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Determination of lethal concentration and antibacterial activity of commonly used disinfectants

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ABSTRACT

The toxic effects of four disinfectants *viz*., copper sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$), methylene blue and malachite green on fish and fish pathogenic bacteria *Aeromonas* sp., *Pseudomonas fluorescens*, *Edwardsiella* sp. and *Flavobacterium* sp. were investigated. Lethal concentration of the disinfectants to fingerlings of *Labeo rohita* was determined in aquarium by standard method. Lethal concentration of copper sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$), methylene blue and malachite green against fish were found in 0.75ppm, 7ppm, 6ppm and 0.5ppm at 21.4hrs, 18hrs, 9.5hrs and 1.40hrs, respectively. Methylene blue at 4ppm and 5ppm concentration inhibited the growth of *Pseudomonas fluorescens* and 6ppm concentration suppressed the growth of *Aeromonas* sp. Copper sulfate ($CuSO_4$) was effective only against *Edwardsiella* sp at concentration of 10ppm and 8ppm. Malachite green repressed the growth of all four tasted bacteria at a concentration of 1ppm. Potassium permanganate ($KMnO_4$) was failed to exhibit any inhibitory effect on the bacteria even at 30ppm concentration.

Keyword: Disinfectant, Lethal concentration, Fish pathogen, Antibacterial activity.

INTRODUCTION

In Bangladesh, large number of fish dies in different fish farms each year due to the outbreak of bacterial and fungal diseases [1]. The major bacterial fish diseases frequently occurred in fish farms of Bangladesh are motile Aeromonad septicaemia, bacterial haemorrhagic septicaemia, edwardsiellosis and columnaris disease (gill rot and tail and fin rot disease) which are caused by motile *Aeromonas* spp., *Pseudomonas fluorescens*, *Edwardsiella* spp. and *Flavobacterium* spp. , respectively [2, 3, 4 ,5]. As preventive and control measures of various fish diseases famers and hatchery owners use different kinds of disinfectants. Copper sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$), methylene blue and malachite green are common chemicals used for eradication of external parasites and fungal diseases in fish [6, 7]. Copper sulfate ($CuSO_4$) is an effective algaecide but it has toxic effect on many fish species. The factors influences the toxicity of copper is related to water quality characteristics such as hardness, alkalinity, pH, and dissolved organic carbon [8]. Increases of these water quality parameters result in decreased copper toxicity and subsequent increase of tolerance by fish, while higher concentrations are required to control algae [9]. Potassium permanganate ($KMnO_4$) possesses toxicity for bacteria and phytoplankton and and are generally used to detoxify fish toxins such as rotenone and antimycin [10, 11]. Methylene Blue is effective against superficial fungal infections of fishes since used against bacterial, fungal and parasite infections. It also kills plants and pond bio-filter bacteria. The drug may be used as an alternative to malachite green for the control of fungus when it is known that the fish to be treated are sensitive. Methylene blue is safe for use with fish eggs and fry for the prevention of fungal infections [12]. As a secondary use, it is effective against some external protozoan's, such as *Ichthyophthirius*, *Chilodonella* and *Costia*. Methylene blue was effective in reducing bacterial load in Tilapia (*Orechromis niloticus*) fingerlings [12]. Malachite green has been widely used for external infection of protozoan and fungal diseases since 1993 but due to its negative effect on fish and aquatic organisms yet it struggle to registered as veterinary drugs [13, 14, 15]. However, in Bangladesh the fish farmers do not know the proper doses of the disinfectants and often apply disinfectants at high doses which may lead toxic effects, even may cause mortality of fish. Unfortunately, no systematic study has yet been conducted to solve the problems. Considering the facts, the present study has been conducted to find out the toxic effects of four disinfectants viz., copper sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$), methylene blue and malachite green on fish and fish pathogenic *Aeromonas* sp., *Pseudomonas fluorescens*, *Edwardsiella* sp. and *Flavobacterium* sp. bacterial isolates.

MATERIALS AND METHODS

Determination of Lethal Concentrations to Fish:

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Table 1. LC50 value of $CuSO_4$, $KMnO_4$, Methylene blue and Malachite green for *Labeo rohita* fingerlings.

