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Full Length Research Paper

Tobacco as an anesthetic for fish handling procedures

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The use of aqueous and alcoholic extracts of tobacco as an anesthetic for fish handling procedures was investigated using the Nile tilapia, *Oreochromis niloticus.* An effective concentration of the anesthetic was defined as one giving a sure state of anesthesia for 75% of the fish after an exposure time of less than 5 min. The effective concentration of the aqueous extract was 4/4.5 g/l. For the alcoholic extract it was 6/7 ml/l. There is a highly significant correlation (r = -0.98, p < 0.01) between concentration and recovery time with the aqueous extract. Recovery times with both preparations were short, 2 - 5 min with the aqueous extract and an average of 10 min with the aqueous extract. Anesthesia with the aqueous extract was recommended over the alcoholic extract as it had a lower effective dose and a comparable recovery time.

Key words: Anesthetic, recovery time, effective dose, tobacco.

INTRODUCTION

Fish are routinely handled when carrying out certain operations such as stripping for gametes, sorting, weighing, treatment for and against disease etc. To reduce the effect of stress, chemical and anesthetics are routinely administered to fish before handling (Ross et al., 1993).

Conventional anesthetics such as tricaine methane sulphonate (MS-222), benzocaine and quinaldine are expensive and not readily available in some third world countries. To solve the problem of scarce and expensive anesthetics, some researchers have experimented with low doses of crude extracts of piscicidal plant material as anesthetics for fish (Eze, 1991; Mgbenka and Ejiofor, 1998). Studies carried out on the use of clove oil, also a natural plant extract as a fish anaesthetic have shown it to be a cheaper, safer and more effective anaesthetic especially at low concentrations in comparision with the conventional chemical anaesthetics (Munday and Wilson, 1997; Soto and Burhauddin, 1995).

Tobacco is the common name of the plant *Nicotiana tabacum* and to a lesser extent *N. rustica*. Nicotine and the related alkaloids contained in tobacco are generally recognized as being narcotic. This property makes it useful as an anesthetic, a pesticide and a fish poison (Aleem, 1983, Agbon et al., 2002). No literature on use of tobacco as an anesthetic for fish in Nigeria was available and it would appear that experimental studies on this subject are rare.

Agbon et al. (2002) studied the toxicity effect of tobacco lead dust extract on *Oreochromis niloticus*, the piscicidal effect of the aqueous extract on *Clarias gariepinus* was studied by Omoniyi et al. (2002).

The criteria used to evaluate tobacco leaf extracts as an anesthetic for use in fish culture and research are adapted from Marking and Meyers (1985) list of characteristics of an ideal anesthetic:

1. It has an induction time of less than 15 min and preferably less than 3 min.
2. Recovery time after its use is short, 5 min or less.
3. It is non-toxic to fish and has a large safety factor.
4. It is easy to handle and not harmful to humans during its normal use.
5. It has no persistent effects on fish physiology and behavior.
6. It is rapidly excreted or metabolized leaving no residues and requiring no withdrawal time.
7. It engenders no cumulative effect or problems from repeated exposure.
8. It is inexpensive.

In this present study, *O. niloticus*, one of the common culturable fish species in Nigeria was exposed to different

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Table 1. Dosage, exposure and recovery times for *O. niloticus* anaesthetized with tobacco (aqueous preparation).

Anesthetic does (g/l)	Time to anesthesia (min)	Time to recovery	Mortality (%)
0.2	Ineffective	-	0
0.5	Ineffective	-	0
1	8.2 ± 0.84	12.4 ± 8.0	0
1.5	4.0 ± 1.15	12.4 ± 3.58	0
2.0	5.2 ± 0.45	8.4 ± 3.25	0
3.0	4.6 ± 0.55	8.4 ± 3.25	0
4.0	3.2 ± 1.10	7.6 ± 3.58	0
4.5	3.2 ± 0.45	7.2 ± 0.45	40
5.0	2.2 ± 0.45	6.2 ± 0.45	60

Values are mean±standard deviation; n = 5 for all, except for concentrations of 4 and 4.5 g/l where n = 15.

concentrations of aqueous and alcoholic extracts of dried tobacco leaves to ascertain its suitability as a safe, cheap and effective anesthetic for fish handling procedures and in research. The specific objectives of the study were to: determine the most effective concentration of alcoholic and aqueous extracts of dried tobacco leaf powder as fish anesthetics and determine which of the two extracts is a more effective fish anesthetic.

MATERIALS AND METHODS

Experimental fish

Healthy adult specimen of *O. niloticus* with an average weight of 14.4 - 30.3 g and an average fork length of 20.46±0.73 cm were procured from TAP a fish farm at Moniya, Ibadan in 3 separate batches of 50 each. They were transported in oxygenated polyethene bags to the Department of Zoology. University of Ibadan where they were acclimated in a small outdoor concrete tank for one week. The fish were not given any artificial food during this period. Fish handling to take weight and length measurements were done after the first series of experiments when the fish were relatively immobile due to the effect of the anesthetic.

