



## Effect of Essential Oil Anesthetics on Water Quality During the Transport of *Etroplus Suratensis* Juveniles

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### Abstract

The fascinating coloration and serene nature of ornamental fish provide a source of blissful tranquillity for people irrespective of age group. Ornamental fish industry can provide ample opportunity for the self-employment of people hailing from rural sector and is a good foreign exchange earner too. A critical aspect of long-distance live fish transportation is the use of anaesthetics to minimize stress and ensure fish welfare. The present study aimed to evaluate the effects of essential oils used as anaesthetics on water quality parameters during prolonged transportation of juveniles of *Etroplus suratensis*. Water samples were collected at intervals of 6, 12, 24, 36 and 48 hours from experimental groups treated with Clove oil, Nutmeg oil, Cinnamon oil and Peppermint oil, along with a control group. Key water quality parameters, dissolved oxygen and carbon dioxide, were measured using standard analytical procedures. Results indicated that Nutmeg oil and Peppermint oil maintained more favourable water quality conditions compared to Clove oil and Cinnamon oil across the tested parameters. These findings suggest that Nutmeg oil and Peppermint oil are more effective in reducing transport-induced stress in ornamental fish, outperforming even Clove oil, which is widely recognized as a standard anaesthetic in the industry.

**Keywords:** Ornamental Fish Industry; Essential Oils; Anaesthetics; Water Quality Parameters.

### 1. Introduction

The fascinating colouration and serene nature of ornamental fish offer tranquillity to people across all age groups. These small, flashy fishes, often with unique movements or feeding behaviours, are kept as pets in aquariums or garden ponds for aesthetic pleasure. The ornamental fish industry has gained momentum globally due to its economic potential, with increasing demand driving the transfer of fish from resource-abundant to resource-deficient areas (Specker & Schreck, 1980) [1-3]. India, particularly Kerala, boasts rich freshwater biodiversity, with many indigenous species valued in the ornamental trade. Brightly coloured fishes from the southern Western Ghats are now integral to the international market. This industry handles the largest volume of live animal transportation globally (Teo et al., 1994). However, the transport process subjects fish to stressors like oxygen depletion, carbon dioxide and

ammonia accumulation and physical injuries due to high-density packing and handling (Schreck et al., 1989; Barton & Iwama, 1991). Anaesthetics are commonly used to reduce handling stress [4]. Anaesthesia, a reversible condition of reduced sensitivity or neuromotor control, can be induced chemically or physically (Summerfelt & Smith, 1990). It plays a crucial role in fisheries and aquaculture for minimizing stress and injuries (Cho & Heath, 2000). Common anaesthetics include MS-222, 2-phenoxyethanol, and benzocaine (McFarland, 1960; Bell, 1964). Clove oil, used for decades, is now widely studied for its efficacy and safety (Endo et al., 1972; Hikasa et al., 1986; Keene et al., 1998). *Etroplus suratensis*, locally known as "Karimeen," is a valuable fish species in Kerala, facing population decline due to overexploitation and habitat loss. Ranching efforts require the transport of juvenile fish.



This study evaluates the effects of essential oil-based anaesthetics on water quality during prolonged transport of *E. suratensis* juveniles [5-7].

## 2. Method

### 2.1. Experimental Fish

*Eetroplus suratensis* commonly called Green Chromide and locally known as “Karimeen” is a fish of great demand in Kerala. On account of their unique coloration and remarkable patterns, they are also valued as ornamental fishes. It is essentially a brackish water fish that has become naturally acclimatized to freshwaters [8].

### 2.2. Essential Oils as Anaesthetics

Clove oil, Nutmeg oil, Cinnamon oil and Peppermint oil are the natural essential oils used in this experiment. The pure natural oils were procured from the Greenleaf Extractions Pvt Ltd., KINFRA HI TECH Park, Kalamassery, Cochin [9].

### 2.3. Experiment

The juveniles of fish *Eetroplus suratensis*, for transportation experiment was collected from a fish farm associated with Kole land, KSK Fish Farm and Hatchery, Kurumbilavu, Thrissur District, during the period of August 2015 – August 2016. Juveniles of size  $8.84 \pm 0.26$  gms were brought for experimentation [10-13]. Experiments are conducted to determine the safety dosage of different anaesthetics for short term exposure. Then experiment with 48 hrs of exposure to the anaesthetics were noted for the four natural oils depending on the result of short-term exposure. Clove oil, Nutmeg oil, Cinnamon oil and Peppermint oil are oils selected for long term exposure [14-17].

