Full Length Research Paper

The anti-oomycetic effects of sodium chloride and potassium permanganate and the toxicity of these compounds to tilapia (*Oreochromis niloticus*) eggs

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The anti-oomycetic effects of sodium chloride and potassium permanganate on the vegetative and zoosporic stages of *Achlya* sp. BKKU 0502, *Saprolegnia diclina* BKKU 0506, *Aphanomyces* sp. BKKU 0508, *Achlya ambisexualis* BKKU 0615 and *Achlya bisexualis* BKKU 0616 were investigated at 25°C. The results showed that the exposure of the samples to 3.0 and 2.5% sodium chloride for 24 h was toxic to the vegetative and zoosporic stages, respectively. Moreover, 200 and 25 ppm of potassium permanganate were effective at killing the vegetative stage at 24 h and the zoosporic stage at 1 h, respectively. The toxicities of 2.0, 2.5 and 3.0% sodium chloride for controlling oomycete activity were determined using tilapia (*Oreochromis niloticus*) eyed eggs for both 1 and 24 h treatments. The results showed that salt concentrations at levels of 2.0% or higher reduced the hatching rate percentage of the treatment groups to a value significantly different from that of the control group (P<0.05). A noteworthy result was that the 3.0% sodium chloride treatment for 24 h produced a 0% hatching rate. The treatment of the eyed eggs with 100, 150 and 200 ppm potassium permanganate to control the oomycete activity had a highly toxic effect on the eggs: the results showed a 0% hatching rate for all of the treatment groups after 1 and 24 h exposures. Therefore, it is not possible to use sodium chloride or potassium permanganate to prevent the activity of oomycetes on tilapia eggs because these two chemicals decrease the hatching rate. Other chemicals that may be safe to use on other edible aquatic organisms will require further investigation.

Key words: Sodium chloride, potassium permanganate, oomycete zoosporic stage, vegetative stage, tilapia eyed eggs.

INTRODUCTION

Aquatic oomycetes are ubiquitous in the natural water supplies of fish hatcheries and often cause serious disease problems for fish culturists (Schreck et al., 1993; Rach et al., 1997, 2005). Outbreaks of oomycete infections on fish and fish eggs continue to cause a big problem in cultured fish. The Saprolegniales such as...
Table 1. Oomycetes isolated from tilapia eggs and used in this study.

<table>
<thead>
<tr>
<th>Isolate species</th>
<th>Location of host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achlya</em> sp. BKKU 0502</td>
<td>Kalasin province</td>
</tr>
<tr>
<td><em>Saprolegnia diclina</em> BKKU 0506</td>
<td>Khon Kaen province</td>
</tr>
<tr>
<td><em>Aphanomyces</em> sp. BKKU 0508</td>
<td>Sakon Nakhon province</td>
</tr>
<tr>
<td><em>Achlya ambisexualis</em> BKKU 0615</td>
<td>Sakon Nakhon province</td>
</tr>
<tr>
<td><em>Achlya bisexualis</em> BKKU 0616</td>
<td>Sakon Nakhon province</td>
</tr>
</tbody>
</table>

Figure 1. The healthy eyed stage of tilapia eggs used in this experiment (1 scale bar=1 mm).

*Saprolegnia, Achlya* and *Aphanomyces* are endemic to all freshwater habitats and they affected fish culture (Noga, 1996). This infection is one problem in brood stock husbandry and it can increase the mortality rate up to 80-100% of incubation eggs (Chukanhom and Hatai, 2004). In case of fish eggs, the infected eggs turned to be white and become dead. In fry, oomycetes start to infect on some part of body surface and then covered the entire body. It causes low productivity of fry and fish cultures (Paxton and Willoughby, 2000). In the past, this problem was solved with the extremely effective chemicals malachite green and formalin (Hansen, 2004). However, due to their toxicity and carcinogenicity to fish, fish eggs and the health of the farmers, the use of these two chemicals was discontinued for oomycete control in edible aquatic animals (Schreck et al., 1993). Many chemicals and drugs have been recommended for use in aquaculture for the prevention and treatment of diseases, and also for improving the water quality (Chinabut, 1997). Standard treatments with sodium chloride (NaCl) and potassium permanganate (KmMnO₄) are now effectively used in aquaculture to treat external parasites (Klinger and Francis-Floyd, 2002; Marecaux, 2006), and these compounds have also been reported to be anti-oomycete agents in fish culture (Schreck et al., 1993; Bruno and Wood, 1999). The aims of the present study were to examine the anti-oomycete effects of sodium chloride and potassium permanganate on (1) the vegetative and zoosporic stages of 3 genera, *Achlya, Aphanomyces* and *Saprolegnia*, and (2) their toxicities in the eyed stage of tilapia eggs.

