

## Production of Monosex Nile Tilapia, *Oreochromis niloticus* Using Seed of *Mucuna pruriens*

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**Abstract:** The present study was aimed to investigate the potential effect of *Mucuna pruriens* on masculinisation of Nile tilapia during its in vivo application through direct feeding and immersion techniques and to determine an ideal concentration for each method for production of all-male tilapia population. Three days old mixed sex juveniles of Nile tilapia (mean weight  $0.025 \pm 0.009$  g; mean length  $1.25 \pm 0.012$  cm) were subjected to dietary treatment with powdered *M. pruriens* seeds (0.0, 2.0, 3.5 and 5.0 g/kg feed) and immersion treatment with aqueous extract of the plant seeds (0.02, 0.035, 0.05 g/l) for one month. For dietary treatment, the highest percentage of survival ( $94.33 \pm 0.33$ ) was obtained in the 5.0 g/kg category, while treatment with 0.05 g/l yielded the highest survival percentage ( $92.33 \pm 0.33$ ) for immersion treatment. Dietary and immersion treatment with the plant material resulted in significantly higher ( $P < 0.05$ ) percentage of males compared to the control groups. Among the fish fed powdered *M. pruriens* seed containing diets, the highest percentage ( $73.33 \pm 0.67$ ) of males was observed in 5.0 g/kg group. During immersion treatment, the highest percentage ( $74.67 \pm 0.33$ ) of males was obtained in 0.05 g/l category, which was significantly higher ( $P < 0.05$ ) compared to all the treatment groups.

**Keywords:** *Mucuna pruriens*, *Oreochromis niloticus*, Phytochemicals, Sex reversal

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### I. Introduction

The Nile tilapia is a well accepted species for culture due to its rapid growth, high tolerance to low water quality, efficient food conversion, resistance to disease, ease of spawning and good consumer acceptance [1]. Because of high fecundity, females generally reproduce at a small size and exhibits stunted somatic growth at higher densities, while male tilapias exhibit faster growth rates and are often the preferred gender for monosex aquaculture [2]. The success of the utilization of steroid hormones to produce monosex populations of tilapia is well documented [3, 4], but because of the potential hazards of such steroids; the use of new chemicals is a potential alternative to be explored [5]. Phytochemicals such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils have been reported to promote various activities like antistress, growth promotion, appetite stimulation, tonic and immunostimulation, and antimicrobial properties in fish culture [6, 7]. These are also reported to block biosynthesis as well as action of estrogen by acting as aromatase inhibitors and antagonists to nuclear estrogen receptor in gonad germ cells [8] and hence may be considered as potential mean for inducing sex reversal in fish. However, there are significant variations regarding the efficacy of different phytochemicals for production of all-male fish population and the potential anabolizing and virilizing effects of such plant extracts need to be clearly documented. The herb, *Mucuna pruriens* has various therapeutic uses and aphrodisiac effects in mammals [9]. It has been reported to possess medicinal values and some works indicated the potential use of *M. pruriens* as alternative source of protein in fish feed [10, 11, 12]. However, no studies have been reported related to its in vivo effect on sex reversal, growth and immunostimulation of fish. Various methods such as oral administration and immersion technique have been adapted for in vivo application of phytochemicals with medicinal values [13]. Therefore, the ideal method of application for *M. pruriens* for commercially feasible induction of sex reversal and growth in tilapia must be determined. Considering these aspects, the objective of the present study was to investigate the potential effect of the plant on the masculinisation of Nile tilapia, to compare direct feeding and immersion techniques as methods for in vivo application of the plant material and to determine an ideal concentration for each method with the plants that might produce maximum percentage of males in tilapia.

### II. Materials And Methods

#### 2.1 Collection of fish seed

Just hatched juveniles of mixed-sex Nile tilapia, *Oreochromis niloticus* (Linnaeus) was collected from the Fish Hatchery of West Bengal Government, oxygen packed and transported to the laboratory.

#### 2.2 Plant extract preparation

*M. pruriens* seeds were procured from the local plant market, washed in sterile distilled water, air-dried in shade and powdered. These powdered plant materials (250 gm) were extracted with 500 ml water in a Soxhlet

apparatus and the extracts were evaporated to dryness under pressure at 45°C using a rotary evaporator and stored under nitrogen at -20°C in amber glass bottle until those were used.