### 2.4. Alterations in Water Quality Parameters

Water quality analysis provides a non-invasive approach to estimate stress in fish. In this study, water samples were collected at 6, 12, 24, 36, and 48 hours

during transportation experiments using clove oil (10 mg/L), nutmeg oil (4.5 mg/L), cinnamon oil (5 mg/L) and peppermint oil (8 mg/L) as anaesthetics, along with a control group. Key water parameters dissolved oxygen and carbon dioxide were monitored. Dissolved oxygen was estimated by the Winkler method (Winkler, 1888) and carbon dioxide by titrimetric method (Leeds, 1891). Data were statistically analyzed using two-way ANOVA in XLSTAT software to assess the effects of anaesthetic type and time interval on water quality variables [18].

## 3. Results and Discussion

### 3.1. Results

The water quality parameters dissolved oxygen and dissolved carbon dioxide are tested prior to experiment [19]. The value for dissolved oxygen in oxygenated water was 18mg/l and dissolved carbon dioxide was 7.59 mg/l.

#### 3.1.1. Alteration in Dissolved Oxygen

The variations in the dissolved oxygen content in water during the experimentation are given in the Table 1 [20-23]. The value of oxygen at 0 hour was 18 mg/l. After 6 hrs of experiment highest value for oxygen of  $15.33 \pm 0.471$  was noted in Clove oil treated fish bag even though the value was decreased from the initial level. The dissolved oxygen level was decreasing as the experiment proceeded. In all experiment groups value of control group was lowest on comparing with other groups. At 48 hours of experiment the highest value for oxygen was seen in Nutmeg oil treated group having a value of  $6.67 \pm 0.471$  followed by Peppermint oil, Clove oil and Cinnamon oil. Control had least amount of oxygen of  $3.67 \pm 0.471$  [24].

**Table 1 Alterations in Dissolved Oxygen During Transportation of *Eetroplus Suratensis* Using Anaesthetics**

ANAESTHETICS WITH CONCS.	0 HR	6 HRS	12 HRS	24 HRS	36 HRS	48 HRS
Control	18	$12.67 \pm 0.471$	$7.67 \pm 0.471$	$6.67 \pm 0.471$	$5.67 \pm 0.471$	$3.67 \pm 0.471$
Clove Oil (10 mg/l)	18	$15.33 \pm 0.471$	$10.33 \pm 0.471$	$8.67 \pm 0.471$	$6.67 \pm 0.471$	$5.67 \pm 0.471$
Nutmeg Oil (4.5 mg/l)	18	$14.67 \pm 0.471$	$11.67 \pm 0.471$	$10.33 \pm 0.471$	$8.67 \pm 0.471$	$6.67 \pm 0.471$
Cinnamon Oil (5 mg/l)	18	$13.67 \pm 0.471$	$12.33 \pm 0.471$	$10.33 \pm 0.471$	$8.33 \pm 0.471$	$5.33 \pm 0.471$
Peppermint Oil (8 mg/l)	18	$14 \pm 0.816$	$11.67 \pm 0.471$	$8.67 \pm 0.471$	$7.67 \pm 0.471$	$6.33 \pm 0.471$

Two-way ANOVA was carried out for the variation in dissolved oxygen during packing and transportation in *Etroplus suratensis* using four different natural oils as anaesthetics and a control group at different time intervals. P-value was less than .05, there was a statistically significant difference in the amount of dissolved oxygen in water across different natural oils used as anaesthetics.  $F(4, 20) = 8.977, P = .0003$ . Descriptive statistics of two-way ANOVA for variations in dissolved oxygen during packing and transportation in *Etroplus suratensis* across different natural oils as anaesthetics given in Table 2.

**Table 2 Descriptive Statistics of Two-Way ANOVA for Alterations in Dissolved Oxygen During Transportation of *Etroplus Suratensis* Using Anaesthetics Across Different Natural Oils as Anaesthetics**

SUMMARY	N	MEAN	STD DEVIATION
CONTROL	6	9.06	5.313
CLOVE OIL	6	10.78	4.909
NUTMEG OIL	6	11.67	4.120
CINNAMON OIL	6	11.33	4.404
PEPPERMINT OIL	6	11.06	4.399

Since the P-value was less than .05, there was a statistically significant difference in the amount of dissolved oxygen in water across different time intervals.  $F(5, 20) = 152.4012, P < .0001$  (P value =  $3.28 \times 10^{-15}$ ). Descriptive statistics of two-way ANOVA for variations in dissolved oxygen during packing and transportation in *Etroplus suratensis*

across different time intervals is given in the Table 3.

