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Fish Anaesthetic Properties of Some Local Plant Material

J.C. Ramanayaka and *N.S.B.M. Atapattu
Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka.

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ABSTRACT

Objective of this study was to evaluate the anesthetic properties of several plant extracts in common carp (*Cyprinus carpio*). Extractions were made by grinding 50 g of plant material with 500 ml of water followed by crushing and filtering. Induction times of anesthesia to four pre-determined stages (S_1-S_4) were recorded for different concentrations of plants extracts. When fish lost the total equilibrium (S_4), it was placed in a fresh water tank and the recovery time was recorded. Each concentration of the plant extract was tested in ten replicate fish. Clove (*Eugnia carioplyllum*), Clotalaria (*Clotalaria* sp), Kalawel (*Derris scandens*) and Kukuru extracts showed high to medium level of anesthetic properties. Clove gave the best induction and recovery times. At 3 ml plant extract/L, fish attained S_4 within 5.5 minutes and recovered within 15 minutes. Even though clotalaria at 2.5ml/L induced S_4 within 4.3 minutes, the recovery time was 64 minutes. Kalawel had a narrow effective range and acted fast. At 2 ml/L fish attained S_4 within 8.7 minutes and recovered in 43 minutes. At 3.75 ml/L Kukuru had 20 minutes induction and 60 minutes recovery time. Welan and Medella (*Barrigtonia racemosa*) showed some anesthetic properties but were less effective due to longer induction and recovery times. Thiththawel (*Anamirla coculus*), Thala (*Corypha ambraculifera*) and Daluk (*Euphorabhia antiqurum*) showed no anesthetic properties but were toxic. It is concluded that anaesthetic properties of clove are comparable with the recommended criteria for being an effective anesthetic.

Key words: fish, anaesthesia

In fish farming, various management practices such as weighing, tagging, stripping and transportation are required to handle fish. Handling of fish out of water is very stressful and even lethal to fish depending on the severity of the stress. In order to minimize the stress of transportation fish should be immobilized. Fish are among the most widely used laboratory animals. Therefore, apart from aquaculture related handling, on many occasions fish are subjected to stressful surgical and experimental procedures. Fish are considered as sentient animals and thus are included in ethical codes. Therefore, from legal and ethical point of view, fish should be anaesthe ized when they are subjected to any stressful handlings.

*Corresponding Author

Chemical anesthetics are now widely used for fish anesthesia. The chemical anesthetics used in fish have changed gradually (Stuart 1985). Many of the first generation anesthetics such as ether, chloroform, menthol, urethane and cocaine are now not in use due to, health hazards, lack of adequate efficacy and adverse physiological effects on fish. The second generation of fish anesthetics are the chemicals such as tricaine, benzocaine and quinaldine. Though these anesthetics are effective, only tricaine has been permitted to use in food fish anesthesia mainly due to environmental and health risks (Marking and Mayer, 1985). This has resulted in a renewed interest to develop 'green anaesthetic' with low environmental and health risks.

Though the term anesthesia is technical, for thousands of years people used plant extract to kill or immobilize fish. These plant extracts are added to water in various forms and ways. The efficacy varies greatly from light sedation to killing. The objective of the present study is to evaluate the fish anaethetic properties of some plant materials commonly used by Sri Lankan fishermen to immobilize or to kill fish.

Extracts of ten plant materials were used for the experiment (Table 1).

The method of extraction was similar for all pant materials except for Thiththa Wel. 50 g of fresh pant material were blended in a kitchen blender (300 rpm) with 500 ml of distilled water.

Then the extract was collected into a flask by crushing and filtering through a piece of cotton cloth. The method of extraction for Thiththa Wel was as follow; 50 g of fresh nut of Thiththa Wel was mixed with 5 ml of coconut oil and then crushed together using a motar and pestle. 55 g of this mixture was then mixed with 500 ml of water and belnded. The extraction was collected by crushing and filtering though a cotton cloth.

