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Anesthetic efficacy of clove oil in the transportation of carp (*Cyprinus carpio*) seed

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Abstract
Clove oil was applied as an aid in seed transportation in plastic bags. The safe concentrations of clove oil (0.02 ppm) calculated using second order polynomial regression was used for this purpose. This concentration was applied for 24, 48 and 72 hrs of transportation with three loading densities of 1000, 1500 and 2000 fish per bag of 3,000 ml freshwater. In 24 hrs transport at high packing density (2000 seed /bag) without clove oil, mortality rate was only 2%. However, there was no mortality during 24 hrs transport in clove oil treatments. In clove oil treated fish there was no mortality upto 48 hrs transportation and at 72 hrs transport only 3.70% mortality was noticed. Whereas in control 3.4 to 58.3% mortality was observed. During transportation experiments a significant reduction in OCR, COR and ammonia excretion rate was also noticed in clove oil treated seed bags.

Keywords: Anesthetic, fish seed, seed transportation, seed mortality, respiratory metabolism

Introduction
The availability of quality seed is a prerequisite for rapid expansion and growth of aquaculture. However, uncertainty in timely seed supply is one of the major constraints. Considering its significance constant efforts have been made to produce large quantities of carp seed every year in increasing trends. For instance, the total fry production in India was estimated at 632 million in 1986-87 which had increased to 18.5 billion in 2002-2003 and in 2015-16 it was over 32 billion. The objective of live fish transport is to maximize loading density and concurrently to maintain the fish in good condition on arrival. As the metabolism during transport is about three times higher than the routine metabolism [1], the existing packaging system focuses on lowering the metabolic rate of the fish, to reduce oxygen consumption and accumulation of acidity, carbon dioxide and ammonia in the transport water. High water temperature reduces the loading density of fish due to the increased metabolic rate of fish. It results in faster bacteria growth and lower dissolved oxygen levels, leading to increased waste production and decreased availability of oxygen to the fish. Hence, the temperature of transport water is usually reduced to the level that the fish can tolerate, that is, 22^{0}C for tropical fish and 15-18^{0}C for temperate species
Transporting live fish seed requires a high professional skill. In live fish transport it is often observed that after handling during transport, and its release, even in oxygen rich waters. There is higher mortality observed during transportation due to prolonged hyper activity produced during the process. In view of this, the modern live fish transport techniques, “the use of anesthetics” find an important place in live fish transport. Anesthetics are useful in lowering the metabolic activity of fish which facilitates the transport of more fish in a given quantity of water for a longer time. Anesthetics are also widely used in routine aquaculture activities to reduce incidence of stress by sedating and immobilizing fish before performing any task in aquaculture. 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 [2]. Biologists and aquaculturists alike have been searching for alternative anesthetics that are less toxic, readily available, efficacious and safe for humans. From this point of view, the present study was conducted to assess the efficacy of clove oil in the safe transportation of fish seed.

2. Material and Methods
To work-out the safe level of clove oil for 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 (1.3±0.01g) 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 safe level (0.02 ppm) of clove oil was calculated through second order polynomial regression between fish survival and clove oil concentration. (Fig.1).

