The use of potassium permanganate against trichodiniasis on milkfish (*Chanos chanos*) fingerlings

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Abstract

Trichodiniasis was noted in an intensive milkfish (*Chanos chanos*) nursery pond in Dumangas, Iloilo, Philippines. It was predominantly caused by a small trichodinid species (body diameter = 23-29 μm) with well-developed denticles, identified as *Paratrichodina* sp. The trichodinid infection resulted in proliferative changes, including clubbing and epithelial hyperplasia of the gill lamellae sufficient to disrupt respiratory function. Tolerance tests of milkfish fingerlings in an earthen pond-simulated environment resulted in a 24 h TL90 value of 1.98 ± 0.25 ppm $KMnO_4$. A treatment of 1.0 ppm $KMnO_4$ was highly efficacious (96%) in eliminating trichodinids on gills with minimal mortality of treated milkfish observed 24 hours post-treatment.

Introduction

Trichodiniasis is an ectoparasitic disease in fish caused by *Trichodina* spp., a group of ciliated protozoans and is one of the most commonly encountered diseases in aquaculture, especially in intensive systems. Frequent occurrence of trichodinids was recently observed among milkfish (*Chanos chanos*) fingerlings intensively-reared in an earthen nursery pond in Dumangas, Iloilo, Philippines (10°49′N, 122°43′E). Heavily infected fish displayed erratic swimming behavior (i.e. extreme flashing), excessive gill and skin mucus production, with moderate to intense erosion of fins. Mortalities were also noted. These disease signs are consistent with trichodinid-infections in fish (Lom and Dyková, 1992).

Preventative measures for controlling trichodiniasis in earthen pond culture systems has not been established for cultured fish species in the Phillipines. As such, fish farmers often rely on anecdotal means of controlling parasites in the ponds. One effective treatment to control trichodinids is the use of potassium permanganate ($KMnO_4$). $KMnO_4$ is a popular chemical application in aquaculture as its reduced form is non-toxic and is biologically unavailable (Boyd, 1979). Its strong oxidizing ability makes it highly effective against ectoparasites in fish, although this oxidizing potential is greatly affected by the presence of organic matter in ponds (Wilkinson, 2002).

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The present study has the following objectives: 1. identify the trichodinid species infecting milkfish in an intensive nursery pond in Iloilo, Philippines; 2. evaluate the histopathological response of gill tissue of trichodinid-infected milkfish; 3. determine the safe level of $KMnO_4$ to milkfish in an earthen pond-simulated environment; and 4. determine the efficacy of $KMnO_4$ in eliminating trichodinids on milkfish in an earthen pond-simulated environment.

Materials and methods

Identification of trichodinid

Milkfish fingerlings (mean body weight (BW), 0.12±0.3 g; mean total length (TL), 25.1±3.3 mm) reared for two months in an intensive nursery pond in Dumangas, Iloilo, Philippines (10° 49′ N, 122° 43′ E) and naturally infected with trichodinids were transported alive to the laboratory of University of the Philippines Visayas (UPV), Miagao, Iloilo. Twenty (20) heavily infected fish were used to prepare gill and skin smears and stained using dry silver impregnation technique (Klein, 1958). Stained images of trichodinids were observed using Motic BA310 biological microscope. This was followed by measurement of adhesive disc features using Image Tool v.2.01. The morphometry is presented following Grupcheva et al (1989).

Histopathology of trichodinid-infected milkfish

For histopathological examination, gills were immediately fixed in cold 10% buffered formalin for 24 h, processed using standard histological techniques, sectioned at 5 μm, and stained with hematoxylin and eosin. Sections were examined using Motic BA310 biological microscope.

