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

R Adharyan Islamy[1]*, Uun Yanuhar[2], Asus Maizar Suryanto Hertika[2]

[1]Master Program of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia
[2]Department of Aquatic Resources management, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang, Indonesia

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.

* Correspondence address:
R Adharyan Islamy
Email : r.adhariyan@gmail.com

Address : Faculty of Fisheries and Marine Sciences,
University of Brawijaya, Veteran Malang, 65145

ISSN. 2087-2852
E-ISSN. 2338-1655

Appearance micronucleus can used as indicator of genotoxic activity in fish body [13,14]. Moreover, occurance of micronucleus in blood erythrocyte can used as of cellular deviation and other genetic demage 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 environtment [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. nilotius*).

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 holded 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 conduted 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, erythrocute 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].

$$\text{Mikronuclei Frequency} = \frac{\textbf{Mikronuclei x (1000)}}{\textbf{Total Cell Counted}}$$

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

ISSN. 2087-2852
E-ISSN. 2338-1655

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

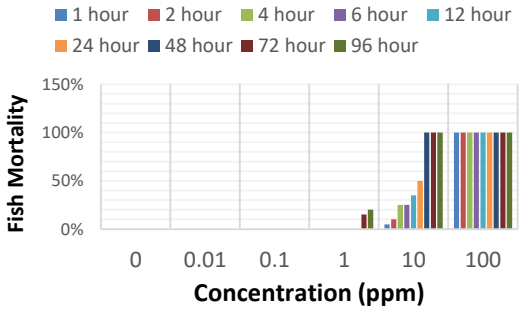

Figure 1. Fish Mortality during critical range test

Definitive test

Base on lower and upper range of methomyl-based pesticide on Tilapia, the variative concentration for definitive test determined base 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%.

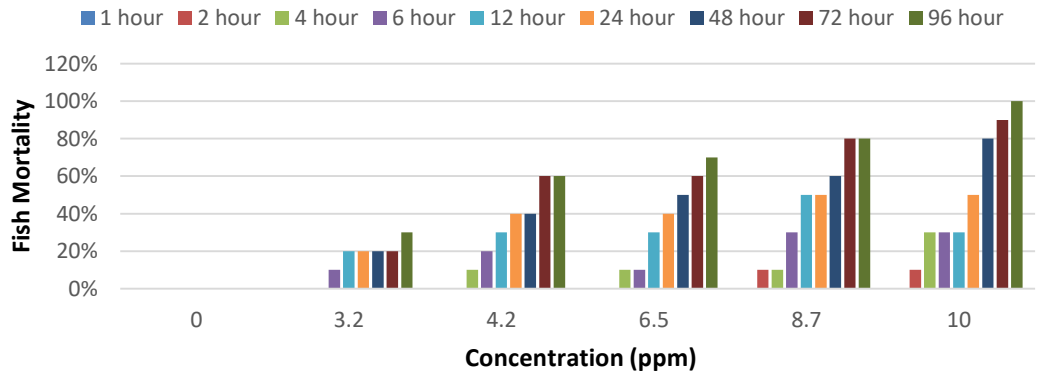

Figure 2. Percentage Mortality of Tilapia (*O. niloticus*) During the Definitive Test

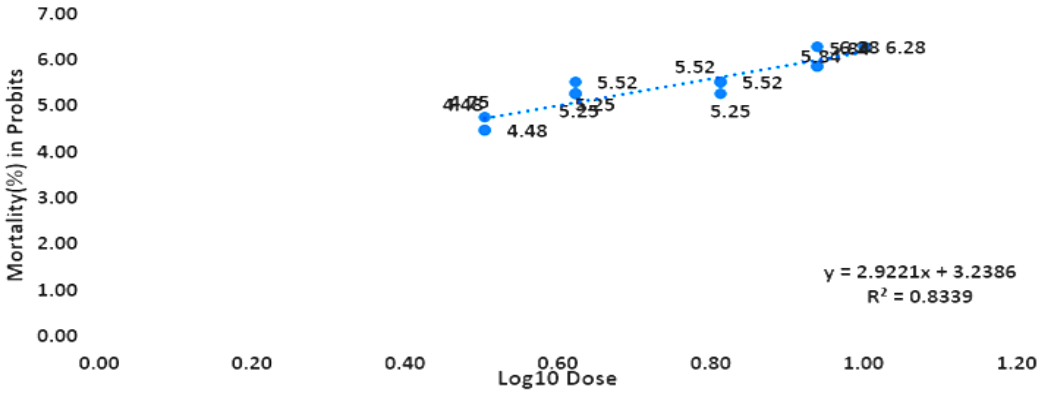

Figure 3. Probit Analysis of LC50-96 hour of methomyl-based pesticide

During 96 hours exposed, lower mortality of Tilapia *(O. niloticus)* occurred at concentration 3.2

ISSN. 2087-2852
E-ISSN. 2338-1655

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 = $\mathbf{2.9221x + 3.2386}$ 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‰, 130‰ and 137‰. At concentration 10 ppm, after 96 hours exposure showed that frequency of micronuclei was increase up to 163‰, 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.

