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Efficacy of tobacco leaf dust (*Nicotiana tabacum*) as an anaesthetic and euthanasia for Rohu (*Labeo rohita*) fingerlings

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Abstract
Tobacco leaf dust, due to its low cost, local availability and complete degradability could be used as a novel and alternative product in anaesthesia and euthanasia procedures for fishes. Utilizing these benefits of tobacco, the present study was carried out to determine the anaesthetic potential of tobacco in rohu fingerlings and also to investigate its efficacy in euthanasia procedure when the individuals are to be sacrificed. Different concentrations of tobacco leaf dust such as 0.00, 0.25, 0.50, 0.75, 1.00, 1.25 g L^{-1} and 0.00, 2.00, 3.00, 4.00, 5.00, 6.00 g L^{-1} were tested for anaesthetic potential and euthanasia in rohu fingerlings (9.46 ± 0.56 cm and 6.29 ± 0.43 g), respectively for 30 minutes in glass tanks (30 L capacity) at a stocking density of 10 fishes/tank among which 0.00 g L^{-1} was used as control. Increase in the concentrations of tobacco leaf dust decreased and increased the induction and recovery times significantly ($p<0.05$) in anaesthesia and euthanasia experiments. 100% mortality of fishes was observed during euthanasia in all the treatments with a variation in induction times while 100% survival rates were noticed in all the treatment groups of anaesthetic experiment. Our results reveal that tobacco would be a cheap and promising natural product to anaesthetise (1.25 g L^{-1}) and euthanize (6.00 g L^{-1}) rohu fingerlings with a rapid induction time for minor operations/handling (where fish will be released back) and for culling/laboratory analyses (where fish will be killed), respectively.

Keywords: Tobacco leaf, *Nicotiana tabacum*, *Labeo rohita* fingerlings

1. Introduction
Aquaculture practices incorporate various activities such as netting, handling, sizing, grading, weighing, transportation, malady treatment and so on. The cultured animal faces a genuine pressure circumstance while carrying out any of the above activity or a blend of these exercises. These stress conditions at last outcomes in the mortality of the fishes following a middle of the road indication of physical damage, loss of appetite and poor growth. As a result, the aquaculturists experience serious monetary misfortunes. To forestall these pressure-prompted mortalities, sedation and anaesthetic technique proves demonstrates to be a legitimate and solid one to empower an assortment of activities. The method is all around performed with the work of medicines known as sedatives and anaesthetics which includes a variety of products of both natural and synthetic origin.

Anaesthesia is commonly characterized as a condition of loss of sensation or obliviousness caused nervous system depression when an external agent is applied (Ackerman *et al.*, 2005; Ross and Ross, 2008) [1, 11] and can be acquainted with fish through physical or chemical strategies. It is a technique of criticalness in aquaculture that outcomes in better and more secure management practices during biometry, blood collection and body investigations due to incomplete and facultative immobilization of fishes (Boijink *et al.*, 2016) [4]. Anaesthetic conditions not just reduce the stressor impression of the fishes yet, also, forestall their nerve signal transmission to the central nervous system (Woods *et al.*, 2008) [13]. It additionally mitigates the corresponding worry stress from handling or confinement stress which makes infection in fishes and restraint of their craving (Silva *et al.*, 2012) [12].

The term euthanasia comes from the Greek language meaning a good death (eu = good, thanatos = death). The term is generally used to depict finishing the life of an individual creature in a manner that limits or wipes out agony and misery (Leary *et al.*, 2013) [10]. Wilful extermination of creatures as a way to end enduring and torment is generally acknowledged as great creature welfare. Up until now, the researchers have tried the adequacy of various narcotics over various fish species. These investigations uncover that a few tranquillizers are costly and inaccessible while some are harmful and effectively accessible. Till date, MS-222 is the only anaesthetic endorsed by the USFDA to be utilized on food fishes which additionally comprises of numerous downsides, for example, mind-boggling expense, low pH and low

adequacy on plasma cortisol control. Hence, looking for safe, successful and cheap narcotic, tobacco is viewed as a novel and cutting-edge narcotic in supplanting the other costly and harmful medications inferable from its attributes like natural origin, cost proficiency, local availability, biodegradability and non-dangerous to fishes and people. Our previous works with rohu fingerlings (Dinesh *et al*., 2017) divulged the calming capability of tobacco during live fish transport while in this investigation we attempted to unfurl the tobacco's viability in anaesthesia and euthanasia procedures for rohu fingerlings.

