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ORIGINAL PAPER

# Effect of potassium ferrate(VI) on survival and reproduction of *Ichthyophthirius multifiliis* tomonts

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**Abstract** *Ichthyophthirius multifiliis* is an important freshwater teleost pathogen that can infect most species of freshwater fish worldwide and often leads to significant economic losses to the aquaculture industry. Potassium ferrate(VI), as a potential therapeutic agent for external protozoan parasite infections, has been proven to kill *I. multifiliis* theronts effectively; however, no information is available on effects of potassium ferrate(VI) exposure to *I. multifiliis* tomonts. This study evaluated the effects of potassium ferrate(VI) on the survival and reproduction of *I. multifiliis* tomonts. The results of experiment 1 showed that potassium ferrate(VI) at concentrations of 2.4, 4.8, 9.6, and 19.2 mg/L resulted in tomont survival rates of 82.2%, 34.2%, 14.6%, and 0, respectively, and significant differences were noted in tomont reproduction between the treatments and the control ($P<0.05$). Additionally, this study was designed to determine the effect of potassium ferrate(VI) toxicity on age of the tomont. The results indicated that encysted *I. multifiliis* was more resistant to potassium ferrate(VI) treatments. In addition, this study was designed to investigate *I. multifiliis* tomont survival and reproduction when collected from infested goldfish in potassium ferrate(VI) solutions at different concentrations (0, 2.4, 4.8, 9.6, and 19.2 mg/L) for 30 min. It was found that the tomont survivals in the treatments were significantly lower than those in the control ($P<0.05$). This study demonstrated that a bath with potassium ferrate(VI) possibly was an effective method to treat ichthyophthiriasis.

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## Introduction

*Ichthyophthirius multifiliis* is an important pathogen of freshwater teleosts occurring in both temperate and tropical regions throughout the world (Matthews 2005). The disease ichthyophthiriasis, caused by *I. multifiliis*, probably results in more damage to freshwater fish populations worldwide than any other eukaryote pathogen (Hines and Spira 1973; Rogers and Gaines 1975; Matthews 2005). The life cycle of this parasite is direct and consists of three stages: an infective theront, a parasitic trophont, and a reproductive tomont (Nigrelli et al. 1976; Noe and Dickerson 1995; Swennes et al. 2006). Both the theront and tomont stages are free living in the life cycle of *I. multifiliis* (Buchmann and Bresciani 2001). Killing the parasite at these stages will stop the reproductive cycle and prevent the spread of the disease (Tucker and Robinson 1990; Schäperclaus 1991).

Potassium ferrate(VI), as a strong and environmentally friendly oxidant, has been used as a dual-function chemical reagent for water and wastewater treatment (Jiang and Lloyd 2002). It possesses many functions, such as coagulation, oxidation, and purification (Sharma 2002). Recently, Ling et al. (2010) reported that potassium ferrate (VI) killed free-living *I. multifiliis* theronts effectively. However, little information is available on the effect of potassium ferrate(VI) on survival and reproduction of *I. multifiliis* tomonts. Such information will be valuable to evaluate potassium ferrate(VI) potential as a therapeutic agent for external protozoan parasite infections.

The main purpose of this study was to determine the effect of varying concentrations of potassium ferrate(VI) on survival and reproduction of *I. multifiliis* tomonts. Next, this study was also to show the effect of potassium ferrate (VI) toxicity on age of the tomont. In addition, *I. multifiliis* tomont survival and reproduction were investigated when collected from infested goldfish in potassium ferrate(VI) solutions at different concentrations for 30 min.

## Materials and methods

### Fish and parasite

Goldfish (*Carassius auratus*), weighing 4.07±0.63 g, were utilized throughout the study. All fish, referred to as "naive fish," were kept in several 300-L opaque tanks and supplied with a constant flow of aerated tap water (flow rate, 1.0–1.5 $Lmin^{-1}$), at 22.0±2°C, pH 6.9±0.4, with dissolved oxygen of 6.2–7.9 mg/L, ammonia content (total nitrogen) of 0.7–1.9 mg/L, and total hardness ($CaCO_3$) of 83.5–108.5 mg/L. They were fed once at 1% body weight daily with commercial fish pellet feed.

