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RESEARCH ARTICLE

Application Of *Asparagus racemosus* Roots for Production Of Monosex Nile Tilapia, *Oreochromis niloticus*

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Abstract

The present study was aimed to investigate the effect of *Asparagus racemosus* root extract for masculinisation of Nile tilapia during its in vivo application through immersion technique. Three days old mixed sex juveniles of Nile tilapia (mean weight 0.025 ± 0.009 g; mean length 1.25 ± 0.012 cm) were subjected to immersion treatment with aqueous extract of the plant roots (0.01, 0.015, 0.02 g/L) for one month. Treatment with 0.015 g/L yielded the highest survival percentage (95.56±1.12). Treatment with the plant material resulted in significantly higher ($P<0.05$) percentage of males compared to the control groups. The highest percentage (90.60±1.56) of males was obtained in 0.015 g/L category, which was significantly higher ($P<0.05$) compared to all other treatment groups. The highest percentage of females (56.92±3.07) was observed in the control group and it was significantly higher ($P<0.05$) than all the treatment categories. The control diet fed group showed no intersex fish while the highest percentage of intersex fish (1.45±1.44) was observed at the concentration of 0.02 g/L of immersion treatment with *A. racemosus*. The study indicates that *A. racemosus* aqueous extract might be used as a potential alternative of synthetic steroids for production of all-male tilapia population.

INTRODUCTION

The Nile tilapia is a well accepted species for aquaculture because of its rapid growth, high tolerance to low water quality, efficient food conversion, resistance to disease, ease of spawning and good consumer acceptance (El-Saidy and Gaber, 2005). Nile tilapia females have high fecundity, generally reproduce at a small size and exhibit stunted somatic growth at higher densities, while male tilapias exhibit faster growth rates and are often the preferred gender for monosex aquaculture (Hines and Watts, 1995). The success of utilization of steroid hormones to produce monosex tilapia populations is well documented (Baroiller and D'cotta, 2001; Strussmann and Nakamura, 2002). However, due to the potential ecological and health-related hazards of such synthetic steroids, the use of plant materials is a potential alternative to be explored (Papoulias et al., 2000). 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 (Citarasu, 2010; Chakraborty et al., 2011). 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 (Rempel et al., 2008) and hence may be considered as potential mean for inducing sex reversal in fish.

But, 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 established. The plant, *Asparagus racemosus* has been reported to have medicinal values, various therapeutic uses and aphrodisiac effects in mammals (Jetmalani et al., 1967; Gaitonde et al., 1969; Dhahanukar et al., 1986; Thatte et al., 1987; Thakur et al., 2009; Mishra et al., 2010; Alok et al., 2013). It was found to stimulate growth in fish as well (Borkar et al., 2014). However, no studies have been conducted to determine its *in vivo* effect on sex reversal and production of monosex fish population. Hence, the objective of the present study was to investigate the potential effect of the plant on the masculinisation of Nile tilapia through immersion technique as method for *in vivo* application of the plant material and to determine an ideal concentration that might produce maximum percentage of males in tilapia.

Materials and Methods

a. 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.

b. Plant-extract preparation

A. racemosus roots 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 45ºC using a rotary evaporator and stored under nitrogen at -20ºC in amber glass bottle until those were used.

c. 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 glass aquaria (40 fish / aquaria) to 4 different treatment groups (0.0, 0.01, 0.015 and 0.02 g/L) for the plant extract and three aquaria were assigned to each treatment group. 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 ± 2ºC) static systems. Water in all aquaria was replaced manually and the fish was kept under similar photoperiod (14 L: 10 D). The fish was fed finely ground (< 500-1000 µm) artificial diet containing 30% crude protein (Tokyu, Japan) at a rate of 20% body weight / day.

d. Sexing of fish

Sexing of the juvenile fish was done by the standard acetocarmine squash technique of gonads (Guerrero and Shelton, 1974). Histological studies of the gonads were also performed.

e. Qualitative phytochemical studies

Qualitative phytochemical analysis of the aqueous extract of *A. racemosus* roots were carried out using standard procedures (Malpani et al., 2011. Kumar and Bhardwaj, 2012. Ray et al., 2013).

f. Statistical analysis

All data are expressed in terms of mean ± 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.

Results

Immersion treatment with aqueous extract of *A. racemosus* root at the concentration of 0.01 g/L resulted in significantly higher ($P<0.05$) survival percentage (95.56±1.12) compared to control, 0.015 and 0.02 g/L treatment categories (Fig. 1). The highest percentage of males (90.60±1.56) was observed at the concentration of 0.015 g/L, which is significantly higher ($P<0.05$) compared to all other groups (Fig. 1). The control group showed the highest percentage of females (56.92±3.07) and it was significantly higher ($P<0.05$) than all the treatment categories (Fig. 1). The control diet fed group showed no intersex fish while the highest percentage of intersex fish (1.45±1.44) was observed at the concentration of 0.02 g/L of immersion treatment with *A. racemosus*. (Fig. 1).

