Antifertility, antioxidants and hemo-immunological effects of Jantar (*Sesbania sesban*) powder on *Oreochromis mossambicus*

By

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2020-2022

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A thesis submitted in partial fulfillment of the requirements

for the Degree of

MASTER OF PHILOSOPHY

IN

ZOOLOGY

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2022

CERTIFICATE

This dissertation ‘‘**Antifertility, antioxidants and hemo-immunological effects of Jantar (*Sesbania sesbans*) powder on *Oreochromis mossambicus*’’.** submitted by **Adnan Khan** is accepted in its present form by the Department of Zoology, Faculty of Biological sciences, Quaid-I-Azam University, Islamabad, as satisfying the thesis requirement for the degree of Master of Philosophy in Zoology.

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Date: _____

Declaration

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of this thesis has been previously presented for any other degree.

ADNAN KHAN

Dedicated to:

My loving parents, siblings, and respected supervisor.

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List of Abbreviations

POD	Peroxidase
SOD	Superoxide dismutase
CAT	Catalase
SS	*Sesbania sesbans*
EDTA	Ethylene diamine tetra acetic acid
BSA	Bovine serum albumin
ALT	Alanine transaminase
AST	Aspartate transaminase
LDH	Lactate dehydrogenase
H_2O_2	Hydrogen peroxide
Hrs	Hours
LPO	Lipid peroxidation
PBS	Phosphate buffer saline
ROS	Reactive oxygen species
TBARS	Thiobarbituric reactive substance
G	Gram
M	Molar solution
µmol	Micromole
mM	Millimole
µg	Microgram
Kg	Kilogram
mg	Milligram
OD	Optical Density
MT	17α-methyl testosterone
FCR	Feed Conversion Ratio
SGR	Specific Growth Rate
WG	Weight Gain
IBW	Initial Body Weight
FWB	Final Body Weight
OAG	oleanolic acid 3-β-D glucuronide

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Acknowledgements

Praise is to Allah, Lord of the Worlds. The Most Beneficent, the Most Merciful, Who is the entire source of knowledge and wisdom endowed to mankind, Who gave me courage and potential to pursue this goal Whom I believe that He never spoils any effort of good deeds. Blessings of ***Allah*** *be upon His* ***Prophet Muhammad (SAW)****", the city of knowledge and blessing for entire creature, who has guided his* ***Ummah*** *to seek knowledge from Cradle to Grave, and enabled me to win honour of life.*

It is a matter of great pleasure to express my sincere regards to my honourable supervisor Prof. ***Dr. Amina Zuberi****, Chairperson, Department of Zoology, for affectionate supervision, inspiring attitude, masterly advice, and encouragement. Without her useful intellectual suggestions, it would have been impossible for me to complete this tedious work.*

I must acknowledge my deepest debt of gratitude to my teachers from Department of Zoology, QAU, Islamabad.

I am thankful to ***Higher Education Commission*** *for providing me financial support for completing my M. Phil degree on behalf of* ***HEC Indigenous 5000 Ph.D. Fellowship Programme.***

My special thanks to ***Dr. Imdad Ullah, and Dr. Muhammad Ahmad*** for making me this research work possible. *I wish to express my deep and sincere gratitude to my most cooperative Ph.D. seniors* ***Mr. Waqar Younas, Mr. Muhib Jan, and Mr. Muhammad Noorullah*** *for their encouragement and personal guidance.*

My heartfelt thanks to my seniors ***Mr. Sami Ullah, Mr. Mashooq Ali, Ms. Zara Naeem, Muhammad Kamran, Samreen Tariq, Sheheryar, Muhammad Abbas, Kiran Aftab,*** *and dear labmates* ***Shawal Khan, Muhammad Aleem Khan,*** *and* ***Rehmana Aslam*** *for their care and moral support****.***

I must acknowledge my debt to my junior lab fellows ***Muhammad Asad, Javeria Shamas, Sadia, Dua Laraib, Waliullah, Andleeb, Muhammad Shahid, Muhammad Alam*** *and* ***Aiman Aziz*** *for their kind help and cooperation during my research work. I am also thankful to them for their nice company and time they provided me with beautiful memories that I will treasure throughout my life.*

I am obliged to ***Sher Ali (FARC, QAU), Ali, and Yasir*** *for their help, and support during my research.*

My heartfelt thanks to my batch fellows ***Arshad Khan, M. Tufail, Tariq Aziz, Inamullah, Asifullah, Sadrudin, Sana Ahmed, Bilal Khan,*** *my dearest friend* ***Aleesha Asghar*** *and other fellows from faculty of Biological Sciences for their nice company, cooperation and help during my research work.*

Words always seem to shallow whenever it comes to my beloved ***Ammi*** *for their constant love, care, and endless support. I am thankful to my father* ***Muhammad Ismail*** *for providing me a chance to prove and improve myself through all walks of life. I owe my heartfelt thanks to my elder sister* ***Sara Akbar****. My younger brothers* ***Bilal Akbar****, and* ***Basit Akbar*** *deserves special mention for their incredible care, love, respect, and prayers.*

A non-payable debt to my loving parents, cousins and family, their wish motivated me in striving for higher education; they prayed for me, shared the burden, and made sure that I sailed through smoothly.

I would like to thank my uncles, ***Col Ilyas Khan, Prof. Dr. Muhammad Iqbal, Shah Nawaz Khan, Arshad Khan, Chan Mubarak, Abdul Qayum, Maqbool Hussain*** *and brother-in-law* ***Waqas Mubarik*** *for prayers and support during my entire student life.*

I am thankful to all those who helped me.

ADNAN KHAN

Abstract

Tilapia is the second most culturable fish after carps. However, wild tilapia (*Oreochromis mossambicus*) poses a significant danger to the freshwater system especially in aquaculture due to its rapid breeding. About 75% of the published research indicates the detrimental effect of tilapia's introduction in aquaculture. Similarly, hormonal administered mono-sex tilapia is also not satisfying the consumer's demand due to its potential hazardous effects. Therefore, this study is executed to explore the antifertility and growth-promoting effects of the medicinal plant *Sesbania sesban* in *O. mossambicus*. A 90-day feeding trial in a replicate of three was conducted in an indoor facility under semi-control conditions. Uniform size, active fry of *O. mossambicus*, average body weight 1.7± 0.4g were equally distributed in 21 glass aquaria (13 fry/aquarium) having well-aerated water. The aquaria were randomly divided into 7 groups, one group (C) was fed 40% crude protein basal diet while others, each of three groups (S1, S2, S3 and R1, R2, R3) were fed a basal diet fortified with graded level i.e., 12.5, 25 and 50g per kg diet of S. *sesban* seed and root powdered. Initially, fish were provided feed at the rate of 7% body weight, three times a day. Afterward, based on the body weight, feeding frequency and ratio was changed. Results indicated a dose-dependent significant effect of both seed and root of *S. sesban* on the growth performance of *O. mossambicus*. However, all pairwise comparisons among groups indicated the most significant effects of *S. sesban* seed compared to roots. The S3 group showed the highest weight gain and SGR and the lowest FCR. In addition to these S *sesban* supplemented diet also showed significant (P>0.05) dose-dependent positive effects on blood indices i.e., S3 group showed the highest RBCs and WBCs count, hemoglobin level, HCT%, MCV, MCH, MCHC; and status of antioxidant enzymes SOD, POD, CAT, and the lowest LPO level. However, metabolic enzymes, AST, ALT, and LDH did not show any significant difference among the treated groups and control group. Both seed and root of *S. sesban* showed dose-dependent negative effects on the GSI of both male and female fish. In male, both roots and seeds at higher doses showed statistically similar effects, i.e., 92% decrease in GSI as compared to the control group. However, in females, the roots of *S. sesban* showed the most significant effect on the GSI, in contrast to plant seeds. The GSI of the R3 group was 94.6% decreased as compared to control, while the S3 group showed 89.8% reduction in GSI. Serum testosterone level also showed a dose-dependent decrease in response to both roots and seeds of *the S. sesban* plant. However, at higher dosage levels, in contrast to roots, seeds of plants most significantly reduced the testosterone level. Based on results, *the S.*

sesban plant could be recommended for improving the growth, health status, and controlling the reproduction of *O. mossambicus*

Introduction

Aquaculture is defined as the rearing of aquatic organisms (animals and plants) including fish in sea water, freshwater, and brackish water (El-Sayed, 2006). Aquaculture was firstly practised in 5th century at China (Pillay and Kutty, 2005). Oysters were reportedly grown intertidally in Japan 3000 years ago, as well as by the Romans almost 2000 years ago (Stickney, 2005; Ampofo-Yeboah, 2013). Research on sterility causing agents is going on to solve the problem of 'Population Explosion' globally. Hormonal drugs are available, but they have side effects. So, the indigenous medicinal plants can be used to produce a suitable product which could be used as alternative for hormonal drugs. (Singh *et al.,* 1990). Globally aquaculture has grown in recent decades as compared to farmed meat production and catch fisheries (FAO, 2003). Fisheries sector is expanding due to rising demand of fish, there is an immediate need for sustainable aquaculture by improving skills and scientific based approaches to meet the demands. In this way, profit and income will be generated and economic goals will be achieved (Asmah, 2008).

