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Use of Clove Oil to Anesthetize Female Wild Guppy (*Poecilia reticulata)* for a Short Duration

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ABSTRACT. *A study was conducted to determine the safe and effective range of clove oil concentrations that induce different stages of anesthesia in female Wild guppies (Poecilia reticulata) with one hour of exposure and also to find out the relationship of total length and weight on induction times. Clove oil (BDH UK- 1.041-1.054 g ml^{-1}) was mixed with fresh water at rates of 20, 25, 30, 35 and 40 µl l^{-1} to make anesthetic baths. Age matched, 96-related female wild guppies were exposed to different concentrations of clove oil for one hour with 16 fish in each concentration. Another experiment was conducted with 48 genetically related 6-weeks old female wild guppies to study the relationship of total length and live weight of fish on induction times using 25 and 30 µl l^{-1} clove oil dosages with a control. Series of behavioural changes ranging from mild sedation, deep sedation and anesthesia were identified. All the fish, which were kept in the concentration of 20 µl l^{-1}, were found either in the stage of deep or mild sedation. Induction times of 25, 30, 35, and 40 µl l^{-1} concentrations differed significantly. The mean induction times observed were 37±2.7, 27±1.6, 9±0.7 and 2±0.3 minutes respectively. Guppies in the first experiment, kept in Clove oil doses of 25 and 30 µl l^{-1} for one hour did not show any mortality. Their average recovery times were 46± 9 sec and 101± 11 sec, respectively. Only 25% of fish, which were in 35 and 40 µl l^{-1} recovered. Induction time correlated positively with total length and live weight at clove oil concentrations of 25 and 30 µl l^{-1} ($p<=0.0001$), at 28^{0}C while total length showed a stronger relationship ($R^2=0.93$ vs 0.80 and $R^2=0.84$ vs 0.75 respectively) than the live weight. Clove oil is proved to be highly effective and easy to use with wild Guppies. Clove oil concentrations of 25 and 30 µl l^{-1} anesthetized wild guppies within 37±3 to, 27±2 minutes at 28^{0}C and they could be handled for 33±3 to 23±2 minutes without any mortality after one hour of exposure. Clove oil concentration of 20µl l^{-1} could be further studied on sedation of wild guppies for an extended period of time.*

INTRODUCTION

Handling of fish for commercial purposes, research studies, transport and treatment, often results in mortalities attributed to stress. The level of stress a fish undergoes may affect its immune response and can make it vulnerable to diseases (Brown 1993). As an attempt to overcome this stress, biologists use a variety of sedatives and anesthetics on fish.

Several chemicals are used to sedate and anaesthetise fish. The anesthetics

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that are commonly used on fish are 3-aminobenzoic acid ethyl ester methanesulphonate (MS-222), carbon dioxide gas (CO_2), and benzocaine (Brown 1993). Among those anesthetics MS-222 is the most widely used and recommended by many countries (Molinero and Gonzales, 1995). MS-222 and benzocaine are expensive anesthetics (Woody *et al.*, 2001) which are not available locally. Eventhough MS-222 is generally recognized as safe for human intake, treated fish must be held for 21 d before releasing to allow the anesthetic to leave the fish body (Woody *et al.*, 2001). CO_2 is considered only partly effective (Woody *et al.*, 2001) although is available locally.

Clove oil is another substance, which has been used for centuries as a topical anesthetic in humans, particularly for pain relief in dental problems. Over the last five years, studies have been carried out on clove oil to determine its effectiveness as an anesthetic for fish (Woody *et al.*, 2001). Clove oil is a mixture of different compounds. It is 85% to 95% eugenol, while the remaining 5 to 15% of the ingredients constitute with isoeugenol and methyleugenol (Tamaru and Lyum, 2000). Clove oil has been shown to be an effective anesthetic on a variety of marine species and same fresh water fish species (Tamaru and Lyum, 2000). However, its efficacy as an anesthetic agent on guppies and other fresh water fish species in Sri Lanka has not been reported.

This preliminary study was conducted to determine the safe and effective range of concentrations that induce different stages of anesthesia with hour of exposure in female wild guppies and to ascertain the relationship of total length and live weight on induction time. The findings of this study will help in procedures involving handling of live fish (experimental, disease diagnosing and treatment) such as blood sampling, microscopic examinations and minor surgeries requiring maintenance of anesthesia and also forms the basis of future studies in anesthetizing fish for a longer period of time.

