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Analysis of Anaesthetic Effect of Natural Oils for Handling Ornamental Fish, Platy

Swapana Johny [1&2] & Dr. N D Inasu[3]
[1]Research Scholar, Sacred Heart College, Thevara. [2]Asst.Prof.Little Flower College, Guruvayur
[3]Former Pro Vice Chancellor, CUSAT.

Abstract: *Ornamental fish culture is fast emerging as a major branch of aquaculture. Indian ornamental fishes with their brilliant colours and unique features need no introduction to the world market. As most ornamental fish are destined for export, the fish must not only be pleasing to look but also robust enough to withstand the long journey by air transportation. Accumulation of carbon dioxide, depletion of oxygen, accumulation of ammonia, physical injury due to hyper activity etc, are some of the important constraints felt during live fish transport. Methods available to reduce the stress caused due to handling include the use of anaesthetics. In this study the efficacy of five different bio oils viz., clove oil, orange oil, nutmeg oil, cinnamon oil and holy basil, as anaesthetics in handling the ornamental fish Platy sps is tested. The concentrations of 40mg/l, 240mg/l, 396mg/l and 663mg/l of clove oil, nutmeg oil, cinnamon oil and holy basil oil gave anaesthetic effect on term exposure. Orange oil failed to reach anaesthetic stage even at higher concentration.*

Keywords: *fish anaesthetics, bio oils, Platy sps., fish transportation.*

1. Introduction

Ornamental fish trade is a multimillion-dollar-industry now-a-days. In the ornamental fish business, the ability to meet customer's needs for high quality fish is always a critical factor. As most ornamental fish are destined for export, the fish must be not only pleasing to eyes but also robust enough to withstand the long journey by air transportation. But there are several constraints in the live fish transport. Plastic bags were first used for transport of fish by tropical fish importers. Nowadays polyethylene bags are used for transport. Accumulations of metabolic wastes occur in high density packing [22, 23].

Fishes are exposed to various stressors during the period of capture, handling and packing procedures [19, 1]. Struggling of the fishes has detrimental effects on their physiology and behaviour and the animals would easily be damaged [15]. This may lead to large scale mortality during the subsequent period of transportation. Accumulation of carbon dioxide, depletion of oxygen, accumulation of ammonia, physical injury due to hyper activity etc, are some of the important constraints felt during live fish transport. Biochemical and physiological changes occur during fish handling and transport. Methods available to reduce the stress caused due to handling include the use of anaesthetics. Anaesthesia is a biological state induced by an external agent, which results in partial or complete loss of sensation or loss of voluntary neuromotor control through chemical or non-chemical means [21]. In fisheries research and assessment as well as in aquaculture operations, anaesthetics are necessary to minimize stress to the fish and to reduce the physical injury during various handling procedures [4].

Prior to anesthesia, the veterinarians recommend starvation for one feeding cycle. A fish with full stomach may regurgitate under anesthesia and clog its gills partially and foul the water.

Anesthetics can be delivered by immersing the fish in a tank or bucket of water containing the anesthetic preferably from the aquatic system from which the fish is collected. All water parameters should be within acceptable range and temperature should be constant. The procedure is then performed as quickly as possible and the fishes are returned to anaesthetic free water for recovery. Respiration will be gradually strengthened, normal upright position will be returned. Even once the fish is recovered and appears outwardly normal it may remain hypoxic (low blood oxygen level) for sometimes. So maintaining adequate aeration of water is important. According to Marking and Meyer [11] an ideal anaesthetic should permit a reasonable duration of exposure, produce anaesthetic within 3 minutes or less, allow recovery within 5 minutes or less and be reasonable at cost.

Mc Farland [13] and Bell [2] studied the importance of anaesthetics in reducing packing stress. The anaesthetics mainly used are MS222, 2-

phenoxy ethanol, benzocaine, etc. Various works have been carried out using these anaesthetics. Though clove oil has been used as a fish anaesthetic for at last the quarter – century [5, 9, 7], it has been the focus of research aimed at establishing its effectiveness and safety for use by both aquaculturists and fish researchers [3, 10].

In the present study we intend to find out the efficacy of five different bio oils as anaesthetics in fish handling. The oils selected were clove oil, orange oil, nutmeg oil, cinnamon oil and holy basil, all of which have good health benefits and available in the market. The study was made on one of the commonly used ornamental fish Platy sps collected from nearby aquarists.