## 2.2 Dietary treatment of fish with powdered plant material

Three days old mixed sex juveniles of Nile tilapia (mean weight  $0.025 \pm 0.009$  g; mean length  $1.25 \pm 0.012$  cm) were randomly allocated into 4 groups (40 fish / group). Three groups were fed diets containing powdered Mucuna seeds at concentrations of 2.0, 3.5, 5.0 g/kg feed, one group was fed control diet without plant powder. The powdered plant materials were mixed thoroughly with the finely ground ( $< 500-1000 \mu\text{m}$ ) artificial diet containing 30% crude protein (Tokyu, Japan). It was then wetted with deionized water, mixed thoroughly, formed with a pelleter (diameter 2 mm), and dried at room temperature. Pelleted feed was pulverized before feeding to the juvenile fish. The experiment was conducted for 30 days and the fish were fed with respective diets at a rate of 20% body weight / day. The aquaria were continuously aerated and maintained in heated ( $T = 27 \pm 2^\circ\text{C}$ ) static systems. Water in all aquaria was replaced manually and the fish was kept under similar photoperiod (14 L: 10 D). This entire experimental set up was conducted simultaneously in triplicate.

## 2.3 Immersion treatment of fish with plant aqueous extract

Three days old mixed sex juveniles of Nile tilapia from the above described stock were randomly assigned in 12 glass aquaria to 4 different treatment groups (0.0, 0.02, 0.035 and 0.05 g/l) for the plant extract. The experiment was conducted for 30 days and the fish were exposed to the plant extract 4 times (once weekly) during this period. The aquaria were continuously aerated and maintained in heated ( $T = 27 \pm 2^\circ\text{C}$ ) static systems. Water in all aquaria was replaced manually and the fish was kept under similar photoperiod (14 L: 10 D). Each aquarium was stocked with 40 fish. The fish was fed finely ground ( $< 500-1000 \mu\text{m}$ ) artificial diet containing 30% crude protein (Tokyu, Japan) at a rate of 20% body weight / day. The experiment was conducted simultaneously in triplicate.

## 2.3 Sexing of fish

Sexing of the juvenile fish was done by the standard acetocarmine squash technique of gonads [14]. Histological studies of the gonads were also performed.

## 2.4 Qualitative phytochemical studies

Qualitative phytochemical analysis of the aqueous extract of *M. pruriens* seeds were carried out using standard procedures [15, 16, 17].

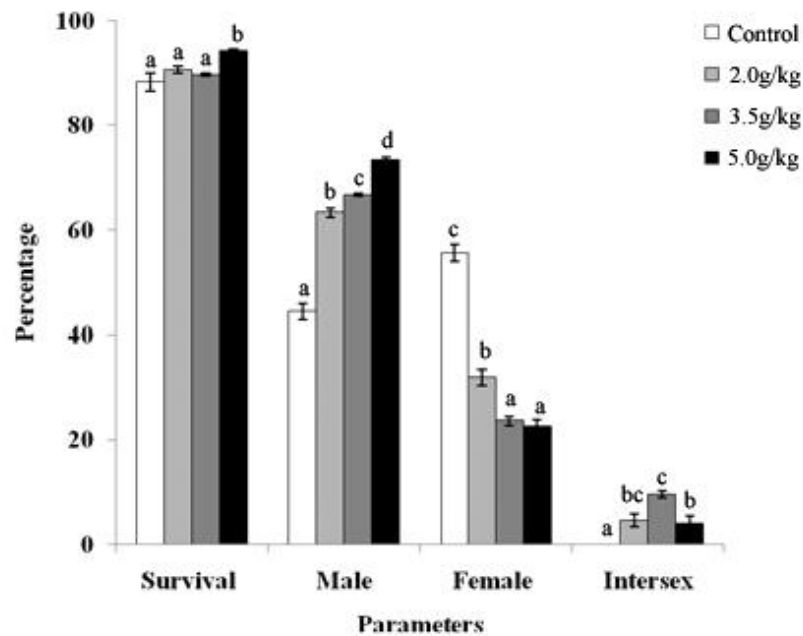
## 2.4 Statistical analysis

All data are expressed in terms of mean  $\pm$  standard error (SE). Treatment effects on different parameters were analyzed by one-way analysis of variance (ANOVA) after checking normality by Shapiro-Wilk's test. Where significant differences were found, a Tukey's test was performed for separating treatment means. All statistical analysis was performed using the SPSS version 11.5 for Windows.