*I. multifiliis* was isolated from goldfish, obtained from a pet shop, and its culture was maintained by serial infestation on goldfish as Ling et al. (2009, 2010) described. The fish were held at 22±2°C in a static 40-L aquarium equipped with an outside biological filter and air stones to maintain enough dissolved oxygen (greater than 5 mg/L).

### Potassium ferrate(VI)

The reagent of potassium ferrate(VI) used in this study was purchased from Xi'an Tian Shun Fine Chemical Plant (Xi'an, Shaanxi, China). In order to obtain an accurate dosage for the test, the potassium ferrate(VI) content of the reagent was measured using both chromate titration and spectroscopy methods (Jia et al. 1999; Jiang and Lloyd 2002). The results suggested that this reagent containing 96% of potassium ferrate(VI) was used throughout this study.

### Toxicity assay

The toxicity assay consisted of four experiments. Experiment 1 was conducted to determine the effect of potassium ferrate(VI) on survival and reproduction of unsettled *I. multifiliis* tomonts. *I. multifiliis* was collected using a method adapted from Cross and Matthews (1993). Several heavily infected fish were placed into filtered aquarium water for 30 min. The trophonts were allowed to escape from the host naturally. One hundred trophonts were distributed to each well ($N$=5) of a 24-well tissue culture plate. After discarding the water in each well, 1 mL potassium ferrate(VI) solution at concentrations of 0, 2.4, 4.8, 9.6, and 19.2 mg/L was added to each well, respectively (preliminary study suggested the concentration 19.2 mg/L resulted in 100% mortality). The solutions were not changed throughout the 20-h experiment, and the 24-well plate with tomonts was incubated at 23.5±0.5°C. At 20 h, the number of dead tomonts was counted under a dissecting microscope (×40 magnification). A dead tomont was determined by the absence of internal cell motility or abnormal cell division. Besides, theronts released by each tomont in each well were enumerated in ten 1- or 2-μL droplets of the theront suspension as Schlenk et al. (1998) described. The mortality and reproduction of tomonts were determined for each well according to Xu et al. (2008). The tomont reproduction was expressed as number of theronts released by each tomont, calculated by total theronts/live tomont. This experiment was repeated five times.

Experiments 2 and 3 were designed to determine the toxicity of potassium ferrate(VI) to *I. multifiliis* tomonts allowed to settle for 2 and 10 h, respectively. The trophonts were collected from infected goldfish as described above. One hundred trophonts were distributed to each well ($N$=5) of a 24-well tissue culture plate and allowed to attach for 2 and 10 h, respectively. Next, the water in each well was discarded, and 0, 2.4, 4.8, 9.6, and 19.2 mg/L potassium ferrate(VI) were added to each well, respectively (1 mL). The 24-well plate with tomonts was incubated at 23.5±0.5°C. The solutions of experiment 2 were replaced by tempered test water after 10 h of exposure to potassium ferrate(VI), and the solutions of experiment 3 were not changed during the experiment. At 20 h after exposure, the mortality and reproduction of tomonts in each well were recorded as described above. Experiments 2 and 3 were repeated five times, respectively.

Experiment 4 was performed for determining tomont survival and reproduction when collected from infested goldfish in potassium ferrate(VI) solutions at different concentrations for 30 min. Twenty-five heavily infected fish were divided into five groups (five fish per group) and exposed to potassium ferrate(VI) solutions at concentrations of 0, 2.4, 4.8, 9.6, and 19.2 mg/L for 30 min in opaque breakers, respectively. Then the fish were placed into five opaque breakers containing filtered aquarium water without potassium ferrate(VI), and the trophonts were collected as described above. One hundred trophonts were distributed to each well ($N$=5) of a 24-well tissue culture plate and allowed to attach. The water in each well was discarded, and 1-mL filtered aquarium water was added to each well. After incubation for 20 h at 23.5±0.5°C, the mortality and reproduction of tomonts were determined as mentioned above. As with experiments 1–3, experiment 4 was also repeated five times.