Experimental set-up

The experiments though carried out in fundamentally, the same manner were done using two different tobacco preparations:

a. An aqueous extract.
b. An alcoholic extract.

Aqueous extract

Aqueous extracts of the tobacco preparations were obtained by agitating pre-weighed amounts of the tobacco powder in a beaker containing 200 ml of water. The solution was thereafter made up to 8 L with acclimation water in a glass trough (120 × 15 × 15 cm). This was done for each concentration in consideration. Each medium was further stirred with a plastic spoon to ensure better dispersal of tobacco. The temperature of the acclimation and test media were taken using a mercury-in-glass thermometer. pH values of the acclimation and test-media were also determined using pH meter. Baseline concentrations were first tried on separate batches of five fish. The reactions of the fish were noted in all experiments and the stage of anesthesia was determined by reference to the anesthesia series of McFarland (1960).

Continuous observation to establish the onset of anesthesia in individual fish was abandoned after 60 min as periods greater than this were considered impractical for routine fish handling procedures. Any of the test fish that lost balance and ceased respiratory movements of the opercula membrane was removed immediately and transferred to about 10 L of tobacco free water. The time of exposure to the tobacco and the recovery time were noted.

The exposure time was defined as the time taken from the moment the fish was exposed to the anesthetic to the moment the respiratory movement of the opercula membrane stopped.

Recovery time was defined as the time taken from the moment the fish was considered anaesthetized until the moment regular respiratory movements were resumed. None of the revived fish were re-used for further experimentation but were kept in another glass aquarium and plastic buckets.

On determining the safest and most effective concentration, the experiment was repeated in triplicates using batches of five randomly selected fish each time.

Alcoholic extract

The temperature of the acclimation and test media were taken and the pH of the same media was taken. The tobacco powder was prepared in the same manner as previously described. 20 g of the tobacco powder were poured into a conical flask and 150 ml of 50% alcohol was poured into the flask. This left the powder well covered. The mixture was stirred, allowed to settle and covered.

This preparation was put away for 48 h for the alcohol to extract the active ingredients from the powder. At the end of the second day, the extract, a very dark brown liquid was filtered through No. 1 Whatman filter paper. The filtrate was used to conduct baseline experiments as previously done using batches of five fish. Specific volumes of the extract were poured out using a measuring cylinder.

This was poured into the experimental glass trough, made up to 8 L with acclimation water and stirred to ensure even dispersal of the anesthetic. Exposure and recovery times were noted as previously done. On determining the safest and most effective concentrations, the procedure was repeated in triplicate. Revived fish were not re-used but were kept in plastic buckets and in a glass aquarium.

Statistical analysis

Data from the experiments were analyzed using Pearson's correlation coefficient to determine the level of interaction (Ogbeibu, 2005) between anesthetic doses, recovery and induction times.

RESULTS

Results of the various tests at different concentrations of the aqueous preparation of tobacco are shown in Table 1. From the experiments, a dose of 4 or 4.5 g/1 of an aqueous preparation of tobacco ensures that over 75% of the fish were anaesthetized within 4 min of exposure. A concentration of 4 g/l gave a shorter recovery time

Table 2. Dosage, exposure and recovery times for *O. niloticus* anesthetized with tobacco (alcoholic extract).

Anesthetic dose (g/l)	Time to anesthesia (min)	Time to recovery (min)	Mortality (%)
1.0	2 - 3*	-	0
2.0	2 - 3*	-	0
3.0	2 - 3*	-	0
4.0	2 - 3*	-	0
6.0	2.4 ± 0.54	5	0
7.0	2 ± 0.55	3	0
8.0	1.6 ± 0.55	3	0
10.0	1.0	-	100

*Fish maintained a peculiar oblique position and did not lay on their side after hyperactive phase.

than a concentration of 4.5 g/l. At concentrations of 5 g/l induction time was shorter though over 60% of the revived fish died. There is a highly significant correlation between the concentrations of the anesthetic and time of induction ($r = -0.89$; $p < 0.01$) with larger doses of the anesthetic having shorter induction times. There is also a highly significant correlation between the time of recovery and dosage ($r = - 0.98$; $p < 0.01$).

Exposing the fish to anesthetic concentrations of 0.2 and 0.5 g/l had no sedative or anesthetic effect on the fish for over 60 min. When fish were introduced into the glass troughs containing the anesthetic at all concentrations, except 0.2 and 0.5 g/l, they initially dashed about very wildly. Next, their swimming decreased, some swam in a convulsive manner, and others remained stationery. Partial loss of equilibrium followed as the fish maintained an oblique posture, next the fish fell flat on their sides on the bottom of the trough and opercula movement was greatly induced. The fish did not respond to tactile or visual stimuli and lay very still. This sequence of events was the same at all concentrations with the only variation being the time taken. On recovery, the fish resumed opercula activity, slowly initially and then becoming more rapid and steady. The fish moved their head and caudal region in a jerky fashion and made efforts to maintain the normal lateral position.