## III. Results And Discussion

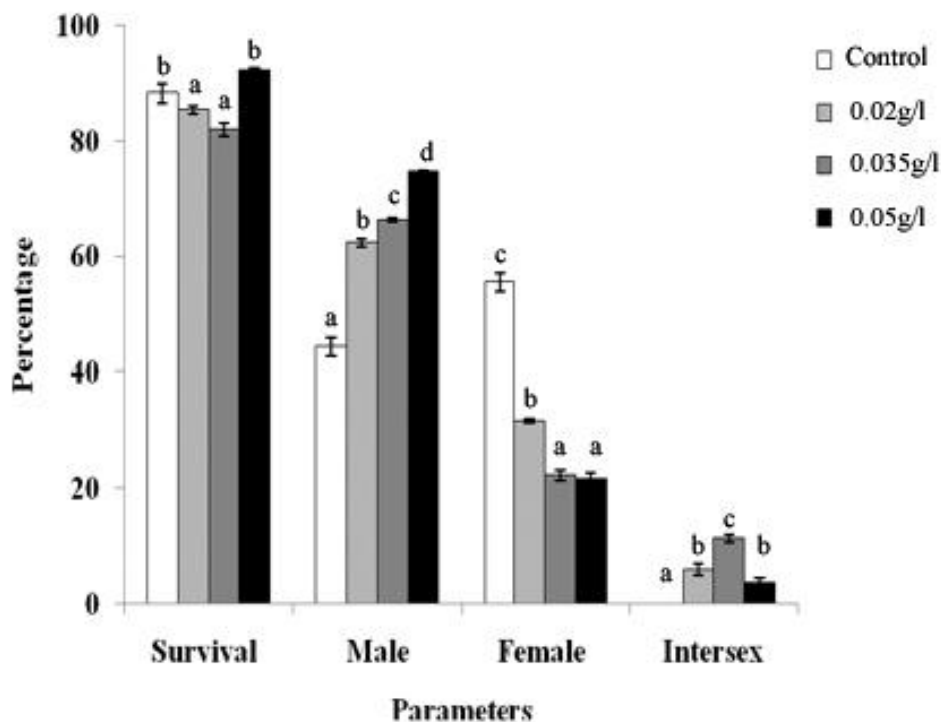
During dietary treatment with powdered *M. pruriens* seeds, treatment with the concentration of 5.0 g/kg resulted in significantly higher ( $P < 0.05$ ) survival percentage compared to other treatment categories and the control group showed the lowest survival percentage ( $88.3 \pm 1.7$ ) (Fig. 1). However, during immersion treatment with the aqueous extract of *M. pruriens* seeds, the control and 0.05 g/l categories showed significantly higher ( $P < 0.05$ ) survival percentage compared to 0.02 and 0.035 g/l groups (Fig. 2). These results indicate that both dietary and immersion treatment with *M. pruriens* seed powder and its aqueous extract, respectively, have no adverse effect on general fish health. In another study as well, toxic changes, stress and changes in behavior were not observed in rats treated with differential doses of ethanolic extracts of *M. pruriens* [18]. In a feeding trial with Nile tilapia, fish fed diets containing differentially processed mucuna seeds for 56 days, showed no mortality for the entire period of experiment [19].

