Evaluation of bitter melon *(Momordica charantia)* seeds powder as a potential infertility inducer in Male Nile tilapia (*Oreochromis niloticus*)

By

Muhammad Alam

Department of Zoology

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad

2023

Evaluation of bitter melon *(Momordica charantia)* seeds powder as a potential infertility inducer in Male Nile tilapia (*Oreochromis niloticus*)

A thesis submitted in partial fulfillment of the requirements for the Degree of

MASTER OF PHILOSOPHY

IN

ZOOLOGY

By

Muhammad Alam

Department of Zoology

Faculty of Biological Sciences

Quaid-i-Azam University

Islamabad

2023

CERTIFICATE

This dissertation **"Evaluation of bitter melon *(Momordica charantia)* seed powder as a potential infertility inducer in male Nile tilapia (*Oreochromis niloticus*)**
". submitted by **Muhammad Alam** 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 Fisheries and Aquaculture.

Supervisor:

_____

Prof Dr. Amina Zuberi

External Examiner: _____

Chairperson _____

Dr. Amina Zuberi

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.

Muhammad Alam

Dedicated to:
This thesis is dedicated to my loving parents, siblings, respected supervisor and my beloved friends for their advice, patience and faith in me

Table of Contents

List of Abbreviations

°C	Celsius
µL	Microliter
µM	Micromolar
FCR	Food conversion ratio
ANOVA	Analysis of variance
HCT	Hematocrit
MCH	Mean corpuscular hemoglobin
SST	Serum separation tube
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
cDNA	Complementary deoxyribose nucleic acid
HBSS	Hank's balance salt solution
Cm	Centimeter
MC	*Momordica charantia*
CQ	Chloroquine
CV	Caudal vein
DEPC	Diethyl Pyro carbonate
MT	Methyl testosterone
dNTPs	Deoxyribonucleotide triphosphate
DO	Dissolved oxygen
FAO	Food agriculture organization
EDTA	Ethylenediaminetetraacetic acid
SGR	Specific growth rate
WG%	Weight gain percent
g/ml	Gram per milliliter
BMSM	Bitter melon seed meal
IBW	Initial body weight
FBW	Final body weight
H_2O	Water
H_2O_2	Hydrogen peroxidase
Hb	Hemoglobin

HCQ	Hydroxychloroquine
HCT	Hematocrit
HN	Hepatocyte nucleus
Hp	Hyperplasia
Hrs.	Hours
HT	Hypertrophy
Ig M	Immunoglobulin
IL-1β	Interleukin 1 beta
Kg	Kilogram
CLA	Conjugated Linoleic Acid
O. niloticus	*Oreochromis niloticus*
LDH	Lactate dehydrogenase.
LPO	Lipid peroxidation
LSD	Least significant difference
M	Molarity
M cm	Molar coefficient
m/v	Mass per volume
Mg	Milligram
mg/L	Milligram per liter
Min	Minute
mM	Millimolar
Mm	Millimeter
mRNA	Messenger ribonucleic acid
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
N	Necrosis
$NADH^+$	Nicotinamide adenine dinucleotide hydrogen
NBT	Nitro blue tetrazolium
NCBI	National center for biotechnology information.
ST	Seminiferous tubule
ND.	Nanodrop
Nm	Nanometer
nmol	Nanomole

O_2	Oxygen
OE	Edema
P	Probability
PCR	Polymerize chain reaction
pH	Power of hydrogen
POD	Peroxidase
RNA	Ribonucleic acid
ROS	Reactive oxygen specie
Rpm	Revolution per minute
S	Sinusoid
SE	Standard error
SOD	Superoxide dismutase
SS	Sinusoidal space
CG	Control group
AZ	Azoospermia
OZ	Oligospermia
TBARS	Thiobarbituric acid reactive substances
TNF-α	*Tumor necrosis factor*
U/L	Units per liter
V	Vacuolization
***β*- actin**	Beta actin

LIST OF TABLE

ACKNOWLEDGEMENTS

In the name of Allah who is the most Beneficent and the most Merciful. All praises to Almighty Allah, the creator of universe. I bear witness that Holy Prophet Muhammad (SAW) is the messenger, whose life is a perfect model for the whole mankind till the Day of Judgment. Allah blessed me with knowledge related to earth. Allah enabled me to complete my work. Without the blessings of Allah, I would not be able to complete my work and to be at such a place.

I am very grateful to my supervisor ***Professor Dr. Amina Zuberi*** *for her excellent mentorship, support, advice and encouragement. Her worthy comments in meetings increased my knowledge and experience, and her keen insight, great experience, and thoughtful reflection on the different aspect of research are outstanding abilities to which I aspire.*

I am especially indebted to ***Mr. Mashooq Ali****,* ***Mr. Muhib Zaman and Waliullah*** *for making me this research work possible.*

I pay my thanks to the whole faculty of my department especially the respected teachers whose valuable knowledge, assistance, cooperation and guidance enabled me to take initiative, develop and furnish my academic carrier.

I especially want to thank all participants, volunteered samples for my work. I think it would be incomplete. I am especially thankful to ***Mr Muhammad Noor Ullah khan, Waqar khan, Adnan khan, Andleeb Ashraf and Shahid khan*** *who was always with me in field work to support, encourage and appreciate me.*

I am thankful to my class fellows, friends and juniors for their help and support. Special thanks to ***Shahryar Sandhu, Saeed Ahmad, Shehla Kanwal, Maryam, Samina Farheen, Faisal khan, Wahaj, Aziz, Fida Ullah, Haroon khan, Shawal khan and Aleem khan,*** *for their practical and moral support given to me during the M.Phil. They helped me a lot and I am unable to forget their support.*

I also acknowledge the help, encouragement, support and prayers of my family which have always been there to help and support me. I cannot forget their love, affection and care.

May ALLAH bless all of them "Ameen"! ***"Muhammad Alam"***

ABSTRACT

Nile tilapia (*Oreochromis niloticus*) is a pond breeder that shows prolific breeding which results in excessive recruitment of fingerlings in grow-out systems, thus leading to overcrowding and resource competition, and ultimately stunted and small-sized fish that are unable to command high market prices. Current research was conducted to investigate the effect of bitter melon (*Momordica charantia*) seed powder on (*O. niloticus*) fertility, which has previously been shown to suppress reproductive capability in mice and rats. O. *niloticus* fingerlings (10.2±2g) were distributed equally into six groups in replica of three. Fish were stocked in outdoor fibreglass tanks having 450 Liters of water with stocking density of 1g/l. Six experimental diets having 35% crude proteins with various doses of *M. charantia* seeds powder (0, 2, 4, 6, 8 and 10g/kg diets) (for C, S2, S4, S6, S8, and S10, respectively) were prepared and fed the Tilapia fingerlings at the rate of 5% of their body weight for 90 days. The results showed that supplementing *M. charantia* seed powder had a significant ($p<0.05$) effect on reproductive performance of *O. niloticus*. Experiment results indicated that gonadosomatic index, serum testosterone was decreased significantly ($p<0.05$) in treated groups. The order of significant decrease is as (C > S2 > S4 > S6 > S8 > S10). Reactive oxygen species (ROS) in blood was decreased while there was a slight increase in antioxidant enzymes (SOD, POD, CAT) of testicular tissues. Histological examination of the testicular tissue revealed that in the control group, sperms were observed to have been released into the lumen of the seminiferous tubules. In contrast, the S10 group displayed the lowest number of sperm within the tubular lumens. Histological observations, GSI and serum testosterone concentration reveals that *M. charantia* seed is an efficient reproductive inhibitor in *O. niloticus* thus could be employed to manage overpopulation in Nile tilapia.

INTRODUCTION

The culturing of aquatic organisms like fish, crustaceans, molluscs and aquatic plants under controlled conditions is referred to as aquaculture (Ntsama *et al*., 2018). Similarly, according to Stickney (2006), aquaculture is the practice of producing aquatic organisms in a controlled or semi-controlled conditions. The history of aquaculture dates back to 2000 BC (Rabanal ,1988) but in the first half of 20th century there was no organized global expansion of aquaculture industry (FAO, 2018). Moreover, during the 2nd half of the 20th century it began to be transformed into a modern science globally. Furthermore, within 25 years or so, it is established as a significant food production industry, with an annual average growth rate of over 8% which accounts for 50% of the global food fish consumption. In addition, this sector is continued to be predominant in developing countries like Asia, which accounts for more than 85% of the global production (De-Silva and Davy, 2010).

Asia, particularly in inland aquaculture, has played a critical role in increasing global production levels and guaranteeing food security. In fact, this region accounted for nearly 92% of all fish farming and seaweed cultivation in 2017 (Naylor *et al*., 2021). As the world's population grows, providing food security has become a critical issue for growth and survival. As per UN forecasts, the world's population will grow by more than one billion people between 2017 and 2030. The combination of population growth and urbanization is expected to raise demand for animal protein sources, particularly meat, during the next two decades. This increasing trend in population growth and urbanization leading to an increase demand for animal-based protein (Tsakiridis *et al*., 2020). Consequently, the production of aquatic food is essential for guaranteeing cheap food security and producing income on a local as well as global scale (Cochrane et al., 2009).

Globally the production of aquaculture and fisheries (excluding algae) has experienced a remarkable surge, witnessing a substantial growth from 19 million tons in 1950 to a record-breaking level of nearly 179 million tons in 2018. This represents an annual increase of 3.3%. Subsequently, there was a slight decline in production in 2019, with a decrease of 1%. However, there was a modest recovery in 2020, with a marginal increase of only 0.3%, bringing the total output of aquaculture and fisheries to 178 million tons (Boyd *et al*., 2022). Recent data indicates that the global production of fisheries and aquaculture is projected to experience a steady growth at a rate of 1.5% in the upcoming years. This growth is expected to lead to an increase in production, reaching a level of 184.6 million tons per

year. The combined value of fisheries and aquaculture production in 2020 was estimated to be approximately 406 billion USD. Out of this total, around 265 billion USD was attributed to aquaculture activities (FAO, 2022). While the production of marine and freshwater fish is experiencing rapid growth, freshwater aquaculture continues to be the dominant sector in the industry. Furthermore, an overwhelming majority of approximately 75% of the global volume of edible aquaculture is comprised of freshwater fish (Edwards *et al.,* 2019). Marine fish make up 3% of the total global aquaculture production. Diadromous fish follow at 6%, crustaceans at 9.6%, molluscs at 23.6%, freshwater fish at 56.4%, and other aquatic animals at 1.4% (FIPS, 2012).

Pakistan, being an agricultural country, possesses abundant natural resources for the aquaculture sector, including both fresh and marine water sources. The country is blessed with an extensive network of rivers, lakes, ponds, and other water bodies, which cover approximately 8,563,820 square kilometres (Aslam, 2008). The water resources available in Pakistan are highly suitable for aquaculture activities. With a coastline stretching over 1,120 kilometres, there are abundant opportunities for coastal fishing. Additionally, shrimp farming has emerged as a significant industry in the country. Pakistan's long coastline along the Arabian Sea offers favourable conditions for shrimp cultivation. In recent years, the shrimp farming sector in Pakistan has experienced remarkable growth and development (Hassan *et al.*, 2022).

Pakistan has achieved the status of being one of the world's top exporters of farmed shrimp, with a particular emphasis on the export of black tiger shrimp. The country primarily cultivates two major species of shrimp: the black tiger shrimp and the white leg shrimp. These species have gained prominence in the aquaculture industry in Pakistan, contributing significantly to the country's export market (Mohsin *et al*., 2017).

In addition, Pakistan possesses an expansive Exclusive Economic Zone (EEZ) extending approximately 350 nautical miles, providing a significant open maritime region under state ownership. Despite the abundance of resources and potential for aquaculture, the fisheries and aquaculture sector in Pakistan has experienced limited progress and development (Zahra *et al*., 2022). The decrease in aquaculture production can be attributed to the lack of attention and awareness among farmers regarding the implementation of advanced extensive and semi-intensive techniques in aquaculture systems. Additionally, coastal and deep-water marine resources have not been utilized for aquaculture purposes. Consequently,

our reliance on natural availability remains crucial for marine-based production. As a result, the projected production from fish farming in the aquaculture system is estimated to range from 179,900 to 180,000 metric tons, while the natural catch is expected to contribute approximately 600,000 metric tons (Mohsin *et al*, 2017).

The prospects of Pakistan's fisheries sector rely on a multitude of factors, including the adoption of sustainable fishing methods, efficient management of fish populations, investments in infrastructure and technology, and the establishment of effective regulations and policies. Furthermore, the future of the aquaculture sector in Pakistan is influenced by additional factors such as environmental conditions, population growth, and economic stability. These factors also contribute to shaping the outlook of the aquaculture industry (Nazir *et al*., 2021).

Countries like China, India, Bangladesh, and Vietnam have experienced notable advancements in aquaculture, primarily due to technological innovations in fish production to meet the growing global demand for fish. Additionally, the ecological and economic feasibility of fish farming has played a significant role in driving this growth (Ogunfowora *et al.*, 2021). Pakistan, however, faces certain challenges in its aquaculture sector. These include inadequate infrastructure and technology, limited investment, ineffective management and regulations, restricted market access, and a lack of knowledge and skills. These shortcomings hinder the progress of aquaculture in Pakistan (Shah *et al*., 2018).

1.1 Tilapia culture

Tilapias are recognized as the second most significant group of fin fish in freshwater aquaculture, ranking just below carps (Waite *et al*., 2014). Tilapia is not only one of the most consumed farmed fish worldwide but also holds the distinction of being the most significant cultured non-cyprinid fish globally. In 2019, the total production of tilapia reached 6.6 million tons, with most of it originating from countries beyond its natural habitat in Africa and the Levant (Bardhan *et al*., 2019). Tilapia has been introduced to nearly 90 countries, including India, where it is utilized for purposes such as aquaculture, game fishing, and pest control (Huang, 2003) Tilapia contains a higher amount of omega-3 fatty acids compared to beef, pork, or chicken. Additionally, tilapia can be sustainably farmed in aquaculture because it can thrive on algae or other plant-based protein feed, eliminating the need for animal-based protein feeds required by wild fish and other farmed fish species (Tower, 2015).

