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# Does Tobacco (*Nicotiana tabacum*) Leaf Dust Save the Life of Rohu (*Labeo rohita*) Fingerlings During Transport?

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## Abstract

Fishes undergo a multi-phase of stress due to multiple stressors involved during transportation which is an inexorable and indispensable operation in aquaculture. The aim of this study is to promote a novel, inexpensive, active and eco-friendly sedative to replace expensive and toxic sedatives used in aquaculture. With this intention, we investigated the efficacy of tobacco leaf dust as a sedative for the transport of rohu (*Labeo rohita*) fingerlings. The experiment of sedative efficacy and simulated transportation was conducted for 12 h in glass tanks (30 L capacity) and plastic bags (75 cm length × 45 cm wide), respectively with different concentrations of tobacco leaf dust such as 0 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm among which 0 ppm was used as control. The fingerlings (6.45 ± 0.68 cm and 3.29 ± 0.52 g) were stocked at a stocking density of 10 fishes/ tank and 30 fishes/ plastic bag in triplicates. The induction and recovery times observed in the anesthetic bath significantly ($p<0.05$) decreased and increased with increase in the concentrations of tobacco leaf dust. The lowest effective dose found to produce induction (≤ 15 min) and recovery (≤ 5 min) was 25 ppm and the same was effective in inducing light sedation in rohu during the behavioral response observation. Mortality rate (15% to 40%) of fingerlings during transportation was significantly higher in control (without sedative) than the sedative doses of tobacco. Also, poor water quality was noticed in control group with the serious changes in hemogram and leukogram of fingerlings. The experimental results revealed the efficacy of tobacco in minimizing the metabolic activity of the fishes and thereby reducing the water quality deterioration and stress during transportation. Therefore, the present study reveals that tobacco leaf dust (25 ppm) could be a futuristic sedative for safe and successful transportation of *L. rohita* fingerlings.

**Keywords:** Rohu; Fish transportation; Sedative; Tobacco; Water quality parameters; Hematological values

## Introduction

Owing to its richness in fish biodiversity that has been distributed widely among different freshwater ecosystems, India is considered to be one of the affluent nations in the world. Aquaculture in India is highly promising and has grown over the last two decades with freshwater aquaculture contributing over 95% of the total aquaculture production. The country shared 4.39 million tons to world freshwater fish production in 2014 [1]. The aquaculture systems in India and its neighboring countries mainly constitute Indian major carps namely Catla (*Catla catla*), Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*). Among the three Indian major carps, rohu is the species of significance preferred in poly-culture systems of carps because of its high growth potential. It is a fast-growing freshwater fish species which belongs to the family Cyprinidae and genus, Labeo and is an important species of great demand by the consumers due to its texture and taste of the carcass.

To maximize and sustain the production, a healthy and quality seed is an essential input. Fish seeds are produced in hatcheries and are supplied to the farms for grow-out culture. But many times the hatcheries are located far away from the culture sites. Therefore, transportation of fish is one of the most significant operations in aquaculture which plays a crucial role in the supply of seeds from hatcheries to grow out farms. The duration of transport varies depending on the distance of farms to which the fishes are transported and is divided into short term (≤ 8 h) or long term (≥ 8 h) with the threshold of 8 h as the distinction between short and long transport [2]. During transport, the metabolic activities of fish are three times more than that of the normal [3]. Stress is an indispensable factor during the transportation of fishes. Rohu is a fish which is highly stressed as it is quite sensitive to the transport stress. Consequently, there is a high mortality rate (about 90%) among transported rohu fingerlings [4], since packing those at high densities get resulted in confinement [5]. Transport duration and the physico-chemical parameters of the transport media determine the severity of transport stress in fishes but it can be minimized with the help of light sedation, i.e., low concentration of an anesthetic. Therefore, in order to reduce the stress-induced mortality, natural or synthetic sedatives and anesthetics can be used to sedate and immobilize fish [6,7].

Sedation is a mild form of anesthesia which puts the fish to sleep by calming and immobilizing their activities. The application of sedatives silences the activity of fishes and is beneficial in lowering the stress-induced mortality during transportation [8]. Sedative and anesthetic substances used for fishes must have rapid induction and recuperation period, safe margin to fish and humans, no physiological and residual effect, local availability and low cost [9]. Several anesthetics have been evaluated and used so far in which some are toxic and inexpensive while some are expensive and less toxic. As of now, MS-222 is the only anesthetic that has a USFDA approval to be used as an anesthetic in food fishes irrespective of its demerits like low pH, low efficacy on plasma cortisol control and expensiveness [10]. To avoid adverse stress effects from anesthetics, knowledge on the optimum concentration of

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an anesthetic for different fish species is more significant [11,12]. For practical use in aquaculture, only a few studies have been conducted in commonly cultured tropical freshwater food fishes.

Tobacco, *Nicotiana tabacum*, is an herbaceous annual or perennial plant in the family Solanaceae (Night shade), grown for its leaves. It is a medicinal plant of remarkable benefits and history of use in traditional Indian medicine as a sedative, antispasmodic, and vermifuge with high alkaloid content mainly nicotine and it is under the utmost care of humans [13]. It has a potential to heal and protect when used effectively but has the ability to harm when abused. India stands 3rd in tobacco production globally [14]. The expensive anesthetics are scarce in developing and under developed countries whereas inexpensive anesthetics are toxic and harmful to fish and humans with more deleterious effects. In search of safe, effective and less expensive sedatives and anaesthetics, tobacco which is of natural origin, cheap, easily available and eco-friendly narcotic could be a novel and futuristic sedative and anaesthetic for fishes during transport and surgeries.