MATERIALS AND METHODS

Female wild guppies which could be bred in abundance were used as experimental animals. During the experiment a number of guidelines recommended by Brown (1993) were followed. Those were 24 h fasting, properly aerated anesthetic bath, maintenance of same temperature as in the breeding tank and the use of properly aerated recovery bath.

The method used was the introduction of clove oil into fish gills through water. Clove oil from BDH UK, which has concentration of 1.041-1.054 g ml^{-1}, was mixed with fresh water at rates of 20, 25, 30, 35 and 40 $\mu l\ l^{-1}$, respectively. Anesthetic solution was prepared by vigorously shaking the clove oil and fresh water in a flask to obtain a whitish immersion.

Experiment was carried out on age matched, 96-related (common father) female Wild guppies. Total length of each fish was taken before the experiment and the fish were divided in to four groups of 24 fish according to the total length (0.5-1.0 cm, 1.1-1.6 cm, 1.7-2.2 cm and 2.3-2.8 cm). During the experiment, each length group was divided in to six groups of four fish. The fish were anesthetized at the same time by placing each group in five glass tanks (100 ml) of different concentrations of clove oil and a control tank (100 ml) with fresh water. Each set of fish was observed for one hour and induction time was noted. Water temperature and pH of each anesthetic tank were recorded before and after the treatment. After one hour of observation, all the fish

were placed in a recovery tank, and time taken for recovery was recorded. These fish were kept in the recovery tank under observation for 72 h.

Second experiment was conducted to study the effects of total length and live weight of the fish on induction times. It was conducted using 25 and 30 µl l^{-1} clove oil concentration with a control. Experiment was carried out using age matched 48-related female Wild guppies grown up to 6 weeks.

During the experiment 16 fish were anesthetized at a time by placing each fish in 100 ml glass tanks filled with the anesthetic with 8 fish in each concentration excluding the control. A control group of eight fish were kept in 100 ml fresh water tank (after shaking vigorously) as a control in the respective time. During the experiment each set of fish was observed for one hour and induction time was recorded. Water temperature of each bath was kept the same as the temperature in the breeding tank. The pH of each anesthetic bath was recorded before and after the treatment. After one hour of observation, all fish were placed in a recovery tank and the recovery times were recorded. Standard lengths of each fish and the length weight were recorded. These fish were kept in the recovery tank under close observation for 72 h.

Results were analyzed using SAS computer package. Mean separation was done using Duncan's New Multiple Range test.

RESULTS AND DISCUSSION

Introduction of anesthetic agent through water is known as immersion anesthesia (Strosskopf, 1993). The substance is absorbed through the gills and travels through the blood stream to the central nervous system (Brown, 1993). The fish then goes through several stages of anesthesia ranging from loss of balance to total immobility and ventilatory arrest (Brown, 1993).

Following series of behavioural changes of fish were identified during the experiment.

i. Slight loss of equilibrium, absence of real attempt to avoid the sides of the tank, slowing of swimming movements, fast or slow swimming with little rest (identified as mild sedation).
ii. Slight or obvious loss of equilibrium, absence or further slowing of swimming movements, sinking to the bottom of the tank most of the time, less resistance when handled (identified as deep sedation).
iii. Complete loss of equilibrium, lie upside down on the bottom of the tank, with little swimming motions, no reaction to being handled, depressed or absent gill ventilation (identified as anesthetized).

Such behavioural changes have been observed by Molinero and Gonzales (1995) with MS-222. None of the fish in control tanks showed any behavioural change.

Average induction times observed for 25, 30, 35, and 40 µl l^{-1} clove oil concentrations were 37±2.7,27±1.6, 9 ±0.7 and 2 ±0.3 minutes respectively. These induction times were significantly ($p<0.01$) different to each other.

All the fish, which were kept in 20 µl l^{-1} concentration, were found either in the stage of deep or mild sedation. Clove oil concentration of 20 µl l^{-1} and lower could be further studied in keeping fish sedated for an extended period of time.

Guppies in clove oil doses of 25 and 30 µl l^{-1} experienced an average recovery times of 46± 9 and 101± 11 sec., respectively. Only 25 % of fish, which were in 35 and 40 µl l^{-1} survived after being transferred to the recovery tanks. All recovered fish were active and no abnormal behaviour could be observed during the 72 h monitoring period.

