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RESEARCH ARTICLE

STANDARDIZATIONS OF CLOVE OIL DOSES AND LOADING WEIGHT FOR LIVE TABLE SIZE FISH TRANSPORTATION

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ABSTRACT

Globally, demands of fish have been increasing due to awareness of peoples about health. Live table size fish marketing is now becoming popular due to preference of consumer andalso fetch higher price in the market. The traditional methods of live fish transportation have significant fish mortality due to transportation stress and thus economic losses. The objective of this study was to standardize the doses of clove oil for handling and transportation as well as loading weight of fish. The presents study has found that optimum doses of clove oil for handling was 0.03-0.05ml/Lfor carp fish species and doses for the transportation of live fish (table size) was rangesfrom 5.0 to7.5 µl/litre of water. The present study showed that the optimum loading weight of fish (approximate 1-2kg size) was 40kg/500 litre water for safe fish transportation. Precaution should be taken to reduce the stress during harvesting, loading, transportation of table size fish. The farmers and venders should follow the recommended practices such as harvesting in the cool time, proper conditioning,optimum loading weight, use of sedatives and aeration in the transportation system to supply healthy table fish for the consumers and mitigate the stress related mortality.

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INTRODUCTION

Live fishes are value-added product that has guarantee the freshness and also fetch higher price than fresh, chilled or frozen goods. Live fish marketing mainly involves the transportation of fish after harvesting from the pond to the fish market in live condition for sale through the auctioneers. Globally live fish trade is well established mainly in most of Southeast Asia and southern Pacific regions (Fabinyi, *et al.*, 2012). Marketing live fish attracts consumers for its quality and ensures better revenue for farmers. Live fish is occupying a specialized segment in both domestic and international markets (Nair *et al.*, 2023). Awareness of food safety and the quality of fish increases the demand for edible live fish around the world. Live fish marketing is now becoming popular due to preference of consumer. However, about 50 to 100% fish mortality was observed in the long-distance-carriage or truck-pool method, carps weighing 300-1500 g without proper transportation protocol (Muzaddadi *et al.*, 2017). Stress leads to decreased immune system function, resulting in sickness and death (Tacchi *et al.*, 2015). Larger fish frequently leads to large-scale mortality due to stress arising out of handling, packing, crowding and physical injury (Singh *et al*., 2004; Basavaraja, 2007). Anesthesia has been extensively proved to be an effective way to minimize stress or physical damage during various aquaculture procedures (Javahery *et al.*, 2012; Baaberoo *et al.*, 2016; Bowker *et al.*, 2019), and a variety of agents have been used to induce anesthesia in aquatic animals, such as clove oil, and eugenol (Zahl *et al.*, 2009; Saydmohammed and Pal, 2009; Pramod *et al.*, 2010; Velisek *et al.*, 2011; Parodi *et al.*, 2012; Mohammadi and Khara, 2015; Balamurugan *et al.*, 2016). Herbal anaesthetic use is increasing in aquaculture (Husaini *et al.*, 2019). Many studies have been demonstrated that clove oil is safe, effective and cheap anaesthetics for handling, transportation and other aquaculture purpose of fish (Javahery *et al.*, 2012; Mitjana *et al.*, 2014; Husen and Sharma, 2015a,b; Hur *et al*., 2019). For the transport of live fish in bulk quantity, anesthetization can be done to reduce stress during handling and maintain the quality of the live fish (Hasan and Bart, 2007; Dheeran *et al*., 2023). Demands of fish have been increasing in the country due to awareness of peoples about health and also increased population of Nepal. Live table size fish have getting higher price in the market (Husen, 2019; Das *et al.*, 2024). For table size live fish, the venders buy fish from fish producing farmers and they sale to local retailors or directly to the hotels and consumers. For the local market, they transported live fish in the hundies with water. For the distant market, they transported live fish in the plastic tank capacity of 200 to 500 litre water loaded on the mini truck (Husen, 2019). There are 1681 fish markets and 22406 farmer's pond sites from which fish marketing are carried out and 69 live fish selling stall for the selling of live fish in Nepal (NFS, 2017). More people want to buy live fish from the stall (Husen, 2019). However, traditional methods of live fish transportation have significant mortality of fish about 10-25% as reported by fish supplier and its cause's economic losses in Nepal (Husen *et al.*, 2024). Therefore, the objective of this study was to develop protocol to reduce the effects of stress related mortality during handling and transportation using organic sedative. This study has recommendedthe fish loading weight as well sedative doses of clove oil for table size fish transportation to reduce the farmers' economic losses due to fish mortality during transportation.