**Table 1** *I. multifiliis* tomont survival and number of theronts released from unsettled tomonts after 20 h of exposure to different concentrations of potassium ferrate(VI)

| Concentration (mg/L) | Tomonts | | Theronts | |
|---|---|---|---|---|
| | Number of dead tomonts | Survival (%) | Number (×1,000) | Theronts per tomont |
| 0 (Control) | 10.2±1.48 | 89.8±1.48a | 32.09±1.86a | 357.52±22.69a |
| 2.4 | 17.8±2.86 | 82.2±2.86b | 23.60±1.02b | 287.54±19.96b |
| 4.8 | 65.8±7.95 | 34.2±7.95c | 9.60±1.09c | 286.72±33.26b |
| 9.6 | 85.4±5.46 | 14.6±5.46d | 3.36±0.83d | 241.81±48.52b |
| 19.2 | 100 | 0e | 0e | 0c |

The solution of each well was not replaced throughout the experiment, and the temperature of incubation was 23.5±0.5°C. Each value was expressed as mean ± S.D. of five replicates, and within a column, the values followed by the different lowercase letters were significantly different ($P<0.05$)

Statistical analysis

All data in this study were analyzed by version 13.0 of Statistical Product and Service Solutions (SPSS). Student–Newman–Keuls Test procedure for multiple comparisons was used to compare tomont survival and reproduction ($\alpha=0.05$). The lethal concentration ($LC_{50}$) with 95% confidence intervals (CI) was determined using the probit procedure of SPSS.

## Results

The results of experiment 1 showed the effect of potassium ferrate(VI) at different concentrations on unsettled *I. multifiliis* tomont survival and reproduction (Table 1). All tomonts were dead in potassium ferrate(VI) solution at 19.2 mg/L, and no theronts were released. Although the tomonts were exposed for 20 h, the lethal effects of the potassium ferrate(VI) were found after 30 min. The range of potassium ferrate(VI) concentrations against tomont survival demonstrated a distinct dose–response relationship. The $LC_{50}$ was 4.14 mg/L potassium ferrate(VI) (95% CI, 2.15–6.33 mg/L). Besides, it was observed that potassium ferrate(VI) at four concentrations (2.4, 4.8, 9.6, and 19.2 mg/L) significantly reduced tomont reproduction ($P<0.05$), while no statistical difference was noted on reproduction between tomonts exposed to the concentrations of 2.4, 4.8, and 9.6 mg/L (Table 1).

In experiments 2 and 3, both the results showed that higher concentrations of potassium ferrate(VI) lead to higher tomont mortality; however, 2.4 mg/L potassium ferrate(VI) demonstrated distinct toxicity to tomonts in experiment 2 (Table 2), while there was no significant difference between the control and 2.4 mg/L potassium ferrate(VI) treatment in experiment 3 (Table 3). Additionally, in one replicate of experiment 3, two tomonts were live and produced theronts with 19.2 mg/L potassium ferrate(VI) treatment, albeit much less than the control. Significant differences in tomont production were noted between the control and tomonts exposed to 2.4–19.2 mg/L potassium ferrate(VI) in experiment 2, while no significance of difference was demonstrated in experiment 3 with the exception of the 19.2 mg/L treatment.

Table 4 shows *I. multifiliis* tomont survival and reproduction when collected from infested goldfish immersed in potassium ferrate(VI) solutions at different concentrations for 30 min. The survivals of tomonts in

**Table 2** *I. multifiliis* tomont survival and number of theronts released from each tomont allowed to settle for 2 h, and the solutions were replaced by tempered test water after 10 h of exposure to potassium ferrate(VI)

| Concentration (mg/L) | Tomonts | | Theronts | |
|---|---|---|---|---|
| | Number of dead tomonts | Survival (%) | Number (×1,000) | Theronts per tomont |
| 0 (Control) | 10.40±1.34 | 89.60±1.34a | 33.46±1.19a | 373.40±10.38a |
| 2.4 | 18.40±1.14 | 81.60±1.14b | 22.52±1.94b | 276.00±23.83b |
| 4.8 | 49.00±2.74 | 51.00±2.74c | 13.70±1.52c | 269.09±31.49b |
| 9.6 | 72.80±2.78 | 27.20±2.77d | 7.76±0.90d | 286.97±39.07b |
| 19.2 | 100 | 0e | 0e | 0c |

Total incubation time was 20 h, and the temperature of incubation was 23.5±0.5°C. Each value was expressed as mean ± S.D. of five replicates, and within a column, the values followed by the different lowercase letters were significantly different ($P<0.05$)

