

Short communication

Antiparasitic Effect of Formalin, Trichlorfon, Hydrogen Peroxide, and Copper Sulfate on the Parasitic Isopod *Caecognathia coralliophila*

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ABSTRACT—*Caecognathia coralliophila* is known as a pathogenic ectoparasite infecting tiger grouper *Epinephelus fuscoguttatus* in a hatchery in Sabah, Malaysia. The effects of copper sulfate, formalin, trichlorfon, and hydrogen peroxide on the survival of *C. coralliophila* larvae were tested *in vitro*. The larvae were exposed to different concentrations of each chemical for 10, 20, 30, 60 min, or 24 h. Trichlorfon was found to be the most effective, killing the parasites within 24 h at 0.2 ppm. Consequently, the toxicity of trichlorfon to tiger grouper was tested. Fish were exposed to trichlorfon at 0.2 ppm for 24 h or 3.2 ppm for 60 min. No fish died in the experiment. Thus, our data suggest that trichlorfon is effective for treating *C. coralliophila* infection in *E. fuscoguttatus*.

Key words: *Caecognathia coralliophila*, trichlorfon, *Epinephelus fuscoguttatus*, tiger grouper, ectoparasite

Parasitic crustaceans commonly affect aquaculture species. Infestation by a parasitic crustacean isopod from the family Gnathiidea, *Caecognathia coralliophila*, was recently reported by Chong *et al*. (2015b). Gnathiid infection in aquaculture systems is not common in Malaysia. However, there have been reports of gnathiid outbreaks in Europe (Marino *et al*., 2004; Hispano *et al*., 2013) and Japan (Yoshida, 1988).

Only the larval stage of *C. coralliophila* is parasitic to the fish whereas the adults are free-living bottom dwellers (Chong *et al*., 2015a). The male is responsible for fertilizing the eggs inside the female while the female is responsible for carrying the eggs and developing them into larvae before the final rupture of the ventral brood pouch to release the larvae (Tinsley and Reilly, 2002). The parasitism of *C. coralliophila* can cause significant problems in hatcheries. Broodstock of tiger grouper, *Epinephelus fuscoguttatus*, and humphead wrasse, *Cheilinus undulatus*, may cease spawning as a result of parasite-associated stress. The larvae of gnathiids can physically damage host tissue (Honma and Chiba, 1991; Honma *et al*., 1991; Hayes *et al*., 2007), cause anemia (Jones and Grutter, 2005), and even induce mortality (Yoshida, 1988; Marino *et al*., 2004; Hayes *et al*., 2011). Therefore the focus of this study is the larvae as it is the parasitic stage on a gnathiid.

Studies of treatments for crustacean isopod infections are more commonly carried out using cymothoidae; however, the study on the control of gnathiids is limited (Hispano *et al*., 2013). The purpose of this study therefore was to evaluate formalin, trichlorfon, hydrogen peroxide, and copper sulfate as potential treatments for *C. coralliophila*. Furthermore, the toxicity of trichlorfon against a marine fish species, *E. fuscogutattus*, was also evaluated.

Materials and Methods

Sampling

Sampling was done in one of the hatcheries in Sabah, Malaysia during an outbreak of the parasites. During the peak of the outbreak, the parasites could be seen very clearly throughout the water column. Larval of *C. coralliophila* were collected using a 250-μm net by randomly sieving for parasites throughout the tank.

Treatments

Ten larvae were randomly chosen, not in any specific developmental stage. And then they were placed in a petri dish each containing 10 mL of chemotherapeutic agent (formalin [Merck], trichlorfon [Bayer], hydrogen peroxide [Merck], or copper sulfate [Merck]) at the concentrations indicated below. Parasites were exposed to each concentration for various time periods: 10, 20, 30, 60 min or 24 h. Immediately after treatment, the parasites were transferred to a new petri dish containing fresh seawater and mortality was assessed. Mortality was confirmed by observing the parasites after 24 h post treatment. The experiment was conducted at 24°C.

Formalin was used at 200, 100, 50, 25, 12.5, and 6.25 ppm; trichlorfon was used at 3.2, 1.6, 0.8, 0.4 and 0.2 ppm; hydrogen peroxide was used at 1,000, 100, 10 and 1 ppm; and copper sulfate was used at 40, 20, 10 and 5 ppm.

Toxicity test

Parasite-free tiger grouper, *E. fuscogutattus* (length

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9–12 cm) were used for the toxicity test. Two fish were placed in 17-L aerated aquarium. The fish were exposed to trichlorfon at a concentration of 0.2 and 3.2 ppm for 24 h and 60 min, respectively. Immediately after the treatment, the fish were transferred into another tank containing fresh seawater and observed for 1 week. Throughout the observation period, fish were fed twice daily and water was changed daily. The experiment was conducted at 24°C with duplicate.