Before the Pandemic, An Overview of Fisheries Sector

Global fisheries and aquaculture output (excluding aquatic plants) hit a new high of over 179 million tonnes of live weight equivalent in 2018. Overall, catch fisheries accounted for 54% of the total, with 96.4 million tonnes, while aquaculture represented for 46% with 82.1 million tonnes. Aquaculture has been the primary engine of increased fish output over the past three decades, but the catch fisheries industry still dominating for several species and essential for local and international food security. China, Indonesia, India, Vietnam, and Peru are the leading producers in 2018, with China, Indonesia, India, Vietnam, and Peru leading the way (FAO, 2021).

As a result of the COVID-19 pandemic, global aquaculture production is predicted to drop by 1.3 percent, with more disruptions possible in 2021 as lockdowns influence supply and demand throughout the sector (FAO, 2021).

Aquaculture's Contribution to Global Food Consumption

Our earth is predicted to contain 9.7 billion people by 2050, putting significant strain on our agricultural systems to adequately feed a rising human population (Sampantamit *et al.,* 2021; Gentry *et al*., 2017). Globally, fish contributes for 15.7% of consumed protein of animal origin, and 6.1% of overall protein consumption. The supply of fish in developing countries was an average of 15.1 kg / capita annually, while in Sri Lanka, Haiti, Chad, Ghana, Iraq, Zimbabwe, Bangladesh, and Kiribati which are poor countries, the supply was 14.4 kg / capita annually (FAO, 2010). The global average yearly per capita intake of fish grew significantly from 19.9 kilogrammes to 20.5 kilogrammes between

2014 and 2019. Globally, 21 kg per capita consumption was expected in 2019. Fish consumption in North America was projected to be 23.7 kilos per capita in that year (Shahbandeh, 2020). (Shahbandeh, 2019). Aquaculture's expansion, on the other hand, is not evenly distributed over the globe, with significant differences between areas and nations in terms of farming practices, production level, producer profile and species composition. According to an FAO assessment in 2010, the Asia–Pacific area accounts for 89.1% of world fisheries production, with China adding 62.3% in 2008 (FAO, 2011).

Fisheries in Pakistan

Fisheries play a significant part in Pakistan's economy, as well as being a considerable source of nourishment, money, and employment. Pakistan has a diverse range of marine and inland water resource. This region's fishing resources offer enormous economic growth potential (Baset, 2020). The rivers, lakes, ponds, and water lodging places cover an area of around 8,563,820 km^2 in Pakistan (Jarwar, 2008; Laghari, 2018). Although there is a large potential for coastal fishing with a 1120 km coast line, there has been no substantial advancement in the fisheries industry. Furthermore, Pakistan controls an open sea region of around 350 nautical miles known as the Exclusive Economic Zone. The practice of aquaculture is the key factor. Extensive and semi-intensive aquaculture systems have only been used in freshwater. As a result, we are completely reliant on natural availability in the marine realm, as marine culture has yet to be fully adopted. As a result, the aquaculture farming system's production is anticipated to be between 179,900 and 600,000 metric tonnes, depending on the natural catch (Minfal, 2012; Laghari, 2018).

Pakistan is an agriculture-based country, with agriculture employing almost half of the working population, either directly or indirectly. This industry accounts for around 25% of GDP and is a significant source of foreign exchange. Fisheries, as a sub-sector of agriculture, is important for economic growth. Overall, 400,000 people work directly in this sub-sector in Pakistan, with another 600,000 working in ancillary sectors (Mohsin et al., 2017). With the passage of time, capture fisheries productivity in Pakistani marine waters is declining. This is due to the continuing overfishing in Pakistani marine environments. The water sector is subject to an open access regime because there is no attention, proper planning is lacking and execution of policy too. As a result, because marine resources are now under stress, overfishing must be prevented to maintain fisheries resources, and there must be a viable alternative for producing fish for human consumption (Mohsin et al., 2017; Memon et al., 2015).

Aquaculture's Contribution to Food Security and Poverty Alleviation

According to FAO (1996), those people having economic as well as physical approach to healthy, safe and enough food for their dietary requirements at all the times, are having the state of food security. It includes 800 million people without appropriate food, 192 million children whose poverty

now indicates a future lack of possibilities and feeding 9,000 million people by 2030. As a result, the (FAO) will need to address these issues efficiently (Shaw, 2007). According to the World Bank (2004), poverty is a multifaceted aspect of failure to meet the basic requirements, supplies uncontrolled, less knowledge and training, nutritional deficiencies, inadequate housing, poor clean water and sanitation, shock vulnerability, violence and crime, and lack of government freedom and voice" (Akiyama, 2005).

Several key causes have thrown the world off course in terms of ending world hunger and malnutrition in all kinds by 2030, dating back far before the COVID-19 epidemic. The sustained drop in world hunger that began in 2005 came to an end in 2014. People affected by the malnutrition slowly boosted until in 2020 the world saw an exceptional complication in its aim to get rid of hunger. Furthermore, progress in lowering child stunting has halted, and adult overweight and obesity are on the rise in both developed and developing nations. (IFAD, 2021). There are an estimated 925 million hungry people throughout the globe, and around 1.4 billion people are living on less than US$ 1.25 per day, putting them in severe poverty (Kanayo, 2012).

Taxonomy of Tilapia

"Tilapia" is the generic name for; Oreochromis, Sarotherodon, and Tilapia. The key distinguishing feature among them is reproductive behaviour. All tilapia species make nests; Oreochromis and Sarotherodon species are mouth brooders (Popma, 1999).

Scientific Classification of Mozambique tilapia (ITIS - Report: Oreochromis mossambicus)

Oreochromis mossambicus (Peters, 1852)- Mozambique mouth breeder

Culture of Tilapia

Marine fishes account for 3% of global aquaculture production, followed by diadromous fishes 6.0%, crustaceans 9.6%, molluscs 23.6%, freshwater fishes 56.4%, and other aquatic animals 1.4% (FIPS, 2012). Tilapia is the world's second most farmed fish, behind carp. Tilapia contains a greater concentration of omega-3 fatty acids than beef, pig, or chicken. Since wild fishes and other farmed fishes require animal source protein-based feed for good growth, so it is sustainable to farm tilapia in aquaculture as this fish can grow well on algae or some other plant source protein feed (Towers, 2015).

Table 1 shows the features of commercially important tilapia species modified from Mair 2001 (Gupta, 2004).

Species	Common name	Characteristics
Oreochromis niloticus	Nile tilapia	Performs well in tropical and subtropical regions; sexual maturity in ponds occurs at the age of 5-6 months; least tolerant to cold water; suited for a variety of farming systems (monoculture and polyculture); good consumer and producer acceptability.
O. aureus	Blue tilapia	Good for rearing in countries with seasonal temperature variations; most cold resistant species; often used in hybridization for monosex tilapia production; sexual maturity in farms occur at 5-6 months.
O. mossambicus	Mozambique tilapia	Early reproduction (reaches sexual maturity at 8-9 cm) and high fecundity; high saline tolerant
O. spilurus	None	Used in marine cage culturing since it is saline tolerant.
O. hornorum (Tilapia urolepis)	Zanzibar tilapia	It is brakishwater tolerant
Sarotherodon galilaeus	Gallilee tilapia	Growth is slow; tolerant to saline conditions.
S.melanotheron	Black-chinned tilapia	Excellent for brackishwater aquaculture; high salinity tolerant; largely being used in extensive aquaculture in Africa.
Tilapia rendalii	Redbreast tilapia	Macrophytes feeder
T. zillii	Redbelly tilapia	Good growth in sea
Red tilapia hybrids	Hybrid origins	Its parental species are good saline tolerant; it is good for brakish-water and seawater; also used in intensive cultures (cages, tanks, raceways), but it is also suitable for farming under low-input conditions; it has a high consumer acceptance due to its colour; its fecundity is low.