Table 2 gives a summary of the dosages, induction time, and recovery time after exposure to various concentrations of the alcoholic extract preparation, pH values of the acclimation water taken before and after all the experiments ranged between 6.0 and 7.0 temperatures of the water measured before and after the experiments had a mean value of 28 ± 1.0°C.

A dose of 6 ml/l ensured that 100% of the fish were deeply anaesthetized within 2 - 3 min. A dosage of 6 ml/l gave a shorter recovery time than 7 ml/l. At concentrations of 10 ml/l, all the fish exposed to the anesthetic were anaesthetized and duly transferred to the recovery tank but none of them recovered. There is a highly significant correlation between the anesthetic dosage and the time to anesthetic ($r = - 0.99$; $p < 0.01$) larger doses of the anesthetic induced anesthesia in shorter period of time.

Concentrations of 1, 2, 3 and 4 ml/l had a peculiar effect on the exposed fish. Fish exhibited partial loss of equilibrium in the first 2 - 3 min, maintaining oblique or sideways positions. This was after an initial phase of hyperactivity when they dashed about vigorously and swarm in a convulsive manner.

At concentrations of 6, 7 and 8 ml/l the fish responded to the anesthetic almost immediately. There was some hyperactivity, followed by partial loss of equilibrium before they lay at the bottom of the trough having lost equilibrium totally.

The fish were promptly removed into the recovery trough at this stage. Recovery was quick and behavioral patterns exhibited on recovery were similar to those shown by the previous set of fish.

DISSCUSSION

The properties required of an anaesthetic will vary with the research objectives. In any case a quick induction and a recovery time which allows for varied manipulations is desirable. In addition anaesthetics should be safe, easy to handle and cheap moreso in a developing country like Nigeria.

The present observation suggests that the tobacco extracts acted as an anesthetic in the fish tested. The sequential progression through the various stages of anesthesia with increasing dose and time and the recovery of anaesthetized fish all followed the patterns of typical fish anesthetic (McFarland, 1960; Marking and Meyer, 1985).

In terms of induction time to anesthesia, the aqueous and alcoholic extracts met Marking and Meyers (1985) first criterion, a dosage of 4 or 4.5 g/l of the aqueous preparation and a dose of 6 or 7 ml/l of the alcoholic extract sufficed for quick anaesthetization of tilapia, moreso, as over 75% of the fish recovered well within 10 min of removal from anaesthetizing solution, time enough to perform most routine fish handling procedures like retrieving gametes, length and weight measurements,

tagging and sex determination. Tobacco appears to meet five of the eight criteria used to define an ideal anesthetic (Marking and Meyer, 1985). Its main advantages lie in its low cost, availability, relative safety to humans and being a natural product.

It is easily biodegradable when compared to inorganic or artificial products. Currently, MS-222 is a restricted substance and there is some concern over the effect it may have on fish and on those consuming fish products exposed to MS – 222 (Summer felt and Smith, 1990).

These observations compare well with the findings of Konar (1970) where a concentration of 5 g/l of nicotine (the active ingredient in tobacco) elicited a high degree of excitability and eventual stupor within 5 - 10 min of exposure. Recovery time in the above experiment was however very long spanning 4 - 6 days.

The results of the current study vary from that of Sado (1985) where the effective dose required to induce anesthesia using quinaldine in three tilapine species including *O. niloticus* was 50 g/l compared with the effective dose of tobacco (using either of the tobacco preparations) needed to anaesthetize the same species was much higher. Also the period of anesthesia was long (over 12 h) as compared with that of the tobacco preparations.

Malstrom et al. (1993) recommended a dosage of 2.5 g/l for quick anaesthetization of halibut from which fish recover in 11 min. Using dosages as low as these did not effect anesthesia in tilapia, using any of the tobacco preparations. Recovery times using 4/4.5 g/l or 6/7 ml/l of the tobacco preparations however compares favorably with the recovery time when administering MS – 222 as in the aforementioned investigations.

In a leaflet accompanying the product Metomidate HCl manufactured by wildlife laboratories USA, the use of this anesthetic was contra-indicated in the anaesthetization of cichlids. According to Stoskpok and Posner (2008), the addition of MS-222 and Benzocaine lowers the pH of water to which is added, to as low as 5. It is recommended that the solution be buffered with sodium bicarbonate to a pH of 7.0 – 7.5. From our results, the introduction of tobacco had no effect on the pH or temperature of the water. This may be attributed to the fact that being a natural product it probably has a lower toxicity when compared to inorganic anesthetics.

Conclusion

The use of tobacco as an anesthetic for *O. niloticus* is a better alternative as it can be safely utilized on them and recovery times are shorter than with other conventional anesthetics. Given that there is a high correlation between the anesthetic dosage and recovery time, smaller dose of the anesthetic could be utilized in inducing sedation for longer period. Thus making tobacco a highly effective, readily available and cheap fish anesthetic with potentially few or no side effects. Aqueous extracts are more effective than the alcoholic extracts.

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