Table 1. Plant materials studied for fish anesthetic properties

Common name	Botanical name	plant part used to get extract
Clove	*Eugnia ariophylum*	dry flower buds
Clotalaria	*Clotalaria spp*	leaves
Kalawel	*Derris scandens*	stem
Kukuru		nut (fruits)
Welan		nut (fruits)
Medella	*Barringtonia raecemosa*	bark
Andu	*Eryagaum foetidany*	whole plant
Thithawel	*Anamirla cocculus*	Nut
Thala	*Corypha ambraculifera*	nut (fruit)
Daluk	*Euphorabia antiquarum*	stem

2-3 months old common carp (*Cyprinus carpio*) of indeterminate sex was used for the experiment. Fish were kept in an outdoor cement tank before being used for the experiment. A pre-trial was conducted to establish the effective dose range of each plant extract.

A glass tank (45 L * 25 W*29.5 H cm) was filled upto 18 cm with fresh tap water so that the tank contains 20 l of water. A measured volume of plant extract was added to the water and mixed thoroughly for 20 seconds. Then a fish was introduced into the tank. The time taken to reach pre determined stages of anaesthesia was taken (Table 2).

Table 2. Stages of anesthesia and their characters (Modified from Stuart 1985).

Stage of anesthesia	Characters used to identify
1	Loss of equilibrium, but fish responded to tactile stimuli
2	reduced response, partial loss of equilibrium, fish can hold on hand, reduced operculum activity
3	loss of response, can pull by posterior fin
4	total loss of equilibrium, fish lay down

As soon as the fish reached to S_4 anesthesia, it was transferred to a glass tank filled with 40 l of fresh tap water. The tank was aerated with normal circuit of aerator. The time taken to gain the complete equilibrium was taken as the recovery time. Each concentration of all the plant extracts was replicated in 10 fish. No fish was reused. The weight of the fish varied from 100-150 g.

Time taken to reach different stages of anesthesia and recovery time from S_4 are given in Table 3.

The effectiveness of the plant extracts was decided on the time taken to reach to S_4 anesthesia and the recovery time. Marking and Mayer (1985) recommended desirable induction and recovery times of anesthesia of fish as 3 and 5 minutes, respectively. None of the tested plant extract met the recommended induction and recovery time regimes. This less effectiveness with respect to induction and recovery times may partly be due to the low concentration of active ingredients in the crude plant extracts we used. Much of the fish anesthetic studies have been conducted in salmonids whose oxygen demand is high (Sedgwick (1985). The transfer of anesthetics to and from the fish takes place via the gills and thus the effectiveness of anaesthetics in these active fishes are higher compared to carps. The observed less effectiveness may be attributed to this fact as well.

Despite the fact that plant extracts are not as effective as an ideal anesthetic should be, a considerable level of anesthetic properties could be found even in crude plant extracts. Clove, Clotalaria, Kalawel and Kukuru extracts showed high to medium levels of anesthetic properties. Clotalaria at 3-3.75 ml/l level gave around 3 minutes induction time. However, recovery time was far exceeded the recommended time. Clove at 3 ml/l gave the closest characters to the ideal anesthetic. Munday and Wilson (1997) and Peake (1998) have also reported the anesthetic properties of clove oil. We observed 5.5 minutes induction time and 15 minutes recovery times at 3 ml/l concentration. Peake (1998) reported 6 minutes of induction and 7 minutes of recovery time for clove oil. Values we observed were more or less similar to the values reported by Gilderbus and Marking (1987) for CO_2 at 240-265 mg/l; i.e 3.1 minutes induction and 16 minutes recovery time. Marking and Mayer (1985) showed that CO_2 was stressful and lethal after repeated exposure because CO_2 acidifies water and reduces desolved O_2. Other adverse

Table 3. Time taken to reach different stages of anesthesia and to recover as affected by the plant extract and the concentration.