Name of the chemical	Dose (ppm)	Mortality rate (in time)	LC50
$CuSO_4$	Control	0%(24hrs)	LC50=21.40hrs
	0.25	0%(24hrs)	
	0.5	17%(18.10hrs),33%(27.40hrs)	
	0.75	33%(18.10hrs),50%(21.40hrs)	
	1	17%(9.45hrs),100%(10.15hrs)	
	2	17%(46min),100%(9.45hrs)	
	3	17%(30min),100%(9.30hrs)	
	4	17%(30min),33%(46min),100%(9hrs)	
	5	33%(30min),100%(7.50hrs)	
	10	100%(2.5hrs)	
	20	100%(1.30hrs)	
	30	100%(50min)	
$KMnO_4$	Control	0%(24hrs)	LC50=18hrs
	5	0%(24hrs)	
	6	17%(6.30hrs), 33%(12.45hrs), 50%(18hrs)	
	7	17%(5.30hrs), 33%(9.45hrs) 100%(21hrs)	
	8	100%(9.15hrs)	
	9	100%(6.30hrs)	
	10	100%(3hrs)	
Methylene blue	Control	0%(24hrs)	LC50/=.30hrs
	5	0%(24hrs)	
	6	17%(3.5hrs), 33%(5.50hrs), 50%(9.30hrs)	
	7	33%(3.30hrs), 50%(6.45hrs), 67%(9.30hrs), 83%(15.30hrs)	
	8	67%(9.30hrs), 83%(10.30hrs), 100%(18hrs)	
	9	17%(20min), 83%(8.50hrs), 100%(10.30hrs)	
	10	83%(5.30hrs), 100%(8.45hrs)	
	15	83%(3.15hrs), 100%(2.45hrs)	
	20	100%(2.30hrs)	
	30	100%(2hrs)	
	40	100%(1.15hrs)	
Malachite green	Control	0% (24hrs)	LC50=1.40hrs
	0.1	0% (24hrs)	
	0.25	17% (17hrs)	
	0.5	33% (1hr), 50% (1.40hrs), 67% (2.40hrs), 100% (3.10hrs)	
	0.75	67% (1hr), 83% (1.40hrs), 100% (2.40hrs)	
	1	17% (15min), 50% (30min), 83% (45min), 100% (50min)	

Determination of lethal concentration of disinfectants copper sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$), methylene blue and malachite green were carried out in fish on aquarium, each of 30 liters water capacity. Individual aquarium was suspended with 6 liters of filtered water. Copper sulfate ($CuSO_4$) solution was prepared at 0.25ppm, 0.5ppm, 0.75ppm, 1ppm, 2ppm, 3ppm, 4ppm, 5ppm, 10ppm, 20ppm and 30ppm concentrations while potassium permanganate ($KMnO_4$) solution was prepared at 5ppm, 6ppm, 7ppm, 8ppm, 9ppm, 10ppm concentrations. The concentration of methylene blue was 5ppm, 6ppm, 7ppm, 8ppm, 9ppm, 10ppm, 15ppm, 20ppm, 30ppm, 40ppm and 50ppm. Doses of malachite green were 0.1ppm, 0.25ppm, 0.5ppm, 0.75ppm and 1ppm. The concentrations were adjusted by measuring the disinfectants in electric balance and dissolve in necessary volume of water. Each aquarium was marked with specific concentration of individual disinfectant. Six fingerlings of *Labeo rohita* with a size range of 8-10 cm were stocked in each aquarium and monitored at a regular interval.

Determination of Inhibitory Effects on Bacteria:

The detrimental activities of the disinfectants against representative of four genera of fish pathogenic bacteria *viz.*, *Aeromonas* sp. isolate E22, *Pseudomonas fluorescens* isolate PuKL2, *Edwardsiella* sp. isolate Eds33and *Flavobacterium* sp. isolate Fxskin5 were determined in *in vitro* condition. At the beginning of the experiment the selected isolates were grown in nutrient broth for 24 hours into a shaker-incubator at 25ºC with a rotation of 100 rpm. Approximately 50µl of bacterial broth suspension was taken on nutrient agar plates inside a laminar air-flow cabinet and bacterial broth culture was spared with L-shaped glass rod by turning the plate clock wise and anti-clock wise. The glass rod was sterilized by sinking glass rod with 70% alcohol and flamed in spirit lamp. Solution of different concentrations of copper sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$), methylene blue and malachite green were prepared in autoclaved distilled water. Then, 1 ml of each solution was taken in individual micro centrifuge tube and 50 µl of each solution was inoculated on the agar plate containing

Table2. Antibacterial effect of Methylene blue, $CuSO_4$, $KMnO_4$ and Malachite green.

