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Immersion of rohu fingerlings in clove oil reduced handling and confinement stress and mortality

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Abstract
The use of anaesthetics becomes essential in the transportation medium for mitigating physiological stress and reducing metabolic rates. Clove oil is now emerging as safe, eco-friendly, effective, and economic fish anaesthetic. We tested the efficacy of clove oil as an anaesthetic for the handling and transportation of rohu, *Labeo rohita* (Hamilton-Buchnan) fingerlings. The lowest effective dose of clove oil that produced induction (≤3 min) and recovery (≤5 min) found was 50µl L^{-1}.The induction times decreased and recovery times increased with increased in the concentrations of clove oil. The effective sedative dose at 5µl L^{-1} of clove oil was found suitable for transportation in plastic bags with pure oxygen up to 12h. The mortality rate (%) of fingerlings was significantly higher (14.4±1.14%) in the control (without sedative) than sedative doses of clove oil ($P<0.05$). The dose at 5µl L^{-1} of clove oil was found to mitigate stress responses with lower glucose level and reduce the deterioration of water quality in comparison to control. The present findings revealed that clove oil is promising to be used as anaesthetic and sedative for handling and transportation of rohu fingerlings. Clove oil is feasible to use in the commercial fish seed transportation due its merits like cheap and safe anaesthetic.

Keywords: *Labeo rohita*, clove oil, anaesthetic, transportation, stress.

1. Introduction
Rohu, *Labeo rohita* (Hamilton-Buchanan, 1822), belongs to the family Cyprinidae. Rohu is tasty fish which fetch higher prices in the market, and its contribution is fourth in total aquaculture production of Nepal. Pond aquaculture is the major contributors to aquaculture production in Nepal. Semi-intensive carp polyculture is the major established aquaculture in Nepal. Rohu is cultured in polyculture with other carps of pond fish farming in Nepal. It is often link to the problem of transport stress and mortality as hatcheries are located far away from the fish rearing ponds. Seed is the most important input in aquaculture in Nepal, and its supply is challenging both in terms of quantity and quality [1].Significance mortality during and post transportation of fish seed (up to 70%) experience by farmers in Nepal [2]. It is speculated that consistent supply of high quality and healthy fish seeds to the fish grower could increase fish production in Nepal [1, 3, 4].To ensure that it is necessary to establish suitable protocol that will avoid handling and transportation stress and its related hazards for fish seed. Use of anaesthetics in proper concentration to reduce stress related hazards during transportation and handling is the one option. It will ensure the supply of healthy fish seed to the fish grower to enhance the fish production in Nepal [5].

Fish transport is one of the most stressful procedures that consists of several potential stressors, such as capture, on-loading, transport, unloading, temperature differences, water quality changes and stocking. Its stress can result in immuno-suppression, physical injury, reduced growth and even death. Use of anaesthetic during handling and transportation could reduce stress responses in fish [6, 7, 8, 9, 10, 11, 12]. Inappropriate concentrations of an anaesthetic may lead to adverse effects such as stress and its related consequences on fish. Therefore, optimum concentration of an anaesthetic should be determined for various fish species to perform aquaculture practices [13, 14, 15]. The basic criteria which should be considered when choosing an ideal anaesthetic includes: efficacy (rapid induction and calm recovery), cost, availability, ease of use, toxicity to fish and humans, and the environment [16, 17, 18, 19, 20]. It is often advisable to identify the lowest effective doses of different anaesthetics in a specified species because the responses to the same anaesthetic may vary considerably among different species [19, 21, 22].

Clove oil is a natural product obtained from the flowers, leaves and stalk of the of *Syzygium aromaticum.* Its major active ingredient is oil eugenol (70-90% of clove oil by weight). It is a dark brown liquid with a rich, aromatic odour and flavour [17]. Many studies have been demonstrated that clove oil is safe, effective and cheap anaesthetics for handling, transportation and other aquaculture purpose of fish [23, 24, 25, 26]. Little researches have been carry out on the cultured fresh and warm water carp fish species, especially for practical use in aquaculture practices [8, 15]. The only one preliminary study related to anaesthetic efficacy of clove oil on rohu fry-fingerlings have been reported but it is not applicable to practical purpose like transportation [27]. This is the first complete study to investigate the efficacy of different concentrations of clove oil as an anaesthetic in rohu fingerlings for handling and transportation purpose. Furthermore, the clove oil was selected for this study because it is cheap, eco-friendly and easily available in the market of Nepal. Hence, the hatchery owner, government farm and farmers of Nepal, and also other countries will easily adopt this technology. This research work will add new innovation for safe transportation technique to increase the fish production in Nepal and also will be applicable to the Asian region. The main objective of this research was to find out appropriate doses of clove oil for rohu fingerlings to use in the handling and transport of this species.