**Table 3** *I. multifiliis* tomont survival and number of theronts released from each tomont allowed to settle for 10 h and then exposed to different concentrations of potassium ferrate(VI) for 10 h

| Concentration (mg/L) | Tomonts | | Theronts | |
|---|---|---|---|---|
| | Number of dead tomonts | Survival (%) | Number (×1,000) | Theronts per tomont |
| 0 (Control) | 12.60±1.82 | 87.40±1.82a | 29.32±2.41a | 335.33±24.26a |
| 2.4 | 10.60±1.52 | 89.40±1.52a | 30.32±3.82a | 338.77±38.96a |
| 4.8 | 40.20±2.28 | 59.80±2.28b | 17.48±1.75b | 292.03±23.65a |
| 9.6 | 55.60±4.39 | 44.40±4.39c | 13.38±2.00c | 300.31±18.60a |
| 19.2[a] | 99.60±0.89 | 0.40±0.89d | 0.06±0.13d | 28.90±64.62b |

Total incubation time was 20 h; the temperature of incubation was 23.5±0.5°C. Each value was expressed as mean ± S.D. of five replicates, and within a column, the values followed by the different lowercase letters were significantly different ($P<0.05$)

[a] Two live tomonts were observed in one replicate and released theronts after 20 h of incubation

four treatments (0, 2.4, 4.8, 9.6, and 19.2 mg/L) were significantly lower than the survival in the control ($P<0.05$). In addition, no statistical difference was observed on tomont reproduction in potassium ferrate(VI) solutions with concentrations ranging from 0 to 19.2 mg/L, and each tomont released approximately 285–310 viable theronts (Table 4).

## Discussion

In this study, high concentrations of potassium ferrate(VI) resulted in more mortality of tomonts at prolonged exposure. In experiment 1, about 65% of tomonts were killed with 4.8 mg/L, and 100% was killed with 19.2 mg/L (Table 1). Ling et al. (2010) reported that potassium ferrate (VI) at the concentration of 4.8 mg/L killed 100% of theronts after 4 h of exposure. These data indicated that tomonts were less susceptible to potassium ferrate(VI) than theronts. Buchmann et al. (2003) findings suggested that the tomonts resisted some substances better than the theronts. Combined with the results of Straus and Meinelt (2009) and Meinelt et al. (2009), it is shown that peracetic acid (PAA) leads to more toxicity to theronts than tomonts. Additionally, we observed that increasing duration of exposure to potassium ferrate(VI) was associated with increased mortalities of tomonts. In the treatment of 19.2 mg/L potassium ferrate(VI), the lethal effects of the potassium ferrate(VI) were found after 30 min, and at 4 h, approximately 80% of the tomonts were dead. The time–response relationship was also found in the study of Ekanem et al. (2004). They reported the effects of crude extracts of *Mucuna pruriens* and *Carica papaya* against *I. multifiliis* and confirmed this relationship. It is therefore necessary and valuable to access the information in order to treat ichthyophthiriasis using potassium ferrate(VI).

The second and third experiments were designed to determine the effect of potassium ferrate(VI) toxicity on age of the tomont. Results showed that age of the parasite distinctly had an influence on potassium ferrate(VI) toxicity because of the development of the cyst. Maclennan (1937) demonstrated that at 26°C, after tomonts left the host fish, it was 2.5 h before the beginning of encystment. According to Matthews (2005) and Meinelt et al. (2009), *I. multifiliis* tomonts encyst within 15 min to 6 h of leaving the host epidermis. In experiment 2, the tomonts were allowed to settle for 2 h, and then exposed to potassium ferrate(VI) for 10 h. The concentration of 2.4 mg/L potassium ferrate(VI)

**Table 4** *I. multifiliis* tomont survival and reproduction when collected from infested goldfish in potassium ferrate(VI) solutions at different concentrations for 30 min

| Concentration (mg/L) | Tomonts | | Theronts | |
|---|---|---|---|---|
| | Number of dead tomonts | Survival (%) | Number (×1,000) | Theronts per tomont |
| 0 (Control) | 12.20±2.86 | 87.80±2.86a | 27.14±1.23a | 309.22±11.91a |
| 2.4 | 54.60±5.55 | 45.40±5.55b | 13.47±1.76b | 296.41±9.83a |
| 4.8 | 73.00±3.39 | 27.00±3.39c | 7.66±0.50c | 285.92±28.42a |
| 9.6 | 77.80±3.03 | 22.20±3.03c,d | 6.84±0.82c | 308.80±12.29a |
| 19.2 | 79.40±2.88 | 20.60±2.88d | 6.35±0.49c | 310.48±21.71a |