Results and Discussion

Formalin is an effective chemotherapeutic agent used to treat various external parasitic infections in fish (Francis-Floyd, 1996; Tonguthai, 2000). The concentrations typically used for prolonged bath treatment are 25–30 ppm (Francis-Floyd, 1996; Tonguthai, 2000), whereas the concentration suggested for short-term bath treatment is 250 ppm for duration of not more than 60 min. However, in this study, formalin was proven effective against *C. coralliophila* only at the concentration of 100 ppm for 24 h treatment. The survival of the parasites was very high at the suggested range of concentration and duration of the treatment (Table 1), therefore formalin is not considered a suitable treatment for *C. coralliophila*.

Hydrogen peroxide is normally used as a bleaching and oxidizing agent but is also used to treat sea lice in cultured salmonids (Bruno and Raynard, 1994). The recommended dosage used in aquaculture for hydrogen peroxide is 1,500 ppm for 20 min (Athanassopoulou *et al*., 2009). Furthermore, in Japan, Hirazawa *et al*. (2016) has shown the effectiveness of hydrogen peroxide against some monogenean parasites of greater amberjack *Seriola dumerili*, at 75 ppm for 30 min. However, according to Bruno and Raynard (1994), the increasing temperature could induce the chemical to become hazardous and compromising the survival of the fish. In this study, at 20 min treatments for all concentrations used, resulted in a very high survival of the parasite. In the present study, hydrogen peroxide killed all parasites only at the 24 h treatment with the concentrations above 100 ppm (Table 2). Considering the use in Sabah that has relatively constant high temperature in comparison with the temperate countries, hydrogen peroxide may not suitable for the treatment for *C. coralliophila*.

Copper sulfate ($CuSO_4$) is commonly used to treat marine fish infected with protozoan parasites (Straus, 2008; Athanassopoulou *et al*., 2009). Straus (2008) concluded that copper sulfate is very effective for treating catfish infected with ectoparasites with the concentration of 1.1 ppm for prolonged treatment. However, the results of the present study indicate that copper sulfate was able to kill the larval *C. coralliophila* at 40 ppm after 24 h (Table 3).

Trichlorfon has been used in aquaculture as an effective antiparasitic chemical for several decades. Brandal and Egidius (1979) used Neguvon for the treatment of salmon lice in farmed salmon in Norway. Hispano *et al*. (2013) succeeded in eliminating *Gnathia maxillaries* in aquaria and suggesting the bath treatment within the range of 0.3–0.5 ppm. In the present study, trichlorfon was used on a trial basis to kill *C. coralliophila*, and the *in vivo* experiment revealed that trichlorfon killed all of the larval parasites, even at a concentration of 0.2 ppm (Table 4). In addition, although trichlorfon was shown to be toxic to marine fish, especially yellowtail, *Seriola quinqueradiata* (Fujita *et al*., 1968), all of the tiger grouper used in the toxicity test survived from the exposure to both 0.2 ppm for 24 h and 3.2 ppm for 60 min.

In summary, the results indicate that formalin, hydrogen peroxide and copper sulfate are only effective to kill the parasite at the concentrations above the safe

Table 1. Numbers of *C. coralliophila* larvae survived in the formalin treatment

	200 ppm	100 ppm	50 ppm	25 ppm	12.5 ppm	6.25 ppm
10 min	10	10	10	10	10	10
20 min	9	10	10	10	10	10
30 min	8	10	10	10	10	9
60 min	4	9	9	10	10	10
24 hours	0	0	4	10	8	9

Table 2. Numbers of *C. coralliophila* larvae survived in the hydrogen peroxide treatment

	1,000 ppm	100 ppm	10 ppm	1 ppm
10 min	10	10	10	10
20 min	10	10	9	10
30 min	9	10	10	10
60 min	5	10	8	10
24 hours	0	0	8	10

Table 3. Numbers of *C. coralliophila* larvae survived in the copper sulfate treatment

	40 ppm	20 ppm	10 ppm	5 ppm
10 min	10	10	8	10
20 min	10	9	10	9
30 min	10	7	10	10
60 min	7	9	9	10
24 hours	0	9	10	9

Table 4. Numbers of *C. coralliophila* larvae survived in the trichlorfon treatment

	3.2 ppm	1.6 ppm	0.8 ppm	0.4 ppm	0.2 ppm
10 min	0	0	1	5	9
20 min	0	0	2	4	6
30 min	0	0	0	3	8
60 min	0	0	0	1	10
24 hours	0	0	0	0	0

limit in the aquaculture industry thus it is not suitable to be used as the treatment. Whereas the trichlorfon is able to kill the parasite effectively in relatively short time within the safe concentration (i.e. 10 min at concentrations above 1.6 ppm). Thus this indicates that among the tested drugs, trichlorfon is most suitable for treatment of *C. coralliophila* infection in *E. fuscogutattus* around Sabah, Malaysia. In the future, more toxicity study shall be conducted to test the suitability of the chemical against the other marine fish.

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