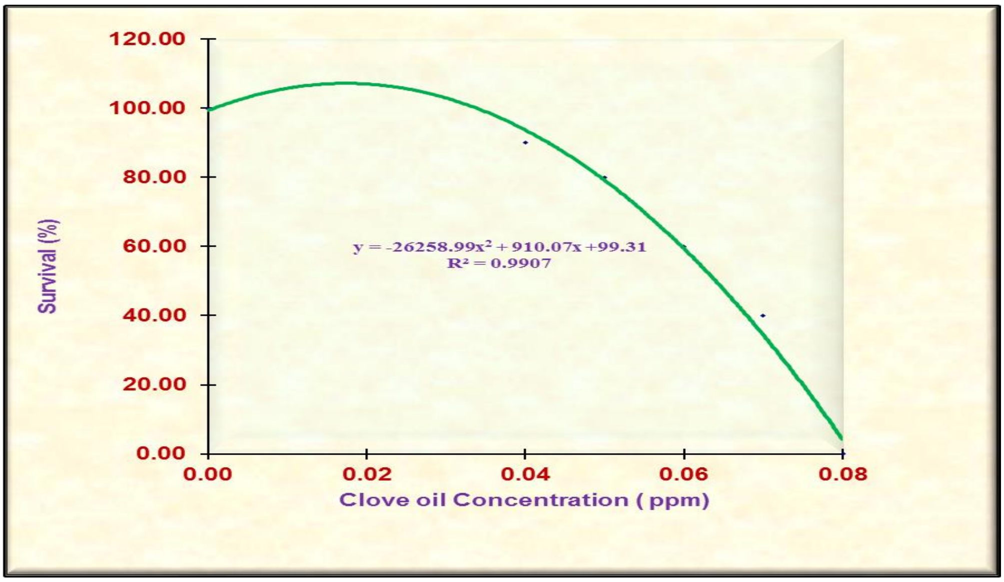


Fig 1: Relationship between clove oil concentration with a survival rate of common carp fry (as described by second order polynomial regression)

The fishes were starved for 24-48 hours to empty their digestive tracts to reduce ammonia production during the transportation. The optimum concentration (0.02 ppm) of clove oil used for fish seed transportation was selected from the results of second order polynomial regression between fish survival and clove oil concentration. The poly-bags of 16 x 29 inches were used for this experiment. The optimal concentration (0.02 ppm) of clove oil was mixed with well oxygenated water and filled in polythene bags (3 liters in each bag). The desired numbers of common carp seed were loaded in poly bags and these oxygenated polythene bags were tied with rubber bands and packed in hard paper canton. The rate of fish seed survived after 24, 48 and 72 hrs of transportation was also recorded. The OCR, COR, AER, RQ, and AQ were estimated after 24, 48, and 72 hours using the following formula:

$$OCR/COR/AER = (I - F)\,\frac{V}{1000}\;x\;\frac{1000}{G}\;x\;\frac{60}{t}$$

Where I = Initial level; F = Final level; V = Volume of water; g = Weight of the fish in grams; t = Experimental duration in minutes

The respiratory quotient (RQ) and ammonia quotient (AQ) were calculated using the following formula:

RQ = volume of CO_2 output/ volume of O_2 consumption

AQ = volume of NH_3 -N excretion/ volume of O_2 consumption

3. Results

The impact of clove oil on seed survival and water quality was evident during 24, 48 and 72 hours seed transportation. The result pertaining to oxygen level, oxygen consumption rate, carbon- dioxide output rate, ammonia excretion rate and seed survival are presented in Tables 1 to 3 and Figure 2.

3.1 Seed Mortality

The results recorded for fish seed mortality are given in Table 1. It is evident from this table that the fish mortality in all the three loading density (i.e.1000, 1500 and 2000 Nos/bag) was nil except 2% mortality in 2000 /bag fish for the transportation duration of 24 hour. However, with the increasing transportation duration, the rate of mortality was also increased. As such a highest (58.30%) mortality in control was noticed during 72 hours transportation experiment. In clove oil treated seed only 3.70% mortality was noticed with highest loading numbers (2000/bag) and transportation duration (72 hours). There was no mortality in 1000 and 1500/bag at 24, 48 and 72 hours transportation experiments in clove oil treated fish. However, in control a notable 3.4 to 27.34% mortality was observed. Thus the results of this experiment showed a significant role of clove oil in the safe transportation of fish seed.

3.2 Effect of Clove Oil on Respiratory Metabolism during Transportation

In seed transportation bags, the level of oxygen in the water decreased with increased transportation time (Table 2). However, the oxygen consumption rate significantly decreased with increasing transportation duration. Still the higher reduction rate was noticed in all the treatments as compared to control. The highest (66.37 mg/kg/hr.) and lowest (21.66 mg/kg hr.) oxygen consumption rate was observed in control and clove oil treated fish at 1000/ bag loading density (Table 2). The loading nos. per bag and oxygen consumption rate had a positive significant ($P<0.05$) relationship as the rate of oxygen consumption increased with increasing nos. of seed.

Table 1: Seed mortality rate (%) in control and clove oil anesthetized common carp fry

S. No	Treatment	Seed Stocking density (Nos)	Duration of Transportation (hrs)		
			24	48	72
1	Control	1000	0.00	0.00	0.00
		1500	0.00	3.4	16.76
		2000	2.00	29.30	58.30
2	Clove oil	1000	0.00	0.00	0.00
		1500	0.00	0.00	0.00
		2000	0.00	0.00	3.70

From Table 2, it would be seen that the level of carbon-di-oxide output were initially on the lower side as compared to 72 hours values. In general, the level of carbon-di-oxide output significantly increased with increasing experimental duration. Still the highest level was recorded in control than clove oil treatment for all the three loading densities. The respective lowest (12.95±0.15 mg/kg/hr.) and highest (30.05±0.015 mg/kg/hr.) values of carbon-di-oxide output (COR) were observed in clove oil treatment at 24 hours(1000 seed /bag) and control at 72 hours (2000 seed/bag).