Evidence of tilapia civilization dates back approximately 4000 years ago, as depicted in pyramidal drawings found in an Egyptian tomb (EL-Sayed, 2006). According to Hutton and Ballarin (1979), modern tilapia farming was initially tested in 1924.The initial excitement surrounding tilapia as a food fish was dampened by unregulated pond breeding practices, leading to issues such as excessive recruitment, stunted growth, and a small percentage of fish reaching marketable size. However, a significant breakthrough occurred in the 1970s with the technique of sex-reversal using hormones. This breakthrough allowed for the production of all-male, mono-sex tilapia, which could be raised to a uniform, commercial size. As a result, the tilapia market started to develop in the 1980s, accompanied by advancements in feed, culturing techniques, and processing methods (Crispi, 2009).

1.2 Purpose of the study

Aquatic scientists and conservation biologists have expressed concern that the introduction of tilapia poses a significant threat to the biodiversity of both brackish water and freshwater ecosystems. More than 75% of published research suggests that the introduction of tilapia has had a detrimental impact on ecosystems (Deines *et al*., 2016). In grow-out systems, the prolific breeding of tilapia leads to excessive recruitment of fingerlings, causing issues such as overpopulation and competition for resources (Fagbenro,2004) As a result, the fish often experience stunted growth and remain small in size, which leads to them fetching lower market prices (Coddington *et al*., 2000).

To address the issues related to excessive tilapia reproduction and population, various techniques are employed in tilapia production systems. These methods encompass periodic harvesting of fry and fingerlings, high-density culture, cage culture, polyculture involving predator fish, heat shock sterilization, and the implementation of all-male culture (Guerrero, 2008). These measures aim to minimize unwanted reproduction and maintain better control over tilapia population dynamics in aquaculture settings (Fortes, 2005).

Currently, the culture of all-male tilapia individuals is a widely adopted practice. This approach helps to control prolific spawning, as males exhibit faster and larger growth compared to females (Baroiller & D'Cotta 2001). Consequently, the use of all-male culture leads to a shortened production cycle in tilapia farming (Megbowon & Mojekwu, 2014).

All-male tilapia populations are typically achieved through various methods, including: (a) Sex-sorting by hand: This involves manually separating male and female tilapia

individuals based on their physical characteristics (Adjabi, 2020). (b) Hormonal sex inversion using androgens: Hormones, such as 17-alpha methyltestosterone, are administered to tilapia fry or fingerlings to induce the development of male characteristics in genetically female individuals (Piferrer, 2012). (c) Environmental manipulation: By subjecting tilapia to specific temperature treatments, it is possible to influence their sex determination. Higher temperatures can result in predominantly male populations, while lower temperatures can lead to a higher proportion of females (Valdivieso, 2020). (d) Genetic/chromosomal manipulation: Techniques such as selective breeding, hybridization, and genetic manipulation can be employed to produce all-male tilapia populations with specific genetic or chromosomal traits (Omeje, 2016). These methods provide options for tilapia farmers to achieve all-male populations, allowing for better control over reproduction and faster growth rates in aquaculture industry (Olufeagba & Okomoda, 2015).

Among the various methods mentioned, hormonal sex reversal using exogenous steroids, particularly 17α-methyl testosterone (MT), has been found to yield a high success rate in masculinizing tilapia populations (Homklin & Limpiyakorn, 2011). The high efficacy of 17α-methyl testosterone (MT) in sex inversion is attributed to its ability to inhibit aromatase activity (Ranjan, 2015). MT hinders estrogen biosynthesis while promoting androgenesis in the developing gonads of tilapia. This mechanism leads to the masculinization of genetically female individuals and the development of male characteristics (Golan & Levavi-Sivan, 2014). As a result of its effectiveness in inducing sex inversion and producing all-male individuals, 17α-methyl testosterone (MT) is indeed the most widely used method in tilapia culture for achieving all-male populations (Pandian & Kirankumar, 2003). The use of MT has proven to be a practical and efficient approach in tilapia farming to control reproduction and optimize production outcomes (Baroiller & D'Cotta, 2018).

It is important to note that the use of 17α-methyl testosterone (MT) in tilapia culture has raised concerns due to its potential carcinogenicity and associated adverse effects on human health and aquatic ecosystems (Mahika *et al*., 2015). These concerns have prompted public discussions and debates regarding the safety and sustainability of MT usage in aquaculture. Efforts are being made to explore alternative eco-friendly methods and practices that minimize potential risks of carcinogenesis and ensure the long-term health of both consumers and the environment in tilapia production (Haitham, 2018).

Prolonged exposure to 17α-methyl testosterone (MT) during the application process can indeed lead to hepatotoxicity and fetotoxicity (Abaho *et al*., 2019). These potential health risks associated with MT usage emphasize the importance of proper handling, dosage control, and adherence to safety protocols to minimize the negative impacts on both human health and the environment. It is crucial to prioritize the well-being and safety of individuals involved in tilapia culture and to explore alternative approaches that offer sustainable and safe solutions (Velazquez & Alter, 2004).

Approximately 30% of the hormone-treated diet administered to fish is estimated to be unavailable for absorption during feeding (Ramirez-Godinez *et al.,* 2013). Interestingly, only around 10% of the hormone present in the consumed diet is utilized for sex inversion in tilapia (Chotisukarn & Limpiyakorn, 2012). Consequently, in closed aquatic environments, hormone residues tend to accumulate due to the presence of active metabolites excreted by treated fish and leachates from uneaten food. The hydrophobic nature of 17α-methyl testosterone (MT) enables it to quickly adsorb onto sediments, facilitating the build-up of hormonal residues in the environment. This accumulation raises concerns about potential ecological impacts and the need for careful monitoring and management of hormone use in tilapia culture to mitigate any adverse effects on the aquatic ecosystem (Malila *et al*., 2015).

The potential exists for unintended release of MT and its metabolites into the aquatic environment through uneaten or unprocessed food, which can have detrimental effects on the endocrine and reproductive systems of non-target aquatic organisms (Tumbokon, & Serrano-Jr, 2017). Due to the aforementioned negative impacts, several countries have implemented regulations that prohibit the use of synthetic hormones in the production of fish food (Chakraborty & Hancz, 2013). Therefore, there is a crucial need for research initiatives aimed at identifying and developing alternatives to synthetic steroids that are environmentally friendly, economically feasible, and socially acceptable.

1.3 Using plants to control Tilapia overbreeding an alternative to MT

In response to the growing need to minimize the negative effects of aquaculture on human and environmental health, plant extracts are emerging as a vital component in fish farming. They serve as alternatives to chemicals, drugs, and hormones (Chakraborty *et al.,* 2013). Organic plant products are considered relatively safe, ecofriendly, biodegradable, cost-

effective, and simple to prepare. Consequently, they are seen as a viable approach to achieving sustainable fish production in aquaculture industry (Mirghaed & Yousef, 2019).

Furthermore, consumers are displaying a growing demand for fish products that are not only of high quality but also free from pollutants and disease, ensuring their safety (Chakraborty *et al.,* 2013). In light of this, the adoption of safe and environmentally friendly practices in fish production is essential to meet the evolving market demands for tilapia. Consequently, there is a need for focused efforts to identify and develop innovative plant-based solutions that can replace synthetic hormones and chemicals in tilapia production (Ahmed & Mohamed, 2019).

Natural compounds found in plants, including flavonoids, tannins, terpenoids, alkaloids, and steroids, play a beneficial role in promoting androgenic and anabolic processes (Chakraborty *et al.,* 2013). Additionally, these compounds contribute to the stimulation of digestion, appetite, and immunity in fish (Kpundeh, & Xu, 2015). Phytochemicals, which include steroidal saponins (Golan *et al.,* 2005) and flavonoids (Tarigan & Widjaja, 2016), disrupt estrogen synthesis by inhibiting the action of aromatase. Phytochemicals can potentially compete with endogenous estrogens for binding sites on the estrogen receptor, leading to the suppression of estrogen biosynthesis (Golan *et al*., 2008). As a result, these phytochemicals can act as "Phyto androgens" exhibiting functional effects similar to testosterone in animals. This leads to an enhancement of male reproductive characteristics (Turan & Akyurt, 2005). The utilization of plant extract phytochemicals to induce masculinization or fertility impairment in fish has proven to be an effective strategy for controlling overbreeding in tilapia (Gabriel *et al.,* 2017). These efforts have yielded positive outcomes in terms of controlling reproductive rates in tilapia populations (Ghosal *et al*., 2021).

It is studied by Kapinga *et al.,* (2019) that feeding Nile tilapia (*O. niloticus*) with a diet containing 2–8 g of Aspilia mossambicensis leaf extract kg−1 of diet resulted in degenerated seminiferous tubules, leading to a reduced number of hatchlings in the experimental fish. The presence of significant quantities of saponins and flavonoids is associated with the antifertility properties of A. mossambicensis. (Musyimi *et al.,* 2007). Similarly, feeding female Nile tilapia for 70 days with a diet containing 1%, 3%, and 6% of bitter kola seed powder, has negatively affected their gonadal development (Nyadjeu *et al.,* 2019). Additionally, Sulem-Yong et al, (2018) observed a significant 83.45% reduction in the

number of eggs spawned by Nile tilapia fed with a diet containing 6% bitter kola seed powder for 44 days, which was attributed to the presence of flavonoids.

Further, inclusion of *Gossypium herbaceum* root bark extracts into the diet of Nile tilapia at a rate of 20 g kg−1 for 70 days, resulted in the erosion of testes' connective tissue and disintegration of seminiferous lobules leading to a reduce milt volume (Akin-Obasola & Jegede, 2016). Similarly, addition of Mangifera indica leaf powder to Nile tilapia diets at 0.5–8.0 g kg−1 for 56 days resulted in reduce hatchling numbers, and complete suppression of spawning occurred at ≥2.0 g kg−1 of diet, (Obaroh and Achionye-Nzeh, 2013). This effect is attributed to the disintegration of sperm cells and rupture of follicles in Nile tilapia due to the saponins present in M. indica extract, which inhibits their reproduction (Obaroh *et al*. 2012).

In addition to the above-mentioned plants or plants extract bitter melon (Momordica charantia) seeds powder has antifertility compounds like alkaloids, flavonoids and saponin (Jerald *et al*., 2012). It is a vegetable belongs to Cucurbitaceae family with various names like bitter gourd, balsam pear, pare, or karela, and is widely grown and consumed in regions including Asia, East Africa, India, and South America (Abascal, 2005). *M. charantia* is native to India and Malaysia, and it has since spread widely throughout tropical, subtropical, and warm temperate regions across the globe. This plant is highly valued for its medicinal properties and its use as a vegetable in promoting human health. It is particularly recognized as one of the most promising plants for managing diabetes (Young *et al*., 2009)

Bitter gourd seed (BGS) is a valuable source of oil, containing around 18.1% to 37.6% oil content, along with protein ranging from 28% to 30%. The oil from BGS is particularly high in Conjugated Linoleic Acid (CLA), a specific type of fatty acid, while its protein is notable for essential amino acids like Methionine, Cysteine, Isoleucine, Phenylalanine, Tyrosine, and Lysine, making it suitable for enhancing food products. BGS also provides essential minerals such as Phosphorus, Potassium, Magnesium, Sulphur, and Calcium, along with flavonoids and phenolic compounds like catechin and gallic acid, all contributing to its nutritional value and potential uses in various foods (Ronny, 2010).

The seeds of bitter melon contain two abortifacient compounds, namely alpha-mormorcharin and beta-mormorcharin. Additionally, they also contain vicine, a pyrimidine nucleoside (DerMarderosian, 2005). The health benefits of bitter melon (*M. charantia*) are closely linked to its strong antioxidant properties due to a variety of compounds found in the

plant. These compounds, such as phenols, flavonoids, isoflavones, terpenes, anthraquinones, and glycosylates, work together to enhance the plant's ability to counteract oxidative stress and provide potential health advantages (Mobaraki, 2014).

In addition to biologically active triterpenes, specific proteins in *Momordica charantia*, like alpha-momordicin, beta-momorcharin, and cucurbitacin B, have been studied for their possible anti-cancer properties, with potential to hinder cancer growth and act as therapeutic agents against cancer (Hassan, 2021). Proteins and peptides play a crucial role in the fruit and seeds of M. charantia, serving as the primary functional components. Various proteins and peptides have been discovered in different sections of M. charantia. These include ribosome inactivating proteins (RIPs), *Momordica charantia* lectin (MCL), Momordica charantia anti-HIV protein of 30 kD (MAP30), α-momorcharin (α-MMC), β-momorcharin (β-MMC), γ-momorcharin, δ-momorcharin, and ε-momorcharin. These compounds possess diverse activities such as RNA N-glycosidase activity, PAG activity, DNase-like activity, phospholipase activity, superoxide dismutase activity, as well as exhibiting anti-tumour, anticancer, immunosuppressive, and anti-microbial effects (Kaur *et al.,* 2009).