No literature on use of tobacco as sedative for fish transport is available and it appears that experimental studies on this subject are rare. The change in water quality parameters such as temperature, pH, dissolved oxygen (DO), dissolved free carbon dioxide ($CO_2$), ammonia ($NH_3$) and nitrite ($NO_2$) is one of the prime factors in causing mortality among the transported fishes [15]. Also, the commercial use of sedative could be enhanced by perceiving the water quality changes occurring when fish are under its influence. Hematological parameters such as total erythrocyte count (TEC), hemoglobin concentration (Hb), hematocrit value (Hct), erythrocyte indices, total leukocyte count (TLC) and differential leucocyte count provide detailed information on general metabolism and physiological status of fish which are usually affected by transportation [16]. Hoseini et al. [11] also demonstrated that blood parameters particularly stress indicators are affected by anesthetic concentrations and their exposure period. This study therefore, determined the efficacy of tobacco leaf dust as a feasible sedative in *L. rohita* and its effect on water quality and hematological parameters of the fish during transport.

## Material and Methods

### Animal housing and design

1000 nos. of *L. rohita* fingerlings of 6.46 ± 0.68 cm mean length and 3.29 ± 0.52 g mean weight were obtained from Maharashtra State Fish Seed Farm, Aarey, Goregaon, Mumbai and the same were used for the experiment. The fishes were disinfected with 5 ppm $KMnO_4$ and were acclimatized in 300 L capacity FRP tanks at the wet laboratory unit, Division of Aquaculture, ICAR- Central Institute of Fisheries Education, Andheri (West), Mumbai, India. The fishes were kept under natural photoperiod and fed twice a day (0900 h and 1700 h) with commercial floating type pellet diet. A completely randomized design was used to carry out the experiment. The experiment was conducted in triplicates for both the transit times of five treatments and for the control as well.

### Tobacco leaf dust preparation

Good quality tobacco leaves were procured from a retailer in Kerala. The authentication of the specimen was accomplished in Department of Botany, St. Xavier's College, Mumbai, Maharashtra (India). The leaves were sun-dried for 7 days and were ground into fine dust (powder) with the help of mixer. The fine ground tobacco leaf dust (7% to 10% moisture content) was then stored in an air-tight container and used for the experiment.

### Experimental setup

**Sedation dose for transportation:** Five concentrations of tobacco leaf dust (25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm) as predetermined earlier from lethal toxicity studies were tested for sedative potential on *L. rohita* fingerlings. The known quantities of tobacco leaf dust were weighed according to each treatment dose and were added to glass tanks with fresh water. The glass tanks with known amounts of tobacco leaf dust were agitated and mixed vigorously. To evaluate the time required for induction, 10 fishes, each of which were placed in individual aquaria, were used for each concentration tested, and each fish was used only once. The maximum observation time was 30 min. The induction and recovery times were studied based on the stages described by Keene et al. [17] and were calculated using a stopwatch. The behavioral response of fingerlings was monitored and noted down for every 2 h interval up to 12 h and simultaneously the survival rate was also checked. The fingerlings were further monitored in recovery tanks for another 24 h to check the survival rate.

**Protocol for simulated transportation:** Prior to the transportation experiment, the fishes were starved for 24 h in FRP tanks. Five concentrations of tobacco leaf dust were followed (25 ppm, 50 ppm, 75 ppm, 100 ppm and 125 ppm) with two transportation times (6 h, 12 h). Only freshwater was added in control without sedatives (0 ppm). The plastic bags (75 cm length × 45 cm wide) were filled with 2 L freshwater and were mixed with each sedation dose in triplicates. 30 fingerlings were stocked (approximately biomass weight of 100 g) per plastic bag. Medical-grade oxygen was filled inside the plastic bag after the squeezing of air from the plastic bag. Water quality was maintained under controlled conditions during the start of the experiment. 12 h was maintained as the total duration of stress. After 6 h and at the termination of the transit time (12 h), all the plastic bags were opened and mortality rate was checked. The fingerlings thus survived were taken from each bag and then were transferred to FRP tanks of 300 L capacity with ample aeration and was further monitored for 24 h to evaluate post-transport mortality. Stress was studied by analyzing the water quality and hematological parameters.

### Water quality parameters

All the bags from each treatment group were examined to assess water quality at 6 h intervals until the end of the experiment. During each sampling, the bags were opened, dead fingerlings were counted and water samples were collected for analysis of different parameters. Temperature and pH was measured using digital thermometer (Fisher Scientific) and pH tester (Eutech Instruments) respectively, while DO (Winkler's method), $CO_2$, total alkalinity and total hardness were measured by titrimetric methods [18]. Total ammonia nitrogen (TAN) (Indophenol blue method), nitrite-nitrogen ($NO_2$-N) and nitrate-nitrogen ($NO_3$-N) were measured following standard methods [18].

### Hematological parameters

Blood from the fishes were drawn with the help of a sterilized 1 ml hypodermal syringe and 28 gauge needles directly from the caudal vein containing 2.7% EDTA (Qualigens, India) as an anticoagulant. TEC and TLC were determined using Neuber's hemocytometer (Feinoptik, Germany) with appropriate diluting fluids (Himedia, India) for TEC and TLC. The Hb concentration was analyzed following the cyanmethemoglobin method using Drabkins fluid (Qualigens, India). Hematocrit capillary tubes were two-third filled with the whole blood and centrifuged in a hematocrit centrifuge at 12000 rpm for 5 min and the percentage of the packed cell volume was determined by the

| Treatment (ppm) | Induction (min) | | | Recovery (min) |
|---|---|---|---|---|
| | Stage 1 | Stage 2 | Stage 3 | Stage 6 |
| 25 | 14.36 ± 0.03[a] | - | - | 0.57 ± 0.02[e] |
| 50 | 13.70 ± 0.08[b] | 28.51 ± 0.11[a] | - | 0.88 ± 0.01[d] |
| 75 | 11.76 ± 0.04[c] | 20.48 ± 0.11[b] | 29.82 ± 0.03[a] | 1.23 ± 0.01[c] |
| 100 | 8.18 ± 0.06[d] | 12.50 ± 0.04[c] | 19.60 ± 0.17[b] | 1.75 ± 0.04[b] |
| 125 | 6.17 ± 0.03[e] | 9.50 ± 0.04[d] | 15.30 ± 0.11[c] | 2.26 ± 0.02[a] |

Values in the same column with different superscripts are significantly (P<0.05) different for each stages of anaesthesia and tobacco leaf dust concentration. One-way ANOVA was used following Tukey's HSD post hoc test in SPSS 16.0.