Materials and methods

Experimental fish and procedures

All experiments were performed at Agricultural Research Centre (Fisheries), (ARS, Fisheries) Pokhara, Kaski, Nepal in indoor carp hatchery facilities. Rohu (*Labeo rohita)* fingerlings were used in this experiment. Fingerlings seeds were collected by fry net from nursery pond of ARS (Fisheries) and kept in cemented tank with regular fresh water supply with subsistence feeding for 7 days. For acclimatization to hatchery condition, the fish fingerlings were kept in 500 L circular plastic tank in indoor carp hatchery facilities for 7 days before experiment with continuous fresh water supply and subsistence feeding. The fingerlings were starved for 24 h prior to the experiments. The fingerlings were transferred to an acclimation aquarium (60 L) 2 h prior to the experiment performance [5].Glass aquaria of (30cm×30cm×50cm) 45L equipped with aeration stone was used in the entire experiment. The water in these aquaria was from freshwater supply to hatchery of ARS (Fisheries) Pokhara.

Anaesthetic efficacy

The anaesthetic selected for these studies was clove oil (Dabur India P. Ltd.). Several concentrations of clove oil were tested. The following final concentrations of anaesthetics were evaluated: 25, 50, 75 and 100 µl L^{-1} of clove oil. Only one concentration of clove oil was tested at a time. Stages of anaesthesia and recovery behaviour were observed according to Table 1 and 2.

Table 1: Stages of anaesthesia modified from [28].

Stage	Description	Behaviour sign
A0	Normal	Active swimming patterns; reactive to external stimuli; normal equilibrium; normal muscle tone.
A1	Light sedation	Reduced swimming activity; slight or total loss of reactivity to visual and tactile stimuli.
A2	Light necrosis	Slightly loss of equilibrium.
A3a	Deep necrosis	Total loss of equilibrium; decreased muscle tone; reactivity to strong tactile stimuli; decreased respiratory rate.
A3b	Surgical anaesthesia	Total loss of reactivity; total loss of muscle tone; low respiratory rate; tail swinging stopped.
A4	Medullary collapse	Respiration ceases, cardiac arrest; death ensure.

Table 2: Stages of recovery modified from [29].

Stage	Behaviour sign
R1	Reappearance of opercula movement; weak muscle tone visible.
R2	Reappearance of swimming activity but still loss of equilibrium.
R3	Partial recovery of equilibrium.
R4	Full recovery of equilibrium; reaction in response to visual and tactile stimuli; still stolid behavioural response.
R5	Total behaviour recovery; normal swimming activity.

Ten fingerlings (4.29±1.25 g, 6.96±0.86 cm; mean±SD) of rohu were individually exposed to each clove oil concentration. When performing the experiments, single fingerling was quietly scooped and transferred from the acclimation tank and immersed to the anaesthetic solution bath (10L) in the experimental aquarium (45 L). After reaching the each stages of anaesthesia, (Stages A1-A3b, Table1) time was noted. At that time, the fingerlings were weighed before transferred to the resuscitation tank containing aerated freshwater only. The maximum exposure time to each concentration of clove oil was 10 min. If no anaesthetic effect for surgical anaesthesia (Stage A3b) was observed during those 10 min, the concentration of clove oil was considered insufficient.

The exposure time for recovery was based on the average induction time (stageA3b) achieved from above experiment. Four concentrations of clove oil were tested. Ten fingerlings of rohu were quietly scooped and transferred from the acclimation tank and immersed to the clove oil solution in the experimental aquarium. After the desirable exposure time of the chosen clove oil concentration, the fingerlings were transferred to the resuscitation aquarium for recovery. The time to intrigue in the recovery stage (R5, Table 2) was recorded for each individual fingerling. Time for induction and recovery was recorded in seconds using electronic stopwatch (12).