The primary metabolites found in *M. charantia* include common sugars, proteins, and chlorophyll. In addition, bitter melon also contains various secondary metabolites include alkaloids, which are nitrogen-containing compounds known for their diverse biological activities. Flavonoids, another group of secondary metabolites, are plant pigments with antioxidant and anti-inflammatory properties. Tannins, which are polyphenolic compounds, are known for their astringent properties and potential health benefits (Daniel, 2014). Methanolic and pet-ether leaf extracts of *M. charantia*, also known as bitter melon or bitter gourd, have been found to contain several vitamins, including B3, B6, Vitamin D, and Vitamin K (Bakare *et al*., 2010)

1.4 Biological Activities

M. charantia has been found to show a wide range of biological effects, including antihyperglycemic, antibacterial, antiviral, antitumor, immunomodulatory, antioxidant, antidiabetic, anthelmintic, antimutagenic, antiulcer, antilipolytic, antifertility, hepatoprotective, anticancer, and anti-inflammatory activities (Jia *et al.,* 2017). The application of bitter melon (MC) extracts in mice effectively decreased the skin inflammation

induced by Propionibacterium acnes (Hsu *et al.,* 2012). Furthermore, extracts from M. charantia showed the ability to reduce cytokine and matrix metalloproteinase-9 levels in inflammation induced by Propionibacterium acnes in THP-1 cells (Huang *et al.,* 2015). In Turkish traditional medicine, the mature fruits of bitter melon are used to extract oil, which is then mixed with sun-warmed olive oil and honey. This preparation is employed for both the prevention and treatment of gastric ulcers (Gürdal, 2013). The extract obtained from bitter melon (MC) fruit has demonstrated significant effectiveness in reducing neuro-inflammation, thereby improving, and alleviating neurodegenerative diseases in rats (Nerurkar *et al.,* 2011).

Moreover, Jerald (2012) found that Momordica charantia seed and pulp extract when administered orally cause infertility in male albino rats and observations of in vivo anti spermatogenic activity have been made for alcoholic seed extract of M. charantia (Naseem *et al.,* 1998). Tests conducted on rats using extracts of M. charantia seeds revealed significant anti spermatogenic activity, resulting in a decrease in the number of spermatocytes, spermatids, and spermatozoa. Moreover, an increase in cholesterol levels and accumulation of Sudanophilic lipids indicated inhibition of steroidogenesis. The alcoholic extract exhibited the most potent anti spermatogenic, anti-steroidogenic, and androgenic activities (Naseem, 1998). The use of *M. Charantia* (bitter melon) seed extracts resulted in infertility in male rats. This infertility was likely caused by the direct toxic effects on the seminiferous tubules and epididymis, as well as the reduction in testosterone levels, which could have an impact on sperm parameters (Tumkiratiwong, 2014).

In addition, Patil (2011) concluded that the seeds of *Momordica charantia* (bitter melon) exhibited an anti-spermatogenic effect, as evidenced by a decrease in the number of spermatogonia, spermatocytes, spermatids, and spermatozoa. Similarly, Tumkiratiwong, (2020) conducted experiments on rats indicating that ethanol extracts of *M. charantia* seeds disrupted the activities of testicular superoxide dismutase (SOD), (POD) and catalase (CAT) enzymes. Thus, the present study was designed with the aim of evaluating the potential antifertility effect of M. charantia seed powder in male Nile tilapia (*O. niloticus*). We hypothesized that dietary inclusion of Momordica charantia seed powder causes infertility in male Nile tilapia (*O. niloticus*), so to prove the hypothesis, 80-days feeding trial was conducted with the following objectives.

Objectives

To determine the effect of an M. charantia seed powder-supplemented diet on:

• Gonadosomatic index (GSI)

• The morphological changes in testicular tissue through microscopy

• Blood testosterone level

• Status of antioxidant enzymes: SOD, POD and CAT activity in gonadal tissue of male Nile tilapia

• Hepatosomatic index (HSI).

MATERIALS AND METHODS

2.1 Fish

Before transferring the fish, raceways were, underwent a thorough cleaning process, whitewashed with lime to prevent bacterial infections and then filled with water. Approximately 220 healthy fingerlings of Nile tilapia (*Oreochromis niloticus*) were acquired for the experiment via live hauling technique in air-tight sealed plastic bags with sufficient oxygen and transported from the National Agricultural Research Centre (NARC) Islamabad to the Fisheries and Aquaculture Research Centre Station at Quaid-i-Azam University (QAU) Islamabad. Before transferring to raceways, fish were tempered by a gradual exchange of water from raceways and treated with a 0.2% KMNO4 solution, to protect against potential diseases or parasite infestations. After transferring to concrete raceways, fish are allowed to acclimatize for about two weeks. During acclimatization, fish were provided basal diet.

2.2 Collection of bitter gourd seeds (BGS).

Ripe fruits of a local variety were procured from a nearby vegetable market of district Swat in April. The fruits were sliced in two halves; seeds were manually extracted from the fruits and underwent thorough washing using water to ensure complete elimination of any plant residues or foreign matter. Afterwards, seeds were air-dried in the shade at room temperature or in an electric oven (SANFA DHG-9053A) on stainless steel trays until moisture-free seeds were attained. Once dried, the seeds were stored in air-tight jars for future utilization in the experiment.

2.3 Preparation of experimental fish feed

A basal feed of 35% crude protein (CP) with graded inclusion (0/kg, 2g/kg, 4g/kg, 6g/kg, 8g/kg, 10g/kg) of *M. charantia* seed powder was formulated based on the nutritional requirement of *O. niloticus* as shown in Table No. 1. Dry feed ingredients including corn gluten, sunflower, wheat flour Fish meal, Rice polish soybean meal, vitamin and minerals premixes were finely grounded. Moreover, the *M. charantia* seed powder supplements were mixed at graded level in the feed ingredients. The whole ingredients were mixed thoroughly following the formulations and dough was prepared by the addition of water. Then, the dough was passed through a meat grinder for pellets formation. The prepared pellets were placed in the oven for drying at 60℃ for 24 hours and then crushed into small crumbled pieces according to fish mouth size. Both the experimental and control diet were packed in separate

airtight Ziplocs to prevent moisture and microbial contamination. During the experiment the fish were fed twice a day at the rate of 5% of their body weight. Six different feed batches contain graded level of *M. charantia* seed powder were prepared i.e., five experimental feeds comprised of *M. charantia* seed's powder and the control diet comprise of basal diet only.

2.4 Experimental design

After 14 days of acclimatization period, a completely randomized 90 days experiment was designed in triplicate. About 200 fish having initial body weight (10.2g±2.0) were equally distributed in 6 fiberglass tanks (33 fish/tank) having 350 L of water with a stocking density of 1.0g/L. Tank were randomly divided into the following six groups. First tank was assigned as a Control group while remaining tanks was assigned as treatment groups i.e., S2, S4, S6, S8 and S10. During the experiment, fish were fed with 35% crude protein diet having graded level of *M. charantia* seed powder inclusion for 90 days at the rate 5% of their body weight. Further, feeding rate was adjusted fortnightly according to their body mass.

Moreover, 35% crude protein diet was fed with different supplements bitter melon (seed powder) are as follow;

Control group (C): Diet without supplement

S2: Fish diet supplemented with 0.2% of seed powder

S4: Fish diet supplemented with 0.4 % of seed powder

S6: Fish diet supplemented with 0.6 % of seed powder

S8: Fish diet supplemented with 0.8 % of seed powder

S10: Fish diet supplemented with 1 % of seed powder

Throughout the experiment, close monitoring of water parameters was conducted. This included regular assessments of temperature, dissolved oxygen (DO) levels, and ph. Water temperature during experimental period ranged between 25-28℃.All tanks were adjacent to each other, thus no noticeable changed in temperature was observed among different tanks

To ensure optimal water quality, partial water replacements were performed every 2 or 3 days. The decision to replace the water was based on continuous monitoring of water quality parameters and turbidity levels. This practice helped maintain a clean and conducive

environment for the fish, as any accumulated pollutants or contaminants were effectively removed. Moreover, the concentration of total ammonia in the water was measured using the API freshwater test kit specifically designed for this purpose. Monitoring the ammonia levels was crucial, as excessive amounts can be harmful to the fish.

2.5 Fish sampling

Before sample collection, the fish were subjected to a 24-hour fasting period. Subsequently, the fish were promptly anesthetized using MS-222 at a concentration of 250mg/L, as described by (Abdel-Tawwab *et al.,* 2020). Measurements of fish length and weight were taken, and blood samples were collected from the caudal vein using a pre-heparinised 3ml syringes.

For haematological analysis, heparinised fresh blood samples (n=9) were collected in EDTA tubes. Serum blood samples (n=9) were collected in serum separation (SST) tubes and then centrifuged at room temperature at 5000xg for 20 minutes. The resulting serums were utilized for biochemical and testosterone hormone analysis.

The Gonadosomatic Index (GSI) was determined by weighing the testes of each male Nile tilapia. The target organ (testicular tissue) was then rapidly submerged in liquid nitrogen (-192℃) and stored at -20℃ for further analysis of antioxidant enzyme activity. After sample collection, the fish were euthanized on an icebox. Testicular tissues were sampled from three fish per tank and fixed in 10% formalin for histopathological examination.

2.6 Growth Performance

Prior to dissection, the length and weight of each fish were recorded, and the total number of fish in each fiberglass tank was determined to calculate the average body weight. To assess the growth performance, the following formulas were utilized:

$$\text{Wt. gain (\%)} = \frac{\text{Final body weight} - \text{Initai body weight}}{\text{Final body weight}} \text{x}100$$

$$\text{SGR (\%)} = \frac{\ln(\text{Final weight}) - \ln(\text{Initial body weight})}{\text{Number of days of experiment}} x100$$

SGR=specific growth rate

Where *ln*=natural log

2.7 Haematological parameter

Haematological indices, including white blood cells (WBCs), red blood cells (RBCs), haemoglobin, haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV), were analysed from fresh blood samples. An automatic hematology analyser (Sysmex hematology analyzer) was used for this purpose. Each sample was analysed in duplicate with a 15-second interval between measurements. The mean values of the duplicate measurements were calculated to obtain the final results for each haematological index.

2.8 Total Proteins determination

The method employed by Lowery et al. (1951) was used for the quantification of total proteins in both the control and treatment groups' plasma. A solution of Standard proteins BSA (bovine serum albumin) was prepared by dissolving 1 mg of BSA in 1 ml of D-H2O in a clean test tube, maintaining a 1:1 ratio. To create standards, this stock solution was diluted with distilled water to achieve a volume of 5 ml in each test tube. Following the dilution process, 2000 µl of proteins were transferred using a pipette to a separate test tube, and 2000 µl of Alkaline CuSO4 reagent were added, thoroughly mixed, and left to incubate for ten minutes at 25℃. 200 µl of Folin-Ciocalteu reagent was introduced into each test tube and incubated at 25oC for another 30 minutes. The absorbance was then measured at 660 nm using a spectrophotometer. A standard calibration curve correlating absorbance values to protein concentration was constructed, enabling the determination of total protein concentration in the unknown sample using a spectrophotometer and the standard curve plot.

2.9 Antioxidant enzymes determination

The testicular tissues were homogenized using a Dounce manual homogenizer (Sigma-Aldrich) in 100 mmol potassium phosphate buffer (PBS) containing 1 mmol ethylenediaminetetraacetic acid (EDTA). The homogenization process was performed to break down the tissue and obtain a homogenous mixture. After homogenization, the samples were centrifuged at 4℃ for 30 minutes at 12,000 × g (Younas *et al.,* 2022). This centrifugation step allowed for the separation of the supernatant, which contains the soluble components of the homogenate, from the pellet, which consists of insoluble materials. The supernatant was carefully collected in an Eppendorf tube, while the pellet was discarded. The collected supernatant samples were stored at -20℃ for further analysis. This storage temperature helps

to preserve the stability and integrity of the samples until they are ready for additional investigations or experiments.

2.10 Superoxide Dismutase (SOD)

Kakkar *et al.* (1984) method was used for SOD activity in testicular tissues. 0.3 ml supernatant and 0.1 mL of 186 µM phenazine methosulphate solution mixed with 1.2 mL of 0.052 mM sodium pyro-phosphate buffer (pH=7.0). Next 0.2 mL of 780 µM NADH+ was added to start the reaction and then added 1mL of glacial acetic acid was to stop further. Colour changes were measured at 560nm wavelength with the help of spectrophotometer (UV 3100 PC). One unit of SOD is defined as the amount of enzymes/protein in mg that hinder the quercetin oxidation by 50%. The results of SOD activity were presented in moles/min/mg protein using a molar coefficient of 6.22103/M cm

2.11 Catalase (CAT) Assay

The activity of Catalase in testicular tissues was calculated following the Chance & Maehly (1955) method. 2500ul of 50 mM Pbs buffer (pH=5), 100 µl 0f supernatant, and 400 µl of 5.90 mM H2O2 were mixed and the reaction mixture absorption was checked at 240 nm wavelength via spectrophotometry. The result was expressed as nmol per min per mg protein with a molar coefficient of 43.6/ M cm.

2.12 Peroxidase (POD) assay

Chance & Maehly (1955) and Bibi (2012) method was used to quantify POD activity in testicular tissues. For the reaction, 2500ul of 50 mM phosphate buffer solution (pH 5), 300ul of 40 mM hydrogen peroxide, 100ul of 20 mM Guaiacol, and 0.1 ml of supernatant were mixed and after 1-minute absorption was checked at 470 nm. POD activity was measured in nmol per min per mg protein with a molar coefficient (of 2.66×104 M-1).

2.13 Testosterone ELISA assay

To measure the serum concentration of testosterone, the Cal biotech, Inc. (CBI) Testosterone ELISA Kit (Catalog No. TE373S) was used. The ELISA (Enzyme-Linked Immunosorbent Assay) technique is employed, which involves specific antibody-antigen interactions for quantitative analysis. The ELISA assay was performed following the protocols provided by the manufacturer (Cal biotech, Inc.). The serum samples were appropriately prepared and processed according to the kit instructions. The ELISA kit

provided the necessary reagents and standards for the measurement of testosterone concentration.