MATERIALS AND METHODS

Oomycetes and culture conditions

Five selected oomycete isolates (Table 1) were cultured on glucose yeast extract agar (GYA), incubated at 25°C to obtain the vegetative stage, and then sub-cultured every month. The actively growing hyphae of the 3 days colony of oomycete isolates were used as oomycete inoculums in all experiments.

Source of eggs and incubation water

Samples of healthy eyed-stage tilapia eggs (Figure 1) were obtained from Khon Kaen Inland Fisheries Research and
Development Center hatchery, Khon Kaen Province, Thailand. The eggs were transported to the Fish Diseases Laboratory, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen province, Thailand. The healthy eggs were washed 3 times with sterilized tap water (STW). The incubation water for the experiments was obtained from the Khon Kaen Inland Fisheries Research and Development Center hatchery. The water was filtered through filter paper (Whatman® Schleicher & Schuell No. 1, Whatman International Ltd., England), and autoclaved before use.

**Oomycetacidal activity of NaCl and KMnO₄**

The minimum inhibitory concentrations (MIC) of NaCl and KMnO₄ that inhibited the growth of the oomycetes were verified to determine the proper oomycetacidal dosages. The commercial grades of NaCl (Univar, Asia Pacific Specialty Chemicals Limited, Australia) and KMnO₄ (EIS, Inter-education Supply Inc. Ltd., Bangkok, Thailand) were used in all the experiments, and prepared at various concentrations immediately before use. Approximately the same amount of hyphae was placed into 10 ml of various concentrations of NaCl (0.5, 2.5 and 5.0%) or KMnO₄ (50, 100, 200 and 400 ppm); the hyphae for the control groups were placed in STW without NaCl and KMnO₄. The vegetative growth of the treatment groups with NaCl and KMnO₄ was compared with the control group, and was observed by the naked eye after 1, 2 and 5 days of inoculation at 25°C. If no growth appeared after 5 days, the hyphae were removed, washed with STW, and then placed on new GY agar plates for 2 days at 25°C to observe the oomycete viability.

**Oomycetacidal activity of NaCl and KMnO₄ against vegetative growth**

The actively growing hyphae was placed into 10 ml of various concentrations of NaCl (1.5, 2.0, 2.5, 3.0%) or KMnO₄ (25, 50, 100, 150, 200 ppm) for treatments of 30 min and 1, 2, 6 and 24 h. The hyphae of the control groups were placed in STW without NaCl (0%) or KMnO₄ (0 ppm) for the same durations as the treatment groups. The mycelia were then removed, washed with STW, and placed on GY agar plates at 25°C. The activities of the specimens containing NaCl and KMnO₄ were compared to the activities of the control groups to determine the oomycete viability within 48 h.

**Oomycetacidal effects of NaCl and KMnO₄ on zoospore germination**

Zoospore suspensions of each isolate were adjusted to 1x10³ spores/ml. A total of 1 ml of NaCl or KMnO₄ solution with 10 times the desired final concentration was added to 9 ml of the zoospore suspension, and the mixture was kept at 25°C for 30 min and 1, 2, 6 and 24 h. At each time point, 120 µl aliquots of the mixture were inoculated onto GY agar. The viability of the zoospores was determined by observing the appearance of the colonies over a 2 day period with the naked eye. The control groups without NaCl and KMnO₄ treatment were also observed.