**Table 1:** Time (min) required for induction and recovery (stage 6) (Mean ± SE) of the anaesthesia using tobacco leaf dust in rohu fingerlings (Maximum observation time - 30 min).

| Treatment (ppm) | Behavioral responses | | | | | | Survival Rate (%) |
|---|---|---|---|---|---|---|---|
| | 2 h | 4 h | 6 h | 8 h | 10 h | 12 h | |
| Control | N | N | N | N | N | N | 100 |
| 25 | LS | LS | LS | LS | LS | LS | 100 |
| 50 | DS | DS | DS + 5% PLE | DS + 5% PLE | DS + 5% PLE | DS + 5% PLE | 100 |
| 75 | DS + 20% PLE | DS + 20% PLE | DS + 30% PLE | DS + 30% PLE | DS + 30% PLE | DS + 40% PLE | 100 |
| 100 | DS + 30% PLE | DS + 50% PLE | DS + 50% PLE | 70% PLE | 80% PLE | 80% PLE | 100 |
| 125 | PLE | PLE | PLE | PLE | PLE | PLE | 100 |

N: Normal; LS: Light Sedation; DS: Deep Sedation; PLE: Partial Loss of Equilibrium (Hyperactive phase).

**Table 2:** Behavioral responses of *rohu* fingerlings to different tobacco leaf dust concentrations at 2 h interval during 12 h exposure period in anaesthetic bath.

| Treatment (ppm) | Mortality rate (%) | | |
|---|---|---|---|
| | 6 h | 12 h | 24 h |
| Control | 20.83 ± 1.14[a] | 38.33 ± 1.52[a] | 15.83 ± 0.59[a] |
| 25 | 0.00 ± 0.00[b] | 0.00 ± 0.00[b] | 0.00 ± 0.00[b] |
| 50 | 0.00 ± 0.00[b] | 0.00 ± 0.00[b] | 0.00 ± 0.00[b] |
| 75 | 0.00 ± 0.00[b] | 0.00 ± 0.00[b] | 0.00 ± 0.00[b] |
| 100 | 0.00 ± 0.00[b] | 0.00 ± 0.00[b] | 0.00 ± 0.00[b] |
| 125 | 1.66 ± 0.56[b] | 3.33 ± 0.95[b] | 0.00 ± 0.00[b] |

Values in the same column with different superscripts are significantly (P<0.05) different. One-way ANOVA was used following Tukey's HSD post hoc test in SPSS 16.0.

**Table 3:** Mortality rate (%) (Mean ± SE) of rohu fingerlings found in polyethylene bags at different durations during simulated transportation experiment at 50 g $L^{-1}$ loading density.

hematocrit tube reader [19]. Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Dacie and Lewis [20]. Differential leukocyte count (Lymphocytes and Neutrophils) was performed with blood smears stained with Giemsa solution. The smears were examined by light microscopy (Olympus, Tokyo, Japan) under oil immersion at 100 x magnification.

### Statistical analysis

All the data were represented as Mean ± SEM (Standard error of mean). The treatment means for induction and recovery stages, mortality rate, water quality and blood parameters were compared using one-way ANOVA followed by Tukey's HSD post hoc for multiple comparisons. Data were analyzed using statistical software SPSS version 16.0 with a level of significance of P<0.05.

## Results

### Induction and recovery, behavioral response and mortality rate percentage

The induction and recovery times (Table 1) observed in anesthetic bath significantly decreased (p<0.05) with increase in concentrations among all treatments. Treatment group with 25 ppm tobacco showed slight delay in induction but a rapid recovery whereas 125 ppm showed quick induction and prolonged recovery times. While observing the behavioral responses (Table 2) of rohu fingerlings in anesthetic bath, treatment with 25 ppm concentration showed light sedation whereas treatment with 125 ppm concentration expressed a phase of excitement and hyperactivity. Control (without sedative) showed normal swimming patterns while intermediate responses were found in other treatments. Mortality rate (%) (Table 3) observed during simulated transportation and post transportation was significantly higher (p<0.05) in control group. No mortality was noticed among the treatments with sedative doses except in treatment with 125 ppm concentration that showed low mortality rates.

### Water quality parameters

The water quality parameters of transport water showed a varying trend throughout the simulated transportation period. Each parameter is represented in Table 4 comprising all the treatments and their values of the respective parameter at different time intervals. The water quality deterioration was highly pronounced in control and to a lesser extent in other treatment groups. Temperature of transport water was significantly higher (p<0.05) in control at both the durations (6 h and 12 h) followed by 125 ppm treatment group. pH levels observed at different time intervals showed a significant difference (p<0.05) with control showing the lowest pH and 25 ppm treatment showing the highest pH respectively. There was a significant reduction (p<0.05) in DO levels in control during the simulated transportation while high DO levels were found in all treatments. During the transportation experiment, the increase in $CO_2$ level was found in all treatments with a significant increase (p<0.05) in control group. Low carbon dioxide levels were found in 25 ppm compared to other treatments. Control showed higher alkalinity values and were significantly different (p<0.05) from other treatments. Water hardness was found high in 100 and 125 ppm treatments and were significantly different (p<0.05) from other treatments. Low levels of ammonia and nitrite were recorded in 25 ppm with significantly (p<0.05) higher amounts of control. High nitrate levels were exhibited by 125 ppm and were significantly different (p<0.05) while low nitrate values were observed in control group.