The rate of ammonia (NH_4-N) excretion in seed transportation bags decreased with increasing time of transportation (Table 2). The highest (16.12) level of ammonia excretion rate (AER) was observed in control, whereas the lowest (5.30±0.05 mg/kg/hr.) was observed in clove oil anesthetized fish at 72 hours. Statistically, the level of ammonia excretion rate in control and treatment were significantly different ($p<0.05$) at 5% level of probability.

The minimum value (0.25 mg/kg/hr.) of respiratory quotient (RQ) was found in clove oil treatment during 24 hour of transportation. Whereas the highest value of 0.97 mg/kg/hr. was also noticed at 24 hour in control with 2000 loading density. The amount of respiratory quotient was found increasing with increased transportation duration. Similar trend was also noticed between clove oil treatment and controls as the level of respiratory quotient were higher in control than clove oil treatment (Table 2). The results of the analysis of the variance (ANOVA) are presented in Table 3. From this (Table 3), it is evident that the level of respiratory quotient in control and clove oil treatment were statistically significant ($p<0.05$).

The results of ammonia quotient (AQ) are presented in (Table 2 & Fig.2). The lowest value (22 mg/kg/hr.) of ammonia quotient was obtained at 1500 nos. /bag loading density with clove oil during 24 hours test. Whereas, the highest (0.53 mg/kg/hr.) AQ values was noticed with highest loading density (2000 Nos/bag) in control at 72 hours. In general, the level of ammonia quotient were higher in control than clove oil treatment at all the loading densities. Further, the level of AQ, increased with increasing transportation duration in all the treatment and control. In control and treatment the differences in ammonia quotient levels were statically significant (Table 3) as the values of F- calculated were significantly higher than tabulated values ($p<0.05$).

Table 2: Respiratory metabolism of control and anesthetized common carp Cyprinius *carpio- communis* in commercial transportation experiments

Time of incubation	O_2 level (mg/l)	O_2 consumption rate (mg/kg/hr.)	CO_2 level (mg/l)	CO_2 output rate (mg/kg/hr.)	NH_3-N level (mg/l)	NH_3-N Excretion rate (mg/kg/hr.)
1000/fry/bag without clove oil						
24 hour	5.55±0.015	66.37±0.015	27.63±0.015	16.99±0.01	0.43±0.0264	16.12±0.015
48hour	3.25±0.026	48.81±0.015	78.4±0.305	23.52±0.020	0.53±0.020	9.93±0.015
72 hour	1.02±0.036	38.63±0.015	137.93±2.596	26.89±0.050	0.65±0.015	8.09±0.015
1000/fry/bag with clove oil						
24 hour	5.82±0.015	29.25±0.015	21.06±0.015	12.95±0.015	0.30±0.005	11.25±0.015
48hour	5.00±0.015	24.75±0.015	61.25±0.251	16.37±0.015	0.47±0.015	8.81±0.015
72 hour	4.58±0.020	21.66±0.015	73.82±0.393	18.39± .015	0.59±0.015	7.34±0.015
1500/fry/bag without clove oil						
24 hour	3.99±0.005	58.19±0.005	48.70±0.005	19.94±0.015	0.54±0.005	13.48±0.005
48hour	4.24±0.015	38.46±0.005	87.66±0.028	21.00±0.015	0.63±0.015	7.86±0.015
72 hour	3.78±0.015	37.64±0.005	167.54±0.005	22.75±0.015	0.74±0.005	6.13±0.015
1500/fry/bag with clove oil						
24 hour	5.48±0.015	35.98±0.005	39.41±0.011	17.97±0.015	0.36±0.005	8.99±0.005
48hour	4.80±0.015	28.98±0.005	70.58±0.005	18.10±0.011	0.51±0.015	6.36±0.015
72 hour	3.48±0.005	23.54±0.005	149.61±0.005	19.42±0.005	0.64±0.005	5.30±0.005
2000/fry/bag without clove oil						
24 hour	3.22±0.025	58.5±0.152	99.1±0.208	20.47±0.015	0.66±0.015	12.37±0.015
48hour	2.87±0.020	32.53±0.015	151.11±1.053	22.66±0.015	0.73±0.020	6.84±0.015
72 hour	1.02±0.015	29.3±0.152	205.67±4.908	30.05±0.015	0.86±0.026	5.35±0.015
2000/fry/bag with clove oil						
24 hour	5.48±0.141	29.75±0.015	62.56±2.159	19.23±0.015	0.42±0.020	7.87±0.015
48hour	2.55±0.015	26.15±0.015	102.16±0.752	21.32±0.015	0.57±0.015	5.37±0.015
72 hour	2.05±0.020	22.93±0.015	182.06±0.902	37.75±0.015	0.70±0.015	5.35±0.015