2. Material and methods

2.1 Experimental animal and design

360 nos. of healthy L. rohita fingerlings of 9.46 ± 0.56 cm mean length and 6.29 ± 0.43 g mean weight were obtained and the same were used for the experiment. The experiment was conducted at the wet laboratory unit, Division of Aquaculture, ICAR- Central Institute of Fisheries Education, Andheri (West), Mumbai, India. A completely randomized design was used to experiment. The experiment was conducted in triplicates for five treatments and the control for both the experiments.

2.2 Preparation of tobacco leaf dust

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 a mixer. The fine ground tobacco leaf dust (7-10% moisture content) was then stored in an air-tight container and used for the experiment.

2.3 Protocol and experiment

In the first experiment, five concentrations of tobacco leaf dust (0.25, 0.50, 0.75, 1.00 and 1.25 g L^{-1}) as predetermined earlier from lethal toxicity studies were tested for anaesthetic potential in *L. rohita* fingerlings. In the second experiment, five euthanasia doses (2.00, 3.00, 4.00, 5.00 and 6.00 g L^{-1}) which were fixed based on the results obtained from the anaesthetic experiment were tested for euthanasia in rohu 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 was 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*. (1998) [9] and were calculated using a stopwatch. The fingerlings were further monitored in recovery tanks for another 24 h to check the survival rate.

2.4 Statistical analysis

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

Table 1: Time (min) required for induction (stage 5) and recovery (stage 6) (Mean ± SE) of the anaesthesia using tobacco leaf dust in rohu fingerlings (Maximum observation time - 30 min)

Treatment (g L^{-1})	Induction (min)	Recovery (min)	Survival rate (%) (24 h)
	Stage 5	Stage 6	
0	-	-	100
0.25	8.21 ± 0.06^{a}	1.52 ± 0.04^{a}	100
0.50	7.43 ± 0.11^{b}	2.25 ± 0.07^{b}	100
0.75	5.36 ± 0.08^{c}	2.97 ± 0.03^{c}	100
1.00	2.25 ± 0.04^{d}	4.41 ± 0.10^{d}	100
1.25	1.02 ± 0.05^{e}	5.03 ± 0.04^{e}	100

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

Table 2: Time (min) required for induction (stage 7) and recovery (stage 6) (Mean ± SE) of the euthanasia using tobacco leaf dust in rohu fingerlings (Maximum observation time - 30 min)

Treatment (g L^{-1})	Induction (min)	Mortality rate (%)
	Stage 7	
0.00	-	0
2.00	2.01 ± 0.03^{a}	100
3.00	1.76 ± 0.02^{b}	100
4.00	1.28 ± 0.05^{c}	100
5.00	0.95 ± 0.09^{d}	100
6.00	0.73 ± 0.03^{e}	100

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

3. Results and discussion

3.1 Induction and recovery and survival rate percentage

The induction and recovery times (Table 1) observed in anaesthetic bath significantly decreased and increased ($p<0.05$), respectively with increase in concentrations among all treatments which were in line with the results obtained by several other workers using different anaesthetics in different fish species (Husen and Sharma 2015; Hoseini and Ghelichpour, 2012; Hseu *et al.*, 1998; Cunha *et al.*, 2011; Balamurugan *et al.*, 2016) [8, 6, 7, 5, 3]. Treatment group with 0.25 g L^{-1} tobacco showed a slight delay in induction but a rapid recovery whereas 1.25 g L^{-1} showed quick induction and prolonged recovery times when compared to the other treatments. The same dose dependent change in induction times was found in the euthanasia experiment (Table 2). There exists a highly significant positive correlation between the induction and recovery times and tobacco concentrations with smaller doses having prolonged induction and quicker recovery times and vice versa (Agokei and Adebisi, 2010) [2]. The control (without anaesthetic) group fishes showed normal swimming patterns compared to the anaesthetised fishes. 100% mortality was observed in the euthanized fishes during the experiment in the anaesthetic bath as the fishes were unable to overcome the effect caused by the higher tobacco concentrations whereas 100% survival was noticed among the treatments during the anaesthetic experiment.

In conclusion, our research findings suggest that tobacco would be a low cost, efficient and non-toxic anaesthetic (1.25 g L^{-1}) and euthanasia (6.00 g L^{-1}) for rohu fingerlings. Further researches have to be carried out in other finfish and shellfish species to unveil the benefits of tobacco in other species and

to utilize them potentially and positively reducing its abuse for humans.

4. References

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