MATERIALS AND METHODS

Experimental fish and procedures: The all experiments were carried out in the years from 2021- 2022. The efficacy test and sedatives doses experiments were performed at Fishery Research Station, Begnas, Pokhara, Kaski, Nepal in the indoor carp hatchery facilities Fish collection and acclimatization were followed according to methods described by Husen and Sharma (2015). Tanks of 400

litrecapacity equipped with aeration stone were used in the entire experiment.

Fish species: For induction and recovery test, common carp (*Cyprinus carpio*), grass carp (*Ctenophyrongodon idella*) and naini (*Cirrhinus mrigala*) were selected. The weight (Mean±SD) of fish species used in the experiment were: Bighead carp (1.32± 0.25kg), Naini (1.23±0.31kg) and common carp (0.99±0.41kg).

Experiment 1: Anaesthetic efficacy test

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 on the fish species (0.025/L to 0.05/L). Anaesthetic efficacy test was performed according to methods described by Husen and Sharma (2015).

Experiment 2: Determination of sedative doses of clove oil for transportation

Sedative doses of clove oil were calculated from results of experiment 1 of induction and recovery test. For sedative doses, clove oil doses tested were: 0.004 ml /L, to 0.02 ml/ L. Ten fish were placed into each of 400 litretank filled with aerated fresh water and containing doses of clove oil. Each clove oil concentration was tested in triplicate. Fish behavioural responses were observed and recorded at 2hr., 4hr.and 6hr. Determination of sedative doses of clove oil for transportation test was performed according to methods described by Husen and Sharma (2015).

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 recorded in all experiments weretemperature, pH, and dissolved oxygen.The water temperature; pH was recorded by digital pH meter (Thermo electronic corporation, Singapore) and dissolved oxygen by Winkler methods (Boyd and Craig, 1992) during the experiments.

Experimental3: Standardization of fish loading weight

The fish loading weight (kg) evaluation experiment was conducted with three loading weight. T1: 40 kg/500 litre water; T2: 50 kg/500 litre water; T3: 60 kg/500 litre water. The fish were harvested with fine net with minimal stress and kept in hapa with flowing water for 12-hour conditioning. Common carp, Bighead carp and Naini of size ranging from (0.9- 1.5kg) were used in this experiment. The ratio of fish species were: 34% for common carp and 33 % Bighead carp and Naini each.

Common salt 0.5 % was added in the transport water of fish. Fish were loaded in to 500 litre plastic tanks according to treatments of loading weight and transported for 10 hours by road in pick up.Continuous oxygen was supplied through pipes in plastic drum. At the end of the trial, fish mortality numbers were noted, initial and final water quality parameters were noted.

Experimental 4: Standardization of optimum sedative doses

Sedative doses were determined as results from experiment 2. Three doses were evaluated: T1- 5.0µl/litrewater; T2- 7.5 µl/litre water; T3- 10.0µl/litrewater. The loading weight was selected on the basis of the experiment 3 (50 kg / 500 litre water plastic tank 500 litre after 12-hour conditioning during 10-hour transportation in the pickup). Common carp, bighead carp and naini of size ranging from (0.9-1.5kg) were used in this experiment. The ratio of fish species were 34% for Common carp and 33 % Bighead carp and 33 % Naini. The sedative doses were mixed in the loaded water as treatments. Common salt 0.5 % was added in the transport water of fish. Continuous oxygen was supplied through pipes in plastic drum. After loading, transported for 10 hours by road in pick up. The fish mortality was recorded. At the end of the trial, fish mortality numbers were noted; initial and final water quality parameters were noted.