Tilapia civilization is said to have begun about 4000 years ago, as seen from pyramidal drawings from an Egyptian tomb (El-Sayed, 2006; Crespi, 2009). Modern tilapia farming was first tested in Africa in 1924, according to Hatton and Balarin (1979). The introduction of tilapia into many regions of the world in the middle of the twentieth century is said to have exploded the tilapia culture (Pillay, 1997; De Silva, 2004; Ampofo-Yeboah, 2013). Tilapias have all the desirable features of an excellent culture fish species, including adaptability to different environments, hardiness, and wide feed acceptance. So, tilapias have risen to become one of the three major commercial fish species (El-Sayed, 2006). The early enthusiasm for tilapia as a food fish was tempered by unregulated pond breeding, which resulted in excessive recruitment, retardation, and a small percentage of marketable-sized fish. In 1970s, techniques of sex-reversal by hormones caused a breakthrough due to which all monosex males were raised to a uniform, commercial size. As a result, tilapia's market developed since 1980s along with feed, culturing, processing advancements (Crespi, 2009). Oreochromis genus dominates the tilapia culture in which China contributes 39.4 percent (Ampofo-Yeboah, 2013).

Aim of Study

Tilapia introductions pose a significant danger to the biodiversity of brackish water and freshwater ecosystems, according to aquatic scientists and conservation biologists. Over 75% of published research indicated that tilapia introductions had a detrimental impact (Deines et al., 2016). Twenty-six percent of nations that have had tilapia introduced have reported varied degrees of environmental consequences (Deines et al., 2016); this number is far greater than the most severe 20% and the average 5% recorded (Gozlan, 2008). Too much fingerling recruitment in grow-out systems leads to overcrowding and resource competition, resulting in stunted and small-sized fish that are unable to fetch attractive market prices (Teichert Coddington, Manning, Eya, & Brock, 2000). To reduce undesired reproduction in tilapia production systems, frequent harvesting of fingerlings or fry, polyculture with predator fish, high-density culture, cage culture, sterilization by heat shock, and all-male tilapia culture are used (Fortes, 2005; Mair & Little, 1991).

To control prolific breeding and get good growth, all male tilapia population is cultured as male grow faster than female and attain good weight, resulting in short production cycle (Baroiller & D'Cotta, 2018; Beardmore et al., 2001; El-Greisy & El-Gamal, 2012; Megbowon & Mojekwu, 2013). Techniques like sex sorting by using hand; using androgens as hormonal sex inversion; chromosomal or genetic modification and environmental manipulation (like temperature treatment) are all used to create all-male populations of tilapia (Angienda, Aketch, & Waindi, 2010; Dauda et al., 2014; Olufeagba & Okomoda, 2015). To yield high level of masculinization, 17α-methyl testosterone (MT) exogenous steroids is used as hormonal sex reversal method (Homklin et al., 2011). The ability of MT to block aromatase activity, inhibiting estrogen production while boosting androgenesis in the differentiating gonads, is linked to its high sex reversal potency (Golan & Levavi-Sivan, 2014). As a

result, in tilapia farming, MT is the most extensively employed strategy for producing all male individuals. The carcinogenicity of MT, as well as its negative impacts on human health and marine environments, continue to be a source of public concern (Abo-Al-Ela, 2018). Fetotoxicity and hepatotoxicity occurs if MT application is exposed for longer period (Velazquez & Alter, 2004; Vick & Hayton, 2001). MT is hazardous to personnel involved in operation of tilapia seed development (Megbowon & Mojekwu, 2013). Another drawback is that during feeding, the hormone administered in diet is unavailable to the fish (Ramirez-Godinez et al., 2013; Vick & Hayton, 2001). For sex inversion, only 10% of the hormone is utilized when administered in diet (Ong, Chotisukarn, & Limpiyakorn, 2012).

Hormone residues accumulate as active metabolites excreted by the treated fish or residues from uneaten food in the confined habitats consequently. The hydrophobic property of MT, which allows it to quickly adsorb onto sediments, aids the buildup of hormone residues (Abaho et al., 2021; Mlalila et al., 2015). The release of MT and its metabolites into the aquatic system from unconsumed or unmetabolized food has the potential to adversely affect nontarget aquatic creatures' endocrine and reproductive systems. (Nian, Tumbokon, & Serrano, 2017). Consumers do not accept that fish which is treated with synthetic chemicals because of safety concerns (Reverter et al., 2014). There is ban on use of synthetic hormones in several countries for use in fish food production due to negative consequences (Chakraborty, Horn, & Hancz, 2014). As a result, attempts to find and create ecologically sustainable, commercially feasible, and socially acceptable substitutes to synthetic steroids are needed.

Use of Plant Materials for Controlling Tilapia Reproduction

To achieve fish production sustainably, the organic plant extracts are easy to prepare, safe and inexpensive relatively. So as an alternative to hormones, drugs and chemicals, fish culture is carried on plant extracts to minimize harmful effects to human and environment associated with aquaculture (Chakraborty et al., 2014; Hoseini, Mirghaed, & Yousef, 2019; Reverter et al., 2014). Accordingly, chemicals and synthetic hormones need to be replaced with novel plant extracts for tilapia production. Thus, by adopting environmentally friendly and safe fish production techniques, market value of tilapia will be promoted (Mehrim et al., 2019; Citarasu, 2010).

Plants possess natural compounds like steroids, tannins, alkaloids, flavonoids, terpenoids which enhance androgenic and anabolic processes, in addition stimulate the appetite, digestion, and immunity (Chakraborty et al., 2014; Citarasu, 2010; Gabriel, Qiang, Kpundeh, & Xu, 2015; Abaho, 2021). Phytochemicals like saponins having steroidal nature (Golan et al., 2008) and another, flavonoids inhibit the action of aromatase thus attenuating estrogen production (Tarigan et al., 2017). Phytochemicals have affinity of binding to estrogen receptors by competing with endogenous estrogens, therefore suppress biosynthesis of estrogen (Golan et al., 2008). Conversely, Phytochemicals also have some functional effects by acting as “phytoandrogens” similar as testosterone possess in

animals, thus they elevate male reproductive characters (Turan & Akyurt, 2005). To control the prolific breeding of tilapia, either fertility impairment or masculinization in fish has been induced by potential use of phytochemicals with good outcomes (Nian et al., 2017). The next section describes the selected species of plant used to avoid prolific breeding of tilapia.

Sesbania sesban

Plant Profile of *Sesbania sesban*

Leaves of SS are of anthelmintic nature, useful in diabetes, and skin diseases. Seeds of SS are stimulant, astringent, and useful in diarrhea, skin infections and itches are treated using seed paste (Yousaf et al., 1994). Previously stigmasta-5, 24 (28)-dien-3-ol-3-O-β-D galactopyranoside, lignins (Hossain et al., 2007), fatty acids and amino acids (Gupta et al., 1989), and an anti-tumor principle kaempferol trisaccharide (El-Sayed, 1991) were isolated by the phytochemical analysis of SS.

Hypothesis

We predict that supplementation of *Sesbania sesban* positively induces infertility, enhances growth performance, antioxidant activity, and haemato-biochemical indices in the *Oreochromis mossambicu*s. So, to prove our hypothesis, our research will focus on following objectives:

Aims and Objectives

- To assist the beneficial effects of *S.sesban* plant by feeding seeds and roots supplemented diet to O. mossambicus for 90 days and govern the effects on
  - Haematological indices (RBCs, WBCs, HCT, Hb etc).
  - Red blood cells indices (MCV, MCH, MCHC).
  - Blood biochemical indices including (AST, ALT, and LDH)
  - Antioxidants enzymes (SOD, POD, CAT) and TBARS
- To assess the effects of S.*sesban* supplementation on growth parameters, gonads development and effects on testosterone hormone level of O.*mossambicus*.

