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Assessment of Safe Dose of Clove Oil for Using as Anesthetics in Aquaculture Operations

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ABSTRACT

The safe concentration of clove oil for aquaculture operations was determined by conducting bioassay tests in static waters. For this purpose five doses (0.04, 0.05, 0.06, 0.07, and 0.08 ppm) were applied in glass aquaria of 28 liters capacity. Applying these concentrations of clove oil for 24 and 48 hours, fish, C. carpio survival rate was compared with control. The estimated LC_{50} values for 24 and 48 hrs. were 0.062 and 0.057 ppm respectively. The second order polynomial regression analysis between fish survival and clove oil concentration proved that a dose of 0.02 ppm is safe for use in fish seed transportation.

Key words: *Anesthetics, Aquaculture, Toxicity, Clove oil, Fry, Transportation, Static water.*

INTRODUCTION

In aquaculture operations, anesthetics are very important because they minimize the stress in fish and reduce physical injury during various handling practices like weighing, length measurement, tagging, sampling *etc*.[1] Anesthetics can help significantly in mitigating physiological stress, reducing metabolic rates, thus reducing oxygen consumption and ammonia and carbon dioxide excretion[2]. The use of anesthetics becomes essential in the transportation medium especially for lowering the metabolic activities[3]. According to Osborn[4] the reduction in consumption of oxygen is caused by an anesthetic that seems to increase the ability of fish to withstand lower concentrations of dissolved oxygen. The gills decrease their functions under anesthesia due to the depression of opercular movements and associated nervous control of breathing[5]. Durve[6] stated that the metabolic rate in anesthetized fish was lowered by nearly half and thereby the weight of fish per unit volume of water could double during transportation.

Anesthetics are widely used in routine aquaculture activities to reduce incidence of stress by sedating and immobilizing fish before performing any task in aquaculture.

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The desirable attributes of anesthetics used for fin fish include, short induction and recovery time, non-toxic to fish and humans, no lasting physiological effects, rapid clearance from the body, high solubility in fresh and salt water, availability and cost effectiveness[7]. Biologists and aquaculturists alike have been searching for alternative anesthetics that are less toxic, readily available, efficacious and safe for humans.

Clove oil is a dark brown liquid resulting from the distillation of flowers, flower stalks, and leaves of clove trees *(Eugenia aromatica)* and used throughout the world for applications ranging from food flavouring to local anesthesia in the dentistry profession[8]. According to Hernani and Tangendjaja[9], it consists primarily of phenol eugenol (70-90%), eugenol acetate (>17%) and kariofilen 5 (12%). It is considered noncarcinogenic, non-mutagenic and "Generally Recognized as Safe" (GRAS) substance by the FDA[8]. Clove oil's properties and its status as a GRAS substance make it an ideal candidate as an anesthetic to use in field of fisheries. In view of the above; the present study was conducted to work-out the safe dose of clove oil for aquaculture operations.

MATERIAL AND METHODS

1.1. Experimental Fish

For the present study Common carp (*Cyprinus carpio communes*) fry with an average weight (0.20±0.01 gm) and average total length (21±0.01 mm) were obtained from Aquaculture Research & Seed Unit, Directorate of Research, MPUAT, Udaipur. Before initiating final experiments, the fishes were acclimatized to laboratory conditions. For this purpose the fishes where kept in fiber glass tank (3 x 1 x 0.75 m) filled with freshwater.

1.2. Clove Oil

Laboratory grade clove oil (Hi-MEDIA) was used for the present studies. Clove oil is a pale yellow liquid distilled from the leaves, buds and stems of the clove tree (*Eugenia caryophyllus*). Its active ingredients are eugenol [α-methoxy-4-2 (2-propenyl)-Phenol] and isoeugenol (4-propenyl-2-methoxy phenol), which can comprise 90–95% of clove oil by weight.

