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ANNESTHETIC EFFECTS OF CLOVE OIL ON SURVIVILITY OF GRASS CARP, *CTENOPHARYNGODON IDELLA* CARP SEED

PAWAN KUMAR*
ICAR- Central Institute of Fisheries Education,
Fisheries Resource Management Division, Off Yari Road, Panch Marg, Versova, Mumbai - 400 061, INDIA
e-mail: pawanfrm@gmail.com

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***Corresponding author**

ABSTRACT

The aim of this experiment was to investigate the possibilities for using of clove oil as anaesthetic effect in Grass carp *Ctenopharyngodon idella*. Each test concentration was tested with 10 fish / tank of grass carp having average fingerlings length (65 ± 0.30 mm) and 3.68 g body weight of 90 days old and advanced fingerlings (10.8 ± 1.25 cm) and 15.5 g body weight of 150 days. The tests were carried out under controlled conditions tested at concentration of 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 μl/L. Experiments were conducted in replicates by adopting standard static bioassay method. The safe application factor equation and safe application rate for the clove oil were also worked out separately for different life history stages of *C. idella*. The 72 hrs LC_{50} value exposed clove oil worked out to be 0.050 and 0.029 μl/L for fingerlings and advanced fingerlings. The water quality is determined by temperature, pH, CO_2, total ammonia, nitrate, and dissolved oxygen in different concentration. Therefore, present study of clove oil for aquaculture purposes have to encourage because this natural anesthetic is becoming more evident as a safe and low cost alternative.

INTRODUCTION

The clove oil is used for a long time in medicine, cosmetics and food industry as a food aromatizer. It has been applied in human medicine as a mild anesthetic from ancient time (Taylor and Roberts, 1999). Clove oil is a dark brown liquid, which is distilled from the leaves, stalks, flower, buds and clove tree *Eugenia caryophyllata* (Keene *et al.*, 1998; Zaikov *et al.*, 2008). An emerging and effication anesthetic use in fish is clove oil, containing the active ingradient Eugenol (4-allyl-2-Methoxyphenol), constitutes 70-95% (Taylor and Roberts 1999; Kurt *et al.*, 2001; Keene *et al.* 1998; Hekimoglu and Ergun, 2012; Fernando, 2014) of the total weight of the base. Clove oil has become a commonly used anesthetic that can serve as an alternative to tricane methanesulphonate in commercial (non food) fish and fish industries in the US and Japan (Hikasa *et al.*, 1986; Mumday and Wilson, 1997). Volume of the informations are available on the effects of anesthetics on fish with particular reference to use of benzocaine, metonidate, tricaine methano sulfonate (MS-222), quinaldine sulfate, phenoxy-ehanol (Soto and Burhanuddin, 1995; Jennings and Looney, 1998; Peake, 1998; Ross and Ross, 2008; Prince and Powell, 2000; Gomes *et al.*, 2001). Several investigations have identified the advantage and disadvantages of clove oil and eugenol and reported these products as safe and effective (Sutili *et al.*, 2014). The studies indicate that clove oil and eugenol can be effective at controlling mites, termites, insects, weedy species and mosquitoes at lower application rates (Vaid *et al.*, 2010; Chintalchere *et al.*, 2013). Anesthetic effect of the clove oil on some aquatic organisms was investigated in such cases as its use in the transfer of fish species used in the food sector (Ross and Ross, 2008). Indian major carp were exposed to varying doses of clove oil (Eugenol) to determine the 96 hrs LC_{50} to observe the recovery time. The researches on clove oil anesthetic effect on *C. idella* are insufficient and limited. In recent years, the pharmacological action of eugenol has been developed to immunological function, central cardiovascular system, digestive system, blood biochemistry and urinary system (Prakash and Gupta, 2005; Kong *et al.*, 2014)

Therefore special attention is paid to clove oil as an anesthetic natural substance in the aquaculture and there is a good reason that this substance is considered as an alternative.