2.14 Gonadal somatic index (GSI)

At the end of the experiment, five fish from each group were selected. The weight of each fish was measured, and their gonadal mass was determined after sacrificing the fish. To calculate the Gonadal Somatic Index (GSI), the following formula was employed:

GSI= Weight of gonads in g/ Weight of fish in g ×100.

2.15 Sperm count

2.15.1 Serial dilution

To produce a standard curve with linear correlation between the cell concentration determined by hemocytometer and the absorbance reading, sperm samples collected by crushing or stripping were serially diluted with serial ratios of 1:2, 1:4, 1:8, 1:16, 1:32, and, if necessary, 1:64. This was performed by mixing the sperm sample from each dilution step with the same volume of HBSS 300 and resulted in a set of five to six times serially diluted samples for each fish. To assure accuracy of the measurements of these diluted suspensions.

2.15.2 Absorbance measurement by use of spectrophotometer

The microspectrophotometer (Nanodrop 1000; www. nanodrop.com) used in this study for absorbance measurements was operated following the instructions provided by the manufacturer. The ‘‘Cell Culture’’ module from the instrument program was used for measuring the absorbance of each sample. Sample volume needed for this microspectrophotometer can be as small as 1 mL; however, as recommended by the manual, a 2-mL sample size was used in this study to ensure that there was proper sample column formation between the upper and lower pedestals. Before loading samples onto the lower pedestal of the instrument, the sperm suspension was mixed by tapping the tubes 10 times each by a finger to provide homogeneity, and the absorbance was measured immediately (within 10 s) to avoid precipitation of sperm. The buffer used for suspending the sperm samples, HBSS 300, was used as a reference blank before measurement of samples. Between measurements, the upper and lower pedestals of the instrument were wiped clean with a dry Kim-wipe. For each sample, the absorbance was measured three times, and an average of these absorbance values was used in data analysis. The absorbance values at wavelengths

across the UV to visible spectra (from 200 to 780 nm) were recorded automatically in single scans by the instrument and were processed using Microsoft Excel (2007 version).

2.16 Reactive oxygen species (ROS)

ROS production was assessed following the protocol outlined by Hayashi *et al*. (2007). Reagent 1 was composed of 1 mg of N′N-Diethyl para phenylenediamine sulphate (DEPPD) dissolved in 10 mL of distilled water. Reagent 2 consisted of 50 µl of FeSO4 stock solution (50 mg ferrous sulphate in 10 mL of sodium acetate buffer, pH 4.8) diluted in 100 mL of sodium acetate buffer. Reagent 1 and Reagent 2 were combined at a 1:25 ratio and kept in the dark for 2 minutes. In a cuvette, 60 µL of tissue homogenate was mixed with 1680 µl of the aforementioned reagent mixture and 1200 µl of sodium acetate buffer. The absorbance was measured at 505 nm using a UV spectrophotometer (Agilent 8453, California, USA). Three readings were taken at 15 second intervals for each sample, and an average value was calculated.

2.17 Testicular tissue histology

To investigate the alterations in gonadal tissues of Nile tilapia, three fish per group were sampled from each of the six groups. The samples were then fixed in a 10% neutral buffered formalin solution to preserve the tissue structure. After fixation, the samples underwent a series of processing steps. They were dehydrated using alcohol, followed by clearing in xylene to remove any remaining water. The dehydrated samples were then embedded in paraffin wax to provide support during sectioning. Subsequently, the paraffin-embedded samples were sectioned into slices with a thickness of 5 micrometres. These serial sections were carefully prepared to ensure a representative representation of the gonadal tissue. Finally, the sections were subjected to staining using hematoxylin and eosin (Spencer et al., 2013). Hematoxylin stains the nuclei of cells blue-purple, while eosin stains the cytoplasm and other cellular components pink.

RESULTS

3.1 Growth performance

Diets containing bitter melon seeds had a significant effect on the growth of fingerlings of *O. niloticus*. This is supported by the clear results presented in Table (3). Analysing the data with a statistical method One-way (ANOVA) showed that diets with different amounts of bitter melon had a significant impact on the final body weight of the fish (n=6, F_{530}=26; P<0.003), %WG (n=3, $F_{5\ 30}$=1.4; P>0.001), %SGR (n=3, $F_{5,30}$=111; P<0.001), WG (n=3, $F_{5\ 30}$=89 ; P<0.001),FCR (n=3, $F_{5\ 30}$=607; P<0.004). Pairwise multiple comparison showed that the fish of control group had the lowest percentage of weight gain (%WG). Following this, the S2, S4, S6, and S8 groups exhibited progressively increasing %WG. On the other hand, the fish group S10 displayed the highest %WG among all the groups.

Additionally, using one-way ANOVA, we found a significant difference in the Feed Conversion ratio (FCR) among the different groups of fish that were given bitter melon seed powder supplements (n=3, F5,30=607, P<0.001). When comparing the results, the fish of control group had larger FCR, followed by the S2, S4, S6, and S8 groups. The S10 group had the most favourable FCR (Table 3).

3.2 Haematological indices

Our research uncovered a notable impact of a diet enriched with bitter melon seed on the blood indices of *O. niloticus*. The analysis using ANOVA demonstrated that the inclusion of bitter melon led to a significant alteration (P<0.05) in the blood parameters following 90-day feeding trial. Statistical analysis indicated a notable increase in RBCs(n=9, F5,48=18.1; P<0.003),WBCs (n=9, F5,48=412; P<0.003), haemoglobin (n=9, F5,48=19.1; P<0.005), PCV (n=9, F5,48=6707; P<0.002), MCH (n=9, F5,48=226; P<0.004), MCHC (n=9, F5,48=321; P<0.001).furthermore there is a small but significant increase in RBC and Hb. However, when employing the Multiple Pairwise Comparison LSD test (using SPSS), the results revealed that the inclusion of 10g of seed show a more pronounced and statistically significant impact (P<0.05) compared to all other groups. (Table 04)

3.3 Gonad-somatic index

In current study, bitter melon seed powder showed the most significant effect on the gonadal somatic index (GSI) of male Nile tilapia (n=9, F5,48=179.2; P<0.003). However,

when employing the Multiple Pairwise Comparison LSD test (using SPSS), the Gonadal Somatic Index (GSI) of S10 fed 10g of seed powder displayed a the most significant decrease ($P<0.05$) compared to all other treatment groups. The order of significant decrease was as follows: C/S0 > S2 > S4 > S6 > S8 > S10, respectively. (Table 05)

3.4 Testosterone concertation

In the present experiment *M. charantia* seed powder fed to Nile tilapia were displayed a significant impact ($n=9$, $F_{5,48}=329.2$; $P<0.003$) on their testosterone levels. By employing One-way ANOVA and performing Multiple Pairwise Comparison LSD tests (using SPSS), the findings indicated a dose-dependent fall in hormonal concentration. Furthermore, the lowest concentration was observed in (S10), followed by (S8). The order of significant decrease was as follows**:** C/S0 > S2 > S4 > S6 > S8 > S10 respectively (Table 06).

3.5 Hepatosomatic index (HSI)

In the current study, the hepatosomatic somatic index (GSI) of male Nile tilapia displayed non- significant but positive impact ($n=9$, $F_{5,48}=1.33$; $P<0.2848$) *M. charantia* seed powder supplements in the diet. However, by using one-way ANOVA and performing Multiple Pairwise Comparison LSD test (using SPSS), the hepatosomatic Somatic Index (GSI) displayed a slight increase that was statistically non-significant ($P<0.2848$) (Table 07)

3.6 Reactive oxygen species (ROS)

In the present experiment, Nile tilapia were fed with *M. charantia* seed powder-supplemented diet displayed a significant impact ($n=9$, $F_{5,48}=329.2$; $P<0.05$) on ROS levels of serum. By utilizing One-way ANOVA and performing Multiple Pairwise Comparison LSD tests (using SPSS), the findings indicated a dose-dependent fall in serum ROS concentration.

Furthermore, considerably lower concentration of ROS was observed in the group fed 10g of seed powder/kg diet (S10), followed by (S8). The order of significant decrease was as follows: C/S0 > S2 > S4 > S6 > S8 > S10 respectively. (Table 08)

3.7 Antioxidant enzymes activity

The levels of antioxidant enzymes in the gonad of Nile tilapia displayed significant variations ($P<0.05$) among all the experimental groups. The present experimental investigation highlighted a substantial impact of bitter melon on the enzyme Catalase ($n=9$, $F_{5,48}=2.61$; $P<0.005$), superoxide dismutase ($n=9$, $F_{5,48}=3.09$; $P<0.005$) and peroxidase ($n=9$, $F_{5,48}=10.9$; $P<0.004$). Furthermore, multiple Pairwise comparison LSD test (utilizing SPSS) demonstrated the most significant increase in antioxidant activity was in the group with 10g of bitter melon inclusion (Table 08)

3.8 Testosterone level

In the present experiment, Nile tilapia were fed with *M. charantia* seed powder displayed a substantial impact ($n=9$, $F_{5,48}=329.2$; $P<0.003$) on their testosterone levels. By employing One-way ANOVA and performing Multiple Pairwise Comparison LSD HSD tests (using SPSS), the findings indicated a dose-dependent fall in hormonal concentration. Furthermore, the lowest concentration was observed in a group with 10g of seed powder supplementation (S10), followed by (S8). The order of significant decrease was as follows: C/S0 > S2 > S4 > S6 > S8 > S10 respectively. Table 09.

3.9 Testicular histology

Testes histology was examined by of a photomicroscope (Olympus, Japan)Histological examination of the testes of *O. niloticus* fed with different levels of bitter melon seed meal (BMSM) in their diets revealed distinct observations. The control group that received 0g BMSM/kg diet exhibited typical testicular tissue structure and even distribution of sperm cells in the lumen of seminiferous tubule (Figure A). Fish fed with 2g BMSM/kg (S2) displayed a smaller number of sperm in the lumen of seminiferous tubule (Figure B). Among those fed 4g BMSM/kg (S4) show a greater number of sperm (Figure C) compare to (S6) and (S8) ie (Figure D) and (Figure E) respectively. Fish fed with 10g BMSM/kg diet(S10) showed least number of sperm in the lumen of seminiferous tubule.

Table 01: Ingredients and formulation of 35% CP diet supplemented with bitter melon (*M. charantia*) seeds powder at graded level

Ingredient	Control	S2	S4	S6	S8	S10
Fish meal (60%)	22	22	22	22	22	22
Soybean (48%)	14.5	14.5	14.5	14.5	14.5	14.5
Rice polish (12%)	15	15	15	15	15	15
Wheat flour (12%)	14.5	14.3	14.1	13.9	13.7	13.5
Sunflower (34%)	10	10	10	10	10	10
Corn Gluten(60%)	14.5	14.5	14.5	14.5	14.5	14.5
Fish oil	05	05	05	05	05	05
Minerals and Vitamins premix	02	02	02	02	02	02
Vit C	0.5	0.5	0.5	0.5	0.5	0.5
CMC	02	02	02	02	02	02
Seed powder	00	0.2	0.4	0.6	0.8	1.0
Total	100	100	100	100	100	100

Table 02: Proximate composition of experimental feed

Ingredient %	Control	S2	S4	S6	S8	S10
Moisture	10.28	10.24	10.17	10.31	10.52	10.29
Crude protein	33.9	34.32	34.25	34.42	35.13	35.44
Crude lipids	12.35	12.17	12.41	12.45	12.16	12.33
Crude fiber	7.07	8.03	8.1	8.05	8.09	8.34
Total ash	12.13	12.23	12.31	12.20	12.15	12.10

Table 03: Growth performance of Nile tilapia feeding graded levels of bitter melon (*M. charantia*) seeds powder supplemented diet for 90 days trial

Parameters	Control	S2	S4	S6	S8	S10	F-value	p
FBW(g)	20.1 ± 1.7^{f}	21.4 ± 1.2^{e}	23.4 ± 1.4^{d}	25.4 ± 1.7^{c}	29.8 ± 1.3^{b}	34.5 ± 1.1^{a}	26573	P≤0.001
NWG	9.6 ± 0.05^{f}	11.3 ± 0.08^{e}	13.1 ± 0.01^{d}	14.8 ± 0.04^{c}	19.2 ± 0.02^{b}	23.9 ± 0.05^{a}	89576	P≤0.001
SGR (%body weight/day)	0.39 ± 0.02^{f}	0.49 ± 0.06^{e}	0.55 ± 0.03^{e}	0.61 ± 0.02^{c}	0.8 ± 0.01^{b}	0.91 ± 0.08^{a}	112	P≤0.001
%WG	100.2 ± 1.6^{f}	112.8 ± 1.7^{e}	126.3 ± 1.8^{d}	140.3 ± 1.4^{c}	181.1 ± 1.9^{b}	223.9 ± 1.3^{a}	1.400	P≤0.001
FCR	2.21 ± 0.5^{a}	2.09 ± 0.3^{b}	1.97 ± 0.8^{c}	1.89 ± 0.7^{d}	1.76 ± 0.5^{e}	$1.\pm69.4^{f}$	184	P≤0.001

The data in table (3) is shown as Mean SE(n=3). To demonstrate pairwise comparisons between groups, one-way ANOVA and post-hoc LSD tests were utilized. Lower-case superscripts indicate significant differences (p=<0.05). the groups include S2 (2-g seeds powder/k g diet), s4 (4g seeds powder/kg diet), s6 (6-g seeds powder/kg diet), s8 (8-g seeds powder/kg diet), s10 (10-g seeds powder/kg diet).

Table 04: Haematological parameters of Nile tilapia fed bitter melon (*M. charantia*) seeds powder supplemented feed for 90 days trial.