| Concentration (ppm) | Water quality parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Temperature (°C) | pH | DO (ppm) | $CO_2$ (ppm) | Alkalinity (ppm) | Hardness (ppm) | Ammonia (ppm) | Nitrite (ppm) | Nitrate (ppm) |
| **0 h transport** | | | | | | | | | |
| Control | 27.35 ± 0.20$^{a}$ | 7.92 ± 0.02$^{a}$ | 16.3 ± 0.04$^{a}$ | 00 ± 0.0$^{a}$ | 73 ± 1.0$^{a}$ | 52 ± 0.20$^{a}$ | 0.25 ± 0.03$^{a}$ | 0.004 ± 0.004$^{a}$ | 0.12 ± 0.03$^{a}$ |
| 25 | 27.35 ± 0.10$^{a}$ | 7.90 ± 0.04$^{a}$ | 16.2 ± 0.05$^{a}$ | 00 ± 0.0$^{a}$ | 72 ± 1.0$^{a}$ | 52 ± 0.10$^{a}$ | 0.25 ± 0.02$^{a}$ | 0.004 ± 0.002$^{a}$ | 0.12 ± 0.01$^{a}$ |
| 50 | 27.35 ± 0.30$^{a}$ | 7.94 ± 0.02$^{a}$ | 16.3 ± 0.02$^{a}$ | 00 ± 0.0$^{a}$ | 72 ± 2.0$^{a}$ | 54 ± 0.10$^{a}$ | 0.25 ± 0.02$^{a}$ | 0.004 ± 0.001$^{a}$ | 0.12 ± 0.00$^{a}$ |
| 75 | 27.35 ± 0.05$^{a}$ | 7.92 ± 0.05$^{a}$ | 16.2 ± 0.15$^{a}$ | 00 ± 0.0$^{a}$ | 72 ± 1.0$^{a}$ | 54 ± 0.50$^{a}$ | 0.25 ± 0.01$^{a}$ | 0.004 ± 0.002$^{a}$ | 0.13 ± 0.02$^{a}$ |
| 100 | 27.33 ± 0.20$^{a}$ | 7.94 ± 0.04$^{a}$ | 16.4 ± 0.01$^{a}$ | 00 ± 0.0$^{a}$ | 74 ± 1.0$^{a}$ | 52 ± 0.20$^{a}$ | 0.25 ± 0.01$^{a}$ | 0.005 ± 0.001$^{a}$ | 0.13 ± 0.01$^{a}$ |
| 125 | 27.34 ± 0.10$^{a}$ | 7.94 ± 0.01$^{a}$ | 16.3 ± 0.05$^{a}$ | 00 ± 0.0$^{a}$ | 72 ± 2.0$^{a}$ | 52 ± 0.30$^{a}$ | 0.25 ± 0.02$^{a}$ | 0.005 ± 0.002$^{a}$ | 0.13 ± 0.02$^{a}$ |
| **6 h transport** | | | | | | | | | |
| Control | 28.75 ± 0.05$^{a}$ | 7.32 ± 0.01$^{c}$ | 9.1 ± 0.30$^{b}$ | 18 ± 0.0$^{a}$ | 114 ± 4.0$^{a}$ | 52 ± 0.10$^{b}$ | 0.76 ± 0.01$^{a}$ | 0.066 ± 0.003$^{a}$ | 0.32 ± 0.02$^{d}$ |
| 25 | 28.15 ± 0.05$^{b}$ | 7.48 ± 0.02$^{a}$ | 13.5 ± 0.10$^{a}$ | 12 ± 0.0$^{c}$ | 89 ± 1.0$^{b}$ | 52 ± 0.50$^{b}$ | 0.27 ± 0.00$^{d}$ | 0.004 ± 0.002$^{c}$ | 0.45 ± 0.02$^{c}$ |
| 50 | 28.30 ± 0.10$^{b}$ | 7.45 ± 0.01$^{ab}$ | 13.2 ± 0.10$^{a}$ | 14 ± 0.0$^{bc}$ | 94 ± 2.0$^{b}$ | 57 ± 0.65$^{ab}$ | 0.37 ± 0.00$^{c}$ | 0.009 ± 0.002$^{bc}$ | 0.65 ± 0.00$^{b}$ |
| 75 | 28.35 ± 0.05$^{b}$ | 7.44 ± 0.01$^{ab}$ | 13.1 ± 0.10$^{a}$ | 15 ± 1.0$^{b}$ | 97 ± 1.0$^{b}$ | 62 ± 0.30$^{a}$ | 0.43 ± 0.01$^{bc}$ | 0.011 ± 0.002$^{bc}$ | 0.68 ± 0.00$^{b}$ |
| 100 | 28.40 ± 0.10$^{ab}$ | 7.43 ± 0.01$^{ab}$ | 13.0 ± 0.20$^{a}$ | 16 ± 0.0$^{ab}$ | 97 ± 3.0$^{b}$ | 62 ± 0.60$^{a}$ | 0.47 ± 0.01$^{b}$ | 0.016 ± 0.001$^{bc}$ | 0.73 ± 0.01$^{ab}$ |
| 125 | 28.45 ± 0.05$^{ab}$ | 7.40 ± 0.02$^{b}$ | 12.8 ± 0.20$^{a}$ | 16 ± 0.0$^{ab}$ | 97 ± 3.0$^{b}$ | 63 ± 0.20$^{a}$ | 0.50 ± 0.01$^{b}$ | 0.018 ± 0.001$^{b}$ | 0.79 ± 0.03$^{a}$ |
| **12 h transport** | | | | | | | | | |
| Control | 29.75 ± 0.05$^{a}$ | 6.91 ± 0.01$^{d}$ | 5.4 ± 0.20$^{c}$ | 32 ± 2.0$^{a}$ | 139 ± 1.0$^{a}$ | 52 ± 0.20$^{b}$ | 0.92 ± 0.02$^{a}$ | 0.082 ± 0.002$^{a}$ | 0.47 ± 0.00$^{d}$ |
| 25 | 28.75 ± 0.05$^{d}$ | 7.28 ± 0.04$^{a}$ | 12.9 ± 0.10$^{a}$ | 14 ± 0.0$^{c}$ | 101 ± 3.0$^{d}$ | 52 ± 0.20$^{b}$ | 0.37 ± 0.01$^{c}$ | 0.004 ± 0.001$^{d}$ | 0.54 ± 0.01$^{d}$ |
| 50 | 28.85 ± 0.05$^{cd}$ | 7.23 ± 0.01$^{b}$ | 12.3 ± 0.10$^{ab}$ | 16 ± 0.0$^{bc}$ | 107 ± 1.0$^{cd}$ | 57 ± 0.25$^{b}$ | 0.42 ± 0.00$^{bc}$ | 0.009 ± 0.002$^{cd}$ | 0.71 ± 0.01$^{c}$ |
| 75 | 29.05 ± 0.05$^{bc}$ | 7.17 ± 0.03$^{c}$ | 11.8 ± 0.20$^{b}$ | 19 ± 0.5$^{bc}$ | 114 ± 4.0$^{bc}$ | 66 ± 0.25$^{a}$ | 0.51 ± 0.00$^{b}$ | 0.013 ± 0.001$^{bc}$ | 0.77 ± 0.01$^{bc}$ |
| 100 | 29.20 ± 0.20$^{b}$ | 7.15 ± 0.03$^{c}$ | 11.3 ± 0.10$^{b}$ | 21 ± 1.0$^{bc}$ | 121 ± 1.0$^{b}$ | 66 ± 0.30$^{a}$ | 0.54 ± 0.01$^{b}$ | 0.018 ± 0.002$^{b}$ | 0.83 ± 0.01$^{ab}$ |
| 125 | 29.25 ± 0.05$^{b}$ | 7.14 ± 0.02$^{c}$ | 11.3 ± 0.30$^{b}$ | 22 ± 2.0$^{b}$ | 124 ± 2.0$^{b}$ | 68 ± 0.60$^{a}$ | 0.55 ± 0.01$^{b}$ | 0.020 ± 0.001$^{b}$ | 0.90 ± 0.02$^{a}$ |
| **Concentration (ppm)** | **Hematological parameters** | | | | | | | | |