Tolerance test

Tolerance tests of milkfish fingerlings to $KMnO_4$ were conducted at UPV, following procedures described by American Public Health Association (2005). Hatchery-reared milkfish fingerlings (mean BW = 0.64±0.1 g) obtained from the Southeast Asian Fisheries Development Center/ Aquaculture Department (SEAFDEC/ AQD), Tigbauan, Iloilo were acclimated for three days under laboratory conditions (salinity = 30 ppt, temperature = 28°C, aeration was provided) at UPV. Three hundred and sixty milkfish were then randomly divided among 18 glass aquaria (20 fish/aquarium) with 20 L sand-filtered seawater, two inches sun-dried soil substrate and gentle aeration. Triplicate treatment groups included 0.5, 1.0, 2.0, 3.0 and 4.0 ppm $KMnO_4$, plus no chemical as control. Test concentrations were preselected to range in 0 to 100% survival. Fish were exposed to $KMnO_4$ for 96 hours and were monitored for survival and fish behavior. Tolerance levels were determined by probability unit analysis performed in SPSS 16.0.

Treatment of trichodiniasis using $KMnO_4$

Experimental evaluation of $KMnO_4$ to treat trichodinid infection was performed with naturally trichodinid-infected milkfish fingerlings (mean BW, 0.12±0.3 g) reared for a month in an earthen pond in Dumangas, Iloilo, Philippines prior to transport to UPV. Fish were stocked in glass aquaria with 20 L sand-filtered seawater and two inches of sun-dried soil substrate. Treatment groups were based on the 24 h TL90 value, which included 0.5, 1.0 and 1.5 ppm $KMnO_4$, plus a no chemical control, all in triplicate (20 fish/replicate). Fish were exposed to test concentrations for 24 hours. Fresh gill wet mounts were examined to determine intensity

and prevalence of trichodinids (Bush et al., 1997) on gills of milkfish before and after treatment with $KMnO_4$. Efficacy of treatments was expressed as percent of trichodinids eliminated after exposure to $KMnO_4$. Mean trichodinid intensities among treatment groups before fish were exposed to $KMnO_4$ were compared by one way ANOVA (P<0.05) while mean trichodinid intensities before and after exposure to $KMnO_4$ within each group were compared by Paired-Sample T-test (P<0.05).

Results and discussion

Identification of trichodinid

All 20 skin smears were negative for trichodinids. A trichodinid, identified as *Paratrichodina* sp., was consistently observed in all the 20 stained gill smears. While there was difficulty in obtaining high-quality stained specimens, the morphology of its denticles was completely revealed in some areas of the specimens providing a strong basis for identification.

The *Paratrichodina* sp. in the present study possessed the key characteristics of the genus, which include a well-developed blade attached almost perpendicularly to the central part and non-interlocking arrangement of the denticles (Lom, 1963; Grupcheva et al., 1989; Lom and Dykova, 1992). Furthermore, our specimens have a slender and triangular blade with very short thorns similar to the *P. obliqua* originally described by Lom (1963) from the marine fish *Crenilabrus griseus* and *Scomber scombrus* in the Black Sea. Similarity can also be noted in their body size range (Table 1).

In the Philippines, specimens from brackish water pond-cultured Nile tilapia *Oreochromis niloticus* in Iloilo were reported as *Tripartiella tilapiae* (Bondad-Reantaso and Arthur, 1989) and possesses denticle features similar to those described here for ours. The key characteristics of the genus *Tripartiella*, having knee-like projection of the blade, well-developed thorns, and a delicate central part that interlocks the denticles (Lom, 1959) were however lacking in their specimens. Furthermore, *T. tilapiae* has previously been reported exclusively from freshwater fish (Lom, 1959; Lom, 1963; Duncan, 1977; Albaladejo and Arthur, 1989; Mohilal and Hemananda, 2012), with the lone exception being the case of Bondad-Reantaso and Arthur (1989). Thus we speculate the trichodinid from the gills of brackish water *O. niloticus* is likely a *Paratrichodina* sp. rather than a *Tripartiella* sp. We recommend further studies on trichodinids from brackish water fish in the Philippines to clarify this issue.

Histopathology of trichodinid-infected milkfish

Histopathological analysis demonstrated proliferative changes in response to trichodinid infections such as clubbing and hyperplasia (Figure 1) of epithelial cells of gill lamellae and interlamellar spaces. This response is consistent with previous reports of fish heavily infected with mobile peritrichs (Bruno et al., 2006). Severe infestation of the parasite on the gill tissue and adherence to and suction of the adhesive disc on the epithelium can result in significant inflammation sufficient to disrupt the respiratory function of the gills.