RESULTS AND DISCUSSIONS

Clove oil doses for induction and handling of carp: The results showed that the time for induction was decreased with the increased doses of clove oil in all tested carp fish species (Fig. 1). Common carp was found to reached surgical anesthesia stage within three minutes and recovery within 5 minutes in the dose of 0.05ml while bighead carp 0.04ml and naini in the dose of 0.03ml (Fig.1-3). In the present study, clove oil concentrations 0.03-0.05ml/L of was found optimum dose to reach surgical anesthesia in carp fish. Clove oil concentrations between 33- 50 µl/ L were sufficient to produce anaesthesia in a majority of fish species (Hamackova *et al.*, 2006; Alam, 2012; Husen *et al.*, 2015; Krasteva *et al.*, 2021). For short time handling during breeding procedure as well as tagging and transportation, fish needed to sedate for easy and stress-free handling. To sedate fish (achieve A2 stage anestheisa), as results of present study of induction and recovery test, fish should be immersed in to clove oil concentrations 0.03- 0.05ml/L only forone minute and then fish should be maintained in the dilute concentration which is only 10% of induction dose. This will ensure the stress mitigations during handling and on farm operations such as short distance transport, breeding, tagging, and administration of drugs. The water quality recorded were: temperature (°C) ranged from 27.3±0.1 to 27.5±0.05, pH 7.3 to 7.4 and dissolved oxygen 6.8±0.05 to 7.2±0.02 mg/L.

Sedative doses of clove oil for transportation: The results of sedative doses of clove oil on three carp fish are presented in the table 4-6. The sedative doses of clove oil at concentration of 5 µl/L–10µl/L tested on Naini (Table 4) and bighead carp (Table5) were found to in stage1 (Table 2) condition up to six hours and recovery were also found within five minutes in this experiment.

Table 4. Behavioural observations of Naini in anaesthetics bath during 6hr. exposure in diluted concentration of clove oil

Doses of Clove oil (µl/L)	Behavioral observations in anesthetics bath (Time)			Recovery time(min)	Survival (%)
	2hr.	4hr.	6hr.		
5.0 (3.8 mg/L of eugnol)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	2.5 - 3.45	100%
7.5 (5.6 mg/L of eugnol)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	2.5- 4.30	
10 (7.5 mg/L of eugnol)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	1.44- 1.45	
15(11.3 mg/L of eugnol)	Within 10 min tested fishes lost equilibrium(discarded)	-	-	-	
20 (15 mg/L of eugnol)	Within 6 min tasted fishes lost equilibrium(discarded)	-	-	-	

Table size common carp were in stage1 (Table 2) condition up to six hours in the sedative doses of clove oil at concentration of 10 µl/L - 15 µl/L, and recoveries were also found within five minutes (Table 6).