| | TEC ($10^6$ mm$^{-3}$) | Hb (g dL$^{-1}$) | Hct (%) | MCV (fl) | MCH (pg) | MCHC (g dL$^{-1}$) | TLC ($10^3$ mm$^{-3}$) | Lymphocytes (%) | Neutrophils (%) |
| **0 h transport** | | | | | | | | | |
| Control | 0.82 ± 0.01$^{a}$ | 3.40 ± 0.15$^{a}$ | 7.40 ± 0.10$^{a}$ | 90.1 ± 0.4$^{a}$ | 40.6 ± 0.1$^{a}$ | 46.2 ± 0.6$^{a}$ | 81.80 ± 0.60$^{a}$ | 95.10 ± 0.35$^{a}$ | 3.50 ± 0.05$^{a}$ |
| 25 | 0.82 ± 0.01$^{a}$ | 3.30 ± 0.10$^{a}$ | 7.35 ± 0.10$^{a}$ | 89.1 ± 0.5$^{a}$ | 40.5 ± 0.0$^{a}$ | 45.4 ± 0.2$^{a}$ | 80.80 ± 0.40$^{a}$ | 94.40 ± 0.05$^{a}$ | 3.55 ± 0.15$^{a}$ |
| 50 | 0.82 ± 0.01$^{a}$ | 3.35 ± 0.15$^{a}$ | 7.50 ± 0.05$^{a}$ | 89.1 ± 0.4$^{a}$ | 40.3 ± 0.2$^{a}$ | 45.2 ± 0.2$^{a}$ | 82.00 ± 1.00$^{a}$ | 94.20 ± 0.50$^{a}$ | 3.60 ± 0.15$^{a}$ |
| 75 | 0.82 ± 0.02$^{a}$ | 3.30 ± 0.10$^{a}$ | 7.30 ± 0.20$^{a}$ | 90.1 ± 0.4$^{a}$ | 40.3 ± 0.2$^{a}$ | 45.2 ± 0.4$^{a}$ | 81.50 ± 0.60$^{a}$ | 94.50 ± 0.10$^{a}$ | 3.75 ± 0.05$^{a}$ |
| 100 | 0.82 ± 0.00$^{a}$ | 3.30 ± 0.20$^{a}$ | 7.30 ± 0.10$^{a}$ | 89.1 ± 0.6$^{a}$ | 40.4 ± 0.2$^{a}$ | 46.2 ± 0.2$^{a}$ | 82.50 ± 0.40$^{a}$ | 95.40 ± 0.10$^{a}$ | 3.60 ± 0.20$^{a}$ |
| 125 | 0.82 ± 0.02$^{a}$ | 3.30 ± 0.15$^{a}$ | 7.40 ± 0.10$^{a}$ | 89.4 ± 0.5$^{a}$ | 40.4 ± 0.2$^{a}$ | 46.2 ± 0.3$^{a}$ | 81.80 ± 0.60$^{a}$ | 94.80 ± 0.15$^{a}$ | 3.60 ± 0.15$^{a}$ |
| **6 h transport** | | | | | | | | | |
| Control | 0.23 ± 0.01$^{b}$ | 0.85 ± 0.05$^{d}$ | 1.55 ± 0.15$^{c}$ | 65.8 ± 1.2$^{d}$ | 36.1 ± 0.1$^{d}$ | 55.0 ± 1.1$^{a}$ | 16.40 ± 1.10$^{c}$ | 65.55 ± 2.95$^{c}$ | 23.15 ± 0.65$^{a}$ |
| 25 | 0.80 ± 0.01$^{a}$ | 3.30 ± 0.10$^{c}$ | 7.05 ± 0.15$^{b}$ | 88.0 ± 0.7$^{c}$ | 41.2 ± 0.2$^{c}$ | 46.7 ± 0.4$^{b}$ | 80.00 ± 1.00$^{b}$ | 94.00 ± 0.40$^{a}$ | 3.60 ± 0.10$^{d}$ |
| 50 | 0.84 ± 0.02$^{a}$ | 3.50 ± 0.10$^{bc}$ | 7.65 ± 0.25$^{ab}$ | 90.5 ± 0.3$^{c}$ | 41.3 ± 0.4$^{c}$ | 45.7 ± 0.2$^{b}$ | 82.40 ± 0.60$^{b}$ | 91.50 ± 0.60$^{ab}$ | 5.05 ± 0.05$^{d}$ |
| 75 | 0.85 ± 0.03$^{a}$ | 3.60 ± 0.10$^{abc}$ | 7.75 ± 0.35$^{ab}$ | 91.1 ± 0.9$^{bc}$ | 42.3 ± 0.3$^{bc}$ | 46.4 ± 0.8$^{b}$ | 85.05 ± 2.65$^{b}$ | 89.35 ± 1.55$^{ab}$ | 9.15 ± 0.25$^{c}$ |
| 100 | 0.86 ± 0.00$^{a}$ | 3.80 ± 0.15$^{ab}$ | 8.40 ± 0.10$^{a}$ | 97.1 ± 0.6$^{ab}$ | 43.8 ± 0.4$^{ab}$ | 45.2 ± 0.5$^{b}$ | 95.25 ± 1.05$^{a}$ | 86.55 ± 1.25$^{ab}$ | 12.05 ± 0.85$^{b}$ |
| 125 | 0.88 ± 0.01$^{a}$ | 4.00 ± 0.10$^{a}$ | 8.70 ± 0.10$^{a}$ | 98.2 ± 0.5$^{a}$ | 45.1 ± 0.6$^{a}$ | 45.9 ± 0.6$^{b}$ | 98.10 ± 0.20$^{a}$ | 84.65 ± 0.85$^{b}$ | 13.65 ± 0.15$^{b}$ |
| **12 h transport** | | | | | | | | | |
| Control | 0.44 ± 0.03$^{c}$ | 1.55 ± 0.05$^{e}$ | 3.30 ± 0.20$^{d}$ | 75.0 ± 0.6$^{c}$ | 35.2 ± 0.8$^{d}$ | 46.9 ± 0.7$^{a}$ | 4.10 ± 2.50$^{d}$ | 39.40 ± 0.90$^{d}$ | 26.25 ± 0.55$^{a}$ |
| 25 | 0.80 ±0.01$^{bc}$ | 3.35 ± 0.15$^{d}$ | 7.15 ± 0.25$^{c}$ | 88.7 ± 1.4$^{b}$ | 41.5 ± 0.4$^{c}$ | 46.7 ± 0.4$^{a}$ | 81.40 ± 1.40$^{c}$ | 94.15 ± 0.75$^{a}$ | 3.90 ± 0.20$^{d}$ |
| 50 | 0.86 ±0.01$^{ab}$ | 3.70 ± 0.20$^{cd}$ | 7.95 ± 0.05$^{bc}$ | 92.4 ± 0.5$^{b}$ | 43.0 ± 0.5$^{bc}$ | 46.5 ± 0.3$^{a}$ | 86.20 ± 1.60$^{bc}$ | 88.45 ± 0.65$^{ab}$ | 7.00 ± 0.30$^{c}$ |
| 75 | 0.90 ±0.02$^{ab}$ | 3.95 ± 0.15$^{bc}$ | 8.40 ± 0.10$^{b}$ | 93.3 ± 1.0$^{b}$ | 43.8 ± 0.7$^{abc}$ | 46.9 ± 0.4$^{a}$ | 91.50 ± 1.10$^{bc}$ | 86.45 ± 1.35$^{abc}$ | 9.80 ± 0.40$^{c}$ |
| 100 | 0.93 ± 0.01$^{a}$ | 4.35 ± 0.05$^{ab}$ | 9.50 ± 0.20$^{a}$ | 102 ± 1.0$^{a}$ | 46.7 ± 0.0$^{ab}$ | 45.7 ± 0.8$^{a}$ | 104.90 ± 1.90$^{ab}$ | 82.90 ± 2.70$^{bc}$ | 13.40 ± 0.10$^{b}$ |
| 125 | 0.94 ± 0.00$^{a}$ | 4.55 ± 0.05$^{a}$ | 9.80 ± 0.10$^{a}$ | 103.6± 2.1$^{a}$ | 48.1 ± 0.5$^{a}$ | 46.4 ± 1.0$^{a}$ | 118.40 ± 3.40$^{a}$ | 80.15 ± 0.95$^{c}$ | 14.75 ± 0.95$^{b}$ |