The clove oil finds its wide application in the transportation of either fish seeds or brood stocks, because the drugs applied reduces the metabolic rate of the animal and reduces the rate of oxygen consumption along with the reduction in the release of the carbon-di-oxide and ammonia to the transporting media. The drugs further makes the fish or animal inactive thereby preventing the chances of injury during transportation and also promote survival rate of the animal. The clove oil most commonly used anesthetic for invasive fisheries research, but few studies have examined the use of low concentrations of clove oil to achieve sedation for fish handling and transportation. Hanggono (2003) was investigated on toxicity of clove oil is recommended as an effective anesthetic for sea bass fry based on good efficacy at 5 ppm for transport purpose and 20 ppm for completely anesthetized. Effective clove oil concentrations for anesthesia induction and recovery (40-80mg/L) for seven out of the eight species. Javahery *et al.* (2012) observed that changes in fish behavior during progressively deeper anaesthesia and the physiological effects of clove oil. Chellapan *et al.* (2013) also suggested that 45 ppm clove oil was the optimum dose of anesthetics for safe transport of Angel fish. Kamble *et al.* (2014) reported that the highest doses (15.10 min) and lowest (2.20 min) induction time were noticed at the dose of 0.04 and 0.08 ppm respectively as anesthetic in common carp (*Cyprinus carpio*) were used in static waters. Dolezelova *et al.* (2011) and Cortes-Rojas (2014) reported the toxicity level of clove oil was tested in the medium lethal concentrations (LD_{50}) at 96 h were (18.2 ± 5.52) mg/mL in *Danio rerio* and (21.7 ± 0.8) mg/mL in *Poecilia reticulata*. Kroon (2015) worked on efficacy of clove oil for anesthesia was examined on eight species of Australian tropical freshwater fish. Several researchers worked on clove oil as a fish anesthetic have been reported it to be regarded as safe for the user, an effective and inexpensive anesthetic. Therefore, clove oil to observe differences in anesthesia onset and recovery times determined, to conduct to determine the proper dosage.

The objective of this study was to assess the efficacy of clove oil to determine the acute toxicity and reduce physiological stress responses in grass carp seed.

MATERIALS AND METHODS

Experimental Animal

The study was conducted at College of Fisheries (OUAT), Rangailunda, Odisha during 2005. Grass carp fingerlings (65 $\pm$ 0.30 mm) 3.68 g weight of 90 days old and advanced fingerlings (10.8 $\pm$ 1.25 cm) 15.5 g weight of 150 days old were employed for the present study. For fingerlings and advanced fingerlings rectangular jar of 10.0 L to 20 L capacity were used. All the trails were run in duplicate with renewal of the medium at every 24 hrs till end of the experiment.

Water Quality Parameters

The temperature, pH, CO_2, total ammonia, nitrate and dissolved oxygen were recorded every hour for the first 12 hrs of the experiment, every 3 hrs for the next 12 hrs and every 6 hrs for remaining 72 hrs. The pH of water was measured using a portable global digital pH meter. The CO_2, total ammonia, nitrate, dissolved oxygen content of water ware estimated define by APHA (1998). The water quality was calculated based on a two –way ANOVA followed by the Tukey Test ($P<0.05$) (Zar, 2006). The data are represented as mean $\pm$ SE.

Preparation of Anesthesia

The experiments for determining the efficiency of clove oil as an anesthetic were carried out under controlled laboratory conditions. The alcoholic extract of clove oil product manufactured by M/S Dabur India Ltd., Uttar Pradesh has been used for the purpose to find out lethality, toxicity effects and LC_{50} value for the herbal formulations. Its anesthetic effect was tested at water temperature of 25^{0}C. Herbal formulation of fish anesthetics clove oil was used individually in the present study. A stock solution of fish anesthetics was prepared in glass water and the required concentration was achieved by the addition of calculated amounts of the stock solution to the test medium. The assessment of stock solution was mixed with the experimental tank water to produces final concentration of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 μl/L.

Statistical analysis

Safe Application Factor Equation (SAFE) and Safe Application Rate (SAR)

Estimated by dividing LC_{50} (the maximum concentration at which all the test animals survived for 96 hrs) by LC_{100} which is the minimum concentration at which all the test animal died within 96 hrs. The safe application rate of an insecticide or biocide is determined by multiplying Safe Application Factor Equation (SAFE) by LC_{50} of 48 or 96 hrs (Basak and Konar, 1977).

Safe application factor equation and safe equation rate for the herbal anesthetics were also calculated individually for grass carp seeds. The LC_{50} value with their 95% confidence limits was also calculated by employing Probit regression analysis (Finney, 1971). The acute lethal toxicity of *C. idella* were carried out individually in duplicate by following the short term static bioassay technique recommended by Sprauge (1971) and APHA (1998). The experiments were concerned in finding out LC_{50} value, before starting the experiment, pilot studies were conducted to select different concentration giving to 10 to 100% mortality.