1.3. Clove Oil Toxicity Assay

To work-out the safe level of clove oil for aquacultural operations especially live fish seed transportation, bioassay studies were conducted using different concentrations of clove oil (0.04, 0.05, 0.06 0.07 and 0.08 ppm). A Stock solution of clove oil was prepared in distilled water by dissolving one ml of clove oil in 99 ml distilled water. The desired concentration was obtained by calculating the required quantity of 1 ml stock solution and adding the same to the experimental media. Eighteen glass aquaria of 28 liter capacity each were used for this experiment. Ten fishes (0.20±0.01 gm) were introduced in each aquarium. Simultaneously, a control was also run as customary in all such studies. The above concentration gave 0 to 100 % mortality during the bioassay. The criterion for deciding of mortality was the absence of the movement of the fish when prodded by a glass rod. The LC_{50} values were obtained by graphical interpolation and probit analysis method[10]. The safe level of clove oil was calculated through second order polynomial regression between fish survival and clove oil concentration.

RESULTS

The results of the present studies for toxicity effects of different doses of clove oil are presented in Tables 1-2 and Figures 1-2. The mean mortality of experimental fish was highest (100 %) in highest dose of clove oil (0.08 ppm). Whereas the mortality rate in other treatment (doses) was 20, 40 and 60 in 0.05, 0.06 and 0.07 ppm respectively. However, there was no mortality in 0.04 ppm and control. Further, a positive correlation between fish mortality and clove oil doses was noticed. The lethal concentrations (LC_{25}, LC_{50} and LC_{100}) values of clove oil are presented in Table 1, it would be seen from this table that the LC_{50} values at 24 and 48 hours were 0.062 and 0.057 respectively. In general, the lethal concentration values decreased with

increased test duration (i.e., 24 to 48 hours). Thus showing a negative relationship between exposure time and lethal concentration values.

The data obtained for fish mortality in different concentrations of clove oil were statistically analysed to calculate the safe level of clove oil. The results obtained from second order polynomial regression analysis are reported in Table 2 and Figure 2. The clove oil concentration and fish survival rate had a significant relationship ($p < 0.05$). The regression analysis showed a positive relationship with coefficient value of 0.9907. The result of ANOVA for dose and survival rate (Table 2) showed a significant relationship as the calculated "F" – 160.66 was much higher than the tabulated "F"- 0.00089 values ($p<0.01$).

The second order polynomial regression for clove oil concentrations and fish survival is shown in (Figure 2). This showed a significant coefficient (R^2 0.9901) values between clove oil dose and survival rate .It would be seen from this Figure 2 that the highest survival rate (100%) was in control and lowest was at 0.08 ppm dose of clove oil. However, the second order polynomial regression clearly described that a dose 0.02 ppm had highest survival rate. Thus this dose can be selected as safe dose for aquaculture operations especially fish seed transportation especially common carp fry.

DISCUSSION

In the present study an attempt to determine the toxicity of clove oil (LC_{50}) and to determine safe concentrations that would more realistically meet field conditions was tried. Initial tests with common carp fry produced a LC_{50} of 0.062 ppm clove oil for a 24 hours exposure (Table 1). Keene *et al*.[11] reported that the 0.5 – 96 hr LC_{50} of clove oil for rainbow trout (20 gm in weight ; 12 cm in fork length) was 65 – 9 ppm. In the other study conducted by Taylor and Roberts[12], the 10 min LC_{50} of clove oil for juvenile white sturgeon, chinook and coho salmon were 526, 62 and 96 ppm, respectively. Thus the results of the present study on common carp and studies conducted on other species by different researchers[11, 12] indicate a tolerance difference of clove oil among species tested.