Group	Control	S2	S4	S6	S8	S10	F-value	P
RBCs(10^6/µl)	1.81±0.12^{d}	1.85±0.14^{cd}	1.88±0.16^{c}	1.93±0.15^{b}	1.96±0.13^{ab}	2.00±0.18^{a}	17.6	P≤0.001
WBCs (10^3/ µl)	3.30±0.05^{f}	3.50±0.03^{e}	3.70±0.04^{d}	4.10±0.01^{c}	4.24±0.06^{b}	4.34±0.02^{a}	412	P≤0.001
Hemoglobin(g/dl)	4.71±0.28^{d}	4.75±0.19^{cd}	4.77±0.23^{bc}	4.78±0.17^{bc}	4.80±0.20^{b}	4.90±0.14^{a}	19.1	P≤0.001
PCV%	27.61±0.7^{f}	28.60±0.4^{e}	29.84±0.9^{d}	30.84±0.3^{c}	31.78±0.6^{b}	32.27±0.3^{a}	6707	P≤0.001
MCV (um^3)	110.2±0.27^{f}	115.5±0.31^{e}	122.2±0.45^{d}	127.4±0.33^{c}	132.7±0.53^{b}	134.0±0.41^{a}	4508	P≤0.001
MCH (pg)	51.7±0.9^{f}	56.4±0.6^{e}	60.2±0.4^{d}	64.5±0.7^{c}	68.1±0.1^{b}	69.2±0.5^{a}	16892	P≤0.001
MCHC(g/dl)	33.5±1.8^{f}	34.7±1.7^{e}	36.5±1.3^{d}	38.1±1.6^{c}	40.1±1.2^{b}	41.6±1.1^{a}	1550	P≤0.001

Data in Table (4) is expressed as Mean SE(n=9). ANOVA and the post-hoc LSD test were utilized to illustrate pairwise comparisons of RBCs, Hemoglobin, PSV, MCV, MCH, MHCH among groups. Lower-case superscripts indicate significant differences (p=<0.05). The groups include: Control (C) (diet without bitter melon seeds powder), S2(2g of seeds powder/kg of diet), S4(4g of seeds powder/kg diet), S6(6g of seeds powder/kg of diet),S8(8g of seeds powder/kg of diet) and S10(10g of seeds powder/kg of diet).

Table 05: Gonadosomatic index of male Nile tilapia fed bitter melon (*M. charantia*) seeds powder supplemented feed for 90 days trial.

Groups	C	S2	S4	S6	S8	S10	F-value	p
GSI	1.26±0.04[a]	1.20±0.07[b]	1.05±0.05[c]	0.91±0.09[d]	0.67±0.06[e]	0.60±0.02[f]	2226	P≤0.001

The data in table (5) is shown as Mean SE(n=9). ANOVA and the post-hoc LSD test were employed to demonstrate the pairwise comparison between groups. Lower-case superscripts indicate significant differences (p=<0.05). the groups include S2 (2-g seeds powder/kg diet), s4 (4-g seeds powder/kg diet), s6 (6-g seeds powder/kg diet), s8 (8-g seeds powder/kg diet), s10 (10-g seeds powder/kg diet).

Table 06: Serum Testosterone concentration of Nile tilapia fed bitter melon (*M. charantia*) seeds powder supplemented diet for 90 days trial

Hormone	C	S2	S4	S6	S8	S10	F-value	p
Testosterone(ng/ml)	3.41±0.1[a]	3.00±0.9[b]	2.57±0.3[c]	2.14±0.7[d]	1.44±0.2[e]	0.91±0.8[f]	79.1	P≤0.001

The data in table (6) is shown as Mean SE(n=9). ANOVA and the post-hoc LSD test were employed to demonstrate the pairwise comparison between groups. Lower-case superscripts indicate significant differences (p=<0.05). the groups include S2 (2-g seeds powder/kg diet), s4 (4-g seeds powder/kg diet), s6 (6-g seeds powder/kg diet), s8 (8-g seeds powder/kg diet), s10 (10-g seeds powder/kg diet)

Table 07: Hepatosomatic index of Nile tilapia fed bitter melon seeds powder supplemented diet for 90 days trials.

parameter	Control	S2	S4	S6	S8	S10	F	P
HSI	0.68 ± 0.04^{b}	0.70 ± 0.08^{ab}	0.71 ± 0.06^{ab}	0.72 ± 0.03^{ab}	0.72 ± 0.07^{ab}	0.73 ± 0.08^{a}	1.33	0.2848

Data in Table (7) is expressed as Mean ± SE (n=9). ANOVA and post-hoc LSD tests were utilized to illustrate pairwise comparisons between the groups. Different Lower-case superscripts within row indicate significant differences (p=<0.05). The groups include: Control (C) (diet without bitter melon), S2 (2g of seeds powder /kg of diet), S4 (4g of seeds powder/kg of diet), S6 (6g of seeds powder/kg of diet), S8 (8g of seeds powder/kg of diet), and S10 (10g of seeds powder/kg of diet).

Table 08: Antioxidant activity evaluated in Nile Tilapia fed bitter melon (*M. charantia*) seeds powder supplemented diet over 90-days trial.

Parameter	C	S2	S4	S6	S8	S10	F-value	p
CAT	62.7±2.8^{c}	63.6±2.7^{bc}	64.9±2.5^{06abc}	65.6±2.3^{abc}	68.1±2.4^{ab}	69.8±2.6^{a}	2.61	0.0805
SOD	55.1±1.6^{b}	57.1±1.3^{b}	59.6±1.9^{ab}	60.8±1.7^{ab}	64±1.1^{a}	65±1.8^{a}	3.09	0.0506
POD	64.1±2.07^{d}	65.2±2.01^{cd}	66.4±2.08^{c}	67.1±2.07^{bc}	68.9±2.05^{ab}	70.8±2.03^{a}	10.9	0.0004
ROS	10.5±0.05^{a}	9.64±0.04^{b}	9.32±0.06^{b}	8.34±0.09^{c}	7.2±0.06^{d}	7.00±0.02^{d}	155	P≤0.001

Data in Table (8) is expressed as Mean ± SE (n=9). ANOVA and post-hoc LSD tests were utilized to illustrate pairwise comparisons between the groups. Lower-case superscripts indicate significant differences (p=<0.05). The groups include: Control (C) (diet without bitter melon), S2 (2g of seeds powder/kg of diet), S4 (4g of seeds powder/kg of diet), S6 (6g of seeds powder/kg of diet), S8 (8g of seeds powder/kg of diet), and S10 (10g of seeds powder/kg of diet).

Table 09: Sperm count of male Nile tilapia fed bitter melon seed powder supplemented feed for 90 days trial.

Parameter	C	S2	S4	S6	S8	S10	F-value	p
GSI	1.26±0.04^{a}	1.20±0.07^{b}	1.05±0.05^{c}	0.91±0.09^{d}	0.67±0.06^{e}	0.60±0.04^{e}	179	0.03
Sperm count (×10^{6})	169 ± 3.2^{a}	132 ± 5.45^{b}	108 ± 7.45^{c}	87 ± 5.23^{d}	64 ± 2.33^{e}	41 ± 4.04^{f}	6477	P≤001

Data in Table (9) is expressed as Mean ± SE (n=3). ANOVA and post-hoc LSD tests were utilized to illustrate pairwise comparisons between the groups. Lower-case superscripts indicate significant differences (p=<0.05). The groups include: Control (C) (diet without bitter melon seeds powder), S2 (2g of seeds powder/kg of diet), S4 (4g of seeds powder/kg of diet), S6 (6g of seeds powder/kg of diet), S8 (8g of seeds powder/kg of diet), and S10 (10g of seeds powder/kg of diet.

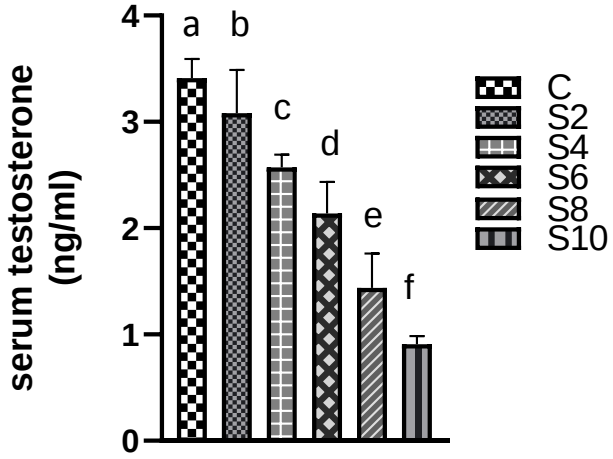

Fig 01: The graph illustrates the blood serum testosterone levels of *O. niloticus* fingerlings fed dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean ± S.E with a sample size (n) of 9.

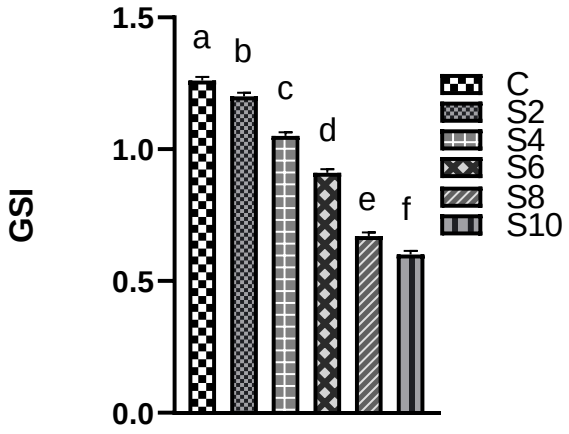

Fig 02: The graph illustrates the GSI of *O. niloticus* fingerlings fed dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

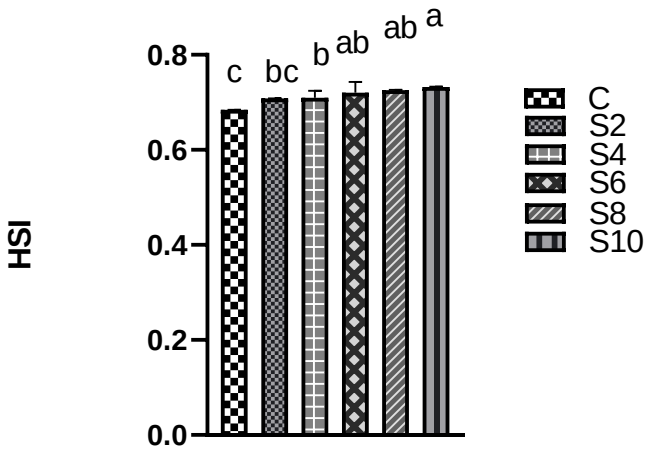

Fig 03: The graph illustrates the HSI of *O. niloticus* fingerlings fed dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

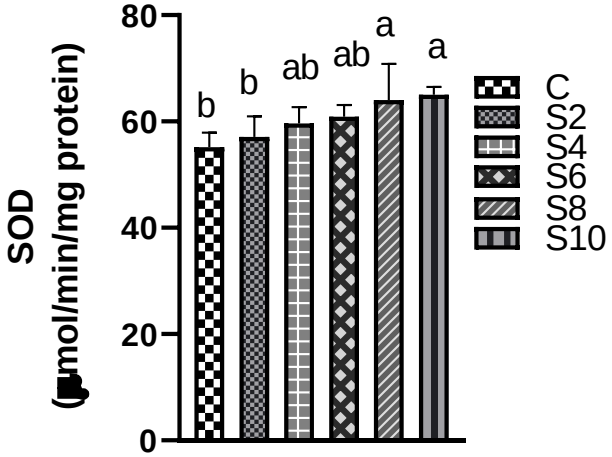

Fig 04: The graph illustrates the SOD activity in the testis of *O. niloticus* fingerlings when fed dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

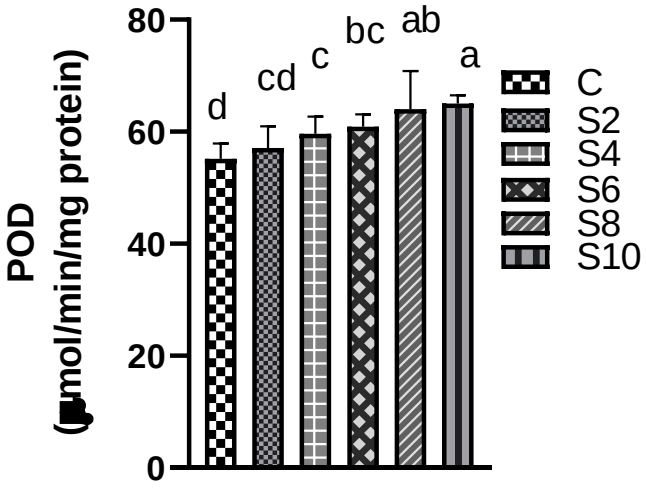

Fig 05: The graph illustrates the POD activity in the testis of *O. niloticus* fingerlings when fed dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

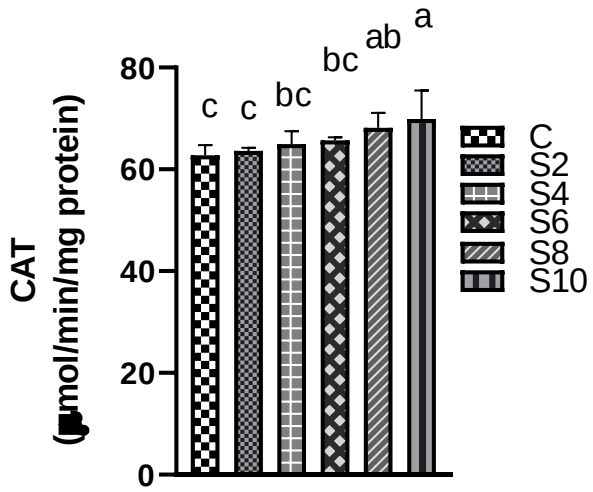

Fig 06: The graph illustrates the CAT activity in the testis of *O. niloticus* fingerlings when fed dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

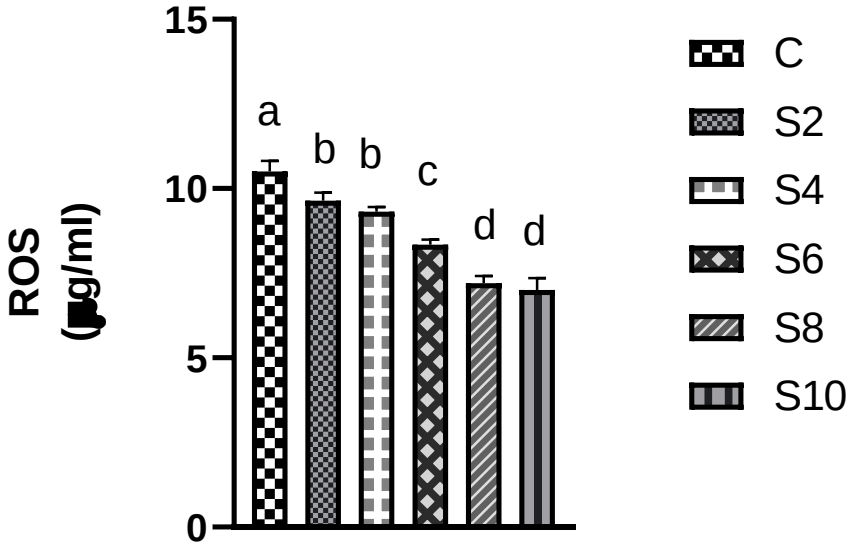

Fig 07. The graph illustrates Ros in the blood of *O. niloticus* fingerlings when fed dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean ±S.E with a sample size (n) of 9.