Sedative dose of clove oil for transportation

Rohu fingerlings (4.29±1.25 g, 6.96±0.86 cm; mean±SD) were used for this experiment. Ten fingerlings were placed into each of glass aquaria (45 L) filled with aerated fresh water and containing doses of clove oil. Clove oil doses tested were: 2.5 µl L^{-1} (1.9 mg L^{-1} eugnol), 5 µl L^{-1} (3.8 mg L^{-1} eugnol) and 7.5 µl L^{-1} (5.6 mg L^{-1} eugnol). Each clove oil concentration was tested in triplicate. A control group of three aquaria without clove oil was used as control. Fingerlings behavioural responses were observed and recorded at 4h intervals up to 12h. After 12h, surviving fingerlings were transferred to recovery tanks and monitored for another 24h.

Simulated transportation procedure

Fingerlings of rohu (6.43±0.78cm, 3.24±0.84 g) were netted from nursery ponds of ARS (Fishery), Pokhara and transferred to a 1 m^2 cemented tanks with constant water inflow and kept there in hapa for 12h. 4×2 factorial design was used with three replications. The treatments groups were doses of clove oil (control, 2.5 µl L^{-1}, 5 µl L^{-1}, and 7.5 µl L^{-1}) with two

transportation times (6h, 12h). Control was maintained without clove oil, only fresh water. Fingerlings were introduced at 30 fish (approximately biomass weight of 100g) per bag in plastic bags (45 cm wide ×95 cm length) containing 2L water, and mixed with clove oil sedation dose in each treatment with replication. The air from the plastic bag was squeezed out and medical-grade oxygen was filled in. The plastic bags were then tied tightly with jute threads. The packing was done with double plastic bags, one being slipped inside the other to ensure the no leakage of air and water. Simulation trials were conducted in the hatchery condition at temperature (28.9±0.14°C). The total duration of confinement stress was 12 h. At six hour and at the end of the transportation period, all plastic bags were opened and dead fingerlings were counted. The survived fingerlings from each bag were transferred to 100 L tanks with constant water circulation and monitored for 24h to evaluate accumulated mortality. Fish mortality rate, glucose levels were measured as indicators of stress.

Blood samplings and glucose estimation

Bloods samples were taken from rohu fingerlings before to transportation (0h), at 6h and at 12h in simulated transportation from each experimental unit for glucose estimation. Ten fingerlings were randomly selected from each treatment replication for glucose estimation. Prior to blood collection, the fingerlings were euthanized with benzoak® vet (20% benzocaine) at 100 mg L^{-1} solution in a glass aquarium of 45 L filled with 10 L water. The blood was collected by severing caudal peduncle of fish fingerling with sharp blade [30]. The blood glucose was measured immediately from the whole blood by commercial "on call plus" blood glucose meter (ACON Laboratories Inc. San Diego, USA) [31].

Preparation of clove oil solutions

Clove oil (75% eugnol) used in this experiment was from Dabur India P. Ltd. It is used for the human medicine. Clove oil (poorly soluble in water) was dissolved in 96% ethanol (ratio of clove oil: ethanol, 1:9). Stock solutions of the clove oil were prepared fresh prior to the start of experiments.

Water quality parameters

Water quality parameters: temperature, pH, dissolved oxygen and ammonia (NH_4^+-N) were measured in all experiments (2.2-2.4). In simulated transportation experiments (2.4), these water quality parameters were measured at 0h, at 6h and at 12h.The water temperature and pH were recorded by digital pH meter (Thermo electronic corporation, Singapore), dissolved oxygen by Winkler methods [32] and ammonia (NH_4^+-N) was analysed using salicylate-hypochlorite method [33] during the experiments.

Statistical analysis

Mortality rate (%) data were square root-transformed before statistical analysis. The treatments means for induction and recovery stages, plasma glucose, and mortality rate and water quality were compared using ANOVA followed by Tukey'S HSD post hoc for multiple comparisons. Data were analysed using statistical software SPSS version15.0 with a level of significance of $P<0.05$.

Results

Anaesthetic efficacy

The water quality (mean±SD) parameters recorded were: temperature, 24.7±0.11°C; dissolved oxygen, 7.16±0.3 mg L^{-1}; ammonia (NH_4^+ -N), 0.002±0.001 mg L^{-1} and pH 7.3 during the entire experiment of induction and recovery. The mean time (min) of anaesthesia stages (A1-A3b) and recovery time on rohu fingerlings are presented in Table 3.