RESULTS

Water Quality Parameters

In the present study, the water temperature treatments with the clove oil concentration throughout this experiment variation was 1-2^{0}C across treatment in the 24-72 hrs period in fingerling and advance fingerling. The pH was significantly higher in 0.01 μl/L group as compared to the fish anesthetized with 0.06 μl/L clove oil**,** while CO_2 exhibited an opposite trend with value significantly higher in the 0.06 μl/L treatment group when evaluated against the 0.1 μl/L concentration. CO_2 value was observed to increase at the experiment ranging from 42.8 to 72.4 μl/L. This result was expected to establish that increase in CO_2 cause a decrease in pH. Total ammonia concentration was markedly higher in the 72 hrs period ranging from 0.02-0.09 mg.L^{-1}. During the experiment the total ammonia increased in all the tanks ranging from 0.02 to 0.08 mg.L^{-1}. Nirtate concentration was higher in the 72 hrs period ranging from 3.4 to 3.6mgL^{-1}. Dissolve oxygen higher in 72 hrs periods ranged from 6.2 to 8.8 mg.L^{-1} concentrations of 0.1 to 0.6 μl/L (Table 1 and 2).

Acute Toxicity of Clove oil

Cumulative percentage of *C. idella* fingerlings exposed to different concentration of clove oil 0.01 to 0.06 μl/L from the experiment (Table 3 and 4) and their corresponding LC_{50} values from 24 to 72 hrs were worked out to be 0.066, 0.061 and 0.051 μl/L respectively (Table 5).

After 4 hrs of exposure, the fingerlings under clove oil stress beyond 0.02 μl/L level exhibited erratic swimming behavior coupled in the exposure period; the experimental animals showed imbalance associated with slow escape reflex and feeble opercular beatings. In case of advanced fingerlings exposed to different concentration of clove oil stress 0.01 to 0.06 μl/L also exhibited the similar type of behavioural response. LC_{50} value from 24 to 72 hrs were worked out to be 0.053, 0.038 and 0.030 μl/L for the advance fingerlings.

Table 1: Assessment of water quality parameters at different clove oil concentrations for fingerlings

Parameters	Time (hrs)	0.1(μl/L)	0.2 (μl/L)	0.3 (μl/L)	0.4 (μl/L)	0.5 (μl/L)	0.6 (μl/L)
Temp (^{0}C)	24	25.0 ± 0.31	25.4 ± 0.62	24.8 ± 0.83	25.7 ±0.45	25.4 ±0.75	25.4 ± 0.12
	48	24.8 ± 0.52	25.7 ± 0.11	24.5 ± 0.65	25.7 ± 0.92	24.8 ± 0.55	25.2 ± 0.10
	72	23.6 ± 0.73	24.6 ± 0.92	24.5 ± 0.63	25.2 ± 0.83	24.3 ± 0.62	24.6 ± 0.96
pH	24	7.72 ± 0.08	7.65 ± 0.11	7.35 ± 0.08	7.52 ± 0.08	7.53 ± 0.08	7.52 ± 0.08
	48	7.65 ± 0.04	7.52 ± 0.01	7.21 ± 0.04	7.03 ± 0.04	7.21 ± 0.04	7.36 ± 0.04
	72	7.25 ± 0.08	7.02 ± 0.04	6.90 ± 0.08	6.88 ± 0.08	6.92 ± 0.08	6.85 ± 0.08
CO_2	24	52.7 ± 2.58	45.8 ± 3.21	45.6 ± 1.52	42.8 ± 2.35	45.3 ± 2.35	55.3 ± 2.25
(mg L^{-1})	48	50.2 ± 1.28	55.6 ± 2.85	62.5 ± 2.34	60.5 ± 1.52	65.6 ± 2.55	66.3 ± 2.41
	72	58.5 ± 0.85	65.8 ± 0.85	67.7 ± 0.00	70.5 ± 1.42	70.3 ± 0.52	72.4 ± 2.35
TotalAmmonia	24	0.03 ± 0.85	0.03 ± 0.25	0.06 ± 0.26	0.06 ± 0.43	0.07 ± 0.65	0.07 ± 0.23
(mg L^{-1})	48	0.04 ± 0.30	0.05 ± 0.31	0.06 ± 0.25	0.07 ± 0.33	0.08 ± 0.26	0.08 ± 0.41
	72	0.06 ± 0.42	0.06 ± 0.21	0.07 ± 0.34	0.08 ± 0.44	0.09 ± 0.27	0.09 ± 0.35
Nitrate	24	3.3 ± 0.56	3.5 ± 0.25	3.2 ± 0.47	3.5 ± 0.75	3.5 ± 0.36	3.4 ± 0.38
(mg L^{-1})	48	3.5 ± 0.55	3.6 ± 0.75	3.3 ± 0.74	3.4 ± 0.77	3.5 ± 0.67	3.4 ± 0.64
	72	3.5 ± 0.88	3.6 ± 0.74	3.4 ± 0.88	3.5 ± 0.26	3.6 ± 0.75	3.6 ± 0.75
Dissolved O_2	24	6.2 ± 1.52	7.5 ± 1.25	7.5 ± 0.25	7.6 ± 0.73	7.8 ± 0.27	7.8 ± 1.05
	48	8.0 ± 0.45	7.6 ± 1.44	7.2 ± 1.15	8.0 ± 0.56	8.0 ± 0.47	7.6 ± 1.14
(mg L^{-1})	72	8.0 ± 0.74	8.0 ± 1.67	7.7 ± 1.15	7.6 ± 0.47	8.0 ± 1.42	8.2 ± 0.42