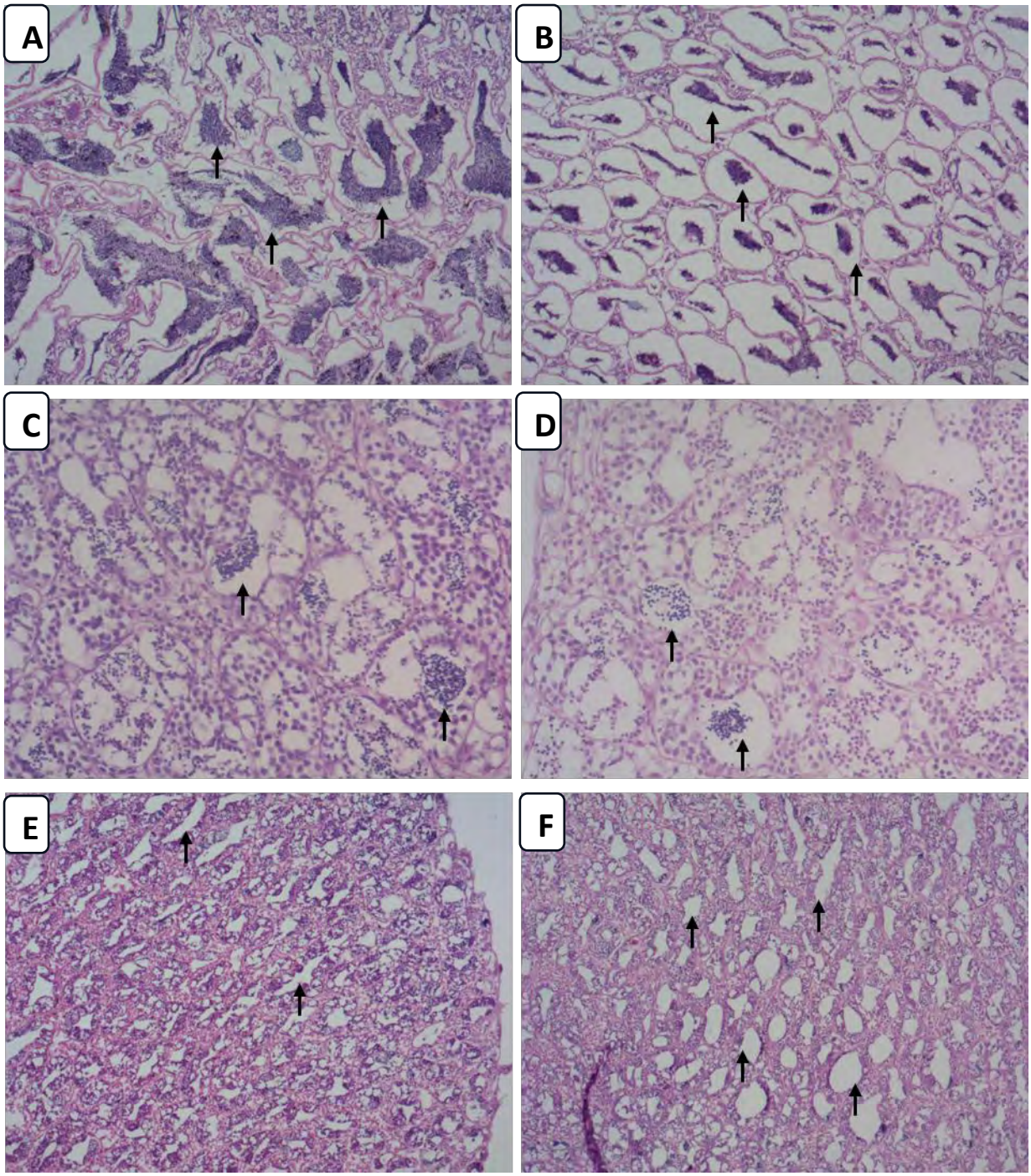

Fig No.8 Histological examination of testicular tissues. (A) Represents CG showing a peak in mature sperms which is stained purple colour. (B) Represents S2 group showing large number of sperm in lumen of seminiferous tubules. (C) Represents S4 group revealing a greater number of sperm in lumen of seminiferous tubules compared to D and E, representing S6 and S8 respectively. While (F) represents S10 exhibiting male gonads that show least number of sperms in seminiferous tubules.

DISCUSSION

Several plants are used for controlling over-breeding in tilapia including *Tribulus terrestris, Mucuna pruriens, and Carica papaya,* among others, have demonstrated promising potential in controlling unwanted breeding in tilapia production systems (Abaho *et al*., 2017). These extracts are primarily administered orally by incorporating them into fish feeds. The main bioactive compounds found in the phytoextracts are saponins and flavonoids, which play a crucial role in inducing sex inversion and impairing fertility in tilapia (Masembe, 2017).

The purpose of this study is to see how *M. charantia* seed powder affect several physiological and histological characteristics of *O. niloticus* when employed to inhibit its prolific reproduction. *M. charantia* effect on fertility, antioxidant of testicular tissues, hormonal profile (testosterone), GSI and testicular tissue effect in Nile Tilapia (*Oreochromis niloticus)* were assessed in this study. Many researches were conducted to examined the bitter melon effect in mice regarded to diabetes, antioxidant activity and infertility factor but few study are available in the case of its effect on fishes. These studies, however, are limited to species other than Nile tilapia.

Medicinal plant is frequently used in aquaculture to control unwanted breeding in tilapia. In fact, when incorporated into the diet, they have been found to be useful in controlling prolific reproduction and inhibit breeding in tilapia (Abaho *et al*., 2017). In a research study, supplementation with bitter melon seed extract resulted in a significant reduction in serum gonadotropins and testosterone concentrations in male rats. Additionally, there was a significant decrease in sperm production compared to the control group when fed to male rats. (Yama *et al.*, 2011). Furthermore, according to a study by S. A. Patil and S. B. Patil (2011), bitter melon seed extracts demonstrated an anti-spermatogenic effect, leading to a reduction in the number of spermatogonia, spermatocytes, spermatids, and spermatozoa in male mice.

The growth metrics encompassing final body weight (Fbw, in grams), weight gain (Wg), specific growth rate (SGR), weight gain percentage (Wg%), feed conversion ratio (FCR), and SGRs, ascertained in *Oreochromis niloticus* (Nile tilapia) fed with bitter melon seed, align with the findings of Dada and Ajilore (2009). Their research previously

demonstrated that *G. kola* exhibits growth-enhancing effects in aquatic organisms. This augmentation of growth can be attributed not solely to the presence of bioflavonoids, recognized as plant growth stimulants within *G. kola* seed. (Jerald *et al*., 2011) also reported the presence of flavonoid in the seeds of *M. charantia.* but also, to their ability to enhance the palatability of the provided feed. The reduction observed in feed conversion ratio (FCR) values among *O. niloticus* (Nile tilapia) nourished with *M. charantia* seed aligns with the findings of Sogbesan and Ugwumba (2008). These researchers postulated that low feed conversion ratios are indicative of improved utilization of provided feed by the fish, in comparison to control groups.

The *M. charantia* seed considerably enhanced haematological values for PCV, MCH, MCHC, and WBC but had little yet significant ($P<0.05$) effect on RBC and Hb. The current study's considerable rise in PCV is consistent with the findings published by Obaroh *et al* (2017) when *O. niloticus* was fed meals with Mangifera indica dosages (0.5-8 mg kg-1). Likewise, Gabriel *et al* (2015) discovered no significant effect of Aloe vera supplementation on *O. niloticus* RBC. However, the current findings contradict those obtained by Fafioye *et al*. (2012) on *O. niloticus* given *A. indica* dosages (0.1-0.5 g L-1), which demonstrated a substantial decrease in Hb concentration, RBC, PCV, and MCH values. Similarly, Saravanan *et al* (2011) observed a substantial reduction in Hb, PCV, MCV, MCH, and MCHC levels in *C. mrigala* following *A. indica* (1.0 g L-1) exposure.

Haematological markers such as Hb, PCV, MCV, MCH, and MCHC are well recognized for indicating erythrocyte state and oxygen carrying capacity in fish (Gabriel *et al.*, 2014). As a result, elevated levels in these parameters suggest erythropoiesis stimulation, which increases the capacity of oxygen transport and strengthens the defence mechanisms against physiological stress (Gabriel *et al.*, 2014). Overall, these findings suggest that feeding *O. niloticus* diets containing and at the dosage used improves immune system because the majority of haematological parameters obtained in this study are within the ranges for healthy *O. niloticus* cultured in a semi-intensive system, as described by Bittencourt *et al* (2003).

Furthermore, Clauss *et al* (2008) demonstrated that the approved PCV range for fish is between 20% and 45%. Fish with PCV values greater than 45% and less than 20% are believed to have polycythaemia due to dehydration and anaemia, respectively. The majority of the PCV levels in the current study were greater than 20% and less than 45%, indicating that the fish were in good health.

Infertility is associated to hormonal disruptions induced by psychological and physiological factors, in addition to oxidative stress. Some chemicals, notably glucocorticoids, have a deleterious influence on the hypothalamic-pituitary-gonadal (HPG) axis and, as a result, spermatogenesis (Chandra *et al.,* 2012). The hypothalamic gonadotrophin-releasing hormone (GnRH) stimulates the anterior pituitary to produce FSH and LH. They both then regulate spermatogenesis and testosterone synthesis in the gonads. As a result, when the HPG axis is disturbed by hormones such as gonadotrophin-inhibiting hormone (GnIH), prolactin (PRL), and cortisol, spermatogenesis suffers (Nargund, 2015). *M. charantia* increases prolactin levels, which affect sperm production while decreasing serum gonadotrophin and testosterone levels (Oremosu *et al.,* 2011).

Reproductive hormones (testosterone, progesterone, and 17-estradiol), play essential roles in the process of sexual development and maturation (Sahafi *et al.,* 2020). Effective reproduction in fish species relies on the controlled release of gonadotrophic hormones (LH and FSH) from gonadotrophs. This process is influenced by the synchronization of environmental and brain cues, enabling successful reproductive functions. Duru *et al* (2011) found that the seed extracts of *M. charantia* decreases serum testosterone and impair various aspects of sexual performance in male mice.

Testosterone is required for the development of spermatozoa (spermatogenesis) and male fertility (Pratis *et al*., 2002). The process of spermatogenesis takes place in the testis's seminiferous tubules, and testosterone is the most abundant androgen present (Knobil *et al*., 1994). Only Leydig cells, peritubular cells, and Sertoli cells express testosterone receptors (the androgen receptor, AR) in the testis. AR is not expressed in mature testicular germ cells (Lyon *et al.,* 1975) and it is not considered that testosterone acts directly on germ cells.

Instead, the Sertoli cell, which surrounds and nurtures germ cells as they grow into spermatozoa, is the primary target of testosterone in the testis (Griswold, 1998). For many decades, testosterone has been recognized to aid spermatogenesis. To effectively support spermatogenesis, Sertoli cells require substantially greater local amounts of androgen (>70 nM) (Endocr, 1988). The physiological requirement for greater amounts of testosterone in the testis is unknown, except that spermatogenesis is terminated in the absence of relatively high levels of testosterone (>70 nM) in the rat, resulting in the generation of few or no sperm (Zirkin *et al*., 1999).

Current trial results show a significant ($P<0.05$) decrease in serum testosterone in dose dependent manner. Compared to present study results, Naseem *et al* (1998) observed that rats treated with *M. charantia* seed alcoholic extract exhibited cholesterol accumulation in steroidogenic cells, which did not convert steroids/cholesterol into testosterone. This barrier is likely to have influenced the current experiment, leading to decreased testosterone secretion and subsequent suppression of sperm production (spermatogenesis). It may be due to the presence of bioactive compounds such as triterpenoids, alkaloids, flavonoids, and glycosides in seed (Jerald *et al.,* 2012) which have been shown to suppress the pituitary-testicular axis and sperm production in male rats (Yama *et al*., 2011). Moreover, the *M. charantia* seed extract suppressed seminal testosterone level and plasma testosterone level in male mice (Tumkiratiwong *et al*., 2014).