The filtered aquarium water was added into each well, and the incubation was at 23.5±0.5°C for 20 h. Each value was expressed as mean ± S.D. of five replicates, and within a column, the values followed by the different lowercase letters were significantly different ($P<0.05$)

significantly reduced the tomont survival and reproduction than the control ($P<0.05$, Table 2). In experiment 3, potassium ferrate(VI) exposure was initiated 10 h after the trophonts left the fish. During this period, it was found that most of the tomonts had already encysted and were dividing. At the end of experiment 3, the results demonstrated that there was no significant difference in tomont survival and reproduction between the control and tomonts exposed to 2.4 mg/L. We considered that whether the tomont finished developing the cyst wall possibly influenced the tomont survival and reproduction with the same concentration of potassium ferrate(VI) exposure. The findings of Meinelt et al. (2009) supported our conclusion. Therefore, it was possibly helpful to decide an effective dose of potassium ferrate(VI) to treat ichthyophthiriasis under laboratory conditions, even under field conditions. However, because it was beyond the scope of this study, it was not possible to draw reliable conclusions on infectivity of theronts produced by surviving tomonts, though the information was crucial to treat ichthyophthiriasis using this chemical. The future study will be done to investigate if tomonts exposed to potassium ferrate(VI) can reproduce infective theronts and infect fish in the second infection cycle.

To control ichthyophthiriasis effectively with this chemical, it would be necessary to apply this chemical repeatedly because of change in potassium ferrate(VI) concentration and water temperature that dictated the duration of the life cycle of *I. multifiliis*. Furthermore, the effectiveness of potassium ferrate(VI) was affected by coexisting ions and easily oxidizable substances in water. Therefore, this study was designed to determine *I. multifiliis* tomont survival and reproduction when collected from infested goldfish in potassium ferrate(VI) solutions at different concentrations for 30 min. Results showed that there were significant differences between the survivals of tomonts in four treatments and that in the control (Table 4). It is therefore indicated that bathing for a short time with a high concentration of this chemical possibly was an effective method to treat ichthyophthiriasis. Importantly, this bath method can still reduce the cost. However, the mechanism that led to a decline in survival of tomonts when collected from infested fish immersed in potassium ferrate(VI) for 30 min needs further study.

Recently, Sudová et al. (2010) have undertaken a study of evaluation of a continuous 4-day exposure to PAA as a treatment for *I. multifiliis*. They suggested that PAA exposure should be continued for one or two additional days or possibly at a higher dose to completely disrupt the development cycle of *I. multifiliis*. Similarly, increasing the bath time and/or dosage possibly resulted in better efficacy of potassium ferrate(VI) for the treatment of ichthyophthiriasis, because the data of this study showed that the bath in 19.2 mg/L potassium ferrate(VI) for 30 min did not result in 100% mortality of the tomonts.

Xu et al. (2008) considered that harvesting trophonts from fish by allowing the parasites to naturally leave live or dead fish can be used to obtain clean and uninjured trophonts. In our study, tomonts were obtained according to the method as described above, but tomont survivals (in the control of the four experiments) were only approximately 90%. The result was close to that of Buchmann et al. (2003). This demonstrated that about 10% of the parasites appeared dead or had abnormal reproduction in the incubation. However, it is unclear what caused this phenomenon.

In summary, results in this study showed that potassium(VI) ferrate solution at concentrations of 4.8 and 19.2 mg/L resulted in the tomont survival rates of 34.2% and 0, respectively, and significantly reduced the tomont reproduction, compared with the control. Additionally, this study demonstrated that age of the parasite distinctly influenced potassium ferrate(VI) toxicity to the tomont. Whether the tomont finished developing the cyst wall had a potential impact on potassium ferrate(VI) toxicity to the tomont. It was noted that tomont survival was obviously reduced when collected from infested goldfish in potassium ferrate(VI) solutions at different concentrations for 30 min. Such a bath method will be possibly more effective in treating ichthyophthiriasis with potassium ferrate(VI), if taking account of this chemical cost.

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