Assessing the Genotoxic Potentials of Methomyl-based Pesticide in Tilapia (Oreochromis niloticus) Using Micronucleus Assay

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Abstract
Pesticides are recognized as serious pollutants in the aquatic environment with the potential to cause genotoxic on the aquatic organism, especially fish. The micronucleus (MN) assay has been used to evaluate genotoxicity of many compounds in polluted ecosystems such pesticides. im of this study to determine genotoxic effect of methomyl-based pesticide on tilapia (Oreochromis niloticus). Fish were exposed to six different concentrations base on range finding test (0 ppm, 3.2 ppm, 4.2 ppm, 6.5 ppm, 8.7 ppm and 10 ppm) of methomyl-based pesticide. The micronucleus were collected from peripheral blood erythrocyte of fish after 96 h exposure. Peripheral blood samples smears were stained with Giemsa, MN frequencies were counted and statistically analyzed using one-way ANOVA. The result of this study showed after 96 hours exposed to methomyl-based pesticide, at concentration 0 ppm causes 0% mortality, at concentration to 3.2 ppm causes 30% mortality, at concentration 4.2 ppm causes 60% mortality, at concentration 6.5 ppm causes 70% mortality, at concentration 8.7 ppm causes 80% mortality, at concentration 10 ppm causes 100% mortality of fish test. Lethal Concentration 50 (LC50 - 96 hours) of methomy-base pesticide towards tilapia (O. niloticus) is 4.015 ppm. Through micronuclei assay during 96 hour exposure of methomyl-based pesticide, the result shows that frequencies of micronuclei in erythrocyte of fish test at concentration at 0ppm is 12‰, 18‰ and 16‰; at concentration at 3.2ppm is 33‰, 26‰ and 29‰; at concentration at 4.2ppm is 41‰, 38‰ and 46‰; at concentration at 6.5ppm is 68‰, 81‰ and 82‰; at concentration 8.7ppm is 133‰, 130‰ and 137‰; at concentration 10ppm is 163‰, 166‰ and 156‰. It revealed that methomyl-based pesticide exposure induced after 96 h significantly (P<0.05) increased genotoxic potentials simultaneous with increased concentration.

Keywords: Genotoxic, Methomyl, Micronucleus Assay, Pesticide, Tilapia.

INTRODUCTION
Pesticides are widely used throughout the world in agriculture to protect crops and in public health to control diseases[1]. Although a type of pesticide aimed to turn off a group or species specific targets, but in substance is poisonous against all of an organism either organism target and non target as in fishes [2].

Methomyl (C5H10N2O2S), S-methyl-1-N-[[methylcarba-moyl]-oxy]-thioacetimidate, is an carbamate pesticides which widely used in many agricultural countries to protect crops or plant against insects because of its broad biological activity, relatively rapid disappearance and high efficiency [3,4]. The entry of the pesticides remains as methomyl into the agricultural irrigation will pollute the environment [5]. Several study about consequences of the methomyl pesticide exposure to fish has done. Where certain concentration exposure of methomyl pesticide can cause acute poisoning to death [6].

Exposure of organisms to xenobiotics such as pesticides, insecticides, herbicides and other synthetic materials is a serious matter in environmental and toxicological chemistry. Cypermethrin, as one of insecticides, is highly toxic to fish and aquatic invertebrates [7].

Fish is common aquatic animal that provide a good model for monitoring the toxicity of pesticide such methomyl in aquatic systems because they are extremely sensitive to pollutants, have the ability to metabolize xenobiotics and exhibit a very high bioaccumulation rate of dissolved chemicals relative to their concentration. Moreover, Fish also used for the potential of mutagenic and carcinogenic study of pesticide contaminants present in aquatic [8-11].

In present study, Micronucleus test in fish erythrocyte used as a sensitive indicator for evaluation and assessment of the genotoxic potential environment [12]. Micronuclei is a secondary nucleus that smallest than primary nucleus.
Appearance micronucleus can used as indicator of genotoxic activity in fish body [13,14]. Moreover, occurrence of micronucleus in blood erythrocyte can used as of cellular deviation and other genetic damage because of pesticides exposure [15].

In this study, we choose Tilapia as animal test because of their potential for future aquaculture [16]. They are hardy, prolific and fastgrowing tropical fishes, low on the food chain, and adaptable to all kind environment [17]. Major producers of Tilapia are developing countries, including China, Indonesia, Philippines, Thailand, Honduras, Ecuador and Costa Rica. The main objective of this study was to determine effect of methomyl-based pesticide at dependent doses against micronucleus frequency on erythrocyte of Tilapia (O. niloticus).

MATERIALS AND METHODS

Treatment Preparation

Materials

Methomyl-based pesticide was purchased from agriculture market in Batu, East Java, Indonesia as Lannate 25 WP. In this work, Tilapia fish (±9-12cm) were purchased from Technical Application Unit of Freshwater Fish (UPT Perikanan Air Tawar), Sumberpasir, Malang, East Java.

