Ikan Nila (Oreochromis niloticus). Jurnal Ilmu Pertanian Indonesia, 21(2), 128–132. https://doi.org/10.18343/jipi.21.2.128

Uyun, H. F., & Indriawati, R. (2013). Pengaruh lama hipoksia terhadap angka eritrosit dan kadar hemoglobin Rattus norvegicus Effect of Hypoxia Duration to the Erythrocyte and Hemoglobin Rattus norvegicus. Mutiara Medika, 13(1), 49–54.

Wartono, W., Suwignyo, R. A., Napoleon, A., & Suheryanto, S. (2018). Insecticides Residue in the Centre of Paddy Field in

Musi Rawas, South Sumatera, Indonesia. E3S Web of Conferences, 68(04014), 1–6. https://doi.org/10.1051/e3sconf/20186804014

Wismaningsih, E. R., & Oktaviasari, D. I. (2016). Identifikasi Jenis Pestisida dan Penggunaan APD pada Petani Penyemprot di Kecamatan Ngantru Kabupaten Tulungagung. Jurnal Wiyata, 3(1), 100–105. http://ojs.iik.ac.id/index.php/wiyata/article/view/77

World Health Organization. (2002). Pesticide Residues in Food (Part I). World Health Organization.

Zhang, J., Wang, X., Zhang, S., Gao, Q., & Li, J. (2013). Effects of melamine addition stage on the performance and curing behavior of melamine-urea-formaldehyde (MUF) resin. BioResources, 8(4), 5500–5514. https://doi.org/10.15376/biores.8.4.5500-5514.