MATERIALS AND METHODS

Collection of Fry

Healthy fry of tilapia, *Oreochromis mossambicus*, were collected from NARC Islamabad and transported to Fisheries and Aquaculture Research Center, QAU, Islamabad. Initially they were placed in cemented tank and acclimatized for 14 days. During this period, they were nourished with 40% protein basal diet.

Feed Preparation

Foods containing 40% crude protein and *Sesbania sesbans* (seeds & roots) used in the experiment was prepared at Fisheries and Aquaculture Research Center. The *Sesbania sesbans* plant was finely grind and then mixed with other finely grind feed ingredient. *Sesbania sesbans* free feed was used for the control treatment. Other treatments included the *Sesbania sesbans'* differential concentration in the diet. All dry ingredients i.e., fish meal, wheat bran, rice polish, sunflower oil, soybean meal, gluten and vitamin mineral premix were mixed according to their calculated concentration. After that *Sesbania sesbans* were added to dry ingredients in their respective diet.

All ingredients were carefully mixed with oil and then little water was added so that dough was formed. Then this dough was passed through meat grinder so that pellets were formed, and these pellets were placed in the oven at 60°C overnight. When pellets fully dried, then these pellets were converted into small pieces according to fish mouth size. Then these small pieces of feed were packed in airtight jars at room temperature. The treatment diets and the control diet (basal feed) were given thrice a day to fish at 7% body weight initially. Then as the fish grew, the proportion of feed was reduced to 5%.

Experimental Design

For a 90 days trial, uniformed size, active and healthy fish were chosen regardless of their sex. Fish were distributed in indoor aquariums at a stocking density of 1.5g/L (12 fish/ aquarium). The aquariums were placed in the FARC, QAU, Islamabad.

Almost 273 fry with mean weight of 1.71g were randomly distributed in triplicate into 21 glass aquaria 90 × 40 × 40 cm, capacity 144L (12 fish/aquarium; stocking density 1.5g/L) and was fed daily for 90-days and the feed percentage was adjusted according to the body weight. During the experiment trial, essential water quality parameters were monitored at weekly interval.

Experiment was divided into seven groups named as Control 0, and treatments T1, T2, T3, T4, T5, and T6 groups. First group 0 was labelled as "Control group" and other six were treatment groups.40% crude protein diet was prepared with or without supplementation as follows.

Group 0: Diet without any supplement (0)
Group 1: Diet with 1.25% seed supplement (T1)
Group 2: Diet with 2.5% seed supplement (T2)
Group 3: Diet with 5% seed supplement (T3)
Group 4: Diet with 1.25% root supplement (T4)
Group 5: Diet with 2.5% seed supplement (T5)
Group 6: Diet with 5% seed supplement (T6)

Each fish feed group was given feed initially at 7% of their body weight which was then adjusted with the growth rate of fish to 5%. Overfeeding was minimized and feeding rate was maintained. Fish from each aquarium were weighed every week.

Water Quality

Aquarium water has been continuously aerated using aerators to keep dissolved oxygen levels near saturation. The aquariums were provided with optimum temperature conditions i.e., 28°C temperature. Temperature and pH of the aquariums was also recorded daily at dawn and dusk. Total ammonia nitrogen, nitrite and nitrate concentrations were measured with the help of ammonia kit (API freshwater master test kit).

Sampling and growth measurement

Fish were kept unfed for 24 hours before its collection for sampling. Fish were then, given anaesthesia immediately, with MS-222(70mg/L). After this fish body weight and length were measured. Blood samples were taken from the fish, blood was stored in EDTA tube for study of haematological parameter and then fish were sacrificed on the ice box. Muscles, gills, intestine, liver, brain and gonads was separated, and weight of gonads were taken and then all of these organs were collected in zip-lock bags which were then fixed in liquid nitrogen and stored in refrigerator at -20°C for further analysis. Gonads were also preserved in 10% formalin for histology. Centrifugation of the heparinised blood was carried out for 15 minutes at 3500rpm and plasma was separated which then placed at -20°C for further haematological analysis.

Growth performance

After 90 days feed trial of tilapia *Oreochromis mossambicus,* parameters such as growth performance, Percent Weight Gain (%WG), Specific Growth Rate (SGR%), Feed Conversion Efficiency (FCE), and Feed Conversion Ratio (FCR) was evaluated using the following formulas.

Weight Gain = FBW-IBW

Where,

FBW= Final body weight of fish

IBW= Initial body weight of fish

%Weight gain (%WG): It was calculated by given formula:

$$WG(g) = Wf(g) - Wi(g))$$

$$WG\% = \frac{Wf(g) - Wi(g)}{Wi\ (g)} \times 100$$

Average daily weight gain (ADG) = Final weight −Initial weight/Days

Survival rate (%) = Ni – Nf/ Ni × 100

Where,

ln= Natural log

Nf = Final number of fish (Fingerlings)

Ni = Initial number of fish (fingerlings)

Specific growth rate

Specific growth rate (SGR) was calculated by the formula given below:

$$SGR\ (\%body\ weight/day) = \frac{\ln Wf(g) - \ln Wi\ (g)}{days} \times 100$$

Haematological parameters

Blood parameter like WBCs, haemoglobin, RBCs, HCT, MCV, MCH, MCHC etc in all the control and treated groups (n=4) were examined by taking the blood from fish caudal region using 0.3ml injection, then poured into purple capped and yellow capped blood tubes immediately and tubes were placed in icebox, transferred to the BioGene Lab, Blue Area,

Islamabad and examined by well calibrated top standing hematology analysis machine (Sysmex Hematology Machine).

Metabolic Enzymes

Aspartate Aminotransferase (AST)

Bergmeyer (1965) method was used to check the activity of AST. Selected tissues homogenates were centrifuged and incubated and then a reagent was used to stop the reaction. Then for 20 minutes, the samples were placed at room temperature. Finally, spectrophotometry was used to analyze the contents at 545nm wavelength.

Alanine Aminotransferase (ALT)

ALT activity was checked by the Bergmeyer (1965) method. The whole protocol was followed, samples were homogenized, and supernatant was collected and proceeded with the protocol. The value of optical density of ALT was noted at 545 nm in spectrophotometer.

Lactate Dehydrogenase (LDH)

The activity of LDH (µM Pyruvate/ mg protein/ hr) was find by following Franciscato *et al*., (2011) method by the production of NADH. The samples were prepared according to the protocol and absorbance was noted at 500 nm.

Estimation of total protein contents

Total protein contents in liver samples were determined by following protocol provided by Lowry et al., (1951). BSA (Bovine albumin serum) was used to draw standard for calculating protein in samples.

Extraction of Antioxidant Enzymes

Antioxidant enzymes activity was determined using liver, and brain tissues of selected fish following the standard protocol for each enzyme.

Catalase Assay (CAT)

Chance and Maehly, (1955) method wasfollowed for the activity of CAT in liver, and brain tissues of selected fish of all the group and absorbance was noted at 240 nm wavelength using spectrophotometer.

Peroxidase assay (POD)

The POD activity was analyzed in the selected tissues of the experimental groups using the method provided by Chance and Maehly, (1955); Bibi, (2012). Reactions were made according to the protocols and optical density (OD) values were noted at 470 nm.

Superoxide Dismutase Assay (SOD)

Kakkar et al., (1984) method was followed with modifications for SOD values. Reaction mixtures were prepared, and color intensity was noted at 560 nm using spectrophotometer.

Lipid Peroxidation Assay (LPO/ TBARS)

Wright et al. (1981) method was followed for testing LPO enzyme activity in liver, and brain tissues of O. *mossambicus*. The protocol was followed and at the end of reaction, OD values were measured at 535 nm.

Gonadosomatic index (GSI)

Fish were collected from aquariums randomly. Then, the weight and length of fish were measured, and the length/weight of fish was noted on paper. Then, the fish were dissected.
Fish dissection: Belly was cut opened from vent (shallow incision), next cut behind gill, fish body was opened and gently removed guts to expose air bladder. Both male and female gonads are located on the top/edge of the air bladder.
Female identification:

1. Ovary forms a point and then narrows to oviduct – thread like.
2. Ovary is angular, has ridge.
3. Granulated.
4. Colour is not a good indicator as it can vary from pink to white.