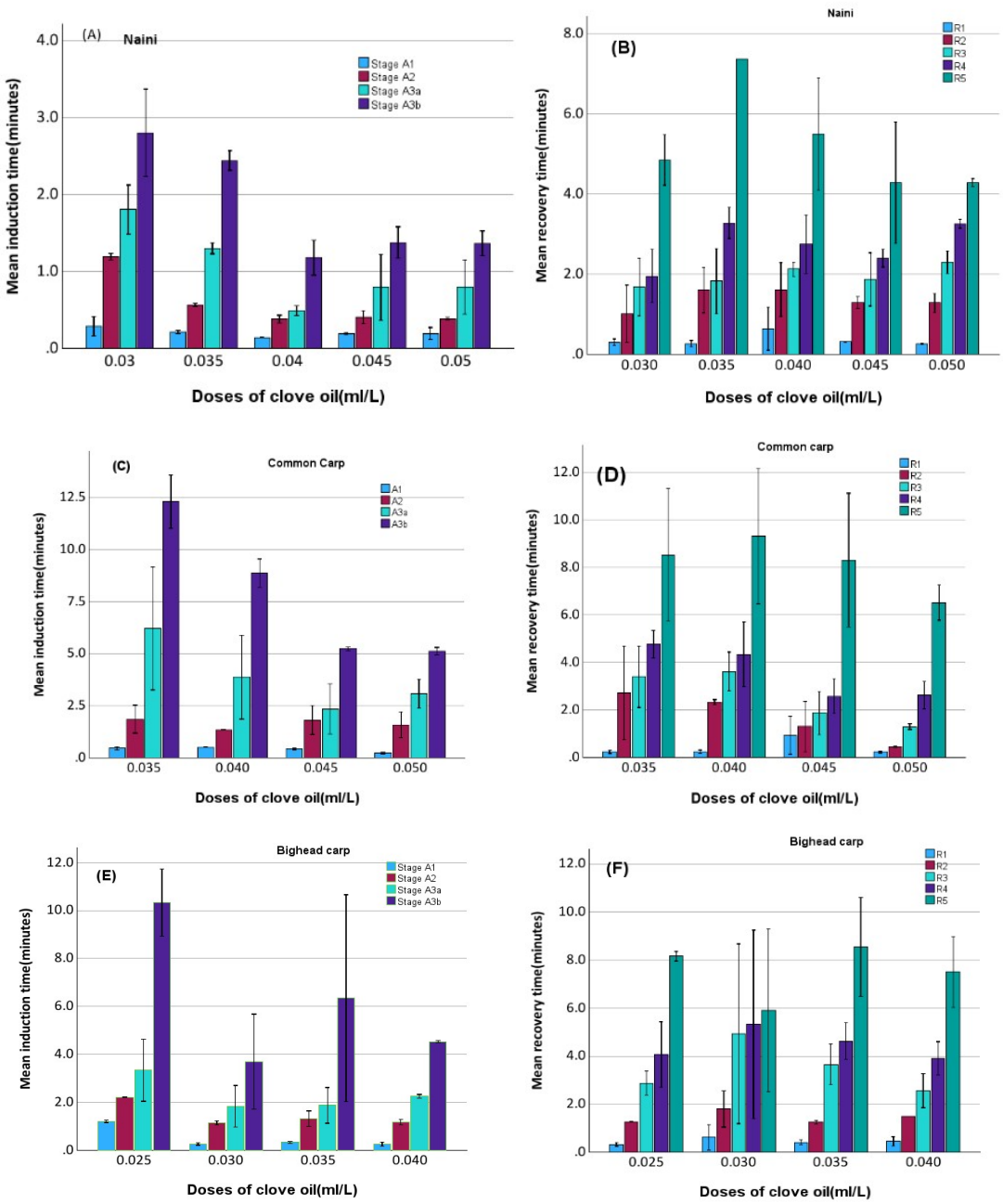

Fig. 1. Results of induction and recovery stages of Naini (A, B), Common carp (C, D) and Bighead carp (E, F)

Table 5. Behavioural observations of Bighead carp in anesthetics bath during 6h exposure in diluted concentration of clove oil

Doses of Clove oil (µl/L)	Behavioural observations in anaesthetics bath (Time)			Recovery time(min)	Survival (%)
	2hr.	4hr.	6hr.		
5.0 (3.8 mg/L of eugnol)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	4.0- 4.5	100%
7.5 (5.6 mg/L of eugnol)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	2.13- 2.15	100%
10 (7.5 mg/L of eugnol)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table2)	4.9-5.0	100%
15(11.3 mg/L of eugnol)	Tested fishes lost equilibrium within 8 minutes (discarded)	-	-	-	-

Table 6. Behavioral observations of common carp in an aesthetics bath during 6h exposure in diluted concentration of clove oil