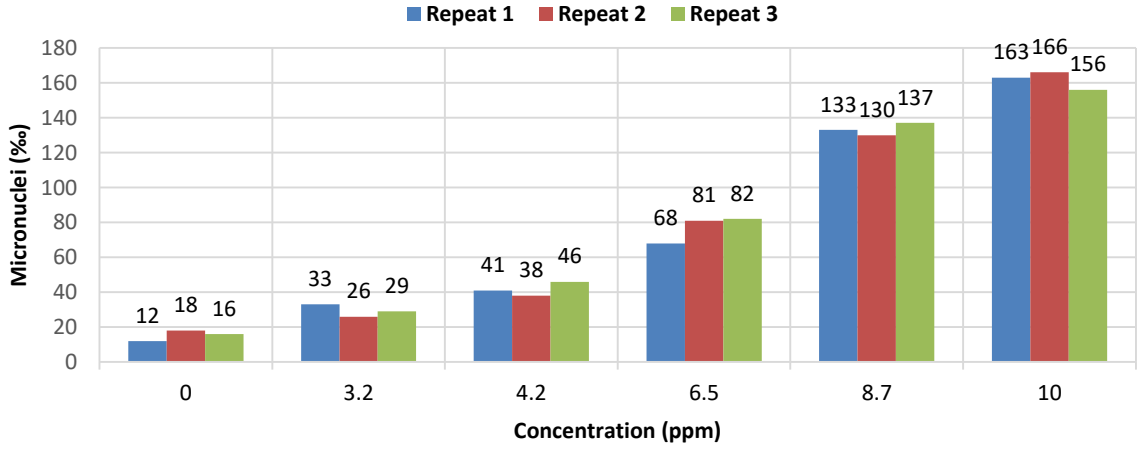

Figure 4. Micronucleus Frequency in Tilapia (*O.niloticus*) Erythrocyte

The lowest micronuclei frequencies were recorded after 96 hours exposure at concentration 0 ppm. The highest micronuclei frequencies were recorded after 96 hours at concentration 10

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 breaked 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 Buctril) 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 methomy-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 3.2ppm 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‰. The frequencies simultanously increase with increased of concentration and time periode of exposure. The increased frequencies of micronuclei in Tilapia erythrocyte meant that genotoxic potential simultaneously increased.

REFERENCES

[1] Sailaja, N., M. Chandrasekhar, P.V. Rekhadevi. 2006. Genotoxic evaluation of workers employed in pesticide production. *Mutat. Res.* 609.74-80.

[2] Supriyono, E. 2005. Studi toksisitas insektisida triklorfon terhadap ikan Nila (*O. niloticus*). Bogor Agricultural University. Bogor.

[3] WHO. 1996. Methomyl environmental health criteria 178. World Health Organization. Geneva.

[4] Meng, S.L., J.Z. Chen, G.H. Hu, C. Song, L.M. Fan, L.P. Qiu, P. Xu. 2014. Effects of chronic exposure of methomyl on the antioxidant system in liver of Nile Tilapia (*Oreochromis niloticus*). *Ecotoxicol. Environ. Saf.* 101. 1-6.

[5] El-Gawad, E.A.A., A.A. Abbass, A.A. Shaheen. 2012. Risks induced by pesticides on fish reproduction. *The Global Journal of Fisheries and Aqua. Res.* 5. 329-338.

[6] Li, H., H. Jiang, X. Gao, X. Wang, W. Qu, R. Lin, J. Chen. 2008. Acute toxicity of the pesticide methomyl on the Topmouth Gudgeon (*Pseudorasbora parva*): mortality and effects on four biomarkers. *Fish Physiol. Biochem.* 34(3). 209-216.

[7] Asztalos, B., J. Nemcsok, I. Benedeczky, R. Gabriel, A. Szabo, O.J. Refaie. 1990. The effects of pesticides on some biochemical parameters of carp (*Cyprinus carpio* L.) *Arch. Environ. Contam. Toxicol.* 19. 275-282.

[8] Da Rocha, C.A.M., L.A. da Cunha, R.H.S. Pinheiro, M.O. Bahia, R.M.R. Burbano. 2011. Studies of micronuclei and other nuclear abnormalities in red blood cells of *Colossoma macropomum* exposed to methyl mercury. *Gen. Mol. Biol.* 34. 694-697.