In current study there is a significant ($P<0.05$) decrease in serum testosterone in treated group compared to control group. this results in decreased sperm count hence sperm production is decreased due to low level of testosterone. Our results from sperm count shows decreased number of sperm in high dose treated groups (S8 and S10) which support it further. (Oshiozokhai *et al.,* 2012) conducted experiment on rats by giving *M. charantia* seed orally their results show a decreased number of sperm in treated groups which further support our results. Similarly, Francis *et al* (2011) performed trial on rats by incorporating ethanolic seed extract of *M. charantia* and found that there is a decrease in sperm count, hence our results are parallel to them.

The gonadosomatic index (GSI) of male fish decreased significantly ($P<0.05$) in treated groups in dose dependent manner as compare to control group. this reduced testicular weight may be due to low number of sperm in their testicular tissue or this reduced testicular weight and volume indicate a wide spread destruction (Abney, 1999). This might also be possible that this reduction in GSI may be due to lack of protein components in these testes (Lohiya *et al*., 2002). Similarly, testicular volume has been demonstrated to be favourably related to testosterone levels as well as testicular function (Mahmoud *et al.,* 2003). This implies that the lower testosterone concentration and testicular volume and weight found in our study suggested both significant testicular damage as well as poor spermatogenesis and male infertility.

In our study seed powder of bitter melon when incorporated into the feed of Nile tilapia shows significant ($P<0.05$) decrease in sperm production compared to control group.

similar research was conducted in mice in which serum gonadotropins, prolactin, and testosterone concentrations, as well as sperm production, were measured. The administration of bitter melon extract led to a significant reduction in serum gonadotropins and testosterone concentrations (Yama *et al*., 2011).

In one experiment by Bhatt & Deshpande (2021) alcoholic extracts obtained from *M. charantia* seeds have been shown to reduce testicular weight and inhibit the process of spermatogenesis in rats. This suppression appears to be linked to a reduction in spermatogonia, spermatocytes, and spermatid populations, signalling a potential disturbance in pituitary gonadotropin production or availability, notably follicle-stimulating hormone (FSH) from anterior pituitary. This study further supports our results.

In similar study by Obasola, & Jegede (2016) observed initial milt volume was higher in the control group but decreased as the amount of *Gossypium herbaceum* in the diet increased. This indicates that higher levels of *G. herbaceum* in the diet led to reduced milt production. The milt count was initially higher in the control group but decreased as the amount of *G. herbaceum* in the diet increased. This suggests that increasing the presence of Gossypium herbaceum in the diet led to a reduction in milt count. Hence results from the current study are similar to them.

Increased intracellular measures of highly reactive oxygen species (ROS) are linked to oxidative stress and can harm lipids, DNA, and proteins during physiological processes in the body (Martínez-Álvarez *et al.,* 2005) and Oxidative stress, caused due to imbalance between ROS production and antioxidant defence, can lead to DNA hydroxylation, protein breakdown, lipid peroxidation, trigger apoptosis, and ultimately cell death (Schieber & Chandel, 2014). Enzymes such as superoxide dismutase, lipid peroxidase, glutathione reductase (GR), catalase (CAT), and glutathione are part of the antioxidant system, as are non-oxidative systems such as vitamin C and E (Mishra *et al.,* 2015).

The most significant elements of the antioxidant system in fish are SOD, CAT, and POD (Alvarez *et al*., 2005). These antioxidant enzymes' primary responsibilities are to eliminate ROS in order to keep the body's internal environment stable (Kroon *et al*., 2017). The activities of SOD, CAT, and POD indirectly reflect the host's ability to eliminate ROS and are often utilized as functional measures for assessing immunological potential (Rodrıguez,& Moullac , 2000). Superoxide dismutase SOD, catalase CAT and peroxidase POD is a type of ubiquitous and important metalloenzymes that efficiently catalysis the

dismutation process of O2 and transforms it into hydrogen peroxide and oxygen for defence against ROS (Zelko *et al*., 2002).

The results of experimental trail in our investigations revealed that the antioxidant system (SOD, POD, and CAT) in Nile Tilapia testicular tissues was significantly ($P<0.05$) elevated in higher doses (s8 and s10) as compare to (s6, s4, s2) as well as to control. These findings showed that enhanced testicular antioxidant enzyme activity was associated to increased oxidative stress. These findings also revealed that *M. charantia* seed powder induced testicular oxidative stress, which provoke the testicular antioxidant system. Thus *M. charantia* seeds disrupted testicular SOD, POD, and CAT activities. A similar outcome was investigated when rats were given seed ethanol extracts of *M. charantia* orally and found that testicular SOD, GSH-Px, and CAT activity were affected by ethanol extracts of *M. charantia* seeds (Tumkiratiwong *et al.,* 2019).

The study conducted by Bucholtz and colleagues (2008) suggests that gonad development is an ongoing process. However, specific histological (tissue structure) features can be used to identify different stages of gonadal growth during the reproductive phase. So, all the changes observed in the tissue structure of the testes in the experimental fish are likely due to the different levels of dietary BMSM (bitter melon seed meal). There was large number of sperm in the lumen of somniferous tubule of control while very few sperm cells were seen in S8 and S10 group. These recent findings support the understanding of the results obtained in male Nile tilapia by Jegede & Fagbenro (2008).

In the current study, we administered doses ranging from 2 to 10 grams per kilogram of body weight for a duration of 90 days. This approach differs from what previous studies have done in terms of dosage and duration of exposure. Similarly, Jegede (2008) found that Aloe vera latex and the leaves of Hibiscus rosa-sinensis, medicinal herbs, also induce similar changes in the testes of Nile tilapia. Likewise, Goyal *et al* (2010) found similar results of changes in gonads of rabbits and rats.

In a similar study Feng (2011) observed significant degeneration and self-digestion (autolysis) of the seminiferous tubules in Nile tilapia (*O. niloticus*) when they were fed a diet containing 2 grams of pawpaw seed powder per kilogram for periods of 15 and 30 days. in other study on anti-fertility conducted by Verma and Chinoy (2002), they administered papaya seed extract through intramuscular injections to male albino rats at a rate of 5 mg/kg

per day for a period of 7 days. This treatment caused a significant reduction in the ability of the epididymal tubules to contract compared to the control group

Conclusion

Utilizing natural plant, specifically bitter melon seed powder, is proposed as an eco-friendly alternative to synthetic hormones for managing excessive breeding in Nile tilapia. Current study indicated that adding bitter melon seed powder in feed has a positive impact on infertility of Nile tilapia. However, it caused a significant decrease in gonadosomatic index (GSI) and serum testosterone levels, and also led to low number of sperm as observed through testicular histology in dose-dependent manner. Furthermore, it facilitated faster growth in the fingerlings and improved their blood parameters. Consequently, we conclude that *M. charantia* seed powder holds promise for controlling overbreeding in Nile tilapia.

Because the testicular tissue wasn't entirely destroyed, further research is necessary to establish a consistent method for producing sterile tilapia using plant-based materials. This is essential to ensure they can be safely used for commercial purposes.

References

Abaho, I., Masembe, C., Akoll, P., & Jones, C. L. (2022). The use of plant extracts to control tilapia reproduction: Current status and future perspectives. *Journal of the World Aquaculture Society*, *53*(3), 593-619.

Abascal, K., & Yarnell, E. (2005). Using bitter melon to treat diabetes. *Alternative & Complementary Therapies*, *11*(4), 179-184.

Abbas HH, Abbas WT. Assessment study on the use of Pawpaw; Carica papaya seeds to control Oreochromis niloticus breeding. Pak J Biol Sci. 2011 Dec 15;14(24):1117-23. doi: 10.3923/pjbs.2011.1117.1123. PMID: 22335051.

Abdel-Tawwab, M., Monier, M. N., Abdelrhman, A. M., & Dawood, M. A. (2020). Effect of dietary multi-stimulants blend supplementation on performance, digestive enzymes, and antioxidants biomarkers of common carp, Cyprinus carpio L. and its resistance to ammonia toxicity. *Aquaculture*, *528*, 735529.

Abney, T. O. (1999). The potential roles of estrogens in regulating Leydig cell development and function: a review. *Steroids*, *64*(9), 610-617.

Adjabi, S., & Adjina, K. *Effect of using Neem extract "Azadirachta indica" on the sexual differentiation of Tilapia "Oreochromis niloticus"* (Doctoral dissertation, UNIVERSITE KASDI MERBAH-OUARGLA).

Ardó, L., Yin, G., Xu, P., Váradi, L., Szigeti, G., Jeney, Z., & Jeney, G. (2008). Chinese herbs (Astragalus membranous and Lonicera japonica) and boron enhance the non-specific immune response of Nile tilapia (Oreochromis niloticus) and resistance against Aeromonas hydrophila. *Aquaculture*, *275*(1-4), 26-33.

Awuchi, C. G., & Okpala, C. O. R. (2022). Natural nutraceuticals, especially functional foods, their major bioactive components, formulation, and health benefits for disease prevention-An overview. *Journal of Food Bioactives*, *19*.

Ayotunde, E. O., Fagbenro, O. A., & Adebayo, O. T. (2011). Histological changes in Oreochromis niloticus (Linnaeus I779) exposed to aqueous extract of Moringa oleifera seeds powder. *Turkish Journal of Fisheries and Aquatic Sciences*, *11*(1).

Bahi, A., Ramos- Vega, A., Angulo, C., Monreal- Escalante, E., & Guardiola, F. A. (2023). Microalgae with immunomodulatory effects on fish. *Reviews in Aquaculture*.

Bain, J., Schill, W. B., & Schwarzstein, L. (Eds.). (2012). *Treatment of male infertility*. Springer Science & Business Media.

Bardhan, A., Sau, S. K., Khatua, S., Bera, M., & Paul, B. N. (2021). A review on the production and culture techniques of monosex tilapia. *International Journal of Current Microbiology and Applied Sciences*, *10*(1), 565-577.

Bittencourt, N. D. L. R., Molinari, L. M., de Oliveira, D., de Abreu Filho, B. A., & Dias Filho, B. P. (2003). Haematological and biochemical values for Nile tilapia Oreochromis niloticus cultured in semi-intensive system. *Haemoglobin (g/dl)*, *10*(3.09), 6-58.

Boyd, C. E., McNevin, A. A., & Davis, R. P. (2022). The contribution of fisheries and aquaculture to the global protein supply. *Food security*, *14*(3), 805-827.

Chakraborty, S. B., Horn, P., & Hancz, C. (2014). Application of phytochemicals as growth- promoters and endocrine modulators in fish culture. *Reviews in Aquaculture*, *6*(1), 1-19.

Chance, B., & Maehly, A. C. (1955). [136] Assay of catalases and peroxidases.

Christopher, U. B., Ajuzieogu, N. A., Umoh, I. A., & Adeolu, A. O. (2021). Effect of pawpaw seed meal using various inclusion level on the gonad structure of Oreochromis niloticus (gift). *International Journal of Aquaculture and Fishery Sciences*, *7*(3), 024

Clauss, T. M., Dove, A. D., & Arnold, J. E. (2008). Hematologic disorders of fish. *Veterinary clinics of North America: Exotic animal practice*, *11*(3), 445-462.

Cochrane, K., De Young, C., Soto, D., & Bahri, T. (2009). Climate change implications for fisheries and aquaculture. *FAO Fisheries and aquaculture technical paper*, *530*, 212.

Crespi, V. (2011). Promotion and Development of Freshwater Aquaculture in Haiti. *FAO Aquaculture Newsletter*, (47), 12.

Dada, A. A., & Ajilore, V. O. (2009). Use of ethanol extracts of Garcinia kola as fertility enhancer in female catfish Clarias gariepinus brood stock. *International Journal of fisheries and Aquaculture*, *1*(1), 5-10.

Daniel, P., Supe, U., & Roymon, M. G. (2014). A review on phytochemical analysis of Momordica charantia. *Int. J. Adv. Pharm. Biol. Chem*, *3*(1), 214-220.

D'cotta, H., Fostier, A., Guiguen, Y., Govoroun, M., & Baroiller, J. F. (2001). Aromatase plays a key role during normal and temperature- induced sex differentiation of tilapia Oreochromis niloticus. *Molecular reproduction and development*, *59*(3), 265-276.

De Silva, S. S., & Davy, F. B. (2010). Aquaculture successes in Asia: contributing to sustained development and poverty alleviation. *Success stories in Asian aquaculture*, 1-14.

Deines, A. M., Wittmann, M. E., Deines, J. M., & Lodge, D. M. (2016). Trade-offs among ecosystem services associated with global tilapia introductions. *Reviews in Fisheries Science & Aquaculture*, *24*(2), 178-191.

DerMarderosian, A., & Beuther, J. A. (2005). The review of natural products. Facts and comparisons.

Balasubramanian, G., Sarathi, M., Kumar, S. R., & Hameed, A. S. (2007). Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp. *Aquaculture*, *263*(1-4), 15-19.

Edwards, P., Zhang, W., Belton, B., & Little, D. C. (2019). Misunderstandings, myths and mantras in aquaculture: Its contribution to world food supplies has been systematically over reported. *Marine Policy*, *106*, 103547.

El-Sayed, A. F. M. (Ed.). (2006). *Tilapia culture*. CABI publishing.

Fafioye, O. O. (2012). Acute and sub-acute toxicities of five plant extracts on white tilapia, Oreochromis niloticus (Trewavas). *International Research Journal of Agricultural Science and Soil Science*, *2*(13), 525-530.