**Toxic effects of NaCl and KMnO₄ on the hatching rate of tilapia eggs**

The experiments were divided into 3 trials as follows: (1) The control group contained STW; (2) the experimental group I contained 2.0, 2.5, and 3.0% NaCl; and (3) the experimental group II contained 1.5, 2.0, and 2.5 ppm KMnO₄. Fifty eggs of each group were maintained in 500 ml of solution with aeration at 27-29°C for 1 and 24 h, respectively. Subsequently, all of the eggs were washed and then transferred to different beakers of sterilized incubation water for seven days until they hatched. Three replicates of each experiment were performed. The hatching rates and percentage of corrected mortality of the eggs were calculated using Abbott’s formula of Barnes et al. (1998).

\[
\text{Percentage of corrected mortality (Pt) = } \frac{Po - Pc}{100 - Pc} \times 100
\]

Where, Po, Mortality rate of test group; Pc, mortality rate of control group.

**Statistical analysis**

The mean hatching and corrected mortality rates of the fish eggs in the control and treatment groups were analyzed using a one-way ANOVA (Zar, 1999).

**RESULTS**

**Oomycetacidal activity of NaCl and KMnO₄**

As shown in Table 2, the treatments with various concentrations of NaCl at 25°C demonstrated that all of the isolates were able to grow in 0 and 0.5% NaCl. The MIC of NaCl was 2.5% for Achlya sp. isolate BKKU 0502, Saprolegnia diclina isolate BKKU 0506, Aphanomyces sp. isolate BKKU 0508, Achlya ambisexualis isolate BKKU 0615 and Achlya bisexualis isolate BKKU 0616. The results of the treatment with KMnO₄ at 25°C showed that the MIC values of each oomycete species were 50 ppm for S. diclina isolate BKKU 0506 and A.

**Table 2. Oomycetacidal effect of NaCl and KMnO₄ dosages on vegetative oomycete isolates at 25°C.**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Concentration</th>
<th>NaCl (%)</th>
<th>KMnO₄ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achlya sp. BKKU 0502</td>
<td>2.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Saprolegnia diclina BKKU 0506</td>
<td>2.5</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Aphanomyces sp. BKKU 0508</td>
<td>2.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Achlya ambisexualis BKKU 0615</td>
<td>2.5</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Achlya bisexualis BKKU 0616</td>
<td>2.5</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Oomycetecidal effect of dosages and exposure times of NaCl and KMnO₄ on vegetative stage of oomycete isolates at 25°C.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Concentration / Exposure time</th>
<th>NaCl (%)</th>
<th>KMnO₄ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achlya sp. BKKU 0502</td>
<td>2.5 / 24 h</td>
<td></td>
<td>50 / 2 h</td>
</tr>
<tr>
<td>Saprolegnia diclina BKKU 0506</td>
<td>2.5 / 24 h</td>
<td></td>
<td>50 / 2 h</td>
</tr>
<tr>
<td>Aphanomyces sp. BKKU 0508</td>
<td>2.5 / 24 h</td>
<td></td>
<td>50 / 24 h</td>
</tr>
<tr>
<td>Achlya ambisexualis BKKU 0615</td>
<td>2.5 / 24 h</td>
<td></td>
<td>150 / 24 h</td>
</tr>
<tr>
<td>Achlya bisexualis BKKU 0616</td>
<td>2.5 / 24 h</td>
<td></td>
<td>200 / 24 h</td>
</tr>
</tbody>
</table>

Table 4. Oomycetecidal effect of dosages and exposure times of NaCl and KMnO₄ on zoospore germination of oomycete isolates at 25°C.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Concentration / Exposure time</th>
<th>NaCl (%)</th>
<th>KMnO₄ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achlya sp. BKKU 0502</td>
<td>2.0 / 24 h</td>
<td></td>
<td>25 / 30 min</td>
</tr>
<tr>
<td>Saprolegnia diclina BKKU 0506</td>
<td>2.0 / 24 h</td>
<td></td>
<td>25 / 30 min</td>
</tr>
<tr>
<td>Aphanomyces sp. BKKU 0508</td>
<td>2.0 / 24 h</td>
<td></td>
<td>25 / 30 min</td>
</tr>
<tr>
<td>Achlya ambisexualis BKKU 0615</td>
<td>2.0 / 24 h</td>
<td></td>
<td>25 / 30 min</td>
</tr>
<tr>
<td>Achlya bisexualis BKKU 0616</td>
<td>2.5 / 2 h</td>
<td></td>
<td>25 / 30 min</td>
</tr>
</tbody>
</table>

