

## Comparison of Treatment Efficacy of Formalin, Bronopol and Bithionol against *Ichthyobodo* sp. in Cultured Olive Flounder, *Paralichthys olivaceus* (Temminck & Schlegel)

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The effectiveness of bronopol and bithionol in treating *Ichthyobodo* sp. infestation in cultured olive flounder was investigated, and compared them with formalin. Bath with formalin at 200 ppm or bithionol at 100 ppm for 1 h showed complete removal of *Ichthyobodo* sp. from all the experimental fish. Baths with lower concentrations of formalin (50 and 100 ppm) or bithionol (25 and 50 ppm) significantly reduced infection intensities of *Ichthyobodo* sp. On the other hand, baths with bronopol or intubation of bithionol at various concentrations were not effective against *Ichthyobodo* sp. infestation.

*Key words* : *Ichthyobodo* sp., Olive flounder, Bithionol, Bronopol, Formalin, Treatment efficacy

Ichthyobodiosis is an important cause of mortality among intensively farmed salmonids (Ellis and Wootten, 1978; Robertson, 1979, 1985; Urawa, 1993) and olive flounder, *Paralichthys olivaceus* (Urawa *et al.*, 1991). It has been suggested that ichthyobodiosis in marine fishes such as plaice, *Pleuronectes platessa*, and winter flounder, *Pseudopleuronectes americanus*, was caused by *Ichthyobodo necator*, which originated from freshwater and adapted to the marine environment (Bullock and Robertson, 1982; Cone and Wiles, 1984). However, it was proposed that a separate species of *Ichthyobodo* may infect marine fishes (Morrison and Cone, 1986; Diamant, 1987). Urawa and Kusakari (1990) demonstrated differences between *I. necator* from chum salmon, *Oncorhynchus keta*, and *Ichthyobodo* sp. from olive flounder by cross-infection experiments, and concluded that *Ichthyobodo* sp. from olive flounder should be regarded as a different species from *I. necator*.

In Korea, infestation of *Ichthyobodo* sp. is respon-

sible for mass mortalities in cultured juvenile olive flounder as in Japan (Urawa *et al.*, 1991). Formalin has been reported to be effective in treating *Ichthyobodo* sp. infestation in cultured olive flounder, but raises safety concerns in relation to the user and to the environment which have led to restrictions on its use. Therefore, in the present study, we tested the effectiveness of bronopol and bithionol in treating *Ichthyobodo* sp. infestation in cultured olive flounder, and compared them with formalin.

In experiment I of the present study, juvenile olive flounder (9-12 cm in body length) infected with *Ichthyobodo* sp. were obtained from a local farm and divided into seven treatment groups, six receiving 50, 100, and 200 ppm of either formalin or bronopol(2-bromo-2-nitropropane-1,3-diol, Sigma) bath, and the 7th serving as a control group. Bath trials were carried out in 50-l aquaria with 10 fish per group which were exposed to the drugs for 1 h. During treatment, the water was held with aeration without change and then completely replaced with fresh, clean water. The control group was subjected to identical treatment without the drug. Five hours

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after the end of the treatment, intensity of infestation of all fish in each group was determined by counting of all parasites in three microscopic fields (100×) in each of two mucus samples, which were taken by gentle scraping 1 cm<sup>2</sup> of the anterior dorsal and posterior dorsal regions of body surface.

In experiment II, juvenile olive flounder were randomly distributed into 7 square tanks (10 fish in each tank) containing 50-l sea water. Among them, four were subjected to static bath treatments of 25, 50 or 100 ppm bithionol (Sigma) and control for 1 h, and then the water was completely replaced. Five hours after the end of the treatment, the parasites were counted by the same method as in experiment I. Fish in the other 3 tanks were anaesthetized with MS222 (Sigma) and were intubated directly into the stomach with either 0 (saline), 100 or 200 mg bithionol/kg of body weight. The treatment efficacy was measured after 24 h by the same method in experiment I. Temperature of water during both experiments was 10 ± 1°C. All data were analyzed using Mann-Whitney's U-test.

Bath with formalin at 200 ppm or bithionol at 100 ppm for 1 h showed complete removal of *Ichthyobodo* sp. from all fish (Table 1, 2). Baths with lower concentrations of formalin (50 and 100 ppm) or bithionol (25 and 50 ppm) significantly reduced infection intensities of *Ichthyobodo* sp. On the other hand, baths with bronopol or intubation of bithionol at all concentrations were not effective against *Ich-*

*thyobodo* sp. infestation (Table 1, 2).