Table 3: Summery of statistical analysis (ANOVA) for respiratory quotient in transportation experiment A: 1000 seed per bag

SOURCE	D.F	SS	M.S.S	F CAL	TAB 0.05	TAB0.01	SEM	CD 5%	CD 1%
24 hrs									
Treatment	1	5.415	5.415	270.750	7.708	21.197	0.008	2.780	0.039
Error	4	0.080	0.020						
48 hrs									
Treatment	1	3.226	3.226	193.600	7.708	21.197	0.007	2.350	0.033
Error	4	0.066	0.016						
72 hrs									
Treatment	1	1.093	1.093	46.930	7.708	21.197	0.008	2.780	0.039
Error	4	0.093	0.023						

B: 1500 seeds per bag

Source	D.F	SS	M.S.S	F CAL	TAB 0.05	TAB0.01	SEM	CD 5%	CD 1%
24 hrs									
Treatment	1	1.411	1.411	6048.64	7.708	21.197	0.008	2.780	0.039
Error	4	0.001	0.002						
48 hrs									
Treatment	1	1.261	1.261	54.848	7.708	21.197	0.008	2.780	0.039
Error	4	0.092	0.023						
72 hrs									
Treatment	1	9.375	9.375	402.360	7.708	21.197	0.008	2.782	0.039
Error	4	0.093	0.023						

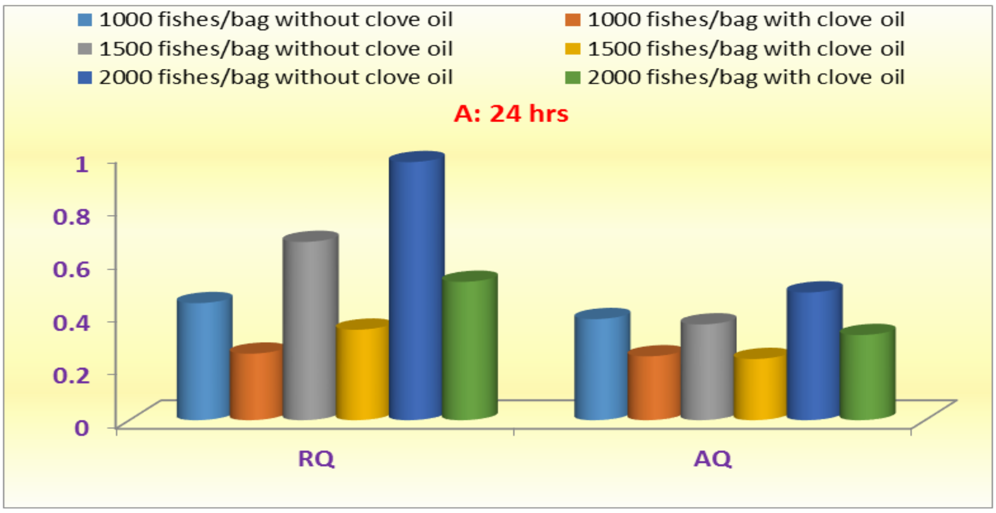
C: 2000 fry/ bag

SOURCE	D.F	SS	M.S.S	F CAL	TAB 0.05	TAB0.01	SEM	CD 5%	CD 1%
24 hrs									
Treatment	1	3.182	3.182	15914.0	7.708	21.197	0.007	2.350	0.033
Error	4	0.0001	0.001						
48 hrs									
Treatment	1	0.020	0.020	102.08	7.708	21.197	0.007	2.350	0.033
Error	4	0.001	0.001						
72 hrs									
Treatment	1	1.766	1.766	75.790	7.708	21.197	0.0074	3.058	0.973
Error	4	0.089	0.022						