**Table 3 Descriptive Statistics of Two-Way ANOVA for Alterations in Dissolved Oxygen During Transportation of *Etroplus Suratensis* Using Anaesthetics Across Different Time Intervals**

SUMMARY	N	MEAN	STD DEVIATION
0 HR	5	18.00	0
6 HRS	5	14.07	1.009
12 HRS	5	10.73	1.861
24 HRS	5	8.93	1.513
36 HRS	5	7.40	1.233
48 HRS	5	5.53	1.168

### 3.1.2. Alteration in Dissolved Carbon Dioxide

The changes in the dissolved carbon dioxide content in water during the experimentation are given in the Table 4. The value of carbon dioxide at 0 hour was 7.59 mg/l. After 6 hrs of experiment the lowest value for carbon dioxide of  $9.32 \pm 0.517$  was noted in Peppermint oil treated fish bag even though the value was increased from the initial level. The dissolved carbon dioxide level was increasing as the experiment proceeded. In all experiment groups value of control group was highest on comparing with other groups. At 48 hours of experiment the lowest value for carbon dioxide was seen in Nutmeg oil treated group having a value of  $68.64 \pm 0.449$  followed by Peppermint oil, Cinnamon oil and Clove oil. Control had highest amount of carbon dioxide of  $75.69 \pm 0.425$ .

**Table 4 Alterations in Dissolved Carbon Dioxide During Transportation of *Etroplus Suratensis* Using Anaesthetics**

ANAESTHETICS WITH CONCS.	0 HR	6 HRS	12 HRS	24 HRS	36 HRS	48 HRS
Control	7.59	$13.05 \pm 0.38$	$27.87 \pm 0.208$	$40.37 \pm 1.012$	$55.99 \pm 0.629$	$75.69 \pm 0.425$
Clove Oil (10 mg/l)	7.59	$9.66 \pm 0.346$	$21.61 \pm 0.389$	$37.39 \pm 0.30$	$51.87 \pm 0.227$	$72.66 \pm 0.429$
Nutmeg Oil (4.5 mg/l)	7.59	$10.05 \pm 0.543$	$20.30 \pm 0.274$	$35.18 \pm 0.331$	$47.27 \pm 0.547$	$68.64 \pm 0.449$
Cinnamon Oil (5 mg/l)	7.59	$11.00 \pm 0.341$	$22.07 \pm 0.285$	$39.14 \pm 0.385$	$51.99 \pm 0.609$	$72.19 \pm 0.813$
Peppermint Oil (8 mg/l)	7.59	$9.32 \pm 0.517$	$20.47 \pm 0.355$	$36.96 \pm 0.446$	$47.27 \pm 0.284$	$70.26 \pm 0.291$

Two-way ANOVA was carried out for the variation in dissolved carbon dioxide in water during packing and transportation in *Etroplus suratensis* using four different natural oils as anaesthetics and a control group at different time intervals. P-value was less than .05, there was a statistically significant difference in the amount of dissolved carbon dioxide in water across different natural oils used as anaesthetics. **F (4, 20) = 11.797, P<.0001.** (P value =  $4.83 \times 10^{-5}$ ). Descriptive statistics of two-way ANOVA for variations in dissolved carbon dioxide during packing and transportation in *Etroplus suratensis* across different natural oils as anaesthetics given in Table 5.

**Table 5** Descriptive Statistics of Two-Way ANOVA for Alterations in Dissolved Carbon Dioxide During Transportation of *Etroplus Suratensis* Using Anaesthetics Across Different Natural Oils as Anaesthetics

Summary	N	Mean	STD Deviation
Control	6	36.76	26.040
Clove Oil	6	33.46	25.562
Nutmeg Oil	6	31.51	23.659
Cinnamon Oil	6	34.00	25.209
Peppermint Oil	6	31.98	24.351

**Table 6** Descriptive Statistics of Two-Way ANOVA for Alterations in Dissolved Carbon Dioxide During Transportation of *Etroplus Suratensis* Using Anaesthetics Across Different Time Intervals

Summary	N	Mean	STD Deviation
0 HR	5	7.59	0
6 HRS	5	10.62	1.499
12 HRS	5	22.46	3.113
24 HRS	5	37.81	2.009
36 HRS	5	50.88	3.687
48 HRS	5	71.89	2.662

Since the P-value was less than .05, there was a statistically significant difference in the amount of dissolved carbon dioxide in water across different time intervals **F (5, 20) = 1425.552, P < .0001** (P value =  $8.31 \times 10^{-25}$ ). Descriptive statistics of two-way ANOVA for variations in dissolved carbon dioxide during packing and transportation in *Etroplus*

*suratensis* across different time intervals is given in the Table 6.