Control without sedative (0 ppm), 25, 50, 75, 100 and 125 ppm = tobacco leaf dust concentrations in transport water during simulated transportation.
DO: Dissolved Oxygen, $CO_2$ = dissolved free carbon dioxide; TEC: Total Erythrocyte Count; Hb: Hemoglobin; Hct = Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration (MCHC); TEC: Total Leucocyte Count
Values in the same column with different superscripts are significantly (P<0.05) different for each parameter. One-way ANOVA was used following Tukey's HSD post hoc test in SPSS 16.0.

**Table 4:** Water quality parameters and haematological parameters observed at different time intervals during simulated transportation of rohu fingerlings.

## Hematological parameters

Blood parameters (Table 4) showed a drastic variation between control and treatments and among treatments as well. The poor hematologic profile was observed in control group fishes at different time intervals during simulated transportation. There was a high reduction in TEC of control group and were significantly different ($p<0.05$) from other treatments. TEC increased insignificantly ($p>0.05$) in all other treatments. High Hb and Hct levels were found in 125 ppm whereas low levels were found in control. MCV levels were high in 125 ppm followed by 100 ppm and low in control and were significantly different ($p<0.05$) from other treatments. A significant ($p<0.05$) increase and decrease in

MCH level was identified in 125 ppm and control group, respectively. At the end of 6 h, control group showed the highest MCHC and is significantly different ($p<0.05$) from other treatments whereas at the termination of the experiment, all the treatments were insignificantly different ($p>0.05$). TLC was found low in control group and high in 125 ppm. There was no significant difference ($p>0.05$) in other treatments. High lymphocyte count was noticed in 25 ppm while low count was observed in control during transportation and were found significantly different ($p<0.05$). Neutrophils were found more in control group and less in 25 ppm exhibiting a significant difference ($p<0.05$) from other treatments.