Immature male identification: Testes are thready throughout, smooth and round, no development or thickness.
Mature male identification: Testes thicken; become white/translucent, smooth, tapers to tail.
Visually identify fish sex. If female or male, then record fish number and sex on datasheet. Then remove and weigh gonads. Zero balance scale was used to measure the weight.

Then Gonadosomatic Index (GSI = (gonad weight (g) / fish weight (g)) ×100) was calculated. (Harstad et al., 2014) (Larsen et al., 2004).

$$GSI = \frac{Weight\ of\ gonads}{Weight\ of\ fishes} \times 100$$

Testosterone ELISA

The Calbiotech, Inc. (CBI) Testosterone ELISA Kit (Catalog No. TE373S) is used for the quantification of testosterone in serum / plasma by Mc Carm, 1985; Ekins, 1984; Paulson et al., 1977 methods.

REAGENT PREPARATION

1. Prepared the Enzyme conjugate (20X)
2. Prepared 1X Wash buffer (1X)
3. All the reagents were kept at room temperature.

ASSAY PROCEDURE

The Calbiotech, Inc. (CBI) Testosterone ELISA Kit (Catalog No. TE373S) procedure was followed. Samples of 50µl standards, control or treated groups were pipetted into the wells, reagents were added, incubation was done, stop solution was added. ELISA Reader was calibrated at 450nm for noting down the absorbance.

Apparent Digestibility Analysis

The apparent digestibility for each nutrient: fats, protein, carbohydrate, and total energy was measured using chromic oxide following the modified protocol of Hanley (1987). Chromic oxide was determined by using the method of Furukawa and Tsukahara (1966).

Calculations

The following formulas were used to calculate apparent digestibility:

Apparent digestibility (%)

$$= \mathbf{100} - [\frac{\%\boldsymbol{Cr2O3\ in\ feed}}{\%\boldsymbol{Cr2O3\ in\ faeces}} \times \frac{\%\boldsymbol{nutrient\ in\ faeces}}{\%\ \boldsymbol{nutrient\ in\ feed}}]$$

The apparent digestibility of the separate components of the diet was calculated according to:

$$\text{AD*test diet} = \frac{\boldsymbol{AD\ total\ diet - (AD\ basal\ diet \times proportion\ of\ basal\ diet)}}{\boldsymbol{proportion\ of\ test\ diet}}$$

(*AD=apparent digestibility)

Statistical analysis

Experiment data was presented as Mean±S.E. Statistix 8.1 version software was used to analyze the data statistically. Comparison between different experimental groups was made by using one way analysis of variances (ANOVA) followed by LSD. Moreover, $P < 0.05$ level was selected as significant different.

RESULTS

Chemical Composition of experimental feeds

In our study of proximate composition of experimental feed, it was found that there was significant ($p < .05$) difference between control and treated groups' moisture content. S3 was highly significant as compared to control.

Moreover, crude proteins, crude lipids, crude fibers, and total ash also showed significance with control group. High values were observed in case of S3 group. although there was non-significant difference in between the treated groups when compared.

Growth Data

In our study, final weight of the fish fed with sesbania sesbans supplemented diet (S1 1.25% sesbania seeds S2 2.5% Sesbania seeds, and S3 5% sesbania sesbans seeds) was significantly ($P < 0.01$) different than the weight of fish fed without any supplemented diet i.e., control. Similarly, weight of fish fed with sesbania sesbans roots supplements (R1 1.25% Sesbania roots, R2 2.5% Sesbania roots, and R3 5% Sesbania roots) was also significantly different as compared to control. Overall, the growth of S3 (13.32±0.8 g) was best among the seed treated while the growth of R3 was best among the root treated groups.

Furthermore, the weight gain (WG) data revealed that there was significant difference between treatment groups as compared to control. No significance difference was observed in seed treated S2 and root treated R2 groups whereas high WG values were showed by seed treated S3(12.28g) and roots treated R3 (10.83g) groups.

Also, significance difference was observed in specific growth rate (SGR) of treatment groups as compared to control values except seed treated S1 and root treated R1 groups which showed non significance to control fed. Highest value was shown by seed treated S3 (2.55% d^{-1}) group.

Feed conversion ratio (FCR) values showed a considerable difference among control and all the treatment groups. Seed treated S2 and root treated R2 groups were non-significant to each other while high significance difference was shown by seed treated S3 and root treated R3 groups.

Study of haematological parameters

In our study three groups (S1, S2, and S3) were exposed to sesbania powdered seeds and other three (R1, R2, and R3) were exposed to sesbania powdered roots. Our hematological

study revealed that RBCs values were significantly ($p < .05$) different among all the treated groups except R1 which was not significantly different compared to the control groups. While highest value of RBCs was recorded in S3 and R3 respectively.

WBCs values also showed considerable difference among all the groups and with the control group as well. Overall highest value of WBCs was noted in the sesbania seeds (S3) treated group while in sesbania roots treated groups R3 was ranked the highest.

While hemoglobin value of S3 group showed a significantly high difference with control group. There was a non-significant difference between control and S1 and S3 groups. Same trend was also recorded among S2, S3, R2, and R3 as well but had a considerable difference with control groups.

Moreover, the HCT values revealed that there was significant difference between S3, R3 and control groups. While there was a non-considerable difference among S1, S2, R1, R2 and control groups. Also, a non-significant difference was noted between S3 and R3 as well.

Furthermore, it was observed in MCV values that there was a non-significant difference between control and R1 group same trend was also found between S1 and R1. As S2, R2 and R3 showed a non-significant difference and S3 and R3 did the same.

MCHC values showed a non-considerable difference among control and S1, S2, R1, R2 and R3 groups. But a significant difference was found between S3 and control group. While in MCH it was found that there was a significant difference of all treated groups compared to control group. Significant difference was also observed between the treated groups as well except few groups where there was not found a significant difference i.e., between S1 and R2.

Plasma Biochemical Parameters

Our biochemical study of blood parameters revealed that in case of LDH, there was no ($p > .05$)significance between the groups. Overall, significant difference was found between S2, S3, and R3 as compared to control. Lowest values were recorded in S3 group.

AST values also showed non considerable difference between S1, R1 and control. S3 and R3 showed the significant difference as compared to control. There was no significance difference among the treated groups. However, the lowest value was noted in sesbania powdered seeds treated (S3) group while in sesbania powdered roots treated (R3) group was recorded the lowest.

Moreover, the ALT values revealed that the groups S1, R1, and R2 showed non significance with control group. However, there was considerable difference between seed

treated S2 and S3 and R3 as compared to control. Seed treated S3 group and roots treated R3 group showed the lowest values.

Gonadosomatic Index (GSI)

For the GSI, there was a high significant ($p < .005$) difference between the treatment and sex and a simple analysis showed the GSI of both males and females was significantly different in the treatment groups compared to control.

Male wild tilapia's GSI values revealed that there was significant difference between seeds treated S2 and S3 groups as compared to control group. While no significance difference was observed between S1 and control group. Root treated R1, R2, and R3 groups showed significant difference as compared to control group.

GSI values of female wild tilapia showed the significant difference between treatment groups and control group except seed treated S1 group and root treated R1 group which were non significance as compared to control. Roots treated R2, R3 and seed treated S2, S3 showed high significant difference as compared to control.

Hormonal analysis

There were significant differences ($p < .05$) among testosterone concentration of experimental groups and when compared to control as well (Table 04). The highest values were recorded in control group while the lowest values were recorded in the seed treated S3 and root treated R3 groups.

Table 1: Proximate composition of the experimental feeds.