Table 3: Times (min) to reach stages of anaesthesia (StagesA1 to A3b) and recovery (R5) for fingerlings (n = 10) of rohu (*Labeo rohita*) anaesthetized with four concentrations of clove oil.

clove oil (µl L^{-1})	Time(min)				
	StageA1	StageA2	StageA3a	StageA3b	Recovery
25	0.85±0.05^{c}	1.4±0.05^{d}	2.36±0.14^{d}	4.26±0.35^{a}	3.84±0.29^{a}
50	0.47±0.03^{b}	0.60±0.02^{c}	0.86±0.07^{bc}	1.14±0.13^{b}	4.1±0.23^{a}
75	0.23±0.01^{a}	0.48±0.01^{b}	0.56±0.01^{ab}	0.63±0.01^{b}	4.34±0.14^{a}
100	0.20±0.00^{a}	0.33±0.01^{a}	0.46±0.02^{a}	0.59±0.02^{b}	4.72±0.23^{a}

All data are presented as mean values ± SEM. For each stages of anaesthesia and clove oil concentration, values in the same column with different letters are significantly different ($P < 0.05$).

The induction time decreased as the concentration of clove oil increased in each stage of anaesthesia while the recovery time increased as the concentration of clove oil increased (Table 3). The lowest effective concentration that produced surgical anaesthesia, stageA3b (≤3min) and recovery (≤5min) in rohu fingerlings was 50 µl L^{-1} of clove oil (Table 3). The induction time to reach surgical anaesthesia (stageA3b) were not significantly difference($P>0.05$) between the concentrations of clove oil 50 µl L^{-1}, 75 µl L^{-1} and 100 µl L^{-1} while 25 µl L^{-1} was significantly($P<0.05$) difference to the rest of clove oil concentration. Recovery from surgical anaesthesia were not significantly difference ($P>0.05$) between the concentration clove oil (Table 3). No mortality of rohu fingerling was observed during the entire experiment.

Table 4: Behavioural observations of rohu fingerlings in anaesthetics bath during 12h exposure in diluted concentration of three doses of clove oil.

Behavioural observations during anaesthesia bath					
Clove oil dose (µl L-1)	4h	8h	12h	Recovery time(min)	Survival (%) at 24h
2.5	Normal; less active than control for swimming pattern	Normal; less active than control for swimming pattern	Normal; less active than control for swimming pattern	0.41±0.02	100
5	*Light sedation	Light sedation.	Light sedation	0.67±0.03	100
7.5	Light sedation (10% fingerlings exhibit slight loss of equilibrium)	Light sedation (10% fingerlings exhibit slight loss of equilibrium)	Light sedation (10% fingerlings exhibit slight loss of equilibrium)	1.87±0.02	100
Control	Normal	Normal	Normal	Normal	100

*Light sedation: Reduced swimming activity, slight or total loss of reactivity to visual and tactile stimuli but without loss of equilibrium.

Sedative dose for transportation

The water quality (mean±SD) parameters recorded were: temperature, 25.5±0.86°C; dissolved oxygen, 7.2±0.4 mg L^{-1}; ammonia (NH_4^+-N), 0.002±0.001 mg L^{-1} and pH 7.3 during the entire experiment.The present study demonstrated that 5 µl L^{-1} clove oil dose was suitable for the light sedation of rohu fingerlings up to 12h without any adverse effects (Table 4). In the higher dose (7.5 µl L^{-1}) of clove oil, ten percentage of fingerlings were slightly loss their equilibrium while lower dose (2.5 µl L^{-1}) was not able to sedate fingerlings. Rohu fingerlings were recovered in very short time in fresh water.

Simulated transportation

The water quality parameter (mean±SD) of source water used in transportation were temperature, 28.9±0.14°C; dissolved oxygen, 7.8±0.6 mg L^{-1}; ammonia (NH_4^+-N), 0.002±0.001 mg L^{-1} and pH 7.8. The mortality rate (%) of rohu fingerling was found significantly higher (14.4±1.14) in the control (without sedative) than sedative doses of clove oil ($P<0.05$). No mortality of fingerling was observed in the dose of 5 µl L^{-1} of clove oil. The mortality rates (%) of fingerlings were also very low (3.3 to 4.4.3%) in the sedative doses (2.5and 7.5 µl L^{-1}) of clove oil (Table 5).

Table 5: Mortality rate (%) of rohu *Labeo rohita* fingerlings found in a simulated confinement experiment at 50g L^{-1} of loading density using plastic bags with pure oxygen.

