



Research Note

Acute Toxicity and Optimal Dose of Clove Oil as Anaesthetic for Blue Hill Trout *Barilius bakeri* (Day)

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Anaesthetics have long been used in handling and transportation of live fish to reduce stress and mortality and to tranquilize fish for biological and aquaculture research (Park et al., 2003; Park et al., 2004; Hur et al., 2005). According to Summerfelt & Smith (1990) and Woolsey et al., (2004) the optimal levels of anesthesia have been determined for fish transportation and general handling. Quite a number of anaesthetics have been used or evaluated for use in aquaculture, but issues such as toxicity, safety, and cost are frequently limiting factors (Ross & Ross, 1999). Though these anaesthetics are effective, only tricaine has been permitted to use in food fish anesthesia mainly due to environmental and health risks. An ideal anaesthetic should meet the following criteria: simple administration, rapid induction of anesthesia, maintenance of the anesthesia state and rapid recovery, effectiveness at low concentrations, a wide range between effective and toxic concentrations, low tissue residues, and low cost (Marking & Meyer, 1985).

There have been few controlled systematic investigations of efficacy and physiologic effects for many of these anaesthetics, and there is a need for more complete and concise ranges of safe and effective concentrations or dosages of anaesthetic agents for fish (Gilderhus, 1987). This has resulted in renewed interest to develop 'green anaesthetic' with low environmental and health risks (Ramanayaka & Atapattu, 2006). A range of crude products and purified compounds are widely used as fish

sedatives viz., clove, wintergreen, or spearmint oils, quinaldine (Neiffer & Stamper, 2009; Danner et al., 2011).

Clove oil has been indicated as an alternative to traditional anaesthetics such as metomidate, quinaldine, and tricaine methanesulphonate because it is a natural oil and safe to use (Sladky et al., 2001). In the last several years, clove oil has been recognized as an effective anaesthetic for sedating fish for a number of invasive and noninvasive fisheries management and research procedures. Many authors in different fish species (Keene et al., 1998; Taylor & Roberts, 1999; Grush et al., 2004; Hamáèková et al., 2004; Roubach et al., 2005; Velíšek et al., 2005ab, 2006; Cunha & Rosa, 2006; Perdikaris et al., 2010) have investigated the efficacy and advisability of clove oil for fish anesthesia. The efficacy is conditioned by environmental and biological factors (Ross & Ross, 1999). The main chemical content of the clove oil is eugenol (70 to 98%) (Veloze, 2002) and has multiple uses mainly in dentistry and medicine as an antiseptic, analgesic and anaesthetic agent (Curtis, 1990; National Toxicology Program, 2002). Many aquaculturists and clinicians add it directly to water baths to achieve the desired effect. It is also an organic substance safe for both environment and user (McGovern-Hopkins et al., 2003). Therefore, it could be a promising anaesthetic agent in aquaculture and fish handling. In the international ornamental fish market, the indigenous fish blue hill trout *Barilius bakeri* (Day) is considered as medium preferred fish and belongs to the family Cyprinidae. In the present study, we assayed the potential of clove oil to anaesthetize *Barilius bakeri* in the course of handling and determine the lethal concentration of clove oil for *Barilius bakeri*.

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Indian blue hill trout *Barilius bakeri* (mean mass and standard length 3.75 ± 1.5 g; 101 ± 1.3 mm) were collected from Chalakkudy river system, and were acclimatized in laboratory for two weeks in square FRP tanks (1000 l) under natural photoperiod and light conditions. The tests were conducted in dechlorinated tap water (dissolved oxygen 7.3 mg l^{-1} , pH 6-7, temperature 27°C , and hardness 152 ppm). The fishes were fed thrice daily with commercial pellet feed and feeding was stopped 48 h prior to the experiment. The stock solution of clove oil (Universal Oleoresins India) was dissolved in 95% ethanol at a ratio of 1:10 (Cho & Heath, 2000). Two sets of experiments were conducted. Eight blue hill trout were used for each concentration and for the control group. In all experiments, diluted clove oil was added directly to the water and the water was vigorously aerated for the experiments. Dilution and experimental protocol were replicated three times.

The first set of experiment was to find out acute toxicities (24 h LC_{50} test) as per Litchfield & Wilcoxon (1949). This experiment was conducted triplicate in glass aquarium containing 5 l of aerated freshwater with different concentrations of clove oil viz., 2, 4, 6, 8 and 10 mg l^{-1} (the concentration lethal to 50% of test animals after 24 h) along with control. Eight fish samples randomly placed in each aquarium. Fish and its behaviour, total mortalities, water temperature, pH and oxygen saturation were monitored (Microprocessor water & soil analysis kit, Model 1160 E1. Environmental and Scientific Instruments, India) and recorded throughout the test and the experiment continued for 24 h under the same conditions to find out the 24 h LC_{50} of *Barilius bakeri*.