Table 2: Assessment of water quality parameters at different clove oil concentrations for advance fingerlings

Parameters	Time (hrs)	0.1 (μl/L)	0.2 (μl/L)	0.3 (μl/L)	0.4 (μl/L)	0.5 (μl/L)	0.6 (μl/L)
Temp (°C)	24	25.5 ± 0.52	25.5 ± 0.61	25.5 ± 0.82	25.7 ± 0.42	25.4 ± 0.73	25.4 ± 0.12
	48	25.2 ± 0.53	25.3 ± 0.10	25.4 ± 0.60	25.3 ± 0.91	25.6 ± 0.55	25.2 ± 0.10
	72	24.8 ± 0.72	24.2 ± 0.92	24.5 ± 0.61	24.2 ± 0.74	24.1 ± 0.06	24.3 ± 0.92
pH	24	7.65 ± 0.08	7.15 ± 0.11	7.03 ± 0.08	7.03 ± 0.08	7.55 ± 0.08	7.52 ± 0.08
	48	7.52 ± 0.04	7.05 ± 0.01	7.22 ± 0.04	6.90 ± 0.04	7.32 ± 0.04	7.32 ± 0.04
	72	7.2 ± 0.06	7.01 ± 0.04	7.82 ± 0.08	6.88 ± 0.08	6.80 ± 0.08	7.87 ± 0.08
CO_2	24	50.7 ± 2.58	45.8 ± 3.21	43.6±1.52	42.8 ± 2.35	45.3 ± 2.35	44.3 ± 2.25
(mg L^{-1})	48	52.2 ± 1.28	48.6 ± 2.85	45.5 ± 2.34	45.5 ± 1.52	45.6±2.55	50.3 ± 2.41
	72	55.5 ± 0.85	58.8 ± 0.85	60.7±0.00	62.5 ± 1.42	65.3 ± 0.52	66.4 ± 2.35
Total	24	0.02 ± 0.24	0.04 ± 0.21	0.06 ± 0.32	0.06 ± 0.23	0.06 ± 0.36	0.07 ± 0.24
Ammonia	48	0.04 ± 0.31	0.06 ± 0.33	0.06 ± 0.25	0.07 ± 0.42	0.07 ± 0.25	0.07 ± 0.34
(mg L^{-1})	72	0.05 ± 0.25	0.06 ± 0.21	0.07 ± 0.36	0.08 ± 0.55	0.08 ± 0.32	0.08 ± 0.35
Nitrate	24	3.3 ± 0.56	3.5 ± 0.25	3.2 ± 0.47	3.5 ± 0.75	3.5 ± 0.35	3.4 ± 0.38
(mg L^{-1})	48	3.5 ± 0.55	3.6 ± 0.75	3.3 ± 0.74	3.4 ± 0.77	3.5 ± 0.67	3.4 ± 0.64
	72	3.5 ± 0.88	3.6 ± 0.74	3.4 ± 0.88	3.5 ± 0.26	3.6 ± 0.75	3.6 ± 0.75
Dissolve O_2	24	7.4 ± 1.52	7.4 ± 1.25	7.5 ± 0.25	7.6 ± 0.73	7.5 ± 0.27	7.6 ± 1.05
	48	7.4 ± 0.45	8.0 ± 1.44	8.0 ± 1.15	7.8 ± 0.56	8.2 ± 0.47	8.8 ± 1.14
(mg L^{-1})	72	7.8 ± 0.74	8.5 ± 1.67	8.2 ± 1.15	8.0 ± 0.47	8.2 ± 1.42	8.4 ± 0.42