Acclimatization

The fishes were held in tank and fed with commercial feed once per day. After 14 days holding periode, fishes were classified into 6 group of 10 fishes, then trasfered and acclimatized into aquarium with aeration system (size 60x30x25cm) for 2 days. If less that 3% of fish population are dead during 48 hours, its mean the Tilapia population treatment that will be considered worthy for testing. But if over than 3% of fish population are dead, the fish should replaced with the new fish from holding tank then reacclimatized for 2 days.

Critical Range Test

This test was conducted to determine the upper range (N) and the bottom range (n) of methomyl-based pesticide on fish test. This section was conducted 96 hours by observed the fish test mortalitas level. The used concentration of methomyl-based based on Guthrie and Perry method [18].

Definitive Test

Devinitive test carried out to determine methomyl-based pesticide concentration that cause 50% mortality of fish population (LC50). Based on critical range test, the concentration of methomyle-base pesticide was 0 ppm, 3.2 ppm, 4.2 ppm, 6.5 ppm, 8.7 ppm dan 10 ppm. The concentration was modified from progressive concentration table of Bowman dan Rand [19] with 96 hours exposure.

Data Collection

Micronuclei Assay

After 96 hours exposure of methomyl-based pesticide, erythrocyte blood from each fish group was sampled and smeared on clean microscope slides. After fixation in absolute methanol for about 20 min, the slides were air-dried and stained with 10% of giemsa for about 25 minutes. Six slides of 1.000 erythrocyte that sampled from each Tilapia (O. niloticus) were scored [20], observed and coding by using microscope (Olympus CX21) with 400X magnification to determine the frequency of micronucleus cell and other different pattern of morphologically altered erythrocyte and then counted as cell per 1000 (%) [21]. The micronucleus frequency then counted base on Betancur formulation [22].

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\text{Mikronuclei Frequency} = \frac{\text{Mikronuclei} \times 1000}{\text{Total Cell Counted}}
\]

Data analysis

Data analysis in this study using probit analysis to determine the relative toxicity (LC50) of chemicals on living organisms. LC50 was base on fish level of Tilapia (O. niloticus) mortality at definitive test after 96 hours methomyl-based exposure. Moreover, the data of micronucleus was analysis using One Way Anova to determine effect of methomyl-based pesticide against current parameter response.

RESULT AND DISCUSSION

Critical Range test

Preliminary test carried out to obtain an upper range and lower range concentration of methomyl-based pesticide against Tilapia. Figure 1 show that after 96 hours exposure causes 100% mortality at concentration ≥10 ppm and 20% mortality at concentration 1 ppm. However, no mortality happened at concentration 0.1 ppm. Base on the data, concentration 0.1 ppm can be used as lower range of methomyl-based concentration and concentration 10 ppm used as upper range.

The preliminary test carried is essential to determine the limits of the range of critical concentration of methomyl-based pesticide (critical range test) against Tilapia (O. niloticus), which became the basis of the determination on the concentrations used in definitive test or toxicity tests. That concentration can cause most of the
Genotoxic Potentials of Methomyl-Base Pesticide in Tilapia (Islamy et al.)

Deaths was close to 50% and lowest mortality approach 50% [23].

**Definitive test**

Based on lower and upper range of methomyl-based pesticide on Tilapia, the variative concentration for definitive test determined based on logarithmic scale of Bowman dan Rand [19] (0 ppm, 3.2 ppm, 4.2 ppm, 6.5 ppm, 8.7 ppm and 10 ppm).

The result of definitive test (Figure 2) showed methomyl-based pesticide exposure was no fish mortality (0% population of fish test) after 96 hours exposure at concentration 0 ppm. During 96 hours methomyl-based pesticide exposure, in concentration 3.2 ppm exposure showed that mortality start happening after 6 hours exposed as much as 10% population of fish and total of fish mortality during 96 hours exposed as much as 30% of population. In concentration of methomyl-based pesticide at 4.2 ppm, fish mortality start happening after 4 hours exposed as much as 10% population of fish and total of fish mortality during 96 hours exposed as much as 60% population of fish. In concentration of methomyl-based pesticide at 6.5 ppm showed fish mortality start happening after 4 hours exposed as much as 10% population of fish test and total of fish mortality during 96 hours exposed as much as 70% population of fish. Concentration of methomyl-based pesticide at 8.7 ppm showed fish mortality start happening after 2 hours exposed as much as 10% population of fish test and total of fish mortality during 96 hours exposed as much as 80% population of fish. Concentration of methomyl-based pesticide at 10 ppm, fish-mortality start happening after 2 hours exposed as much as 10% population and total of fish mortality during 96 hours exposed as much as 100%.