Doses of Clove oil (µl/L)	Behavioral observations in anesthetics bath			Recovery time(min) Range	Survival (%)
	2hr.	4hr.	6hr.		
5.0 (3.8 mg/L of eugnol)	reactive to external stimuli, normal equilibrium	neither normal nor sedation stage, reactive to external stimuli, normal equilibrium	reactive to external stimuli, normal equilibrium	0.55- 1.05	100%
10 (7.5 mg/L of eugnol)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table 2	Stage A1 light sedation (as Table2)	3.45-3.48	
15(11.3 mg/L of eugnol)	Stage A1 light sedation (as Table2)	Stage A1 light sedation (as Table 2)	Stage A1 light sedation (as Table2)	2.1- 4.47	
20 (15 mg/L of eugnol	Both of fishes lost equilibrium within 15 minutes (discarded)	-	-	-	-

On the basis of this results, the optimum doses of clove for the transportation of table size fish for Common carp range from 10 to 15 µl/litre of water, for Bighead carp range from5 to7.5 µl/litre of water, and for Naini range from 5 to10 µl/litre of water. The safe dose of clove oil for transportations of common carp fish seed found was 0.02ppm (Saini *et al*. 2018), on rohu 5 µl/litre (Husen and Shrama, 2015b), on Gaint gourami fingerlings administration of clove oil as an anesthetic for transportation was a concentration of 0.010 ml / L with a duration of transportation of 7 hr. (Lili *et al.,* 2020).

Fish loading weight: The results of present study showed that when loading weight of fish increased, fish mortality was also found to increase. All procedure was done to reduce stress in the present study. However, the crowded condition was found to causes higher mortality (Fig.2). The survival rates of the fish decreased following times in transport duration and post transport, and following increased loading densities (Dinh and Nguyen, 2022). Transportation is a strong trigger for stress in fish (Manuel et al., 2014), with atypical environmental conditions, including temperature, pressure, and agitation. The optimum fish weight for the loading was 40kg/500 litre water. The recommended guidelines for transporting live fish at a maximum loading was 75 kg/500 liter for 10 hr. transportations at temperature below 25°C for carp (Rajts and Shelley, 2020). The transport loading weight varies greatly depending on the species, size, and developmental stage, at loading weight of up to 25 kg/500 liter is advised for salmon (Das *et al.*, 2024).

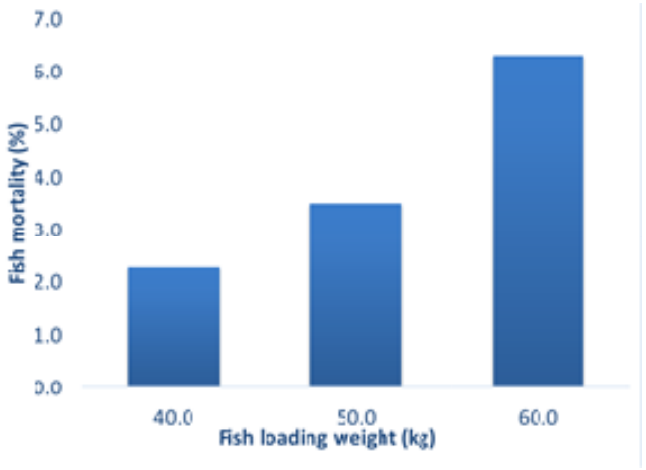

Fig. 2. Fish mortality rate (%) in variable loading weight (kg), during 10 hr. transportation and fish was loaded in the500-litre water plastic tank (12 hr. conditioning)