Duration (h)	Mortality rate (%) of rohu fingerlings in the control group and in different treatments groups (dose of clove oil, µl L^{-1}).			
	Control[a]	2.5	5.0	7.5
6	3.3±0.0[a]	0.0±0.0	0.0±0.0	0.0±0.0
12	8.03±1.13[b]	3.3±0.0	0.0±0.0	3.3±0.0
24	3.73±0.03[a]	1.13±1.13	0.0±0.0	0.0±0.0
Total mortality [b]	14.4±1.13[a]	4.43±1.13[b]	0.0±0.0[c]	3.3±0.0[b]

Values are presented as means %(± SE)

[a]Means (± SE) (dose × time) with different letters for each column are significantly different (ANOVA, HSD, $P<0.05$).

[b]Values means% (± SE) within row (Total mortality) followed by different letters are significantly different (ANOVA, HSD, $P<0.05$)

Table 6: Glucose level of rohu *Labeo rohita* fingerlings found in a simulated confinement experiment at 50g L^{-1} loading density using plastic bags with pure oxygen.

Duration(h)	Glucose level of rohu fingerlings in the control group and in different treatments groups (dose of clove oil, µl L^{-1}).			
	Control	2.5	5.0	7.5
0	88.11±10.48[a]	88.11±10.48[a]	88.11±10.48[a]	88.11±10.48[a]
6	143.0±10.08[b]	112.6±2.37[b]	98.88±6.67[b]	117.0±5.42[b]
12[a]	164.66±16.06[bx]	124.78±10.59[by]	123.11±6.38[by]	123.33±5.37[by]

All values are presented as means (±SE).

Different superscript (a,b,c) in the same column indicate significant difference in transportation duration ((ANOVA, HSD, $P<0.05$).

[a]Different superscript (x,y) in the same row indicate significant difference in the treatment doses (ANOVA, HSD, $P<0.05$).

The glucose level of rohu fingerling was significantly higher in the control (164.66±16.06 mg dL^{-1}) than sedative doses of clove oil (123.11-124.78 mg dL^{-1}) at the end of simulated transport ($P<0.05$). Glucose level of fingerling increased with increased in the confinement duration (Table 6) in each treatment. Dissolve oxygen, and pH were found significantly lower while ammonia concentration was higher in the control than the sedative doses of clove oil at the end of simulated transport ($P<0.05$). Dissolved oxygen and pH level were decreased while ammonia level was increased with increased in the confinement duration (Table 7).

Table 7: Dissolved oxygen, pH and ammonia sampled in a simulated confinement experiment at 0h, 6h and 12h

Duration(h)	DO concentration (mg L^{-1}) in the control group and in different treatments groups (dose of clove oil, µl L^{-1}).			
	Control	2.5	5.0	7.5
0	17.9±0.17[a]	17.9±0.17[a]	17.9±0.17a	17.9±0.17[a]
6	8.8±0.20[b]	13.6±0.08[b]	13.8±0.08b	13.5±0.08[b]
12[a]	4.9±0.10[cx]	8.5±0.05[cy]	9.2±0.05[cz]	8.5±0.05[cy]
pH level				
0	7.8	7.8	7.8	7.8
6	7.0	7.3	7.3	7.3
12	6.9	7.03	7.03	7.03
Ammonia concentration(mg L^{-1})				
0	0.002±0.001[a]	0.002±0.001a	0.002±0.001a	0.002±0.001[a]
6	0.187±0.002[b]	0.134±0.001b	0.132±0.004b	0.138±0.009[b]
12[a]	0.226±0.003[cx]	0.183±0.003[cy]	0.183±0.003[cy]	0.183±0.003[cy]

All values are presented as means (±SE).

Different superscript (a,b,c) in the same column indicate significant difference in transportation duration ((ANOVA, HSD, $P<0.05$).

[a]Different superscript (x,y,z) in the same row indicate significant difference in the treatment doses (ANOVA, HSD, $P<0.05$).

Discussion

The ideal anaesthetic criteria's are that anaesthesia should be induced rapidly (≤3min) with minimum associated hyperactivity or other stress and recovery should be rapid (≤ 5 min) in clean water with no other undesirable features [16, 17].The present findings slightly differ but comparable to the results obtained by [27] at concentration of 50µl L^{-1} of clove oil in the induction times(min) (stageA3b) and recovery times(min) for rohu fingerlings.The slight difference could be due to difference in temperature and active ingredients (eugnol %) in the products of the clove oil. Similar effective dose (50 µl L^{-1} of clove oil) was found for induction (≤3min) and recovery (≤5min) in fingerlings of Shabut (*Barbus grypus*) [34]. Similarly, clove oil concentrations between 33-50 µl L^{-1} are sufficient to produce anaesthesia in a majority of fish species [35, 36]. However, Salmonids needs lower (20-30 µl L^{-1}) and Sturgeons higher (70 µl L^{-1}) concentration of clove oil to produce anaesthesia [36].The present findings on rohu fingerling that induction times decreased and recovery time increased with increased in the concentrations of clove oil are

agreement with previous studies in rohu and also in other fish species [11, 12, 27, 34, 37, 38].