2. Materials and Methods

2.1. Materials Used:

Materials used for the experiment include five bio oils like clove oil, orange oil, holy basil oil, nutmeg oil and cinnamon oil. The ornamental fish taken for this experiment is Platy (Xiphophorus sps.), a fish belonging to the tropical livebearers under the Family Poeciliidae.

2.2. Experimental Method:

The experimental fish, Platy weighing between 210mg – 690mg, was procured from local aquarists and bio oils from extraction unit. The collected fishes were brought to the laboratory and acclimatized in the aquarium for 10 days prior to experiment and fed with commercial pellet feed. Preliminary studies were conducted with all five oils to evaluate the effect of all oils at a random concentration, based on which experimental concentrations were fixed.

As Oils will not readily mix up with water, they are diluted with Ethanol (95%) in the ratio 1:9 (Oil: Ethanol) to prepare the stock solution. Table 1gives the concentrations of the Oils used for the experiment.

Table 1: Concentrations of bio oils used for experiment

Sl. No.	Clove Oil (mg/l)	Nutmeg Oil (mg/l)	Cinnamon Oil (mg/l)	Orange Oil (mg/l)	Holy Basil Oil (mg/l)
1	30	24	198	213	468
2	40	40	330	320	585
3	50	80	363	355	624
4	70	240	396	390	663
5	100	280	429	604	702

Water quality parameters of the experimental tanks as well as acclimatization tank are analyzed. The Table 2 gives the range of four parameters checked during the experiment.

Table 2: Water quality parameters of experimental water

Parameters	Range
pH	6.8- 7.2
Temperature	27.5^{o} – 28.2^{o}C
TDS	112 -114
Salinity	90.6 -92.6 ppm

Feeding was stopped 24hrs prior to the experiment. Fishes were netted from acclimatization tanks and placed in the experimental bags with anaesthetic solution of specific concentration. Three fishes are taken for each concentration of oil for the experimental procedure. In this experiment the anaesthetic efficacy of bio oils for short term exposure are recorded by checking the induction time. During the experiment the behavior of the fish are observed and the time of anaesthetic induction and recovery time measured using stopwatch. Immediately after reaching stage 5 or after 10 minutes of anaesthetic exposure are transferred to recovery tanks with anaesthetic free fresh water. Each set of fish are used only once. The time taken for induction and recovery are recorded for each concentration of all types of oils under experimentation. The stages of induction were asessed following the method of Summerfelt and Smith [21] Table 3.

Table 3: Stages of induction of anaesthesia in fish

Stages of Induction	Description	Behaviour response of fish
0	Normal reaction to external stimuli	Opercular rate and muscle tone normal.
I	Light sedation, slight loss of reactivity to external stimuli	Opercular rate slightly decreased: equilibrium normal
II	Deep sedation, total loss of reactivity to all but strong external stimuli	Slight decrease in opercular rate, equilibrium normal
III	Partial loss of equilibrium	Partial loss of muscle tone, erratic swimming, increased opercular rate, reactivity only to strong tactile and vibration stimuli

IV	Total loss of equilibrium	Total loss of muscle tone and equilibrium, slow but regular opercular rate, loss of spinal reflex
V	Medullary collapse	Respiratory movement ceases

3. Result

Anaesthetic depths can be determined by assessing activity, reactivity to stimuli, equilibrium, muscle tone and respiratory and heart rates. Induction usually takes 5-10 minutes and is marked by a decrease in caudal fin strokes, swimming, respiratory rate and reaction to stimuli. There is loss of equilibrium and the righting reflex is poor. No response to a firm squeeze at the base of the tail confirms a loss of reaction to stimuli and resulting general anaesthesia [8, 20] . Stages of anaesthetization include induction, maintenance and recovery. The stage achieved usually depends on the dose and the length of exposure.

Different oils administered at different concentration ranges ended up in anaesthetic induction. The time of induction is the period from the time when as experimental fish is placed in the anaesthetic tank, to the time it does not respond to external stimuli and the recovery time is the period from the time when an anaesthetised fish is placed in a recovery tank to the time it recovers from anaesthetization with full equilibrium motion [16]. The recovery time started from few seconds to few minutes according to the concentration and types of anaesthetics used.

The lowest effective concentration is the concentration that produces general anaesthesia within 3 minutes and allows recovery within 10 minutes [6, 24]. Tables 4 to 8 give the concentration, progressive anaesthetic induction time and recovery time of Clove oil, Nutmeg oil, Cinnamon oil, Orange oil and Holy basil oil.