4. Discussion

The main purpose of anesthetic in fish transport is to lower metabolic rates and thus reduce oxygen consumption and ammonia as well as carbon dioxide production [3-5]. Apart from the reduction in oxygen consumption, the use of anesthetics for fish transport also controls hyperactivity, thus preventing undue injuries. In this study, common carp fry were treated with a safe dose (0.02 ppm) of clove oil in the 24, 48 and 72 hrs transport test.

Mortality was only found in 48 and 72 hrs of transport for control. In clove oil treated fishes there was no mortality during 24, 48 and 72 hrs except only 3.70% mortality at 72 hrs in treatment having the highest numbers (2000/bag) of seed (Table 1). Perhaps the factor that caused mortality was the deterioration of water quality such as dissolved oxygen depleted or increased total ammonia. This was attributed to increased metabolic rate resulting from increased physical activity under crowded conditions and limited supply of dissolved oxygen. Water quality is obviously an important factor in determining of fish transported in closed conditions. Most water quality degradation occurs rapidly within the first hour or two after packing [6].



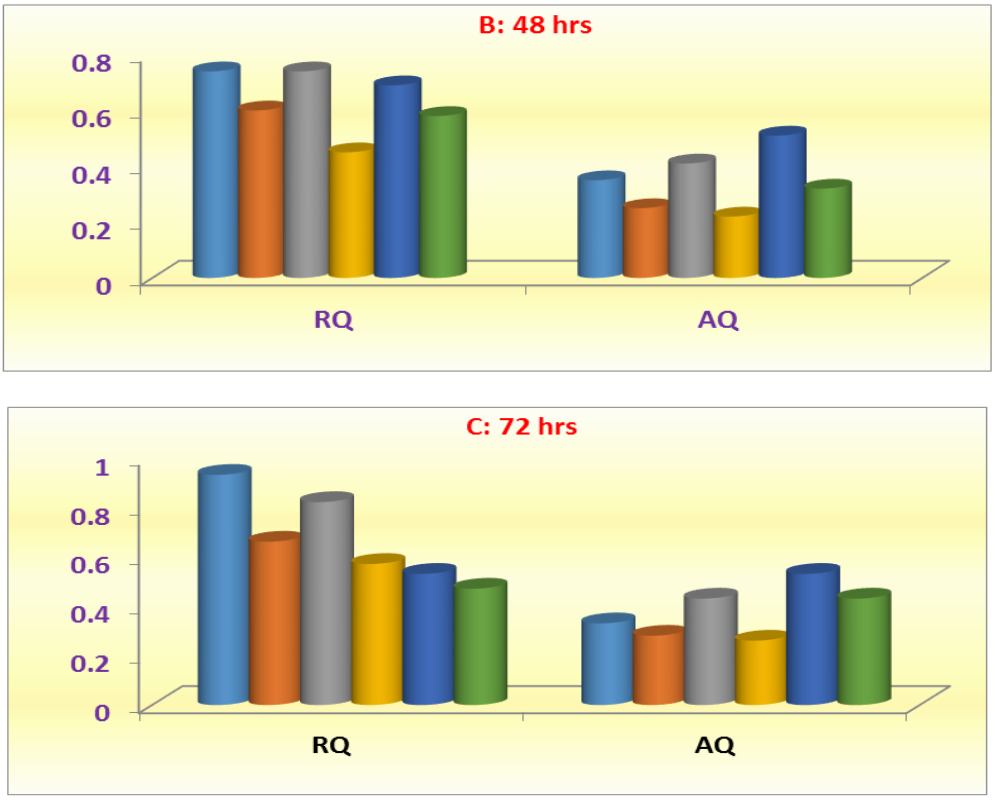


Fig 2: Mean values of respiratory & ammonia quotient in control and anesthetized common carp at different time intervals in transportation experiments

In the present study, both control and treatment groups, indicating a massive increase of carbon dioxide in the water (Table 2). Still the higher increase was in control than treatment groups. As ambient carbon dioxide levels rise, blood CO_2 increases (hypercapnia) and the oxygen carrying capacity of hemoglobin begins to decrease due to the Bohr Effect [3]. Therefore, the highest CO_2 concentration conceded with the highest mortality rate might be due to the Bohr Effect as suggested by Wedenmeyer [3].