Disinfectants		*Aeromonas* sp. (E22)	*Pseudomonas fluorescens* (PuKL2)	*Edwardsiella* sp. (Eds33)	*Flavobacterium* sp. (Fxskin5)
Methylene blue	4ppm	-	13ppm	-	-
	5ppm	16mm	15ppm	-	-
	6ppm	20mm	20ppm	-	-
$CuSo_4$	5ppm	-	6mm	11mm	-
	8ppm	-	-	7mm	-
	10ppm	-	-	-	-
$KMnO_4$	15ppm	-	-	-	-
	20ppm	-	-	-	-
	30ppm	-	-	-	-
	0.1ppm	-	-	-	-
	0.25ppm	-	-	-	-
Malachite green	0.5ppm	-	-	-	-
	0.75ppm	-	-	-	-
	1ppm	7mm	6mm	8mm	8mm

spread culture inoculums. The plate was remarked with solution concentration and bacterial isolate no. The plates were then kept in an incubator at 25°C for 12 hours. After 12 hours of incubation, the organism was considered sensitive if there was zone of complete inhibition around the inoculated solution. The organism was considered resistant if there was no zone of inhibition.

RESULTS AND DISCUSSION

Aquaculture is an important economic arena of Bangladesh. But, incidence of different diseases including the bacterial diseases limits the production of fish in aquaculture facilities. Different disinfectants are traditionally used for prevention and control of fish diseases. However these disinfectants are toxic [17] and may even cause death of fish. In the present study, lethal concentration of copper sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$), methylene blue and malachite green for fingerlings of *Labeo rohita* were found 0.75 mg/L, 7.00 mg/L, 6.00 mg/L and 0.50 mg/L at 21.4 hrs, 18.0 hrs, 9.5 hrs and 1.4 hrs, respectively. Bills (1974)[16] reported the LC_{50} value of malachite green to be 2 mh/L for rainbow trout fingerlings.

Inhibitory effects of four disinfectants to representative of four major bacterial fish pathogen was examined in *in vitro* condition. Experimental data suggested that *Pseudomonas fluorescens* isolate PuKL2 at 4 mg/L and 5 mg/L and *Aeromonas* sp. isolate E22 at 6 mg/L concentrations of methylene blue were unable to multiply. *Edwardsiella* sp. isolate Eds33 was inhibited by 10 mg/L and 8 mg/L $CuSO_4$. *Flavobacterium* isolate Fxskin5 was found to be resistant to copper sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$) and methylene blue at all concentrations but sensitive only against malachite green at 1ppm concentration. Potassium permanganate ($KMnO_4$) was failed to inhibit any of these four types of bacteria at all tested concentrations, whereas malachite green inhibits the growth of all of the bacteria at a concentration of 1ppm. Only methylene blue was capable to eradicate *Pseudomonas fluorescens* and *Aeromonas* sp. bellow the lethal concentration. So, disease in fish associated with *Pseudomonas fluorescens* and *Aeromonas* sp. can be treated with methylene blue without any adverse effects on fish. [12]. Two important algaecides Copper sulfate ($CuSO_4$) and potassium permanganate ($KMnO_4$), were failed to show its efficacy bellow the lethal concentration. Copper sulfate ($CuSO_4$) was effective against *Edwardsiella* sp. isolate Eds33. at 10 ppm and 8 ppm, far away from lethal concentrations (0.75 mg/L at 21.4 h). So, copper sulfate ($CuSO_4$) should not be used for prevention and control of fish disease associated with *Edwardsiella* spp. Potassium permanganate ($KMnO_4$) was ineffective to inhibit any of these four types' of bacteria even at 30mg/L concentration. So, potassium permanganate ($KMnO_4$) should not be considered for health management of bacterial disease of fish.

SUMMARY AND CONCLUSION

The experiment was conducted to detect the lethal concentrations for fish and antibacterial activity of four commonly used disinfectants in Bangladesh. In the present study, it was found that some disinfectant specially methylene blue and CuSO4 were active against *Aeromonas* sp. and *Pseudomonas fluorescens* at lower than lethal concentration. Findings of the present study will be helpful for fish farmers for prevention and control of bacterial fish diseases.

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