According to Metcalfe[13], there are several chemical, physical and biological factors that influence the toxicity of chemicals to fish, including the properties of the chemical in water, the water quality conditions, the route of exposure and the species and life stage of the fish being tested. A chemical can be toxic to a fish in two possible ways. It may affect tissues on the surface of the organism (e.g. gill epithelium) or the chemical may enter the organism and cause toxicity. Most of fish anesthetics are delivered in water, absorbed through gills and rapidly enters the arterial blood from where it is very short route to the central nervous system[14]. Hikasa *et al*.[15] reported that clove oil caused the decrease in respiratory rates. The decrease in respiratory rates caused by clove oil based on the inhibition of respiratory center in the medulla oblongata in relation to depression of central nervous system. In this study, common carp fry exposed to clove oil (0.04 – 0.08 ppm) showed shallow breathing before dead. The dead fish exhibited opening mouth and opercula.

Fish species differ widely in their sensitivity to the toxic effects of chemicals. Sprague[16] noted that there may be greater variability in toxicity associated with the test species than associated with differences in test conditions. Some of this variability can be attributed to differences in metabolic rates and physiology of the fish. The physiological difference among the test fish may be cause the different of LC_{50} value. There is no simple definition of efficacy of anesthetics in fish, and many papers that were published regard efficacy as the ability to handle the fish[6]. This is highly a subjective variable, dependent on the handler, the fish, the procedure to be carried out, and the number of other parameters. Clearly one must also consider biological and environmental factors when administering or comparing studies dealing with anesthetic agents[14]. Biological factors include species, the stage of life cycle and age,

size and weight, lipid content, body condition and disease status. All these factors affect the metabolic rate and therefore the pharmacokinetics of the anesthetic compound. Environmental factors including temperature and pH also affect the metabolic rate in fish, in addition to changing the uptake across the gills, and therefore increase or decrease the efficacy of an anesthetic agent.

Mortality of experimental fish fry exposed to various concentrations of clove oil during LC_{50} test is shown in Fig. 2. The mean LC_{50} of clove oil found to be 0.062 ppm (Table 1).The results of toxicity test of clove oil on common carp fry showed a regular negative correlation between concentrations and exposure time. A comparison of clove oil concentration and survival percentage, using second order polynomial regression (Fig.2) showed that 0.02 ppm of clove oil could be more safe/applicable as proper sedative concentration for transportation. Farid *et al*[15] have studied on the use of clove oil and suggested that a dose of 0.01 ppm is most suitable for anesthetizing *Labeo rohita*. The clove oil concentration of 0.01-0.03 ppm was also found safe for *Heteropneustes fossilis*[16]. The safe clove oil concentrations suggested for the two freshwater fishes by these researchers[15.16], are well within the range of clove oil concentration as calculated using second order polynomial regression.

Table 1: LC_{50} values of clove oil estimated at different exposure times

S.No	Clove oil concentration (ppm)	No of Fishes Exposed	LC	
			24 hrs	48 hrs
1	0.04	10		
2	0.05	10	**0.052***	**0.047***
3	0.06	10	**0.062****	**0.057****
4	0.07	10	**0.083*****	**0.078*****
5	0.08	10		

$^{*}LC_{25,}$ $^{**}LC_{50,}$ $^{***}LC_{100}$

Table 2: Regression statistics : second order polynomial regression statistics between clove oil concentration and mortality rate in common carp

Multiple R	0.9954					
R Square	0.9907					
Adjusted R Square	0.9845					
Standard Error	4.6068					
Observations	6					
ANOVA						
	df	***SS***	***MS***	***F***	***Significance F***	
Regression	2	6819.664	3409.832	160.6667	0.00089	
Residual	3	63.66906	21.22302			
Total	5	6883.333				
	Coefficients	***Standard Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
Intercept	99.31655	4.580406	21.68292	0.000215	84.73965	113.8934
X Variable 1	910.0719	235.2169	3.869076	0.030546	161.5068	1658.637
X Variable 2	-26259	2885.862	-9.09919	0.002805	-35443.1	-17074.9