Clove oil concentrations of 25 and 30 µl l^{-1} seemed to be the safe margins in anesthetizing female wild guppies for purposes which require short period (20 minutes) of anesthesia. Even though the concentrations of 35, and 40 µl l^{-1} were lethal for female wild guppies when kept for one hour, effectiveness of these concentrations could be studied using a shorter exposure period as in the study on sockeye salmon by Woody *et al.*, (2001).

Clove oil doses of 25 and 30 µl l^{-1} were selected to be with the next experiment, which had the advantage of anesthetizing fish quickly with a safe recovery after one hour of exposure.

Induction times in relation to length and weight of fish

Total length and live weight of female wild guppies used in the second experiment ranged from 0.5 cm to 2.8 cm and 0.04 g to 0.26 g, respectively. The induction times observed ranged from 23 to 55 minutes for the 25 µl l^{-1} concentrations and 20 to 35 minutes for 30 µl l^{-1} concentration with the mean induction times of 37±2.6 and 27±1.8 minutes respectively. None of the fish in control tanks showed any such behavioural change.

Regression analyses indicated a significant positive correlation of induction time with total length and live weight of fish at both clove oil concentrations of 25 and 30 µl l^{-1} ($p<0.0001$). Induction times have increased proportionately with increasing total length and live weight (Fig. 1, Fig. 2). Total length of fish showed a stronger relationship with the induction time for both 25 and 30 µl l^{-1} clove oil concentrations ($R^2=0.93$ and $R^2=0.84$, respectively) than live weight ($R^2=0.80$ and $R^2=0.75$, respectively).

Keeping the guppies in Clove oil doses of 25 and 30 µl l^{-1} for one hour at 28 °C after induction did not cause any mortality. They recovered with an average recovery times of 44 were and 100 sec. respectively. Fish deaths were not observed during 72 h monitoring period.

These observations may lead to reveal appropriate methods for anesthetizing freshwater fishes of Sri Lanka for different purposes. Clove oil which is not well known or widely used, could become an alternate to the other anesthetics (MS-222, Benzocaine) which are hazardous, expensive, hard to come by in developing countries and sometimes less effective (Woody *et al.*, 2001)

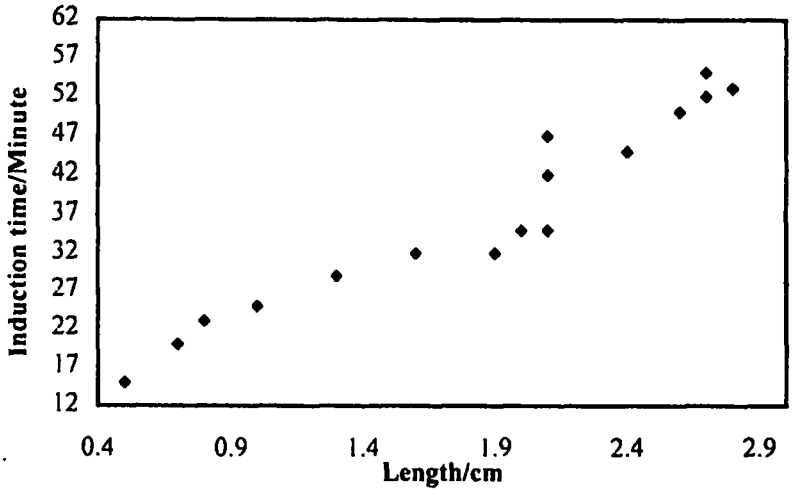

Fig. 1. Relationship between total length and induction time at clove oil concentration of 25µl l^{-1} and 30µl l^{-1}.