Oomycetecidal effects of NaCl and KMnO₄ on vegetative growth

As shown in Table 3, the oomycetecidal dosage of NaCl for use against the vegetative growth of Achlya sp. isolate BKKU 0502, S. diclina isolate BKKU 0506, Aphanomyces sp. isolate BKKU 0508, A. ambisexualis isolate BKKU 0615 and A. bisexualis isolate BKKU 0616 was 2.5% for a 24 h treatment. A concentration of 3.0% NaCl effectively killed all of the isolates after 24 h of exposure. The oomycetecidal dosages of KMnO₄ for the vegetative growth are also shown in Table 3. The treatment with 50 ppm KMnO₄ was effective for the hyphae of both Achlya sp. isolate BKKU 0502 and S. diclina isolate BKKU 0506 after 2 h of exposure and for Aphanomyces sp. isolate BKKU 0508 after 24 h of exposure. The oomycetecidal dosages of KMnO₄ were 150 and 200 ppm against A. ambisexualis isolate BKKU 0615 and A. bisexualis isolate BKKU 0616, respectively, after 24 h of exposure.

Oomycetecidal effects of NaCl and KMnO₄ on zoospore germination

Table 4 shows that the oomycetecide dosages of NaCl for the zoosporic stage were 2.0% against Achlya sp. isolate BKKU 0502, S. diclina isolate BKKU 0506, Aphanomyces sp. isolate BKKU 0508, A. ambisexualis isolate BKKU 0615 and A. bisexualis isolate BKKU 0616 after 24 h of treatment and 2.5% against A. bisexualis isolate BKKU 0616 after 2 h of treatment. The oomycetecidal dosages of KMnO₄ for the zoosporic stage were 25 ppm against Achlya sp. isolate BKKU 0502, S. diclina isolate BKKU 0506, Aphanomyces sp. isolate BKKU 0508 and A. ambisexualis isolate BKKU 0615 for a 30 min exposure and against A. bisexualis isolate BKKU 0616 for a 1 h exposure.

Toxic effects of NaCl and KMnO₄ on the hatching rate of tilapia eggs

As shown in Table 5, the untreated control trial (0% NaCl) showed the highest mean percentage hatching rate, 96.6%, for both the 1 and 24 h exposures. The treatment groups exposed to 3 selected concentrations of NaCl (2.0, 2.5 and 3.0%) for 1 h showed a decreased mean percentage hatching rate: 86.6, 80.6 and 70.6%, respectively. In contrast, the treatment groups exposed to 2.0, 2.5 and 3.0% NaCl for 24 h showed a decreased mean percentage hatching rate: 71.7, 37.3 and 0%, respectively. The results showed decreased mean percentage hatching rates corresponding to the increased salt concentrations, and the control group differed significantly from the treatment groups (P<0.05, one-way ANOVA). The results for the KMnO₄ treatments showed that 100, 150 and 200 ppm KMnO₄ caused a 0% hatching rate after exposures of 1 and 24 h (Table 6).
Table 5. Mean percentages of hatching and mortality rates of the eyed egg stages after exposed with various concentrations of NaCl at 27-29°C for 1 and 24 h.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1 h Hatching</th>
<th>Corrected mortality</th>
<th>24 h Hatching</th>
<th>Corrected mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% NaCl</td>
<td>96.6</td>
<td>0</td>
<td>96.6</td>
<td>0</td>
</tr>
<tr>
<td>2.0% NaCl</td>
<td>86.6</td>
<td>10.3</td>
<td>71.3</td>
<td>26.1</td>
</tr>
<tr>
<td>2.5% NaCl</td>
<td>80.6</td>
<td>16.5</td>
<td>37.3</td>
<td>61.3</td>
</tr>
<tr>
<td>3.0% NaCl</td>
<td>70.6</td>
<td>26.9</td>
<td>0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The different letters in the same column means statistically significant difference between treatments (P<0.05, one-way ANOVA).