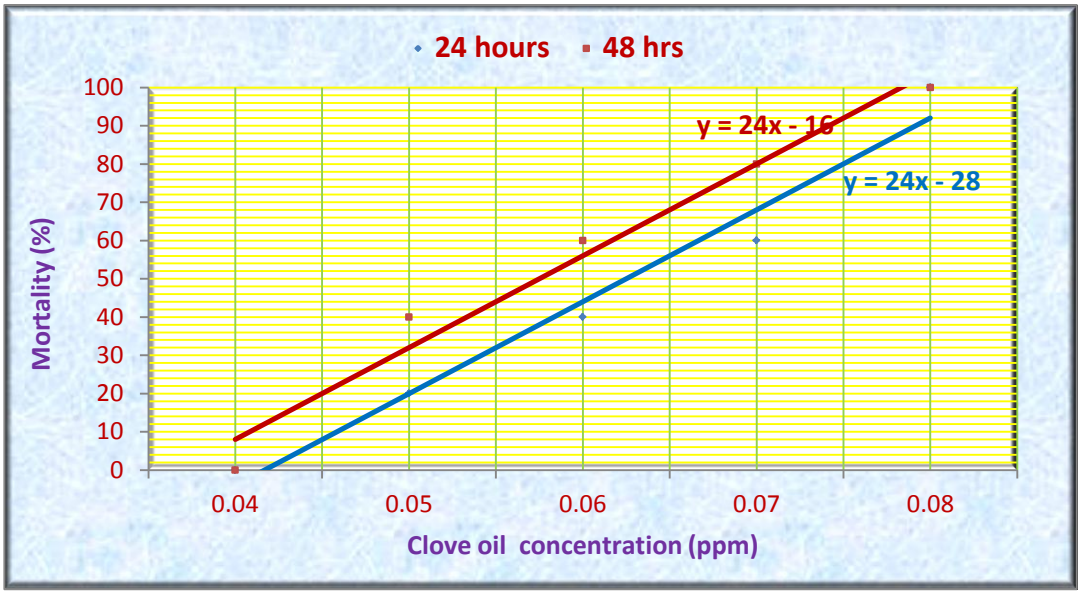

Fig. 1: Estimation of lethal concentration of clove oil

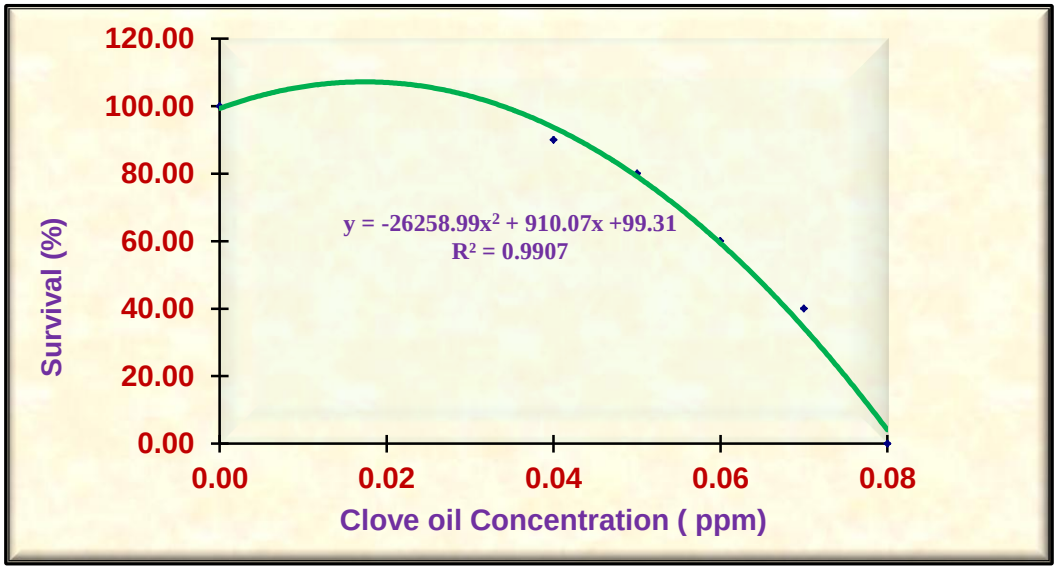

Fig. 2: Relationship between clove oil concentration with survival rate of common carp fry (as described by second order polynomial regression)