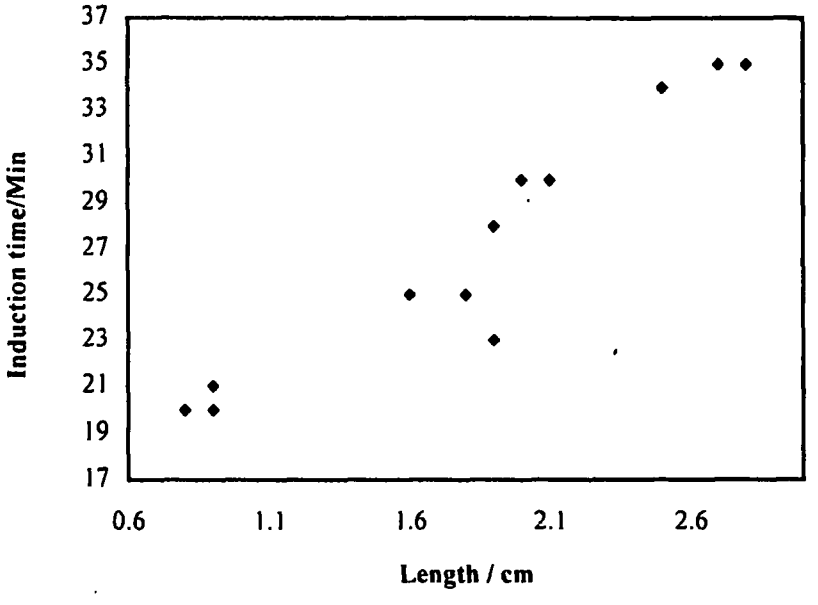

Fig . 2. Relationship between total length and induction time at clove oil concentration of 30µl l^{-1}.

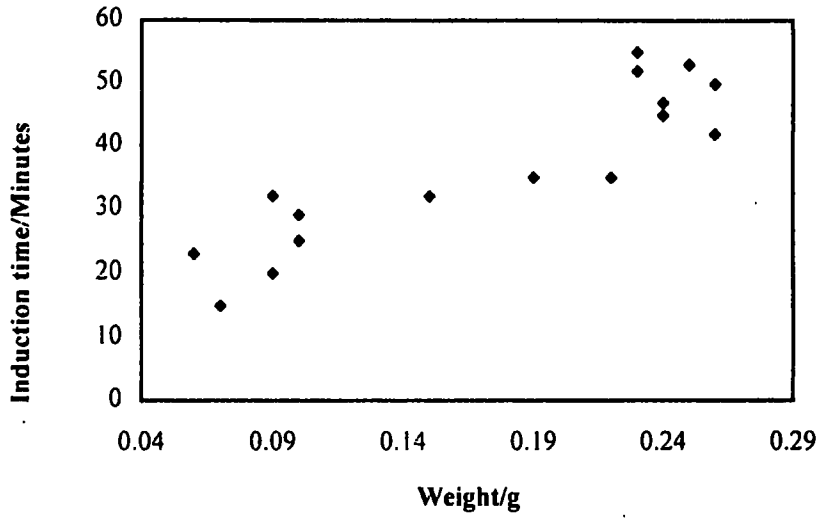

Fig. 3. Relationship between live weight and induction time at clove oil concentration of 25µl l^{-1}.

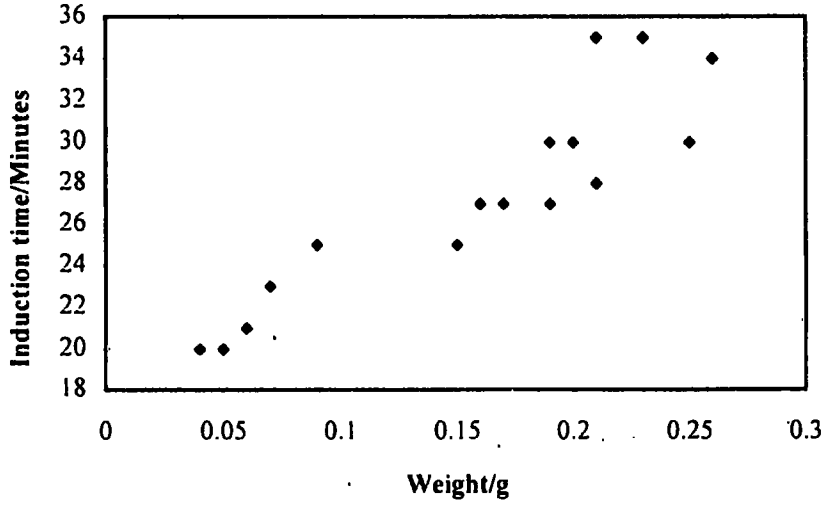

Fig. 5. Relationship between live weight and induction time at clove oil concentration of 30µl l^{-1}.

CONCLUSIONS

Clove oil was effective as an anesthetic that can be easily used with female wild guppies. Clove oil concentrations of 25 and 30 µl l^{-1} anesthetized wild guppies within 37 and 27 minutes at 28°C, and the fish could be handled for 33 to 23 minutes with 100% survival. Both total length (0.5 – 2.8 cm) and the live weight (0.04 -0.26 g) exhibited a significant positive correlation with induction times of female wild guppies at 28°C. Stronger correlation was observed between the total length and the induction

time. Clove oil concentrations of 20 μl l^{-1} and below should be further studied for keeping wild guppies sedated for an extended period of time.

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