Table 4: Stages of anaesthesia induced by clove oil in fish

Concentration of Clove oil (mg/l)	Time in minutes										Recovery time in minutes
	1	2	3	4	5	6	7	8	9	10	
30	0	0	1	1	2	3	3	4	-	-	3
40	0	1	2	3	4	5	-	-	-	-	5
50	2	4	5	-	-	-	-	-	-	-	5
70	3	5	-	-	-	-	-	-	-	-	8
100	5	6	-	-	-	-	-	-	-	-	Dead

Table 5: Stages of anaesthesia induced by nutmeg oil in fish

Concentration of Nutmeg oil (mg/l)	Time in minutes										Recovery time in minutes
	1	2	3	4	5	6	7	8	9	10	
80	0	0	0	0	0	0	0	0	0	1	1
120	0	0	0	0	1	1	2	2	2	2	2
160	0	0	0	1	1	1	2	3	3	3	3
240	0	1	2	3	3	4	5	-	-	-	5
280	4	5	-	-	-	-	-	-	-	-	6

Table 6: Stages of anaesthesia induced by cinnamon oil in fish

Concentration of Cinnamon oil (mg/l)	Time in minutes										Recovery time in minutes
	1	2	3	4	5	6	7	8	9	10	
198	0	0	0	0	0	0	0	1	1	1	2.5
330	0	0	1	1	2	2	3	-	-	-	3
363	0	1	2	3	3	-	-	-	-	-	3.5
396	0	1	2	3	4	4	-	-	-	-	4.5
429	0	1	4	5	-	-	-	-	-	-	5.5

Table 7: Stages of anaesthesia induced by orange oil in fish

Concentration of Orange oil (mg/l)	Time in minutes										Recovery time in minutes
	1	2	3	4	5	6	7	8	9	10	
213	0	0	0	1	1	1	1	1	1	1	
320	0	0	0	1	1	1	1	1	1	1	
355	0	1	1	1	1	1	1	1	1	1	
390	0	1	1	1	1	1	1	1	1	2	1
604	1	1	1	1	1	1	1	1	2	2	2

Table 8: Stages of anaesthesia induced by holy basil oil in fish

Concentration of Holy Basil oil (mg/l)	Time in minutes										Recovery time in minutes
	1	2	3	4	5	6	7	8	9	10	
468	0	0	0	0	0	1	1	2	2	2	
585	0	0	0	1	1	2	2	3	3	4	2
624	0	1	1	2	2	3	4	4	4	4	2.5
663	1	2	2	3	4	-	-	-	-	-	3.5
702	1	2	3	4	4	5	-	-	-	-	4

According to these results the effective concentrations of different oils in the induction of anaesthesia in Platy appeared to be in different

ranges. The time taken to reach anaesthesia or maximum of 10 minutes induction was recorded for all concentrations of the different bio oils used. The effective concentrations of all the oils with their anaesthetic stage, induction time and recovery time are given in Table 9.

Table 9: Effective concentrations of oils inducing anaesthesia

Bio oils	Concentration	Stage	Induction time	Recovery time
Clove oil	40mg/l	IV	5min	5min
Nutmeg oil	240mg/l	IV	6min	5min
Cinnamon oil	396mg/l	IV	5min	4.5min
Orange oil	604mg/l	II	10min	1min
Holy basil oil	663mg/l	IV	5min	3.5min

4. Discussion

The ornamental aquatic industry is undoubtedly the industry which moves the largest numbers of animals globally. In live-fish transport, anaesthetics are useful in lowering the metabolic activity of fish, which facilitates the transport of more fish in a given quantity of water for a longer time. On using the earlier mentioned five different bio oils as anaesthetics, it was noticed that the fish show a definite course of behaviour in the anaesthetization effect. Most of the fishes used in the experiment recovered within 5 min. Recovery time increased with longer exposure time. At higher concentrations, the time taken to reach stage IV was decreased, but more recovery time was needed. But these conditions varied with different bio oils as they had different level of anaesthetizing property.

In the case of Clove Oil, at a lower concentration it took more time to reach stage IV but recovered soon. The increased concentration caused reduced induction time. With early anaesthetic induction more recovery time was taken. At a higher concentration of 100mg/l the fish entered stage V and resulted in death. Studies from the literature show that clove oil and its effect have been well studied and it is extensively used in the ornamental fish industry for a long time as a harmless, human friendly and cheap anaesthetic. It is a medicine used as topical anaesthetic for humans [18]. Latest studies by Sajan *et al*, [16] shows that Clove oil is highly effective as a fish anaesthetic with potentially no side effects, which is safe for both fish as well as man and is also less expensive.