### 3.2. Discussion

Transporting live fish over extended durations and in high densities presents major challenges including mechanical stress, physical injuries and rapid deterioration of water quality due to accumulation of metabolic wastes such as ammonia and carbon dioxide (Leitritz, 1969; Cole et al., 1999; Lin et al., 2012). Key water quality parameters affected during transport include dissolved oxygen, pH, temperature, ammonia and carbon dioxide (Crosby et al., 2011). Sedation using anaesthetics has been recognized as an effective strategy to reduce fish activity and metabolic rates, thereby minimizing stress and lowering the production of metabolic byproducts (Cooke et al., 2004; Coyle et al., 2004; Ross & Ross, 2009; Pramod et al., 2010). The duration of confinement during transport directly influences the degradation of water quality (Ostrensky et al., 2016). Previous studies report variable effects of anaesthetics on water quality. For instance, Clove, Mint and Camphor oils were associated with higher dissolved oxygen in *Amphiprion ocellaris* during transport (Ostrensky et al., 2016), while Dominic (2014) observed elevated oxygen uptake and carbon dioxide accumulation in *Etroplus maculatus*. Conversely, Clove oil reduced dissolved oxygen in *Epinephelus* spp. after 10 hours of transport (Suprato et al., 2017). In the present study on *Etroplus suratensis*, dissolved oxygen levels declined over time across all groups, with the control showing the lowest levels. At 6 hours, Cinnamon oil showed the highest oxygen concentration, followed by Clove and Nutmeg oils. At 48 hours, Nutmeg and Peppermint oils maintained the highest dissolved oxygen levels, indicating reduced oxygen uptake due to anaesthetic-induced metabolic suppression. Carbon dioxide accumulation poses a critical risk during transport, as elevated levels can lower blood oxygen-carrying capacity and cause mortality, even in oxygen-rich environments. Dominic (2014) reported CO<sub>2</sub> concentrations as high as 81.88 mg/L in untreated groups. Anaesthetic treatments with Clove oil, 2-phenoxyethanol and Lemongrass oil significantly reduced CO<sub>2</sub> accumulation. Similarly, Suprato et al.,



(2017) reported increased CO<sub>2</sub> after prolonged exposure to Clove oil in *Epinephelus* spp. Consistent with earlier findings, the present study found lower carbon dioxide levels in anaesthetic-treated fish. Nutmeg oil demonstrated the greatest efficacy, with the lowest CO<sub>2</sub> levels ( $68.64 \pm 0.449$  mg/L at 48 hours), followed by Peppermint, Cinnamon, and Clove oils — all performing better than the control ( $75.69 \pm 0.425$  mg/L). The progressive increase in CO<sub>2</sub> over time was notably less pronounced in the Nutmeg oil group, reinforcing its effectiveness in reducing metabolic activity during transport. In conclusion, the use of natural anaesthetics such as Nutmeg, Peppermint, Cinnamon and Clove oils can mitigate stress and maintain better water quality during prolonged transportation of *Etroplus suratensis*. Among these, Nutmeg and Peppermint oils were most effective in preserving higher dissolved oxygen and minimizing carbon dioxide buildup, indicating their potential as efficient, natural alternatives for fish sedation during transport.

### Conclusion

Live fish transport is a critical component of the ornamental fish industry, where maintaining fish health and minimizing mortality during transit is essential. The use of anaesthetics during transportation has been shown to reduce stress and improve survival rates at the user end. The present study evaluated non-invasive stress indicators—specifically dissolved oxygen and carbon dioxide levels—in transported fish packed with different natural anaesthetics: Clove oil, Nutmeg oil, Cinnamon oil and Peppermint oil, along with a control group without anaesthetics. Measurements were taken at 6-hour intervals over a 48-hour period. Dissolved oxygen levels declined progressively in all groups over time, while carbon dioxide concentrations increased. Among the treatments, Nutmeg oil maintained the highest dissolved oxygen level ( $6.67 \pm 0.471$  mg/L), followed closely by Peppermint oil. The control group consistently recorded the lowest oxygen levels, indicating rapid oxygen depletion due to fish metabolism without sedation. Conversely, carbon dioxide accumulation was highest in the control, while the lowest levels were observed in the Nutmeg oil group. Overall, the

results suggest that Nutmeg and Peppermint oils are more effective in mitigating stress during live fish transport than Clove oil, a widely recognized anaesthetic in the ornamental fish industry.

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