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Antifungal Activity of Processed Sulfur Solution as Potential Eco-Friendly Disinfectant against Saprolegnia parasitica and Its Safety in Freshwater-Farmed Fish

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Abstract : Some chemicals such as malachite green, methylene blue, and copper sulfate had been used frequently as disinfectants controlling fungal infection in aquaculture. However, their carcinogenicity, mutagenicity and teratogenicity were reported in mammals. After their accumulation in food fish and its consumers was confirmed, concerns about public health has resulted in enhanced monitoring and increased demand for eco-friendly treatments. Therefore, this study aimed to evaluate safety to fish and efficacy of sulfur solution processed by effective microorganisms (EM-PSS) against Saprolegnia parasitica, for use of a potential aquatic fungicidal disinfectant. The natural sulfur purchased from Kawah Ijen volcano, East Java, Indonesia was processed by the liquid mixture consisting of following twelve effective microorganisms (Rapha-el®; Lbiotech, Jeonnam, Korea), Lactobacillus parafarraginis, L. paracasei, L. harbinensis, L. buchneri, L. perolens, L. rhamnosus, L. vaccinostercus, Acetobacter lovaniensis, A. peroxydans, Pichia fermentans, Candida ethanolica, Saccharomycopsis schoenii isolated from fermentation process of oriental medicinal herbs including green tea, privet, and puer tea. The material was applied to in vitro antifungal activity test for Saprolegnia parasitica using agar dilution method. In addition, an acute toxicity test was performed on carp (Cyprinus carpio), eel (Anguilla japonica), and mud fish (Misgurnus mizolepis) for 96 hours. After three species of fish (n=15) were accustomed to experimental water environment for three days, the EM-PSS was added to each tank as final concentrations to be 0 to 500 ppm. The fish were taken into necropsy, and the histological sections of the gill, liver, and spleen were counter-stained with hematoxylin and eosin (H-E). And hence, no observed effect concentration (NOEC) of the solution was used for taking a medicinal bath for mudfish infected by Saprolegnia parasitica in practice. The result of in vitro antifungal activity test showed the growth inhibition of the fungus at 100 ppm, which and the lower concentrations occurred no fatal case in any fish species tested until the end of the examination. The 125 ppm of the solution, however, resulted in 13.3 %, 13.3 %, and 6.3 % of mortality in carp, eel, and mudfish, respectively. But both 250 and 500 ppm of the solution leaded lethality to all population of each fish species within 24 hours. Besides, H-E staining also showed no specific evidence for toxicity in fish at lesser than 100 ppm of EM-PSS. On the other hand, as a result of field application of the solution, no growth of fungal mycelium was found in fish bodies from gross observation 5 days post treatment. In conclusion, 100ppm of EM-PSS resulted in inhibition and treatment of Saprolegnia parasitica infection. In addition, the use of EM-PSS lower than 100 ppm is safe for fish. Therefore, EM-PSS could be used as aquatic fungicide, and also may be possible to be a potential eco-friendly disinfectant in aquaculture.

Keywords : antifungal activity, effective microorganism, toxicity, saprolegnia, processed sulfur solution
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