The results of the present study indicated that ichthyobodiosis in olive flounder could be treated effectively by bath with either formalin or bithionol. Tojo *et al.* (1994) reported that a 25 ppm bath with bithionol for 3 h daily on 2 consecutive days was 100% effective against *I. necator* infestation on rainbow trout, *Oncorhynchus mykiss*. In the present study, bath treatment with bithionol at 25 ppm for 1 h removed over 75% of *Ichthyobodo* sp. from the skin of olive flounder, and this was not statistically different from the result obtained when bathing in 50 ppm bithionol. Although a 100 ppm bithionol bath completely removed all the parasites, fish showed some stress responses such as rapid gasping, loss of equilibrium and then listlessness. On the other hand, bathing in 200 ppm formalin for 1 h was 100% effective and caused no stress responses. The efficacy of formalin bath was dose-dependent, and 500 ppm formalin bath was significantly less effective in reducing *Ichthyobodo* sp. numbers, when compared to 100 ppm formalin bath. Intubation of bithionol in the present study was not effective against *Ichthyobodo* sp. infestation on olive flounder, similarly to the findings of Tojo and Santamarina (1998) with *I. necator* infestation on rainbow trout. Bronopol (2-bromo-2-nitropropane-1,3-diol) is a biocide widely used as a preservative in medical and pharmaceutical products, cosmetics and shampoos

**Table 1.** Treatment efficacy of formalin and bronopol baths at various concentrations for 1 h for *Ichthyobodo* sp. infestation in olive flounder, *Paralichthys olivaceus*

Drugs	Concentration (ppm)	Parasite number (mean ± SD)
Formalin	50	131.4 ± 56.1*
	100	64.4 ± 48.5*
	200	0*
Bronopol	50	1242.0 ± 1068.1
	100	2420.0 ± 1944.0
	200	1990.0 ± 717.1
Control	-	1405.0 ± 1454.8

★: Significantly different from control group (P<0.01)

**Table 2.** Treatment efficacy of either bithionol bath at various concentrations for 1 h or bithionol intubation against *Ichthyobodo* sp. infestation in olive flounder, *Paralichthys olivaceus*

Bithionol	Concentration	Parasite number (mean ± SD)
Bath (ppm)	25	17.4 ± 25.0*
	50	21.4 ± 45.6*
	100	0*
Bath control	-	132.6 ± 74.3
	Intubation (mg/Kg of B.W.)	100
	200	21.8 ± 30.6
Intubation control	saline	18.4 ± 25.6

★: Significantly different from control group (P<0.05)

(Bryce *et al.*, 1978; Toler, 1985). Recently, bronopol was used as an anti-fungal agent for rainbow trout and was effective in protecting predisposed fish from infection by *Saprolegnia parasitica* when administered as a daily bath treatment at 15 ppm and greater (Pottinger and Day, 1999). In the present study, however, bronopol showed no treatment effect against *Ichthyobodo* sp. infestation up to 200 ppm for 1 h.

In conclusion, the data reported here indicate that bithionol, can be used effectively at low concentrations to control *Ichthyobodo* sp. infestation in marine cultured olive flounder as *I. necator* infestation in rainbow trout.

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## 양식 넙치에 기생하는 *Ichthyobodo* sp.에 대한 Formalin, Bronopol 및 Bithionol의 치료 효과

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본 연구에서는 bronopol과 bithionol을 이용하여 양식 넙치에 기생하는 *Ichthyobodo* sp.의 치료효과를 조사하였으며, 그 효과를 formalin과 비교하였다. formalin 200 ppm 혹은 bithionol 100 ppm에서 1 시간동안 약욕한 결과 모든 실험어에서 100% 치료효과를 나타내었으며, 이보다 더 낮은 농도(formalin 50 ppm, 100 ppm 및 bithionol 25 ppm, 50 ppm)에서의 약욕에 의해서도 *Ichthyobodo* sp.의 감염강도를 유의적으로 감소시켰다. 그러나 bronopol을 이용한 약욕 및 bithionol의 경구투여는 대조구에 비해 유의적인 치료효과를 나타내지 않았다.

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*Key words* : *Ichthyobodo* sp., Olive flounder, Bithionol, Bronopol, Formalin, Treatment efficacy