Tolerance test

The tolerance level of milkfish fingerlings to $KMnO_4$ at different exposure times (Table 2) are generally higher than earlier studies on acute toxicity of $KMnO_4$ to milkfish fingerlings. For instance, the 24 h TL50 in the present study is

Table 1. Morphometry of *Paratrichodina* sp. recovered from gills of milkfish (*Chanos chanos*) fingerlings obtained from Dumangas, Iloilo, Philippines and two reports of *P. obliqua*.[a]

	***Paratrichodina* sp.** **Present Study (n= 18)**	***P. obliqua*** **Lom (1963)**	***P. obliqua*** **Grupcheva et al. (1989)**
Diameter of:			
Body	26 (23-29)[b]	23 (21-26)	25 (20-31)
Adhesive disc	19 (17-21)	16 (15-19)	13 (11-18)
Denticular ring	10 (9-11)	8 (6-10)	6.9 (5.7-8.1)
Number of denticles	24 (23-25)	22 (19-26)	20 (18-21)
Dimension of denticle:			
Blade	2.9 (2.5-3.3)	3.3	2.3 (1.9-2.9)
Center	1.0 (0.7-1.2)	1.0	1.1 (0.5-1.5)
Thorn	1.3 (0.9-1.6)	1.5	1.0 (0.5-1.5)
Span	5.1 (4.4-5.8)		4.3 (3.8-4.8)
Width of border membrane	1.5 (1.2-1.9)	1.8	1.5-1.9
Host	*Chanos chanos*	*Crenilabrus griseus*	*Trachurus mediterraneus ponticus*
Location	Gills	Gills	Gills
Locality	Iloilo, Philippines	Black Sea, Rumania	Sozopol, Bulgaria

[a] All units are presented in micrometer (μm) except number of denticles
[b] Mean (range from minimum to maximum)

2.53 (±0.20) ppm while Cruz and Tamse (1989) reported 1.49 (±0.04) ppm. This increase in tolerance level of milkfish fingerlings to $KMnO_4$ is attributed to the presence of soil in our set-up, which is readily oxidized by $KMnO_4$ hence competitively reducing the available manganate ion concentrations to affect the fish.

Compared to other fish species, milkfish fingerlings appear to have less tolerance to $KMnO_4$. For *O. niloticus* fry and fingerlings, the 96 h TL50 to $KMnO_4$ is reported at 2.9 ppm and 3.3 ppm, respectively (Dureza, 1995). Goldfish *Carassius auratus* is also more tolerant to $KMnO_4$ with a reported 96 h TL50 of 3.60 ppm (Marking and Bills, 2011). This implies the treatment dose of $KMnO_4$ against trichodinids on other fresh- or seawater fish, which are mainly based on their specific tolerance level, may not be equally safe when used in milkfish fingerlings. In the present study, the maximum level of $KMnO_4$ tested is 1.5 ppm based on (or lower than) the 24 h TL90 value (1.98±0.25 ppm).