Transportation and handling procedures consists of several potential stressors, such as capture, on-loading, transport, unloading, temperature differences, water quality changes and stocking [10, 24, 39]. It has caused stress responses and mortality in rohu fry-fingerlings [30, 40, 41, 42, 43, 44]. Anaesthetic agents are added to the water at low doses to sedate fish prior to transport and mitigate stress responses [17, 30, 43, 45, 46]. This reduces metabolic rate and hence oxygen demand, reduce general activity, increase ease of handling, and mitigate the stress response [17, 47, 48]. Fish handling and transport need desired level of sedation which is reduced reaction to external stimuli, reduced swimming activity but without loss of equilibrium [45]. Low dose of anaesthetics (clove oil) application during transportations was found to improved the survival of rohu fingerlings in comparison to control (without anaesthetic) of the present findings is good agreement to previous studies on rohu fry-fingerlings [30, 42, 43, 44] and It is also consistent in other fish species [46, 49, 50, 51]. Compare to present finding of mortality rate (14.4%), higher mortality rate (30-42.5%) have been reported during transportation of rohu fingerlings [30,42, 44,52]. This is due to difference in the time (h) of conditioning, containers used, transportation system and duration of transportation, methods and medium of transportation, and packing density in the previous studies during transportation of rohu fry-fingerlings. The present findings of effective sedative dose at 5 µl L^{-1} of clove oil (3.8mg L^{-1} eugnol) on rohu fingerling is good agreement to previous studies which concluded that the doses between 2-5 mg $L^{-1}$of clove oil are safe to use in transport of several fish [9, 45, 50, 53, 54, 55]. However, lower dose of clove oil at dose of 1-1.3 mg L^{-1} are suitable for the transportation on other fish [56, 57] and higher dose (15 mg L^{-1}) of clove oil is recommended to be used in transport post larvae of the freshwater prawn, *Macrobrachium rosenbergii* [58].

Stress is an energy-demanding process, and the production of glucose (as secondary stress response) provides energy to cope with the stressful situation [40, 59]. Glucose commonly changes after subjecting fish to stressors such as capture, handling, or transport [60, 61, 62]. Quantification of glucose level can provide valuable information regarding the severity and duration of the stress responses [63]. Cortisol level, glucose level and mortality rate have been used as stress indicators in fish [30, 42, 49, 64].In the present study, glucose levels were increased with the increased duration of confinement stress of rohu fingerlings was due to increased energy demand caused by confinement stress. Confinement due to packing in small quantity water is stressful to rohu fingerlings. Its stress response was found to increase in the cortisol level, glucose level and decrease in chloride and glycogen with increased transport duration [30, 40, 41, 42].The increase of blood glucose level in response to various stressors, like transportation, confinement and handlings have been reported also in other fish species [49, 64, 65, 66]. The glucose levels in the control due to confinement stress response of the present findings on rohu fingerlings are agreement with the glucose levels (150-250 mg dL^{-1}) on other fish after transport [49, 67]. The glucose levels of rohu fingerlings were high in the control group in comparison to sedative doses of clove oil in the present study was due to confinement stress and hyperactivity of fish fingerlings. However, addition of clove oil in the transporting water was found to calm rohu fingerlings and thus less stressed, resulting low glucose levels in the sedative treatments. The addition of clove oil in transporting water, glucose and cortisol levels were found lower in comparison to control due to mitigation of the transport stress responses in fish [9, 54].

Conclusions

The present finding demonstrated that the lowest effective dose of clove oil 50 µl L^{-1} was found suitable for handling and 5µl L^{-1} for transportation purpose of rohu fingerlings.The effective sedative dose of clove oil (5µl L^{-1}) was found to mitigate the stress responses and improves the survival during transportation of rohu fingerlings.Commercial use in the transportation of fish seed of clove oil is feasible as it is economic, effective, safe and an eco-friendly anaesthetics.

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