CONCLUSION

The outcome of this research has been the standardization of appropriate concentration of clove oil for live fish seed transportation and other aquaculture procedures. The result of the present study has clearly indicated that a dose of 0.02 ppm of clove oil is safe for fish. This dose did not cause fish mortality, ensured fish stay calm without loss of equilibrium and reduced the metabolic rate. Thus, clove oil treatment @ 0.02ppm appear to have promise as an effective and safe anesthetic for use on food fishes. However, until further studies are conducted regarding physiological effects, it should be used with caution and at the lowest concentration necessary to induce anesthesia.

REFERENCES

1. Cho, G.K. and Heath, D.D., Comparison of tricaine methanesulphonate (MS222) and clove oil anesthesia effects on the physiology of juvenile chinook salmon *Oncorhynchus tshawytscha* (Walbaum). *Aquaculture Res*.**31:** 537–546 (2000).

2. Sandodden, R., Finsted, B., Transport stress in Atlantic salmon (*Salmo salar* L.), *Aquaculture Research*, **32:** 87–90 (2001).
3. Sado, E. K., Influence of the anesthetic quinaldine on some tilapias. *Aquaculture*, **46:** 55–62 (1985).
4. Osborn, P. E., Some experiments on the use of thioura -cil as an acid in holding and transporting fish. *Progressive Fish-Culturis*, **13:** 75–78 (1951).
5. Randall, D.J. and Hoar, W.S., Special techniques, anesthesia, fish physiology. *Academic Press, New York*. **6:** 511–523 (1969).
6. Durve, V.S., Anesthetics in the transport of mullet seed. *Aquaculture*. **5:** 53–63 (1975).
7. Marking, L.L. and Meyer, F.P., Are better anesthetics needed in fisheries? *Fisheries*. **10:** 2–5 (1985).
8. Nagababu, E. and Lakshmaiah, N., Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. *Journal of Biochemistry Pharmacology*, **43:** 2393–2400 (1992).
9. Hernani and Tangendjaja, B., Analysis mutu minyak nilam dan minyak cengkeh secaa kromatografi. *Media Penelitian Sukamandi*, **6:** 57-65 (1988).
10. Finery, D. J. Probit Analysis: a Statistical Treatment of the Sigmoid Response Curve, 2nd edition Cambridge University Press, London. (1964).
11. Keene, J.L., Noakes, D.L.G., Moccia, R.D. and Soto, G.C., The efficacy of clove oil as an anaesthetic for rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Res*.**29:** 89-101. (1998).
12. Taylor, P.W. and Roberts, S.D., Clove oil: an alternative anesthetic for aquaculture. *North Am. J. Aquacult*.**61:** 150-155 (1999).
13. Metcalfe, C.D. Toxicology, *In* Ostrander, G.K. ed. The Laboratory Fish. *Academic Press, London*. pp 617 – 630 (2000).
14. Ross, L.G. and Ross, B., Anesthetic and Sedative Techniques for Aquatic Animals. 2 nd ed. Blackwell Science Ltd., *Great Britain*. (1999).
15. Hikasa, Y., Takase, K., Ogasawara, T. and Ogasawara, S., Anesthesia and recovery with tricaine methanesulfonate, eugenol and thiopental sodium in the carp, Cyprinus carpio. *Jpn. J. Vet. Sci*. **48:** 341–351 (1986).
16. Sprague, J.B. Factors thatmodify toxicity,.*In* G.M. Rand and S.R. Petrocelli, eds. Fundamentals of Aquatic Toxicology: Methods and Applications. *Hemisphere Publishing Corporation, New York*. pp 124–163 (1985).