Time taken to reach different stages of anesthesia and recovery (Min)

Plant extract	Concentration (ml/l)	S1	S2	S3	S4	Recov-ery
Clove[1]	1.5	4.30	7	12	22	7
	2	4.5	5.3	7	11	9
	2.5	3	4	5	6	14
	3	3	4	5	5.5	15
Clotalaria[1]	1.25	1.1	3.6	8.4	17.7	60
	2.5	0.6	1.3	2.1	4.20	64
	3.75	1.3	1.4	2.3	3.3	84.3
	5	1.2	1.6	2.1	3.2	116.1
Kalawel[1]	2	2.7	4.1	5.6	8.7	43
	2.25	2.4	3.6	5.5	8.1	57.1
	2.5	2	3.9	6.2	8.6	80.5
	2.75	2	3.4	4.4	6.2	161.2
Kukuru[1]	1.25	21	31	33	50	10
	2.5	7	10	21	33	17
	3.75	7	9	17	20	60
	5	7	10	18	20	90
Welan[1]	5	27.8	44.9	75.3	102.	24.2
	7.5	11.6	19	25.1	35.7	40.9
	15	5.9	9.2	13.7	20.1	53.7
Medella	5	20	31.7	42.5	53.7	106.7
	15	9.8	14	17.3	23.4	135.3
Andu[2]	2.5	41	75			
	5	25	46.6			
Thithawel[3]	5	35	46.6	56.6	66.6	
	10	18.6	25	33	45.3	
Thala[3]	2.5	22.6	33.3	42	50.6	
Daluk[3]	5	13.6	22.6	38.6	43	

[1]Means of ten fish. No fish died
[2]Studied only up to S_2
[3] Fish died

effects of CO_2 include erythrocute swelling,
increased lactic acid levels and reduced oxygen partial pressure in blood. Since many countries have now banned chemical anesthetics in aquacultural practices clove can be recommended as an effective anaesthetic agent for food fish.

Some doses of Clotalaria and Kalawel showed fairly effective induction times. However, both of these plant extracts had much longer recovery times. Kalawel could be identified as the most effective in inducing anaesthesia. Also it had narrow dose range of effectiveness. Even at 2 ml/l concentration it caused S_4 anaesthesia within 8.7 minutes but took 43 minutes to recover. The effectiveness of Kalawel is comparable with chemical anesthetic Choral Hydrate at 0.9g/l (Stuart 1985). Kukuru also showed some anesthetic properties. 3-3.75-5 ml/l concentration caused S_4 in 20 minutes. Interestingly concentrations of 1.25 and 2.5 ml/l of kukuru and 5 ml/l of welan had shorter recovery times than their respective induction times. No chemical anaesthetic has shown to have this property. The reason for this peculiarity is not clear.

Welan and Medella can also be categorized as slow acting plant extracts. To achieve S_4 in 20 minutes both welan and medella required as high dose as 15 ml/l. The recovery times were also high for these plant extracts. The longer recovery times of these extracts may be helpful when fish are transported for a long distance under anesthesia. Stuart (1985) compiled the chemical anesthetics suitable for fish transportation under anesthesia. Anesthetic properties of Welan and Medella fairly comparable with the chemicals metomidate and etomidate at 0.75 and 1 mg/l. Andu was the least effective. Since it took 46 minutes to reach S_2 at 5ml/l, we assumed that anesthetic properties of andu was not study worthy. Thiththawel, Thala and Daluk showed no anesthetic properties. They were found to have toxic effects than anesthetic properties (as claimed by indigenous knowledge) since all the fish exposed to above plant extracts did not recover but died.

Fish were observed for five days after the completion of the study. No fish anaesthetized with Clove, Cloralaria, Kalawel, Kukuru, Welan, Midella and Andu died during that period and fish showed normal activity indicating that those plant extracts have no adverse effects. However, how these plant extracts affect the physiological processes are not clear. Therefore, further experiments need to be conducted to establish such effect, if any, if fish are to be anaesthetized using these extracts, for physiologica experiments. Anaesthetic properties of Clotalaria, Kalawel and Kukuru were found to be sufficient enough for many aquacultural practices. Welan and medella could be useful for fish transportation. Andu, thala, thithawel and daluk should not be used as an anaesthetics. Further researches are needed to identify the active ingredients and possible effects on physiology of the fish.

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