Optimum sedative doses for live table fish transportation: The results showed that maximum mortality of fish during 10 hr. transportation was only 0.3 % when clove oil doses were added at the rate of 5.0µl/litre (3.8 mg/L of eugnol) of water in the transportation water (Fig.3).The results showed that the minimum mortality of live fish(table size) was found at the doses of clove oil which ranges 5.0-7.5 µl/litre (3.8-5.6 mg/L of eugnol) of water. However, mortality was above 2 % without clove oil addition in the transportation water (Fig.4).The addition of clove oil in the transportation water was to found to mitigate the stress and results low mortality. Anesthesia reduces metabolic rate, need for oxygen, activity, response to stress and relax fish (Brown, 2011; Skar *et al.,* 2017; Akbari *et al*., 2010), and enabling fish to be transferred in higher densities more efficiently. Many researchers found that clove oil and clove powder have found to mitigate stress and reduces fish mortality during transport and recommended as effective, safe and cheap anesthetics (Velisek *et al.*, 2011; Husen and Sharma, 2015a,b; Hoseini *et al.*, 2015; Mohammadi and Khara, 2015; Balamurugan *et al.*, 2016). The water quality recorded were: temperature (°C) ranged from 27.3±0.1 to 28.3±0.03, pH 6.9 to 7.3 and dissolved oxygen 6.5±0.05 to 6.8±0.02 mg/L.

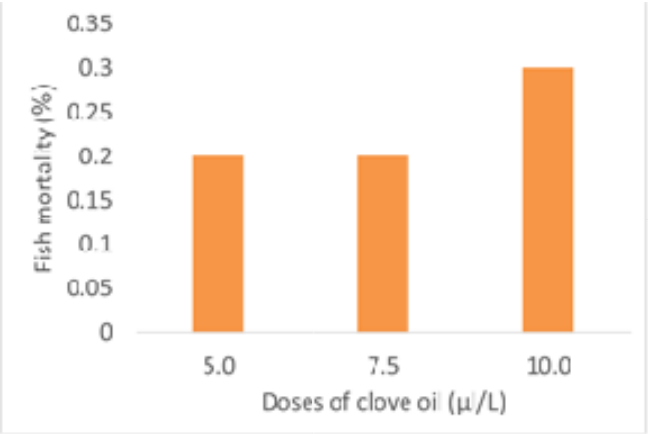

Fig. 3. Sedative doses and fish mortality rate (%) in the loading weight of 40 kg/ 500 litre water plastic tank with addition of clove oil sedative after 12 hr. conditioning during 10 hr. transportation in the pickup

Awareness of food safety and the quality of fish increases the demand for edible live fish and its trends been increasing in Nepal. Fish retains fresh quality when it is alive. Therefore, fish handling and transportation procedure should be done carefully and also avoid the stressful process from fish harvest till end of destination. The produced product should require an efficient handling process as well as an effective transport facility to ensure an increased survival rate.Intense stress response has a negative influence on survival rate and muscle quality of fish, intense stress response generally accelerates the deterioration of muscle quality (Roth and Skåra, 2021; Li *et al.*, 2020; Peng *et al.*, 2023). Therefore, during live table size fish transportation,precautions must be taken to mitigate the degree of stress response throughout the transportation, such as maintaining a low temperature in the water tank, adding anesthetics to the water, or adding a modest quantity of salt (Husen*et al.*, 2015;Wang *et al.*, 2021; Biswal *et al.*, 2021) to supply healthy fish for consumers as well as to ensure losses due to fish mortality.

CONCLUSIONS

Live fishes guarantee for freshness and fetch higher price in the market due to preference of consumers. Marketing live fish attracts consumers for its quality and ensures better revenue for farmers. Live fish needs to transport from the production's sites to retailer's shops. To ensure for delivery of freshness and quality of live table size fish, safe procedure for transportation should be followed such as pre-transport and during transportation procedure such as seining with fine net, conditioning fish prior transportation, carefully hauling, using sedatives and salt, maintaining low temperature and providing aeration in tank are some precautions which are compulsory for stress free live table fish transportation. This study has recommended to use optimal fish loading weight 40kg/500 litre of water and clove oil doses 5.0-7.5 µl/litre (3.8-5.6 mg/L of eugnol) of water for safe fish transportation.

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