Components%	C	S1	S2	S3	R1	R2	R3
Moisture	10.32	10.33	10.35	10.36	10.34	10.35	10.36
Crude protein	32.25	32.30	32.37	32.44	32.29	32.34	32.40
Crude lipid	13.21	13.23	13.27	13.31	13.22	13.25	13.31
Crude fibre	7.05	7.12	7.15	7.17	7.09	7.13	7.16
Total ash	14.41	14.60	14.72	14.82	14.51	14.66	14.72
S. *sesban* seeds (g/kg)	-	12.5	25	50	-	-	-
S. *sesban* roots (g/kg)	-	-	-	-	12.5	25	50

Mean values of treatment groups as compared to control are shown in the table. *S. sesban* is added as a supplemented diet. C, control; S1, S. *sesban* seeds 1.25%; S2, S. *sesban* seeds 2.5%; S3, S. *sesban* seeds 5%; S4, S. sesban roots 1.25%; S5, S. *sesban* roots 2.5%; S6, S. *sesban* roots 5%.

Table 02: Effect of dietary supplementation of S. *sesban* seeds and roots on growth performance of Mozambique tilapia.

	Control	S1	S2	S3	R1	R2	R3	F	P
IBW(g)	1.72 ± 0.2^{bc}	1.75 ± 0.1^{ab}	1.76 ± 0.3^{a}	1.74 ± 0.1^{c}	1.70 ± 0.2^{ab}	1.69 ± 0.3^{a}	1.73 ± 0.4^{c}	4.86	1.0
FBW (g)	7.43 ± 0.1^{d}	9.20 ± 0.2^{c}	10.99 ± 0.7^{b}	13.32 ± 0.8^{a}	9.21 ± 0.6^{c}	11.0 ± 0.9^{b}	12.56 ± 0.9^{a}	28.4	0.01
WG (g)	5.71^{e}	7.45^{d}	9.23^{c}	11.58^{a}	7.51^{d}	9.31^{c}	10.83^{b}	21.3	0.01
WG%	324^{e}	331^{e}	660^{b}	1195^{a}	382^{de}	660^{bc}	498^{cd}	38.7	0.007
SGR% d^{-1}	1.01^{e}	1.27^{de}	1.70^{c}	2.3^{a}	1.2^{e}	1.5^{cd}	2.0^{b}	24.4	0.001
FCR	2.35^{a}	2.11^{c}	1.72^{d}	1.33^{g}	2.21^{b}	1.88^{d}	1.46^{f}	119	0.001

Data were calculated as Mean±S.E. (n= 9). Small letters in the subscript which are different, have the mean value considerably different ($P < 0.05$) in the rows. ANOVA followed by LSD was used to compare the IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio between the groups.
C, control; S1, S. *sesban* seeds 1.25%; S2, S. *sesban* seeds 2.5%; S3, S. *sesban* seeds 5%; S4, S. sesban roots 1.25%; S5, S. *sesban* roots 2.5%; S6, S. *sesban* roots 5%.

Table 03: Hematological indices of Mozambique tilapia after treated with dietary supplementation of *Sesbania sesban.*

Blood Indices	Control	S1	S2	S3	R1	R2	R3
RBCs (10^6/µl)	0.25 ± 0.008^{e}	0.27 ± 0.004^{d}	0.31 ± 0.004^{b}	0.33 ± 0.003^{a}	0.26 ± 0.003^{de}	0.29 ± 0.09^{c}	0.32 ± 0.08^{a}
WBCs (10^3/µl)	3.44 ± 0.13^{e}	3.52 ± 0.11^{d}	3.73 ± 0.02^{c}	4.11 ± 0.01^{a}	3.53 ± 0.08^{d}	3.75 ± 0.02^{c}	3.97 ± 0.01^{b}
Hemoglobin (g/dl)	4.62 ± 0.08^{g}	4.64 ± 0.08^{e}	4.68 ± 0.11^{c}	4.75 ± 0.12^{a}	4.63 ± 0.07^{f}	4.66 ± 0.10^{d}	4.71 ± 1.0^{b}
PCV/HCT%	27.56 ± 1.17^{b}	28.29 ± 0.26^{b}	29.44 ± 1.42^{ab}	31.56 ± 1.49^{a}	28.60 ± 0.26^{ab}	27.90 ± 1.42^{b}	30.50 ± 1.49^{ab}
MCV (µ$m^{3)}$	109.7 ± 0.8^{e}	115.3 ± 1.6^{d}	123.7 ± 2.0^{bc}	129 ± 2.1^{a}	114.8 ± 2.8^{de}	121.4 ± 1.7^{c}	128.2 ± 0.8^{ab}
MCH (pg)	50.8 ± 1.5^{f}	56.5 ± 1.2^{de}	62.1 ± 1.3^{bc}	68.6 ± 1.1^{a}	54.4 ± 0.95^{e}	59.4 ± 1.6^{cd}	63.3 ± 0.8^{b}
MCHC (g/dl)	$33.1^{b}\pm2.4$	$34.7^{b}\pm1.4$	$37.6^{ab}\pm0.89$	$40.2^{a}\pm1.4$	$33.8^{b}\pm1.8$	$35^{b}\pm1.5$	$37.8^{ab}\pm2.1$

Values were represented as Mean±S.E. (n= 9). Mean values in different small letters in the subscript are considerably different ($P < 0.05$) in the rows. ANOVA followed by LSD was used to compare activity of RBCs, WBCs, Hemoglobin, PCV/HCT, MCV, MCH, and MCHC among different groups. RBCS, red blood cells; WBCs, white blood cells; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

C, control; S1, S. *sesban* seeds 1.25%; S2, S. *sesban* seeds 2.5%; S3, S. *sesban* seeds 5%; S4, S. sesban roots 1.25%; S5, S. *sesban* roots 2.5%; S6, S. *sesban* roots 5%

Table 04. Serum analysis of Mozambique tilapia after feeding with dietary supplementation of S. *sesbans*.

Blood Indices	C	S1	S2	S3	R1	R2	R3
LDH (U/L)	22.0±0.53^{a}	20.8±0.54^{ab}	19.5±0.64^{bc}	19.0±0.64^{c}	21.1±0.70^{ab}	20.7±0.0.63^{abc}	19.6±0.65^{bc}
AST U/L	67.1±1.0^{a}	64.7±0.8^{abc}	62.0±0.6^{cde}	60.0±1.3^{e}	65.7±0.9^{ab}	63.0±1.6^{bcd}	61.0±1.2^{de}
ALT U/L	38.0±1.1^{a}	36.0±1.6^{ab}	33.0±1.7^{bcd}	31.0±1.0^{d}	36.9±0.7^{a}	35.1±1.5^{abc}	32±1.6^{cd}

Data were shown as Mean±S.E. (n= 9). Average values in different small alphabets were significantly ($P < 0.05$) different in the rows. ANOVA followed by LSD was used to compare the activity of LDH, AST, and ALT among different groups. ALT, Alanine transaminase; AST, Aspartate transaminase; LDH, Lactate dehydrogenase.

C, control; S1, S. *sesban* seeds 1.25%; S2, S. *sesban* seeds 2.5%; S3, S. *sesban* seeds 5%; S4, S. sesban roots 1.25%; S5, S. *sesban* roots 2.5%; S6, S. *sesban* roots 5%

Table 05: Comparison of Gonadosomatic Index (GSI) of the experimental groups.

Sex	C	S1	S2	S3	R1	R2	R3	F	P
Male	0.72^{a}	0.51^{b}	0.47^{bc}	0.05^{d}	0.33^{c}	0.07^{d}	0.05^{d}	32.4	0.005
female	2.93^{a}	1.37^{ab}	0.47^{b}	0.30^{b}	2.1^{ab}	0.27^{b}	0.16^{b}	2.1	0.003

Values were noted as Mean±S.E. (n= 9). Mean values in the subscript shown by different alphabets are having significance difference ($P < 0.05$) in the rows. ANOVA followed by LSD was used to compare the GSI between different groups. GSI, gonadosomatic index.

C, control; S1, S. *sesban* seeds 1.25%; S2, S. *sesban* seeds 2.5%; S3, S. *sesban* seeds 5%; S4, S. sesban roots 1.25%; S5, S. *sesban* roots 2.5%; S6, S. *sesban* roots 5%.

Table 06: Effect of dietary supplementation of S. sesban on testosterone level of male Mozambique Tilapia.

Hormone	C	S1	S2	S3	R1	R2	R3	F	P
Testosterone (ng/ml)	6.85^{a}	6.82^{a}	5.99^{b}	0.59^{f}	4.97^{c}	2.20^{d}	1.41^{e}	201	0.004

Values were compared as Mean±S.E. (n= 9). Mean values in different small letters in the subscript are significantly different ($P < 0.05$) in the row. ANOVA followed by LSD was used to compare the testosterone level in the plasma of different groups.