Discussion

The high survival percentage of fish in different treatment categories indicates that immersion treatment with aqueous extract of *A. racemosus* root have no adverse effect on general fish health. Acute toxicity study with aqueous extract of *A. racemosus* in rats showed no fatality even with the highest dose of 3200 mg/Kg (Kumar et al., 2010). Oral administration of suspension containing lyophilized aqueous extracts from *A. racemosus* roots at a dose of 200 mg/kg body weight showed pronounced anabolic effects such as significant weight gains in the body and reproductive organs, significant reduction of mount latency, ejaculation latency, post ejaculatory latency, intromission latency, and a considerable increase of mount frequency and penile erection in male albino rats (Thakur et al., 2009). The plant also aids in the treatment of neurodegenerative disorders and in alcohol abstinence-induced withdrawal symptoms (Thakur et al., 2009). It has adaptogenic potency, antioxidant, immunostimulant, anti-dysepsia and antitussive effects. Oral administration of powdered root of *A. racemosus* was reported to produce leucocytosis and predominant neutrophilia along with enhanced phagocytic activity of the macrophages and polymorphs in rats (Alok et al., 2013).

Although the present work has indicated that immersion treatment with aqueous extract of *A. racemosus* 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 and terpenoids in the aqueous extract of *A. racemosus*, while alkaloids, carbohydrate, glycoside and flavonoid are not present in the extract (Table 1). Five steroidal saponins have been isolated from the roots of *A. racemosus* (Gaitonde et al., 1969). These phytoconstituents might be responsible for the masculinisation effect of the extracts. Nile tilapia fry fed diets supplemented with *Quillaja* saponin was reported to show significant change in sex ratio in favor of males (Francis et al., 2002). However, the masculinisation potency of saponin might not exclusively be due to the androgenic activity of the chemical, but also because of its inhibitory effect on the enzyme aromatase (Golan et al., 2008). A variety of pathways have been postulated to be associated with functional mechanisms of phyto-compounds causing both masculinisation and feminization at different concentrations (Chakraborty et al., 2014). Further analysis is required to deduce the functional mechanisms behind the masculinisation potency of the plant.

The results derived from this study signify 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. However, 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.

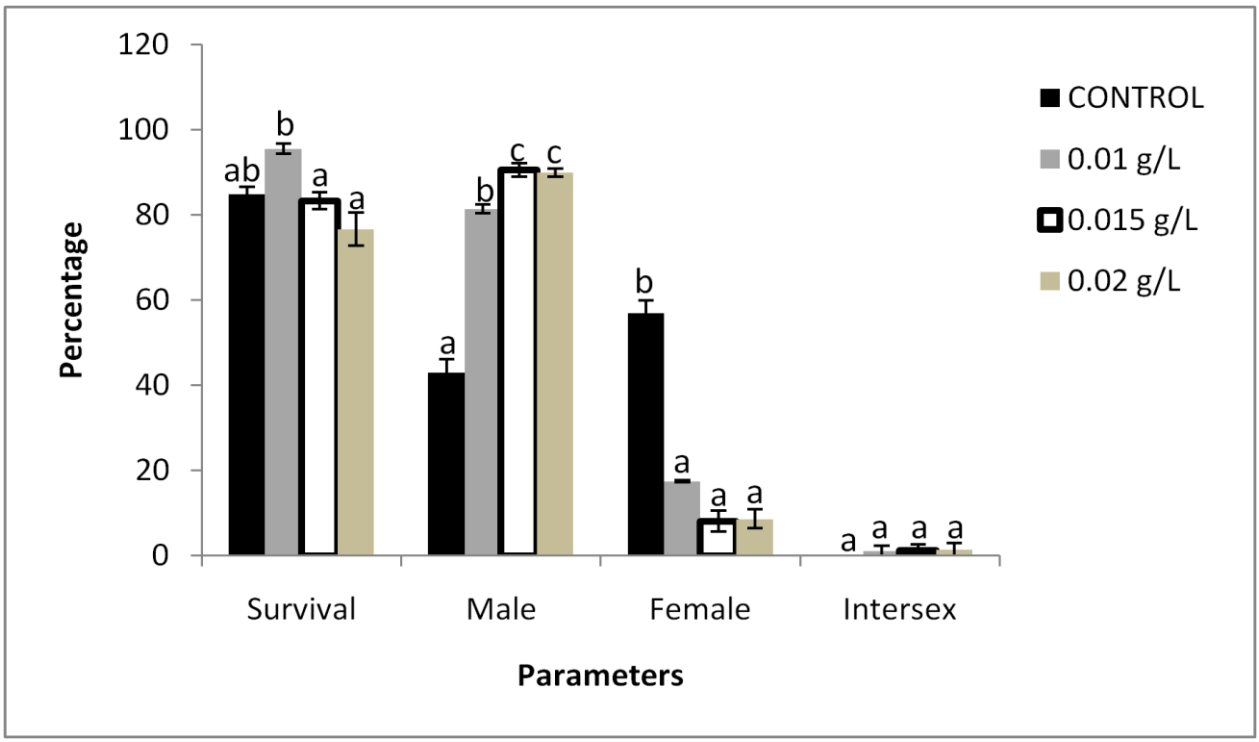

Fig. 1: Percentage of survival, male, female and intersex during immersion treatment with aqueous extract of *Asparagus racemosus* roots. Different alphabets above columns mark significant difference (P<0.05) in means.

Phytochemicals	Present (+)/ Absent (-)
Alkaloid	-
Carbohydrate	-
Flavonid	-
Glycoside	-
Saponin	+
Steroid/ Terpenoid	+
Tannin	+

Table 1: Qualitative analysis of phytochemicals in aqueous extract of *A. racemosus* root.

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