Table 3: Cumulative % mortality of *C. idella* fingerlings exposed to different concentration of clove oil

Concentration (μl/L)	Replication	24 hrs	48 hrs	72 hrs
Control	R1	00	00	00
	R2	00	00	00
0.01	R1	00	00	00
	R2	00	00	00
0.02	R1	00	05	25
	R2	00	05	25
0.03	R1	10	20	40
	R2	05	20	40
0.04	R1	10	30	50
	R2	10	20	50
0.05	R1	15	40	70
	R2	15	40	70
0.06	R1	30	60	100
	R2	30	50	60

The fingerlings and advanced fingerlings of *C. idella* exposed to different concentrations of clove oil exhibited abnormal swimming pattern with irregular movement. The Safe Application Factor Equation (SAFE) and Safe Application Rate (SAR) for clove oil were calculated individually for fingerlings and advanced fingerlings of *C. idella*. The safe application rate for fingerlings and advanced fingerlings were workout to be 0.019 and 0.008 μl/L for clove oil (Table 6).

DISCUSSION

Water Quality Parameters

In the present study, the water temperature variation was 1-2ºC across concentration treatment in the 24-72 hrs periods. In the different clove oil concentration water temperature was significantly higher in 24 - 48 hrs period for fingerling and advance fingerling (Table 1 and 2). Temperature is an important factor in determining the rate of physiological processes in ectothermic animals such as ûsh and thus plays an important role in the processes related to the uptake and elimination of drugs. This leads to a higher oxygen demand that is met by enhanced respiration, increased cardiac output and increased blood ûow through the gills (Nilsson and Sundin, 1998; Webber *et al.,* 1998). Poorer oxygen solubility due to rising water temperature leads to an additional need to enhance respiration and blood row. Reduced induction time at higher water temperatures has also been demonstrated in common carp (*Cyprinus carpio*), rainbow trout, fathead minnows (*Pimephales promelas*) and Atlantic halibut anaesthetised with MS-222 and in Atlantic halibut anaesthetised with benzocaine (Houston and Woods 1976; Sylvester and Holland 1982; Hikasa *et al.,* 1986; Zahl *et al.,* 2011). The rapid changes induction time seen at higher water temperature may be related to higher basal metabolic rate at higher temperatures (Clarke and Johnston, 1999; Zahl *et al.,* 2012) and the corresponding increase in oxygen demand leading to increased respiration and circulation. Furthermore, water temperature was constant avoiding another variant of ammonia toxicity. The water pH decreased after the 72 hrs in the research tank probably as a result of CO_2 accumulation. pH and CO_2 exhibited opposite behavior with pH decreasing during experiment, while CO_2 value were observed to increase. The pH influences the toxicity of several substances in the forms of un-ionized and ionized. At low pH, un-ionized ammonia represents a small portion of the total ammonia (Boyd, 1982). The present study total ammonia levels were increased during different concentration but the levels of un-ionized ammonia were decreased due to the reduction in the pH and probably were did not toxic to the fish.