For Nutmeg Oil, the lower concentrations showed very less anaesthetic property; slightly starting after 10 minutes. On reaching a concentration of 240mg/l it showed stage IV at 6 minutes of induction. But next concentration induced anaesthesia immediately on transferring to anaesthetic water, which is not a good sign of anaesthetic. So here the most suitable concentration for anaesthetic induction on short term exposure is 240mg/l. There is no reference about nutmeg oil used as anaesthetic in literature. Nutmeg Oil has many health benefits including sedative property in human beings and the one of the component in it is eugenol as in clove oil. And this was the reason we tried this as an anaesthetic. Nutmeg oil is also easily available and cheap as that of clove oil. For transportation of fish, it has to be in water for long hours during the transport. During that period lesser concentration of anaesthetic is preferred; in such a case a concentration of 160mg/l can be used.

In our experiments with Cinnamon Oil, the findings showed least anaesthetization in lower concentrations. Higher concentration gave more anaesthetization. A concentration of 396mg/l of oil gave stage IV in 5 minutes and recovered in 4.5 minutes, which is an ideal time period for short term anaesthetization. In this case also there is no literature on using cinnamon oil as anaesthetic in fishes. We selected this because the oil is rich in eugenol. This compound is used as an anesthetic in dentistry along with its various human health benefits. But during these experiments only higher concentrations has got influence on fishes, so we have to check with stress as well hematological parameters whether it has any ill effects after being transported using this as an anaesthetic.

In the case of Orange Oil the results showed no complete anaesthetization in fishes. Very high concentration resulted only in stage II of anaesthesia after 10 minutes of induction, which is not suitable as an anaesthetizing agent. Orange oil is also has got a sedative use, along with its many other health benefits which made us to select it in our study. But it failed to be an effective anaesthetic.

The study on Holy Basil Oil revealed only higher concentrations of oil has anaesthetic effect on fishes. The concentration of 663mg/l gave stage IV in 5 minutes and recovered in 3.5 min. since the concentration is too high we have to conduct more studies pertaining to its blood parameters and other biochemical contents or its ill effects. In this case also there is no literature regarding the anaesthetic effect of Holy Basil Oil usage as fish anaesthetics. Based on its multitudes of health benefits in human beings as well as its sedative property with the presence of Eugenol as one of it component oil made us to select it for our study. It indeed has

anaesthetic property but at high concentrations only.

From the entire study it was revealed that the bio oils containing Eugenol as its component has got anaesthetizing effect. Here in our study only orange oil which is not carrying Eugenol didn't give anaesthetic effect in fish. Clove oil is widely used as anaesthetic in fishes during their transport and has well defined literature collections. But the other three viz., nutmeg oil, cinnamon oil and holy basil oil were found to be having anaesthetic effect but with higher concentration levels. So we need further studies to check with the ill effects in using it as well how the internal systems in fishes are tuned to it.

5. Conclusion

In the ornamental fish business, the use of modern packaging technology for air transport to increase fish loading densities and improve the post shipment survival is critical to the business. During the transportation there is a need to enhance the stress resistance of fish, maintain them in good conditions and reduce them to the fish during transport, thereby ensuring good survival on and after arrival at destination. An effective method is to use anaesthetics and cut down the excretion of ammonia and carbon dioxide by the fish.

Understanding the importance using natural oils as anaesthetics instead of expensive chemicals we intended to find out the efficacy of different bio oils as anaesthetic in fish transport. Based on this the experiments were conducted in a common ornamental fish, Platy, using Clove oil, Nutmeg oil, Cinnamon oil, Orange oil and Holy Basil oil. Clove oil was taken as it is a proved anaesthetic and others are not been used yet and not seen in any literature. All oils except orange oil showed anaesthetic effect. The oils other than Clove oil the concentrations were higher when we compare with that of clove oil. Hence more studies are needed to find out whether there will be any ill effects in having it in higher concentrations. More studies on stress factors and hematological components to be found out before finalizing the anaesthetic effect of it. All the oils selected were having lots of health benefits for human beings in field of medicine. Hence forth we conclude hoping it will be good enough as an anaesthetic also and could be widely used in fish transportation in the Ornamental fish industry.

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