Dissolved oxygen in control reached a critical range of 1.02 – 1.58 ppm. On the other hand, in treated groups, dissolved oxygen varied 2.05 – 5.82 ppm. It suggested that clove oil reduced the oxygen consumption of common carp fry. The results of the O_2 consumption rate (Table 2) has also proved that the rate of oxygen consumption was higher in control than clove oil treated groups. Guo *et al.* [7] found that 2-phenoxyethanol was more effective than the other anesthetics (quinaldine sulphate, MS 222 and metomidate) in suppressing oxygen consumption by platy fish (*Xiphophorus maculates*).

Total ammonia is the main waste product of protein metabolism in fish with un-ionized ammonia being the most toxic [8]. Within the closed system of fish transport, the main sources of ammonia are the excretion by fish as a normal part of their metabolism and the breakdown of protein in dead fish. Clove oil significantly reduced excretion ammonia of common carp fry during transport (Table. 2). Teo *et al.* [9] recommended that reducing the excretion of ammonia by the fish could be done in two ways i.e. starving them before packing and by the application of anesthetic to reduce motor activities of the fish. Application of ion exchange material such as clinoptilolite to reduce accumulation of ammonia in transport water was also suggested by Bower and Turner [10].

Loading density in transport is determined by many factors such as the respiration rate of fish, water temperature, transport duration, size of fish, *etc*. Several anesthetics have been used to increase the loading density of fish distribution units as well as transport duration. In this experiment, clove oil, at 0.02 ppm concentration, is suggested to increase both duration transport and packing density of common carp fry. Addition of this concentration in water transport rendered fish stay calm, reduced fish activity without loss of equilibrium and also reduced fish metabolism resulting in less carbon dioxide and ammonia accumulation and hence improved fish survival rates. Clove oil appears to act as an anesthetic in common carp fry.

5. Conclusion

From the results of the present experiments, it can be concluded that 0.02 ppm clove oil seem to be the optimal concentration for the anesthetization for safe transportation of seed. In term of cost effective, clove oil is much less expensive than other chemicals. In India, its price is around Rs 5000 per 1 liter. However, the price of MS 222 is approximately Rs 25000 per kg. Whereas, quinaldine price is Rs 6000 per kg. Beside the low cost of application, clove oil being the herbal product is safe for fish and consumers. On the other hand the chemical anesthetics especially MS-222 is carcinogenic and unsafe for human.

6. References

1. Froese R. Relationship between body weight and loading densities in fish transport using the plastic bag method. Aquaculture and Fisheries Management. 1988; 19:275-281.
2. Marking LL, Meyer FP. Are better anesthetics needed in fisheries?. Fisheries. 1985; 10(6):2-5.
3. Wedenmeyer GA. Transportation and handling. In: W. Pennel and B.A. Barton (Eds.), Principles of Salmonid Culture, Elsevier, Amsterdam, and the Netherlands. 1996, 727-758.

4. Ross LG, Ross B. Anesthetic and Sedative Techniques for Aquatic Animals. 2 nd ed. Blackwell Science Ltd., Great Britain, 1999.
5. Gilderhus PA, Marking LL. Comparative efficacy of 16 anesthetic chemicals on rainbow trout. North Am. J Fish. Manage. 1987; 7:288-292.
6. Kamble, AD, Saini VP, Ojha ML. The efficacy of clove oil as anesthetic in common carp (*Cyprinus carpio*) and its potential metabolism reducing capacity. International Journal of Fauna & Biological Studies. 2014; 1(5):1-9
7. Guo FC, Teo LH, Chen TW. Effect of anesthetics on water parameters in a simulated transport experiment of platy fish, *Xiphophours maculates* (Gunther). Aquaculture. 1995; 26:265-271.
8. Berka R. The Transport of Live Fish: A Review. EIFAC Technical Paper. 1986, 48.
9. Teo LH, Chen TW, Lee BH. Packaging of the guppy, Poecilia reticulata, for air transport in a closed system. Aquaculture. 1989; 78:321-332.
10. Bower CE, Turner DT. Ammonia removal by clinoptilolite in the transport of ornamental freshwater fishes. Prog. Fish-Cult. 1982; 44:19-23.