C, control; S1, S. *sesban* seeds 1.25%; S2, S. *sesban* seeds 2.5%; S3, S. *sesban* seeds 5%; S4, S. sesban roots 1.25%; S5, S. *sesban* roots 2.5%; S6, S. *sesban* roots 5%.

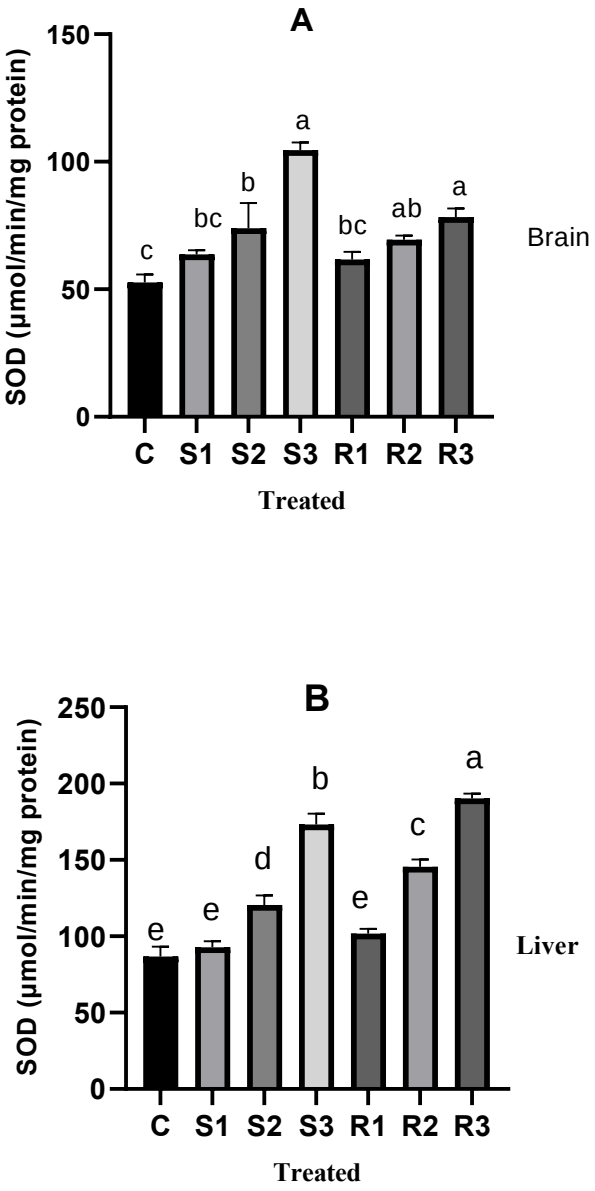


Fig. 01: Graph A and B represents superoxide dismutase activity in brain and liver of *O. mossambicus* fingerlings exposed to dietary powdered seeds and roots of *Sesbania sesbans* in different concentrations. Each bar represents Mean±S.E. (n=9). Means with different alphabet were significantly different ($p < 0.05$).

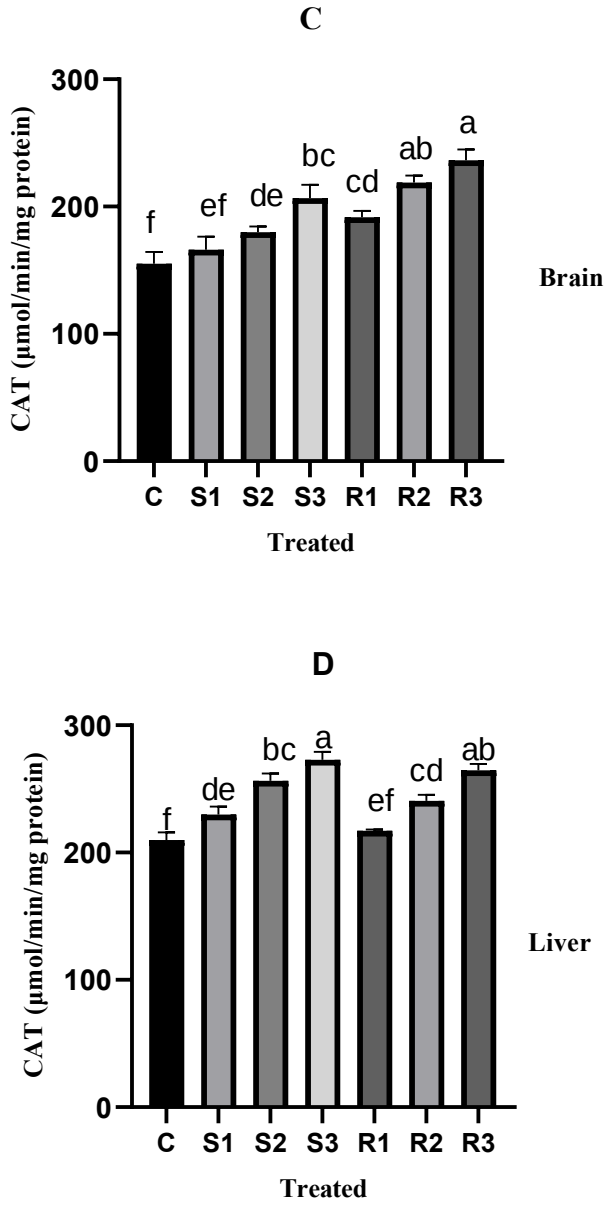


Fig. 02: Graph C and D shows catalase activity in brain and liver of *O. mossambicus* fingerlings when exposed to dietary supplementation of powdered seeds and roots of *Sesbania sesban* in different concentrations. Each bar represents Mean±S.E. (n=9). Means with different alphabet were significantly different ($p < 0.05$).

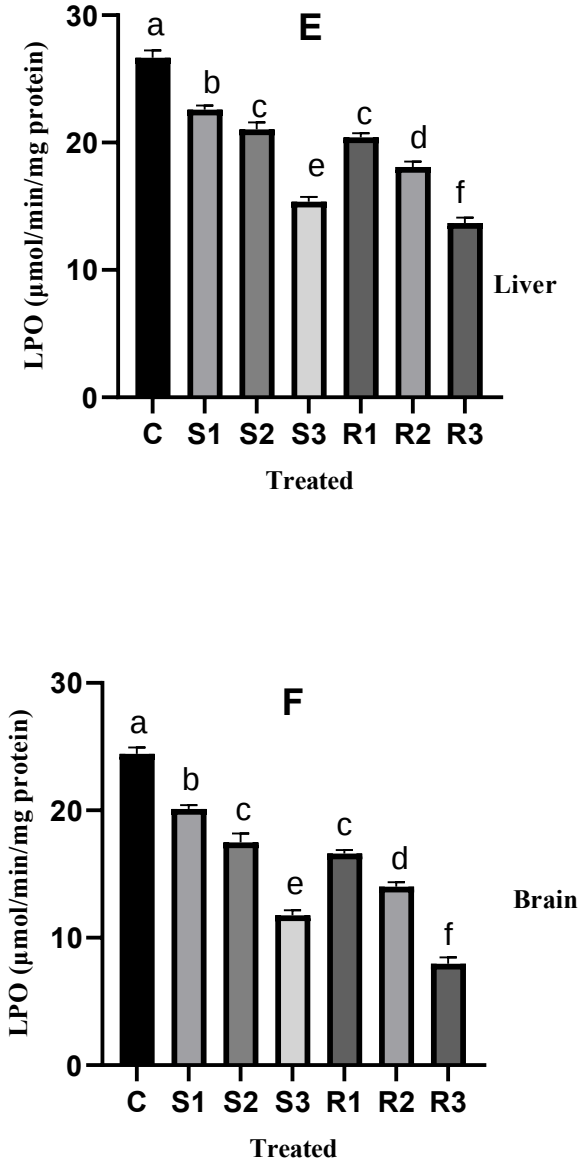
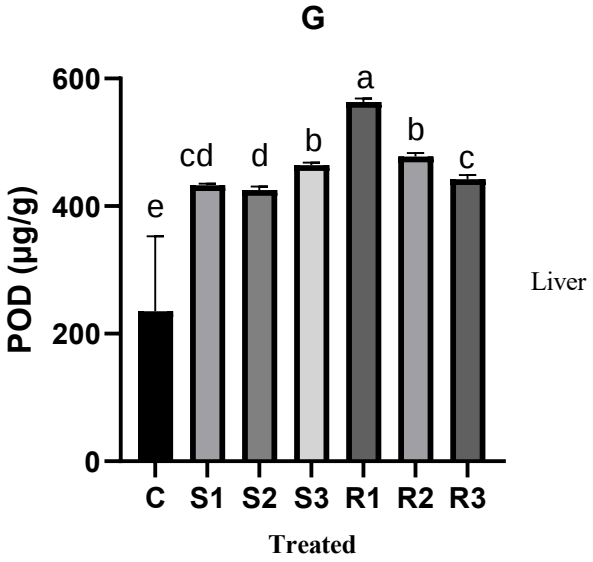