During 96 hours exposed, lower mortality of Tilapia (*O. niloticus*) occurred at concentration 3.2.
ppm (30% population) and the higher occurred at concentration 10 ppm (10% population). Moreover, in each doses (3.2, 4.2, 6.5, 8.7, and 10 ppm), lower mortality occurred in beginning of methomyl-based exposure (1 hour exposed) and higher mortality occurred in the end of exposure (96 hours exposed). The data showed that toxicity of methomyl-based exposure against Tilapia increased simultaneously with increased of doses and time exposure. A chemical toxicity increased against organism simultaneously with increasing of dose and time of exposure [24].

Base on the result of probit analysis that show in Figure 3, can be find out of LC50 96 hours methomyl-based against Tilapia \((Oreochromis niloticus)\). Line equation \(Y = 2.9221x + 3.2386\) and the value of LC50 96 hours is 4.015 ppm that causes 50% death of the population of fish test.

Micronuclei assay

In the present study, the micronuclei test in fish usually based on erythrocytes and it observed that there was a basal level of measurable spontaneous micronuclei formation in \(O. mossambicus\) which was also observed in most of the fish species [11]. Micronuclei assay provide information as a simple bioindicator for chromosomal aberrations not available from other methods: (i) the consolidated effect of a variety of environmental stresses on the health of an organism, population, community, and ecosystem (ii) warning of harmful effects to human health based on the responses of wildlife to pollution, and (iii) the effectiveness of remediation efforts in decontaminating waterways [25]. Counting of micronuclei is faster and less demanding of technical than scoring of chromosomal aberrations, the micronuclei assay has been widely used for chemicals screening that cause these types of damage and also it demonstrate that micronuclei test in fish can be used for the genotoxicity assessment in environment [26]. In the present study micronuclei frequencies in the fish peripheral blood erythrocytes after 96 hours exposure in different concentration of metomyl pesticide (Fig. 4) show significant increase in micronuclei frequencies (\(p<0.05\)).

The result of micronuclei assay on fish against exposure of methomyl-base show in Figure 4. At concentration 0 ppm of methomyl-based pesticide, frequencies of micronuclei was 12‰, 18‰ and 16‰. At concentration 3.2 ppm, after 96 hours exposure showed that frequencies of micronuclei was increase up to 33‰, 26‰ and 29‰. At concentration 4.2 ppm, after 96 hours exposure showed that frequencies of micronuclei was increase up to 41‰, 38‰ and 46‰. At concentration 6.5 ppm, after 96 hours exposure showed that frequencies of micronuclei was increase up to 68‰, 81‰ and 82‰. At concentration 8.7 ppm, after 96 hours exposure showed that frequencies of micronuclei was increase up to 133‰, 166‰ and 156‰. The micronuclei frequencies in tilapia \((O. niloticus)\) erythrocyte were significantly increased (\(p<0.05\)) simultaneously increased concentration and ime of exposure of methomyl-based pesticide.
ppm. The micronucleus assay test in tilapia erythrocyte has been used for accessing genotoxic potential [27]. It also has been used for the detection of broken strand in aquatic species [28]. Figure 4 showed that genotoxic potential of methomyl-based pesticide against erythrocyte of Tilapia (O. niloticus) increased simultaneously with increased concentration. The concentrations and the exposure period of pesticide may be the reason for relatively high micronuclei frequencies recorded in pesticide treated fish. The present study also reports that dose and time dependent of some pesticide exposure (Chlorpyrifos, malathion, cypermethrin, lambda-cyhalothrin and Bucril) can increase micronuclei induction in the peripheral blood erythrocytes of fish (O. mossambicus) [27].

CONCLUSION

Through definitive test, fish test after 96 hours exposed by methomyl-based pesticide, at concentration 0 ppm causes 0% mortality, Increasing concentration to 3.2 ppm causes 30% mortality, Increasing concentration to 4.2 ppm causes 60% mortality, Increasing concentration to 6.5 ppm causes 70% mortality, Increasing concentration to 8.7 ppm causes 80% mortality, Increasing concentration to 10 ppm causes 100% mortality of fish test. Lethal Concentration 50 (LC50 - 96 hours) of methomyl-base pesticide towards Tilapia (O. niloticus) is 4.015 ppm.

Through micronuclei assay during 96 hours exposure of methomyl-based pesticide, the result show that frequencies of micronuclei in erythrocyte of fish test at concentration at 0ppm is 12%, 18% and 16%; at concentration at 3.2 ppm is 33%, 26% and 29%; at concentration at 4.2 ppm is 41%, 38% and 46%; at concentration at 6.5 ppm is 68%, 81% and 82%; at concentration 8.7 ppm is 133%, 130% and 137%; at concentration 10 ppm is 163%, 166% and 156%. The frequencies simultaneously increased with increased of concentration and time period of exposure. The increased frequencies of micronuclei in Tilapia erythrocyte meant that genotoxic potential simultaneously increased.

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