[9] Fazio, F., C. Faggio, S. Marafioti, A. Torre, M. Sanfilippo, G. Piccione. 2013. Effect of water quality on hematological and biochemical parameters of *Gobius niger* caught in Faro lake (Sicily). *Iran J. Fish Sci.* 12. 219-231.

[10] Fazio, F., S. Marafioti, A. Torre, M. Sanfilippo, M. Panzera, C. Faggio. 2013. Haematological and serum protein profiles of Mugil cephalus: effect of two different habitats. *Ichthyol. Res.* 60. 36-42.

[11] Al-Sabti., K, C.D. Metcalfe. 1995. Fish micronuclei for assessing genotoxicity in water. *Mutat. Res.* 343. 121-135.

[12] Nilza, L.R., M.M. Ligia, O.S. Dense, B.P. Rassia, V.N. Celso, U.N. Tania, A.F. Benicio, P.F. Benedito. 2003. Haematological and biochemical values for Nile Tilapia (*Oreochromis niloticus*) cultured in semi-intensive system. *Acta Scientiarum Biol. Sci.* 2(2). 385-389.

[13] da Silva, T., S.F. Carmem. 2006. Micronucleus test and observation of nuclear alterations in erythrocytes of Nile tilapia exposed to waters affected by refinery effluent. *Mutat. Res.* 605. 87-93.

ISSN. 2087-2852
E-ISSN. 2338-1655

[14] Bhatia, A., Y. Kumar. 2014, Relevance of microscopic indicators of chromosomal instability in routine reporting of malignancies. *Diagn. Cytopathol.* 42. 181-188.
[15] da Silva Augusto, L.G., S.R. Lieber, M.A. Ruiz, C.A. de Souza. 1997. Micronucleus monitoring to assess human occupational exposure to organochlorides. Environ. Mol. Mutagen. 29(1). 46-52.
[16] G.H. Yue, H.R. Lin, J.L. Li. 2016. Tilapia is the Fish for Next - Generation Aquaculture. International Journal of Marine Science and Ocean Technology. 3(1), 11-13.
[17] Ng WK, Romano N .2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. Reviews in Aquaculture 5(4): 220-254.
[18] Guthrie, F.E., J.J. Perry. 1980. Introduction to environmental toxicology. Elsevier. New York.
[19] Bowman, W.C., M.J. Rand. 1980. Textbook of pharmacology, 2nd Ed. Blackwell Scientific Publications. Oxford. 16.17-16.31.
[20] Osman, G.M., M. Koutb, A.H. Sayed. 2010. Use of hematological parameters to assess the efficiency of quince (*Cydonia oblonga* Miller) leaf extract in alleviation of the effect of ultraviolet -A radiation on African catfish *Clarias gariepinus*. *J. Photoch. Photo-bio. B.* 99. 1-8.
[21] Güner, U., F.D. Muranlı. 2011. Micronucleus test, nuclear abnormalities and accumulation of Cu and Cd on *Gambusia affinis* (Baird and Girard, 1853). *Turk. J. Fish Aquat. Sci.* 11. 615-622.
[22] Betancur, I.P., J.A. Palacio Baena, M.C. Guerrero. 2009. Micronuclei testapplication to wild tropical ichthyic species common in two lenticenvironments of the low zones in Colombia. *Actual Biol.* 31. 67-–77.
[23] Husni, H., M.T. Esmiralda. 2011. Acute toxicity test liquid waste know the goldfish (*Cyprinus carpio* Lin). Research report. Department of Environmental Engineering. Andalas University. Padang.
[24] Ahmed, M.K., M. Habibullah-Al-Mamun, M.A. Hossain, M. Arif, E. Parvin, M.S. Akter, M.S. Khan, M.M. Islam. 2011. Assessing the genotoxic potentials of arsenic in tilapia (*Oreochromis mossambicus*) using alkaline comet assay and micronucleus test. *Chemosphere.* 84. 143-149.
[25] Villela, I.V., I.M. de Oliveira, J. da Silva, J.A. Henriques, 2006. DNA damage and repair in haemolymph cells of golden mussel exposed to environmental contaminants. *Mutat. Res.* 605. 78-86.
[26] Dixon, D.R., A.M. Pruski, L.R.J. Dixon, A.N. Jha. 2002. Marine invertebrate ecogenotoxicology: a methodological overview. *Mutagenesis.* 17. 495-507.
[27] Naqvi, G.E-Z., N. Shoaib. M.A. Aisha. 2016. Genotoxic Potential of Pesticides in the Peripheral Blood Erythrocytes of Fish (*Oreochromis mossambicus*). *Pakistan J. Zool.* 48(6). 1643-1648.
[28] Mitchelmore, C.L., J.K. Chipman. 1998. DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. *Mutat. Res.* 399. 135-147.

ISSN. 2087-2852
E-ISSN. 2338-1655