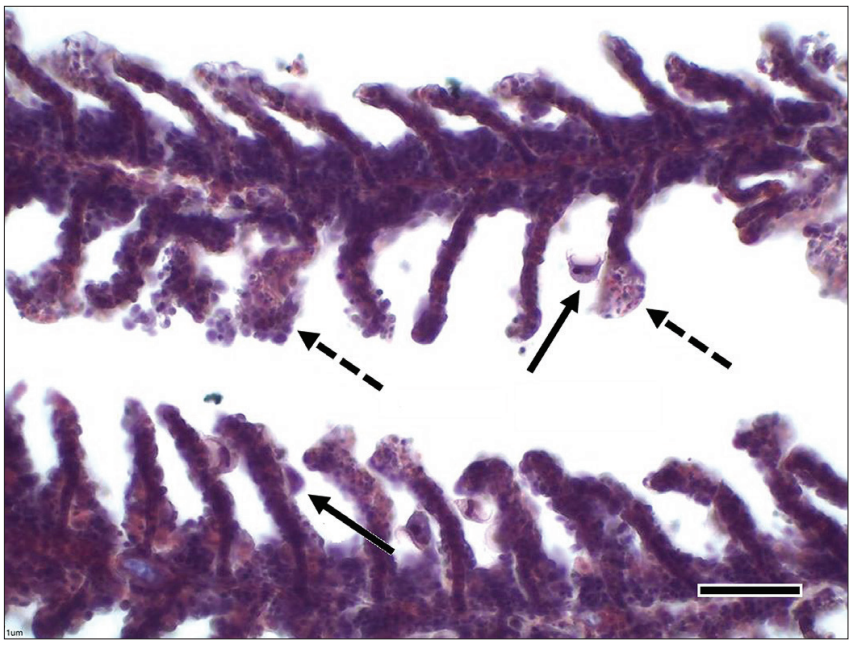

Figure 1. Milkfish gills infected with trichodinids (solid arrows) showing clubbing (dashed arrows) at the tips of gill lamellae (H & E, scale bar = 50 μm).

Table 2. Tolerance levels of milkfish (*Chanos chanos*) fingerlings to $KMnO_4$ at various exposure time.

Exposure time (h)	TL90 (ppm) (95% CL)	TL50 (ppm) (95% CL)
24	1.98 (±0.25)	2.53 (±0.20)
48	1.96 (±0.30)	2.50 (±0.25)
72	1.97 (±0.35)	2.51 (±0.05)
96	1.96 (±0.35)	2.51 (±0.30)

CL, confidence limit
n per treatment = 60; fish body weight = 0.64±0.1 g; fish total length = 35.2 ± 3.4 mm
salinity = 30 ppt; temperature = 28±0.12°C

Treatment of trichodiniasis using $KMnO_4$

All treatments significantly decreased the number of trichodinids on gills of milkfish (Table 3). In particular, 100% efficacy was observed at 1.5 ppm $KMnO_4$ treatment wherein both intensity and prevalence of trichodinid dropped to zero. However, a corresponding fish mortality of 20% was recorded. This high mortality may be due to the toxicity of $KMnO_4$ that might have been amplified when the manganate ions acted on trichodinid-damaged gill epithelial tissues of the infected fish. A lower treatment of 1.0 ppm $KMnO_4$ was also effective and produced only minimal mortality.

Our findings report $KMnO_4$ to be effective in eliminating trichodinids at a lower dose compared to previous records, even when the

Table 3. Efficacy of 24-hour potassium permanganate ($KMnO_4$) treatment against trichodinids on milkfish fingerlings.

$KMnO_4$ (ppm)	Trichodinid Prevalence (%)		Trichodinid Mean Intensity[1]		Efficacy of treatment (%)	Mortality after 24-h treatment (%)
	before treatment	after 24-h treatment	before treatment[2]	after 24-h treatment[3]		
Control	100	100	26.9 (±15.3)	18.0 (±8.6)	33.2	0.0
0.5	100	100	29.2 (±24.7)	3.4 (±3.8)*	88.2	0.0
1.0	100	22.2	25.3 (±13.2)	1.0 (±1.5)*	96.1	3.9
1.5	100	0	21.1 (±12.7)	0.0*	100.0	19.6

[1] average trichodinid cells per gill of fish (±standard deviation).
[2] mean trichodinid intensities among treatment groups before treatment are not significantly different ($P<0.05$).
[3] values with asterisk (*) are significantly different with the mean trichodinid intensity before treatment ($P<0.05$)
fish body weight=0.12±0.0 g; fish total length = 25.1±3.3 mm; fish per treatment = 60; sampled fish per treatment = 9; salinity=30 ppt; temperature=29.0±0.1 °C; D.O.=7.2±0.2 ppm; pH=7.4±0.0.

aquaria were supplemented with a soil substrate. Previous research found $KMnO_4$ to be effective against trichodiniasis on *O. niloticus* fingerlings at 2.0 ppm permanent bath (Younis et al, 2009). A continuous bath of 2.0 to 4.0 ppm $KMnO_4$ had also been recommended for application in ponds (Chakroff, 1976; Noga, 2010).

This current work suggests a 24 h bath of 1.0 ppm $KMnO_4$ is effective to control trichodiniasis on milkfish fingerlings, although follow up treatments at the same or lower concentration may be required to completely eradicate trichodinids from the system.

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