The second set of experiments was to find out the optimal dose of clove oil determined by the concentration range of clove oil. The expected concentration was the lowest level that causes 100% mortality and the highest level that causes 0% mortality. Eight fish were exposed to each of different concentrations of clove oil of 2, 4, 6, 8 and 10 mg l^{-1} . Each concentration had three replicates. Experiment was conducted in glass aquarium containing 5 L of water with continuous aeration. A logarithmic spaced series of clove oil concentrations between the highest concentration that killed no fish and the lowest concentration that killed all fish within 24 h were used as the optimal dose. During the 24 h test period, changes in physiological

parameters of fish and fish mortality figures were recorded after which the fish were moved to fully aerated fresh water.

Based on tests, the 24 h lethal concentration (LC_{50}) of clove oil was determined as 8 mg l^{-1} . 100% mortality rate was observed at clove oil concentrations of 10 and 12 mg l^{-1} (Fig. 1). It was observed that *Barilius bakeri* exposed to 12 mg l^{-1} died within 2 h of exposure. Velišek et al. (2005a) reported that the determination of acute toxicity of clove oil is important for its usage in fish anaesthesia, appropriate treatment concentration for anaesthetic baths and also for possible contamination of the water environment by such anaesthetic (Velišek et al., 2005a). Velišek et al. (2005a) reported that the rainbow trout is the most sensitive fish species, with LC_{50} values for clove oil of 81.1 mg l^{-1} for 10 min. Acute toxicity values of clove oil expressed as 10 min LC_{50} were 74.3 mg l^{-1} for carp (Velišek et al., 2005b) and 76.70 mg l^{-1} for the European catfish (Velišek et al., 2006). Comparable values were reported by the same authors for Chinook salmon (*Oncorhynchus tshawytscha*) at 62 mg l^{-1} , higher for coho salmon (*O. kisutch*) at 96 mg l^{-1} and for white sturgeon (*Acipenser transmontanus*) at 526 mg l^{-1} . Keene et al. (1998) reported that the 0.5 – 96 h LC_{50} of clove oil for rainbow trout (20 g in weight ; 12 cm in fork length) was 65 – 9 ppm. In a study conducted by Taylor & Roberts (1999), the 10 min LC_{50} of clove oil for juvenile white sturgeon, chinook and coho salmon were 526, 62 and 96 ppm, respectively.

Safe and optimal anaesthetic dosage required for inducing sedation with least mortality after 24 h for *Barilius bakeri* was determined as 4 mg l^{-1} . The

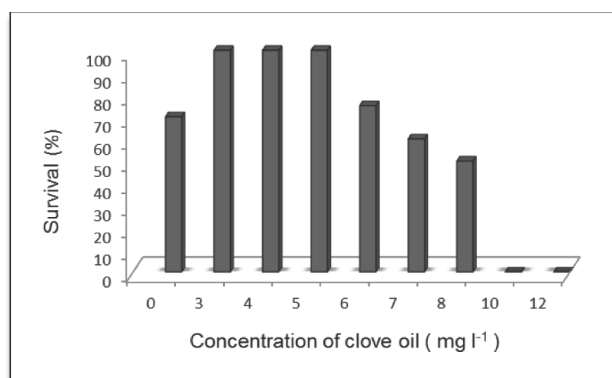


Fig.1. 24 h LC_{50} of *Barilius bakeri*

highest concentration of clove oil that caused 0% mortality was 5 mg l⁻¹. The lowest concentration of clove oil that caused 100% mortality was 10 mg l⁻¹. According to Logarithmic method: 10; 8; 6 & 4 mg l⁻¹ of clove oil was plotted against percentage survival on logarithmic-probability paper to obtain the expected death (percentage survival) (Fig. 1). The optimal dose calculated as per the method of Gilderhus & Marking (1987), was carried out to test the ability of clove oil as a good anaesthetic with the same species. Endo et al. (1972) showed that clove oil is effective in Crucian carp (*Carassius carassius*) and Hikasa et al. (1986) showed that it provides effective anaesthesia in common carp (*Cyprinus carpio*) at 25-100 mg l⁻¹. Soto & Burhanuddin (1995) found a dose of 100 mg l⁻¹ to be effective in rabbit fish. One of the criteria that proper anaesthetic in fish should meet is its safety at treatment concentrations (Marking & Meyer, 1985). Roubach et al. (2005) found that exposure of tambaqui (*Colossoma macropomum*) to 65 mg l⁻¹ of clove oil was sufficient to induce an anaesthetic state. The anaesthetic properties and dosages of clove oil have been tested on different fishes (Griffiths, 2000). Prince & Powell (2000) recommended a concentration of 30 mg l⁻¹ clove oil for effective and safe anaesthesia of adult rainbow trout. The optimal dose of clove oil in the range of 250–300 mg l⁻¹ at water temperature of 18°C was reported by Park et al. (2008).

Stone (1999) and Kildea et al. (2004) have assessed the advantages and disadvantages of clove oil as a fish anaesthetic. Most of them have reported it to be safe and effective anaesthetic but it has a narrow margin of safety. Care must be taken when using high concentrations of clove oil for induction, because ventilatory failure may occur rapidly. A combined view of the findings leads to the recommendation that, for the size of blue hill trout used in the present study, clove oil, with fast induction and rapid recovery times offers a manageable, safe anaesthetic alternative, which can be useful in aquaculture and fish handling activities. In future clove oil would be in use as an anaesthetic in Indian blue hill trout for long distant transportation.

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