Table 4: Cumulative % mortality of *C. idella* advance fingerlings exposed to different concentration of clove oil

Concentration (μl/L)	Replication	24 hrs	48 hrs	72 hrs
Control	R1	00	00	00
	R2	00	00	00
0.01	R1	05	10	20
	R2	05	10	20
0.02	R1	10	30	25
	R2	10	20	25
0.03	R1	10	20	40
	R2	15	20	40
0.04	R1	10	30	50
	R2	10	20	50
0.05	R1	30	40	70
	R2	30	40	70
0.06	R1	50	80	100
	R2	50	70	100

Acute toxicity of clove oil

The performance of the fingerlings belongings to the different age groups shows that, the advance fingerlings were comparatively more sensitive to clove oil than the 90 days old fingerlings. The comparison between the LC_{50} values for fingerlings and advanced fingerlings for 24, 48 and 72 hrs reveals that, there is gradual decrease in the concentration of clove oil to bring out desired changes in the test animals. Clove oil shows an immediate effect on the fish even when it is used at low concentration when compared to such chemicals as MS-222 (Keene *et al.,* 1998). The induction and recovery time from anesthetic effect of clove oil for fingerlings were found out to be 2 min and 5 mins whereas for advanced fingerlings the same was found out to be 1.5 min and 3 min at a concentration of 50 μl/L. However, its recovery time after anesthesia is much longer than the other anesthetics. As the concentration of anesthesia increases the time of transition to induction stage shortens (Ross and Ross, 2008).

Prince and Powel (2000) suggested that, a concentration of 30-40 mg/L of clove oil is effective to bring out deep anesthetic effect on adult rainbow trout's. In contrast, Peake (1998) suggest that, it is safe to use clove oil at a rate of 80-100 μl/L of water for anesthetizing non-salmonoid fishes but always a lower concentration of clove oil 40 mg/L is preferred to minimize recovery time. Kamble *et al.* (2014) reported that the efficacy of clove oil in different doses (0.04, 0.05, 0.07 and 0.08 ppm) were used in static water for common carp. The highest (15.10 min) and lowest (2.20 min) induction time were noticed at the dose of 0.04 and 0.08 ppm respectively. Peake (1998) indicated the concentration of 60 $ml.L^{-1}$ efficient for pike. Similar results were also obtained by several workers on rainbow trout (Anderson *et al.,* 1997; Akhlaghi and Mirab, 1999; Waterstart, 1999). In research, silver catfish anesthetized with eugenol at 50 mg/L presented significantly lower plasma cortisol level than control fish (Sutili *et al.,* 2014). Kong *et al.* (2014) reported that anesthetic concentration of eugenol on *Anguilla reinhardtii* (20-120 mg/L); *Cynoglossus semilaevis* (10 mg/L); *Lctalurus punctatus* (61 mg/L); *Pseudosciana crocea*

Table 5: Lethal toxicity (LC_{50}) values for *C. idella* fingerlings and advance fingerlings exposed to different concentration of clove oil

Stage	Replicates	24 hrs Lc_{50} (μl/L)	Slope 'b'	48 hrs Lc_{50} (μl/L)	Slope 'b'	72 hrs Lc_{50} (μl/L)	Slope 'b'
Fingerlings	R1	0.070(0.058-0.072)	10.72	0.064(0.062-0.083)	3.70	0.052(0.047-0.070)	9.31
	R2	0.062(0.052-0.068)	9.25	0.058(0.054-0.080)	3.65	0.050(0.046-0.068)	9.19
	Mean	0.066(0.055-0.070)	9.98	0.061(0.058-0.081)	3.67	0.051(0.046-0.069)	9.25
Advanced fingerlings	R1	0.054(0.048-0.064)	8.18	0.035 (0.027-0.057)	2.45	0.030(0.023-0.039)	5.65
	R2	0.053(0.047-0.063)	8.17	0.041(0.026-0.056)	2.50	0.030(0.022-0.038)	5.65
	Mean	0.053(0.047-0.063)	8.17	0.038(0.026-0.056)	2.47	0.030(0.022-0.038)	5.65

Value in parenthesis represents 95% confidence limit. R1 and R2 = Replicates

Table 6: Showing the safe application rate of clove oil on fingerlings and advanced fingerlings of *C. idella*

Stages	LC_0 (μl/L)	LC_{100} (μl/L)	72 hrs LC_{50} (μl/L)	SAFE	SAR
Fingerlings	0.022	0.022	0.050	0.42	0.019
Advanced Fingerlings	0.012	0.042	0.029	0.28	0.008

(3-40 mg/L) and *Spinibarbus sinensis* (12-30 mg/L). Much of the information is not available on the levels of clove oil required to bring out acute anesthetic effects to different life history stages of carp seed of *C. idella* and other tropical fishes. However, the result obtained in the present investigation is at Parr with the results obtained by the earlier workers on the temperate water fishes.

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