In feeding experiment with *M. pruriens* seed powder, the highest percentage of males ( $73.33 \pm 0.67$ ) was observed at the concentration of 5.0g/kg, which is significantly higher ( $P < 0.05$ ) compared to all other groups. Fish fed diets containing plant material at any concentration showed significantly higher ( $p < 0.05$ ) percentage of males than the control group (Fig. 1). The control group showed the highest percentage of females ( $55.6 \pm 1.6$ ) and it was significantly higher ( $P < 0.05$ ) than all the treatment categories. However, there was no significant difference ( $P > 0.05$ ) in percentage of females among the different groups fed diets containing *M. pruriens* seed powder (Fig. 1). The control diet fed group showed no intersex fish while the highest percentage of intersex fish ( $9.67 \pm 0.67$ ) was observed in tilapia fed diets containing powdered *M. pruriens* seeds at the concentration of 3.5 g/kg (Fig. 1).



**Figure1:** Percentage of survival, male female and intersex during dietary treatment with *Mucuna pruriens* seed powder. Different alphabets above columns mark significant difference ( $P < 0.05$ ) in means.

Similar results were obtained during immersion experiment with aqueous extract of *M. pruriens* seed as well. Here also, treatment with the highest concentration of 0.05 g/l resulted in the highest percentage of male ( $74.67 \pm 0.33$ ), which was significantly higher ( $P < 0.05$ ) compared to all other groups (Fig. 2). Moreover, treatment with all concentrations of the extract resulted in significantly higher ( $P < 0.05$ ) percentage of males compared to the control (Fig. 2). All the treatment categories except the control group showed intersex with both male and female gonadal tissue and the highest percentage of intersex ( $11.33 \pm 0.67$ ) was observed in 0.035 g/l treatment group (Fig. 2).



**Figure2:** Percentage of survival, male female and intersex during immersion treatment with *Mucuna pruriens* seed powder. Different alphabets above columns mark significant difference ( $P < 0.05$ ) in means.

The ethanolic extract of *M. pruriens* seeds has been reported to significantly increase testosterone, LH, FSH and prolactin hormone levels, levator ani muscle weight, sperm count and motility in infertile obese mutant rat models [20]. It has been found to increase libido in men due to its dopamine inducing properties [21, 22]. Ethanolic extracts of *M. pruriens* seed produced a significant and sustained increase in the sexual activity of normal male rats and improved mount, intromission and ejaculation and decreased latencies at a particular dose [9, 18]. The plant has been reported to possess anti-depressant potency and libido enhancing effect, has an antispasmodic, antipyretic and anti-inflammatory activity, and has also been used to manage Parkinson's disease, anti toxin for snake bites [23, 24, 25]. Results emanating from this study indicate a dose dependent masculinisation effect of *M. pruriens* on Nile tilapia. As the highest treatment concentration of 5.0 g/kg feed and 0.05 g/l produced the maximum percentage of males among the different treatment categories in this study (Fig. 1, 2), further analysis with increased concentration might be required to achieve 100% sex reversal with *M. pruriens*.

Although the present work has indicated that dietary and immersion treatment with powder and aqueous extract, respectively, of *M. pruriens* might induce high rate of masculinisation, whether this potency is caused by increase in androgen level cannot be deduced as the serum testosterone level was not measured during the study. Qualitative analysis for phytochemicals revealed the presence of tannins, saponins, terpenoids, steroids and flavonoids in the aqueous extract of *M. pruriens* seeds, while alkaloids and carbohydrates are not present in the extract. These phytoconstituents might render the androgenic activity of the extracts. A variety of pathways have been postulated to be associated with functional mechanisms of phyto-compounds causing both masculinisation and feminization at different concentrations [26]. Further analysis is required to deduce the functional mechanisms behind the androgenic potency of the plant.

The results emanating from this study indicate that the plant might be used as an alternative method to produce all-male tilapia population in an environment-friendly manner using a natural product. The plant showed higher percentage of males during immersion treatment with aqueous extracts compared to dietary treatment with powdered plant material. But, no conclusive remark can be made regarding the best mode of application for the plant material as the concentrations used for both methods were not same. Dietary treatment might seem more practical approach for large scale production of monosex tilapia under field condition. Moreover, the highest percentage of males produced by the plant material was found to be well below the ideal requirement of 100% male population. Thus, further studies would be required to establish an ideal treatment regime for production of all-male tilapia population using the plant material and to provide conclusive evidence regarding its efficacy to be used as a sex-reversal agent in tilapia culture.

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