Fig. 3: Graph E and F represents the lipid peroxidase activity in liver and brain of *O. mossambicus* fingerlings when exposed to dietary powdered seeds and roots of *Sesbania sesban* in different concentrations. Each bar represents Mean±S.E. (n=9). Means with different alphabet were significantly different ($p < 0.05$).



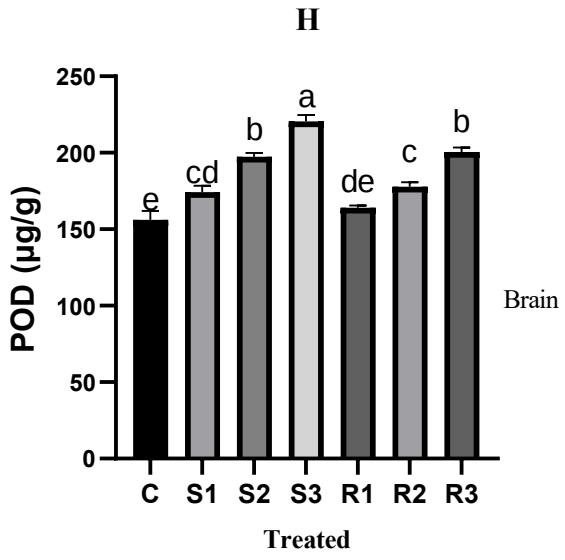


Fig. 4: Graph G and H represents the peroxidase activity in liver and brain of *O. mossambicus* fingerlings when exposed to dietary powdered seeds and roots of sesbania sesbans in different concentrations. Each bar represents Mean±S.E. (n=9). Means with different alphabet were significantly different ($p < 0.05$).

DISCUSSION

The introduction of tilapia has a negative impact on biodiversity of freshwater as well as brackish water ecosystems. Over 75% of publications related to tilapia introduction revealed a harmful impact (Deines et al., 2016). Many countries had reported environmental problems related to ecosystems caused by the introduction of tilapia (Gozlan, 2008). Similarly, hormones like testosterone are administered to tilapia fish for getting all the male populations. This mono-sex technique is not so efficient and acceptable to the consumer demand because of the use of synthetic hormones. Homklin et al., 2011 used 17α-methyl testosterone (MT) exogenous steroids for sex reversal method to yield high level of masculinization. Velazquez and Alter, 2004 showed that if MT is administered to tilapia for longer period, it caused fetotoxicity as well as hepatotoxicity. Megbowon & Mojekwu, 2013 said that MT is dangerous for the persons involved in the operation of tilapia seed production.

As the seed powder of sesbania sesbans disrupted the uterine structure, stopped the implantation, and inhibited the ovarian function in albino female rats (Saptarshi et al., 2017) while the roots extracts have spermicidal activity due to an active substance, oleanolic acid 3-β-D glucuronide (OAG), isolated from roots (Das et al., 2011). So, S. *sesban* seeds and roots were used for causing the infertility in the wild tilapia populations since there was not any previous study on the use of S. *sesban* plant materials on the reproductive behavior of the fish.

The result of our present study revealed that sesbania sesbans powdered seeds and roots had a positive effect on growth performance as indicated by the higher weight gain in the experimental groups of our study. Seeds powder dietary supplemented feed of S3 group and roots powder dietary supplemented feed of R3 group showed the high growth rate, high specific growth rate, and low FCR values, thus indicating the potential use of S. *sesban* seeds and roots as a protein source too. Similarly, Devi et al., 1997 showed the high growth performance attained using sesbania sesban leaf meal in the feed of fish as dietary ingredients. Similar findings were shown by (Farghaly et al., 2022) on growth performance and meat quality of the lambs when they were given *sesbania sesbans* fodder during the experimental period and their growth significantly improved. The crude protein contents in different seeds of Sesbania were from 29 to 33%, crude carbohydrates 44 to 47%, crude lipids 4 to 6%, crude fibre 11 to 16%, and gross energy 19 to 20 KJ/g. however, there were antinutritional factors in different seeds of Sesbania which included the tannins, phenols, condense form of tannin, saponin, and phytic

acid (Hossain and Becker, 2001). In our study, there was good growth performance shown by the fish, but further studies are needed to evaluate the chemical parameters of Sesbania sesban plant materials. In this way, we will be able to analyze the effect of antinutritional factors present in the plant and its potential negative impact.

Many plant origin materials act as natural antioxidants that inhibit the generation of oxygen anions through trapping the free radicals inside body (Chakraborty and Hancz, 2011). Our study revealed that there was increase in antioxidants enzymes SOD, POD, CAT, TBARS activities as fish was exposed to dietary *Sesbania sesban* seeds and root powder which were consistent with the immune system of the fish. This suggested that the S. sesban plant materials can enhance the antioxidant enzymes activity of the Oreochromis mossambicus in all the treated groups particularly in 50g/kg dietary supplemented S3 and R3 groups as shown in the figure. Similar results from another study (Mani et al., 2011; Kathiresh et al., 2011) suggests that saponin, tannin, phenolic compounds, anthocyanins are present in sesbania sesbans seeds and flowers. There compounds exhibit scavenging activity against superoxide anions, hydroxyl radicals, and DPPH radical.

In our study, the hematological indices of blood slightly increased as compared to control group. similar results were reported from Ologhobo (1992) that the most common blood variables consistently influenced by diet are the RBCs, HCt, Hb, and glucose levels. WBC indicates an improvement of the health status of the acutely infected fish (Adewaye *et al*., 2005) This study revealed that WBC count increased significantly in the group administered 50 g kg of S. *sesban* seeds and roots, which would be attributed to the organism's body defence against infection and tissue damage. Gabriel *et al*., 2007 also recorded the high number of WBCs after treatment tilapia with plant extracts.

Gonadosomatic index (GSI) values of treatment groups S3 and R3 were significantly different as compared to control group which indicates that the sesbania sesban seeds and roots included in the dietary supplementation of feed at higher concentration i.e., 50g/kg feed were more effective. Similar findings were obtained by Kareem *et al*., 2016 when tilapia was fed the seed extracts of *Carica papaya* which was effective in lowering the GSI values and quantification of few mature gametes in the gonads of both male and female by delaying gonadal maturation in both sexes. C. *camphora* and A.*indica* significantly reduced the GSI in

female tilapia but these extracts were not much effective as compared to our study. In contrast, Ramirez et al., 2017 showed that there are no significant differences in GSI in the males when fed with dietary extracts of *Passiflora incarnata.* So, S. sesban is the most effective plant specially its seeds and roots are highly effective to cause gonadal changes in both sexes of *Oreochromis mossambicus* without any potential hazard to the tilapia's health condition.

Conclusion

It is concluded from the present study that eco-friendly and bio-degradable herbal extracts might replace the synthetic hormones in the infertility of O. *mossambicus*. It was observed that dietary administration of S. *sesban* seeds and roots at 50g/kg had shown significant effect in the infertility. In addition, S. *sesban* increased the growth performance, antioxidants activity and hematological indices. It was also noted that the gonadosomatic index and testosterone level decreased in the treated groups. Since the gonads were not 100% destroyed so further studies would be required to form a standard treatment protocol for production of sterile tilapia using plant materials to make it suitable for commercial use.

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