Table 6. Mean percentages of hatching and mortality rates of the eyed egg stages after exposed with various concentrations of KMnO₄ at 27-29°C for 1 and 24 h.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1 h Hatching</th>
<th>Corrected mortality</th>
<th>24 h Hatching</th>
<th>Corrected mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm KMnO₄</td>
<td>96.6</td>
<td>0</td>
<td>96.6</td>
<td>0</td>
</tr>
<tr>
<td>100 ppm KMnO₄</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>150 ppm KMnO₄</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>200 ppm KMnO₄</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

DISCUSSION

Oomycete infections of eggs are prevalent in many fish species reared in hatcheries, and the management of oomycete infections has traditionally depended on the prophylactic or therapeutic administration of chemicals. Sodium chloride has been recognized as a safe prophylactic or therapeutic administration of chemicals. Potassium permanganate has been used to treat external pathogens, including fungi, bacteria and certain parasites (Noga, 1996; Francis-Floyd and Klinger, 2002). In this study, the oomycetecidal effects of KMnO₄ on the vegetative and zoosporic stages varied among the isolates and exposure times, with lower concentrations of KMnO₄ (25 ppm) and shorter chemical exposure periods (30 min and 1 h) having a stronger effect on the zoosporic stage than on the vegetative stage. The exposure to 200 ppm KMnO₄ for 24 h was effective in controlling both the vegetative and zoosporic stages of all control groups (P<0.05). A noteworthy result was that the exposure to 3.0% NaCl for 24 h produced a 0% hatching rate. Therefore, the hatching rate percentage of the tilapia eggs was strongly affected by the concentration of salt and the exposure time. This finding is similar to the results of Edgell et al. (1993) who reported that salt solutions may cause egg deaths at levels of 2.5% or higher. According to Martinez-Palacios et al. (2004), it is possible that high salinities have an inhibitory action on the movement of the fish embryo due to the high osmotic impact on the perivitelline layer. Yamagami (1988) stated that hatching success is affected primarily by the level of chorionase activity and embryo movement. In this study, a longer chemical exposure period (24 h) proved to be harmful for all of the eggs. A shorter chemical exposure period could reduce the stress on the egg and allow the safe application of the chemical (Rach et al., 1997, 2000a, 2000b).

The results of this study show that, although oomycete control is more effective at higher salt concentrations, the salt solutions also produced egg death at levels of 2.0% NaCl or higher, with the hatching rate percentage of the treatment groups exposed to 2.0, 2.5 and 3.0% NaCl for 1 or 24 h differing significantly from that of the untreated
of the oomycete isolates. However, this result does not agree with the findings of Yuasa et al. (2000) who reported that 200 ppm KMnO₄ was toxic to Achlya, Aphanomyces and Pythium at 6 h and toxic to the genus Saprolegnia after 12 h treatments. Marking et al. (1994) reported that KMnO₄ was an oomyceticide for the inhibition of a cultured oomycete (in vitro) at 50 ppm and was toxic to eggs at 150 ppm for 1 h exposures at 12°C. However, the treatment of eyed-stage eggs with 100, 150 and 200 ppm of KMnO₄ for both 1 and 24 h resulted in a 0% hatching rate. Prior to this study, the scientific community felt that it was not possible to use NaCl and KMnO₄ to prevent the invasion of oomycetes of tilapia eggs because it was believed that these 2 chemicals would decrease the hatching rates. From this result, it may be suggested that if the tilapia eggs are often bathed with these two chemicals at high concentrations, that is, 3.0% NaCl and 200 ppm KMnO₄ for 30 min or less than 1 h, may inhibit the growth of the aquatic oomycetes without harming the eggs, which require further investigation.

Conclusion

The MIC of NaCl against five zoosporic-stage oomycete isolates was 2.5% for 2 h of treatment, whereas a concentration of 3.0% NaCl was toxic to the vegetative stage after 24 h of treatment. This study found that 25 and 200 ppm KMnO₄ were effective in killing the zoosporic and vegetative stages at exposures after 30 min and 24 h, respectively. The toxicity of 2.0, 2.5 and 3.0% NaCl was high for the eyed eggs, decreasing the percentage hatching rate. In addition, 25, 50, 100, 150, and 200 ppm KMnO₄ had strong toxic effects on the eggs, resulting in a 0% hatching rate. These results are important because many hatcheries mistakenly assume that NaCl and KMnO₄ only affect oomycete viability, yet these compounds are also toxic to tilapia eggs.

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REFERENCES


