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Detoxification enzymes changes of tilapia (*Oreochromis niloficus*) exposed to waterborne Benomyl

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Introduction

Fungicides are used on a large scale, i.e. in agricultural and horticultural practice. As a result of this use, these chemicals can contaminate the surface-water directly or indirectly. The benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, has a relatively low mammalian toxicity but it is highly to very highly toxic to fish. Numerous studies have been conducted to determine the toxicity and associated residues of benomyl and carbendazim in soils, plants and mammals. Certain toxic xenobiotics cause oxidative stress by inducing the generation of ROS and weakening the antioxidant defense system of cells. Banks and Soliman (1997) found that benomyl induces lipid peroxidation and glutathion depletion when fed to rats. Benomyl was also responsible for a time-and dose-dependent microtubular disorganization in rat primary hepatocyte cultures which was reversible, once benomyl was removed, and benomyl also acted as a potent glutathione-depleting agent (Hess and Nakai, 2000; Howlett and Schiest, 2000). However, little information has been published about the toxicity of this compound to aquatic organisms. Therefore, we determined the 96hr-LC_{50} of benomyl and investigated the detoxification enzymes alterations of tilapia (*Oreochromis niloficus*) in response to sub-lethal concentrations of benomyl under the laboratory conditions.

Materials and Methods

Tilapia (16.35±1.22 cm, 71.61±12.05 g) were obtained from a fish farm in Pukyong National University. After acclimatization, the fish were separated into four experimental lots with 10 animals each. The fishes were exposed to test solutions of the different concentration from 0.5, 1, 2, 4, 8, 16 mg L^{-1}

of benomyl for determining 96hr-LC_{50}. Thereafter, fishes were exposed to benomyl chronic test solution; 100, 200 and 400 μg L^{-1} to investigate the detoxification enzyme. At the end of each period, fish were anesthetized and examined. Hepatic microsome were prepared by homogenizing in buffer with several passes of a teflon pestle (099C K4424, Glas-Col, USA). The hemogenate was centrifuged (9,000 g for 20 min, MIKRO22R, Hettich, Germany) at 4℃, supernatant was collected, and ultra-centrifuged at 100,500g for 60 min using a Centrikon T-1190 Ultracentrifuge (Kontron Instruments, Italy) at 4℃ to obtain a cytosol. The cytosolic glutathione S-transferase (GST) activity was analyzed by the kinetic method of Habig *et al.* (1974) with CDNB (1-chloro-2,4-dinitrobenzene) as the substrate. Total superoxide-dismutase (SOD) acitivity was assayed according to the method of Elstner et al., (1983) by color reaction that was the same as in the O_2^- measurement. One enzyme unit is defined as 50% inhibition of the colorimetric reaction. All data were subjected to an analysis of variance (ANOVA) with the factors benomyl and control to determine the significant differences followed by an LSD test at 5 and 1% level.

Results

The levels tested were below the 96hr-LC_{50} of approximately 3.86 ㎎ benomyl L^{-1} for tilapia. According to other study, the 2day LC_{50} to Salmo is 0.48 ㎎ L^{-1} (Canton J. H., 1976), the 96hr-LC_{50} of blugill fry is 1.3 ㎎ L^{-1} (Donald and Charles, 1986).

A significant change of GST level in 100 μg benomyl L^{-1} group was not observed for 7 weeks. In 200 μg L^{-1} group, a significant increase was observed after 5 weeks' exposure, but, it was not significant after 7 weeks. Also, the highest group; 400 μg benomyl L^{-1} showed a significant increase until 5 weeks' exposure, but, it was not significant after 7 weeks. The GST level of rockfish exposed benomyl was affected a dose-dependent manner, but no a time-dependent manner.

In 100 and 200 μg benomyl L^{-1}, the SOD activities was not significant during total exposure period. Only, the 400 μg benomyl L^{-1} showed a significant increase of SOD after 5 weeks ($P<0.05$) and 7 weeks' exposure ($P<0.01$). The SOD level of rockfish exposed benomyl was affected a dose- and time- dependent manner.

References

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