## Discussion

In this present study, the induction and recovery times significantly decreased and increased, respectively with increase in concentrations of tobacco leaf dust. The results indicate that the increase in tobacco concentration reduced the induction time and prolonged the recovery time in fishes. There is a highly significant correlation between the concentrations of tobacco and time of induction and recovery with larger doses having shorter induction times and prolonged recovery [21]. The recovery time is usually faster at lower concentrations of anesthetic and it becomes more prolonged as the concentration increases [7]. Several workers have also obtained the above results using different sedatives and anesthetics in other fish species [5,12,22-24]. The findings from the present investigation reveal that application of tobacco leaf dust have notable effects on the fish behavior during induction and recovery. The behavioral responses observed in *L. rohita* fingerlings during 12 h exposure period in the anesthetic bath were in line with the results reported in Nile tilapia [21] and Thai magur [25] at higher anesthetic doses. Several studies have envisaged that sedation can decrease transport and post-transport mortality [26-28]. The highest mortality of rohu fingerlings occurred when no sedative was used. Fish mortality in control resulted from poor water quality and poor hematologic profile of the fingerlings in confined space. There was no immediate and delayed mortality in tobacco leaf dust treatment groups due to the effectiveness of sedatives that have helped in maintaining better environmental conditions which in turn assisted in less consumption of oxygen by reducing metabolic activity and hyperactivity and thus eliminating undue injuries. However, little mortality was observed in 125 ppm, due to the continuous hyperactive phase caused by the sedative.

The water quality parameters were kept constant and were within the acceptable levels as reported by Bhatnagar and Devi [29] indicating that the experimental condition was suitable for transport. After the experiment, variation in the reported result of monitored parameters was noticed which may be associated with the transportation in a closed container (polyethylene bag). A significant increase in water temperature was observed for control group after transport, which was attributed to the transport condition as well as the heat generated by the fishes as a result of metabolic activity. *L. rohita* fingerlings transported without sedative (control group) showed low pH as a result of $CO_2$ accumulation. The production of more $CO_2$ from respiration lead to the formation of more carbonic acid and subsequent dissociation yield more hydrogen ions turning water acidic [30]. Low pH for the control group compared with that for the sedative treated groups indicates the efficacy of sedative in reducing carbon dioxide excretion and therefore metabolic activity of the fish. Kutty [31] and Das et al. [15] stated that elevated respiration rate due to the hyperactivity of fishes during initial phase contribute to reduced DO tension in water. Low DO observed in control group could be due to high consumption of oxygen resulted from increased temperature and metabolic rate of the fingerlings over the duration. The reduction in oxygen consumption could be due to either a decrease in the rate of oxygen uptake and/or a decrease in metabolism caused by the effect of tobacco leaf dust. The $CO_2$ content in transport water showed a gradual increase with transport duration. Hypercapnia in control group was attributed to the concomitant increase in metabolic rate of the fishes during transport. Next, to control, $CO_2$ was also found high in 125 ppm and 100 ppm which could be due to the hyperactivity phase of the fishes caused by the sedative. The $CO_2$ increase in other sedative treatments during the initial hours may be due to the stress caused by capture and handling before the start of the experiment but declined and subsided towards the end of the experiment indicating the efficacy of the sedative. The results corroborate with the previously simulated transport experiments conducted in Tiger barb [28], Southern Platyfish [32] and Winter Flounder [33]. Boyd [30] indicated that ammonia released by fish into water reacts with water molecules to form ammonium ($NH_4^+$) and hydroxyl ($OH^-$) ions and further, the hydroxyl ion reacts with $CO_2$ to produce $HCO_3$ which results in alkalinity. The results obtained from the present experiment showed a significant increase of total alkalinity in control groups which confirms the phenomena explained by Boyd [30] as there had been a continuous addition of ammonia and $CO_2$ to the ambient water from the excretion and respiration of the fingerlings, respectively. Hardness values in control group and in all treatment groups were within the optimum range of fishes as reported by Bhatnagar and Devi [29]. Control group transported without sedative showed a significant increase in ammonia which was attributed to the increase in metabolic rate of the fishes and release of excretory products. Sedative treatments decreased the metabolic activity of *Labeo rohita* fingerlings and hence ammonia was found low compared to control which conforms with the results obtained by Park et al. [33], Pramod et al. [28], Becker et al. [34] and Husen and Sharma [5] using other sedatives. The increase in metabolic activities coupled with the exciting phase of the fishes caused by the higher concentrations of tobacco leaf dust led to the increase of ammonia in the respective treatment groups. An increase in $NO_2$-N concentration in water is considered toxic to fish [15]. The increased $NO_2$-N concentration observed in control group was found lethal. The results indicate that the increase could be due to the release of excretory products and metabolites by the fishes caused by transport stress coupled with the nitrification of ammonia. Nitrite was found low in treatment groups due to the sedative efficacy in reducing the release of fecal matter by the fishes and thereby reducing the $NO_2$ concentrations in transport water. Where ammonia and nitrite are toxic to the fish, Nitrate is harmless and is produced by the autotrophic *Nitrobacter* bacteria combining oxygen and nitrite. Major chemical constituents identified in tobacco are nicotine, nor-nicotine, anabasine, myosmine, anatabine, nitrate, sorbitol [35]. The results from the current study indicate that the increase in $NO_3$-N concentrations in the sedative treatments could be attributed to the increased nitrate content found in tobacco leaf dust which increased the $NO_3$-N concentrations in water over the duration. Low $NO_3$ was found in control which may be due to poor bacterial degradation of toxic metabolites such as ammonia and nitrate.

Hematological profile changes of peripheral blood have often been used as a stress indicator, though results are equivocal. Serious changes in hemogram of fishes were found in control group due to stress caused by transportation. Erythrocyte value is a function of oxygen absorption and transportation within a cell and depletion in count may weaken the fish and lead to death. The significant decrease in circulating erythrocytes in control group may be due to the poor water quality

found which agrees with the results reported in Gilthead Sea Bream [36] and in *Tilapia zilli* [37]. Similar results were also acquired by Ahmed et al. [38] and Ishikawa et al. [39] in *O. niloticus* when exposed to poor water quality. These findings underline that water quality influences hematological parameters. In stressed fish, an increase in RBC is often observed [40]. The significant increase in the no. of red blood cells in treatment groups with higher concentrations of tobacco leaf dust (125 ppm and 100 ppm) may be due to the higher metabolic demand stress caused by the hyperactivity of the fishes in the respective treatments. The release of the catecholamine is primary stress response causing erythrocytes to swell and spleen to release new erythrocyte to blood [41]. Hyperactivity initiated the release of catecholamine and cortisol to promote the increase in oxygen demand in the tissues, leading to a quick differentiation and proliferation of erythrocytes in fishes of higher treatment groups. Certain blood variables like hemoglobin and hematocrit are considered as auxiliary stress response indicators [42]. The results showed a significant reduction of hemoglobin content in control group which could be due to handling and transport stress as well as due to methemoglobinemia [43,44] caused by the increased nitrite, found in control group. During stress situations, elevated hemoglobin contents increase the oxygen carrying capacity of blood and thus, supply oxygen to the major organs, in response to higher metabolic demands [45]. Thus, the results reveal that hyperactivity of fishes enhanced the blood hemoglobin level in 125 ppm and 100 ppm in order to meet the higher metabolic demands. As stress is an outcome of transport procedures, it could have decreased the Hct values in the control group. These considerations suggest a hemodilution caused by osmoregulatory disturbance [42,46]. Hct values increased significantly during the transportation in treatment groups with higher concentrations of tobacco leaf dust (125 ppm and 100 ppm) which were similar to that reported in common carp [47]. Hrubec and Smith [48] stated that Hct values increase was a result of splenic contraction and RBC swelling. Hct can also vary in fish according to their swimming performance. Wilhelm Filho et al. [49] observed that active fish species presented higher Hct, Hb and RBC when compared to less active fish species. Therefore, the results reveal that increased no. of erythrocytes and hyperactivity of the fishes was the reason for the increased Hct values of fishes in 125 ppm and 100 ppm treatments. Erythrocyte indices namely, MCV, MCH and MCHC measure the volume, weight and the concentration of hemoglobin respectively [50]. Fluctuation in these indices corresponded with values of TEC, Hb and Hct observed. Stress is thought to be responsible for leucopenia in fish [51]. The significant decline in the circulating WBC in control group of the present study may be due to an increase of plasma cortisol concentration which is a glucocorticoid hormone that can act as an immunosuppressive. In the present investigation, the increase in WBC (leukocytosis) may have resulted from the excitation of the defense mechanism of the fish to counter the acute stress effect caused by the higher concentrations of the leaf dust. Pulsford et al. [52] also detected an increased number of leukocytes, particularly phagocytes and damaged cells, in peripheral blood of dab, *Limanda limanda*, when subjected to an acute stress. Lymphocytes are the most common leukocytes found in a healthy teleost, and they represent an important function in the cell immunity of fish [53]. Lymphocytes and neutrophils from the present study showed a significant decrease and increase in control group, respectively which resembles the results reported in slender seahorse [23], *Cyprinus carpio* [54], and Channel catfish [55]. The decline of lymphocytes in control group may be due to the stress of capture and transport which led to increasing in plasma cortisol inducing lymphocyte migration from blood to tissues and thereby decreasing the circulating lymphocytes in the blood. Wiik et al. [56] suggested that transport and handling stress leads to the elevation of plasma cortisol that is reported to have a direct cytolytic effect on lymphocytes. Also, a significant decrease and increase of lymphocytes and neutrophils in the treatment group (125 ppm and 100 ppm) was observed confirming the results reported in Rainbow trout [57] using a high dose of clove oil. It could be due to increase in stress hormones caused by the hyperactivity of the fishes during transportation. No significant changes occurred in treatments up to 75 ppm concentration indicating that the sedative is beneficial to reduce stress. Neutrophilia in control group could be due to the secondary effects of stress in fish, as a consequence of the stress-related release of catecholamine during transportation. In general, acute stress induces both neutrophilia and lymphopenia in fish and that these changes are due to cortisol and norepinephrine, which induce leukocyte migration from blood to tissues and vice-versa [52]. Thus our findings are in agreement with Wendelaar Bonga [41], who has reported that stress causes a rapid increase in neutrophils and a reduction of lymphocytes in peripheral blood. It is noteworthy that throughout the experiment, there were no significant changes in water quality and blood parameters in tobacco leaf dust concentration of 25 ppm which implies the efficacy of the sedative at the particular concentration. Hence, tobacco use in the transport of rohu and other fishes is suggested because of its stress reducing capacity during transport even at low concentrations.

## Conclusion

On the whole, the present study suggests that tobacco leaf dust of up to 75 ppm concentration can be used as a sedative for rohu fingerlings transport with a slight modification of fish behavior and its hemogram. But the tobacco leaf dust is effective in inducing light sedation in rohu at a concentration of 25 ppm. Therefore, it has been concluded that *L. rohita* fingerlings can be successfully transported up to 12 h using 25 ppm tobacco leaf dust as a tranquilizing agent without any change in the transport environment and health of fish. In general, tobacco is less expensive compared to other synthetic sedatives. Therefore, the study would be helpful to fish farmers to prevent the mortality of seed during transportation using *N. tabacum* leaf dust as an alternative, eco-friendly and novel natural product to synthetic, harmful and expensive sedatives, due to its cost effectiveness, easy availability, narcotic at low dose and biodegradability.

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