Fagbenro, O. A. (2004, September). Predator control of overpopulation in cultured tilapias and the alternative uses for stunted tilapias in Nigeria. In *Proceedings of the Sixth International Symposium on Tilapia in Aquaculture* (pp. 634-647).

Gabriel, N. N., Qiang, J., He, J., Ma, X. Y., Kpundeh, M. D., & Xu, P. (2015). Dietary Aloe vera supplementation on growth performance, some haemato-biochemical parameters and disease resistance against Streptococcus iniae in tilapia (GIFT). *Fish & Shellfish Immunology*, *44*(2), 504-514.

Gabriel, N. N., Qiang, J., He, J., Ma, X. Y., Kpundeh, M. D., & Xu, P. (2015). Dietary Aloe vera supplementation on growth performance, some haemato-biochemical parameters and disease resistance against Streptococcus iniae in tilapia (GIFT). *Fish & Shellfish Immunology*, *44*(2), 504-514.

Gürdal, B., & Kültür, Ş. (2013). An ethnobotanical study of medicinal plants in Marmaris (Muğla, Turkey). *Journal of Ethnopharmacology*, *146*(1), 113-126.

Hasheesh, W. S., Marie, M. A. S., Abbas, H. H., Eshak, M. G., & Zahran, E. A. (2011). An evaluation of the effect of 17α-Methyltestosterone hormone on some biochemical, molecular and histological changes in the liver of Nile Tilapia, Oreochromis niloticus. *Life Science Journal*, *8*(3), 343-358.

Hasleton, P., & Flieder, D. B. (Eds.). (2013). *Spencer's Pathology of the Lung*. Cambridge University Press.

Hassan, H. U., Siddique, M. A. M., Abdel-Aziz, M. F. A., Gabo, K., Ahmed, A. E., & Parvez, M. S. (2022). Assessment of growth characteristics and the survival rate of pacific white shrimp litopenaeus vannamei (boone, 1931), reared with different stocking densities in the coastal ponds, sindh, pakistan. *JAPS: Journal of Animal & Plant Sciences*, *32*(6).

Horax, R., Hettiarachchy, N., Kannan, A., & Chen, P. (2010). Proximate composition and amino acid and mineral contents of Mormordica charantia L. Pericarp and seeds at different maturity stages. *Food chemistry*, *122*(4), 1111-1115

Hosseinzadeh Sahafi, H., Dehghan Madiseh, S., Koohilai, S., Hamidinejad, M., & Velayatzadeh, M. (2020). Plasma levels of sex steroids (testosterone, progesterone and 17β-estradiol) in Rohu Carp Labeo rohita broodstock from Khuzestan, Iran. *Iranian Journal of Fisheries Sciences*, *19*(6), 3063-3074.

Huang, W. C., Tsai, T. H., Huang, C. J., Li, Y. Y., Chyuan, J. H., Chuang, L. T., & Tsai, P. J. (2015). Inhibitory effects of wild bitter melon leaf extract on Propionibacterium acnes-induced skin inflammation in mice and cytokine production in vitro. *Food & function*, *6*(8), 2550-2560.

Huang, Y. K., Lin, K. H., Chen, H. W., Chang, C. C., Liu, C. W., Yang, M. H., & Hsueh, Y. M. (2003). Arsenic species contents at aquaculture farm and in farmed mouthbreeder (Oreochromis mossambicus) in blackfoot disease hyperendemic areas. *Food and Chemical Toxicology*, *41*(11), 1491-1500.

Immanuel, G., Uma, R. P., Iyapparaj, P., Citarasu, T., Punitha Peter, S. M., Michael Babu, M., & Palavesam, A. (2009). Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia Oreochromis mossambicus. *Journal of fish biology*, *74*(7), 1462-1475.

Jarwar, A. A. (2008). A status overview of fisheries and aquaculture development in Pakistan with context to other Asian countries. *Aquaculture Asia*, *13*(2), 13-18.

K Chandra, A., Sengupta, P., Goswami, H., & Sarkar, M. (2012). Excessive dietary calcium in the disruption of structural and functional status of adult male reproductive system in rat with possible mechanism. *Molecular and cellular biochemistry*, *364*, 181-191.

Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase.

Kapinga, I. B., Limbu, S. M., Madalla, N. A., Kimaro, W. H., Mabiki, F. P., Lamtane, H. A., & Tamatamah, R. A. (2019). Dietary Aspilia mossambicensis and Azadirachta indica supplementation alter gonadal characteristics and histology of juvenile Nile tilapia (Oreochromis niloticus). *Aquaculture Research*, *50*(2), 573-580.

Kroon, F., Streten, C., & Harries, S. (2017). A protocol for identifying suitable biomarkers to assess fish health: A systematic review. *PloS one*, *12*(4), e0174762.

Laghari, M. Y. (2018). Aquaculture in Pakistan: Challenges and opportunities. *International Journal of Fisheries and Aquatic Studies*, *6*(2), 56-59.

Lavens, P., & Sorgeloos, P. (1996). *Manual on the production and use of live food for aquaculture* (No. 361). Food and Agriculture Organization (FAO).

Lee, S., Eom, S., Kim, Y., Park, N., & Park, S. (2009). Cucurbitane-type triterpenoids in Momordica charantia Linn. *Journal of Medicinal Plants Research*, *3*(13), 1264-1269.

Lohiya, N. K., Manivannan, B., Mishra, P. K., Pathak, N., Sriram, S., Bhande, S. S., & Panneerdoss, S. (2002). Chloroform extract of Carica papaya seeds induces long-term reversible azoospermia in langur monkey. *Asian Journal of andrology*, *4*(1), 17-26.

Lyon, M. F., Glenister, P. H., & Lynn Lamoreux, M. (1975). Normal spermatozoa from androgen-resistant germ cells of chimaeric mice and the role of androgen in spermatogenesis. *Nature*, *258*(5536), 620-622.

Mahmoud, A. M., Goemaere, S., El-Garem, Y., Van Pottelbergh, I. N. G. E., Comhaire, F. H., & Kaufman, J. M. (2003). Testicular volume in relation to hormonal indices of gonadal function in community-dwelling elderly men. *The Journal of Clinical Endocrinology & Metabolism*, *88*(1), 179-184.

Mariappan, B., Kaliyamurthi, V., & Binesh, A. (2023). Medicinal plants or plant derived compounds used in aquaculture. In *Recent advances in aquaculture microbial technology* (pp. 153-207). Academic Press.

Martínez-Álvarez, R. M., Morales, A. E., & Sanz, A. (2005). Antioxidant defenses in fish: biotic and abiotic factors. *Reviews in Fish Biology and fisheries*, *15*, 75-88.

Martínez-Álvarez, R. M., Morales, A. E., & Sanz, A. (2005). Antioxidant defenses in fish: biotic and abiotic factors. *Reviews in Fish Biology and fisheries*, *15*, 75-88.

McLachlan, R. I., O'Donnell, L., Meachem, S. J., Stanton, P. G., De Kretser, D. M., Pratis, K., & Robertson, D. M. (2002). Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. *Recent progress in hormone research*, *57*(1), 149-179.

Mishra, B., & Chandra, M. (2022). Evaluation of phytoremediation potential of aromatic plants: A systematic review. *Journal of Applied Research on Medicinal and Aromatic Plants*, *31*, 100405.

Mlalila, N., Mahika, C., Kalombo, L., Swai, H., & Hilonga, A. (2015). Human food safety and environmental hazards associated with the use of methyltestosterone and other steroids in production of all-male tilapia. *Environmental Science and Pollution Research*, *22*, 4922-4931.

Mobaraki, A., & Moghaddam, T. N. (2014). Karela, a Plant with Many Properties. *International Journal of Agriculture Innovations and Research*, *3*(1), 2319-1473.

Mohsin, M., Mu, Y., Mehak, A., Memon, A. M., Noman, M., & Nazir, K. (2017). Aquaculture in Pakistan: Status, opportunities and challenges.

Mohsin, M., Mu, Y., Memon, A. M., Kalhoro, M. T., & Shah, S. B. H. (2017). Fishery stock assessment of Kiddy shrimp (Parapenaeopsis stylifera) in the Northern Arabian Sea Coast of Pakistan by using surplus production models. *Chinese Journal of Oceanology and Limnology*, *35*, 936-946.

Mondal, H., Chandrasekaran, N., Mukherjee, A., & Thomas, J. (2021). Viral infections in cultured fish and shrimps: current status and treatment methods. *Aquaculture International*, 1-36.

Nargund, V. H. (2015). Effects of psychological stress on male fertility. *Nature Reviews Urology*, *12*(7), 373-382.

Naseem, M. Z., Patil, S. R., Patil, S. R., & Patil, S. B. (1998). Antispermatogenic and androgenic activities of Momordica charantia (Karela) in albino rats. *Journal of Ethnopharmacology*, *61*(1), 9-16.

Naseem, M. Z., Patil, S. R., Patil, S. R., & Patil, S. B. (1998). Antispermatogenic and androgenic activities of Momordica charantia (Karela) in albino rats. *Journal of Ethnopharmacology*, *61*(1), 9-16.

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., ... & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, *591*(7851), 551-563.

Nazir, K., Yongtong, M., Hussain, K., Kalhoro, M. A., Kartika, S., & Mohsin, M. (2016). A study on the assessment of fisheries resources in Pakistan and its potential to support marine economy.

Nazir, M. A., Anjum, K. M., Naseer, J., Anjum, A., Durrani, A. Z., Usman, S., ... & Usman, M. (2021). Effects of Dietary Fish Oil Replacement by Soybean Meal on Performance and Physiology of Rainbow Trout, Oncorhynchus mykiss. *Pakistan Journal of Zoology*, *53*(1).

Nerurkar, P. V., Johns, L. M., Buesa, L. M., Kipyakwai, G., Volper, E., Sato, R., ... & Nerurkar, V. R. (2011). Momordica charantia (bitter melon) attenuates high-fat diet-associated oxidative stress and neuroinflammation. *Journal of neuroinflammation*, *8*, 1-19.

Ntsama, I. S. B., Tambe, B. A., Takadong, J. J. T., Nama, G. M., & Kansci, G. (2018). Characteristics of fish farming practices and agrochemicals usage therein in four regions of Cameroon. The Egyptian Journal of Aquatic Research, 44(2), 145-153.

Nwangwu, D. C., Yisa, M., & Bake, G. G. (2015). The potentials of phytoestrogenic plants in tilapia production–mini review.

Nyadjeu, P., Angoun, J., Ndasi, N. P., & Tabi-Tomedi, M. E. (2019). Effect of Garcinia kola seeds supplemented diet on growth performance and gonadal development of Oreochromis niloticus juveniles breed in ponds. *Fisheries and Aquatic Sciences*, *22*(1), 1-8.

Obaroh, I. O., & Nzeh, G. C. (2013). Antifertility effect of some plant leaf extracts on the prolific breeding of Oreochromis niloticus. *Academic Journal of Interdisciplinary Studies*, *2*(12), 87.

Obaroh, I. O., Keta, J. N., & Bawa, D. Y. (2014). Haematological indices of Oroechromis niloticus fed crude extract Mangifera indica leaf. *European J Biotechnol Biosci*, *2*, 15-20.

Ogunfowora, L. A., Iwuozor, K. O., Ighalo, J. O., & Igwegbe, C. A. (2021). Trends in the treatment of aquaculture effluents using nanotechnology. *Cleaner Materials*, *2*, 100024.

Omar, E. A., Yousef, M. I., Srour, T. M., & Mansour, A. T. E. (2014). Effect of dietary natural phytochemicals on sex-reversal, growth performance, feed utilization and body composition of Nile tilapia (Oreochromis niloticus) fry. *Journal of the Advances in Agricultural Researches*, *19*(3), 428-441.

Opiyo, M. A., Jumbe, J., Ngugi, C. C., & Charo-Karisa, H. (2019). Different levels of probiotics affect growth, survival and body composition of Nile tilapia (Oreochromis niloticus) cultured in low input ponds. *Scientific African*, *4*, e00103.

Pandey, G., Madhuri, S., & Mandloi, A. K. (2012). Medicinal plants useful in fish diseases. *Plant Archives*, *12*(1), 1-4.

Pandian, T. J., & Kirankumar, S. (2003). Recent advances in hormonal induction of sex-reversal in fish. *Journal of Applied Aquaculture*, *13*(3-4), 205-230.

Patil, S. A., & Patil, S. B. (2011). Toxicological studies of Momordica charantia Linn. Seed extracts in male mice. *Int J Morphol*, *29*(4), 1212-1218.

Puri, M., Kaur, I., Kanwar, R. K., Gupta, R. C., Chauhan, A., & Kanwar, J. R. (2009). Ribosome inactivating proteins (RIPs) from Momordica charantia for anti viral therapy. *Current Molecular Medicine*, *9*(9), 1080-1094.

Ravikumar, R., Krishnamoorthy, P., & Kalidoss, A. (2010). Antidiabetic and antioxidant efficacy of Andrographis paniculata in alloxanized albino rats. *International Journal of Pharmacy and Technology*, *2*(4), 1016-1027.

Rodrıguez, J., & Le Moullac, G. (2000). State of the art of immunological tools and health control of penaeid shrimp. *Aquaculture*, *191*(1-3), 109-119.

Rommerts, F. F. G. (1988). How much androgen is required for maintenance of spermatogenesis?. *Journal of Endocrinology*, *116*(1), 7-9.

Saravanan, M., Ramesh, M., Malarvizhi, A., & Petkam, R. (2011). Toxicity of neem leaf extracts (Azadirachta indica A. Juss) on some haematological, ionoregulatory, biochemical and enzymological parameters of Indian major carp, Cirrhinus mrigala. *Journal of Tropical Forestry and Environment*, *1*(1).

Savaya, A., Glassner, H., Livne-Luzon, S., Chishinski, R., Molcho, J., Aflalo, E. D., ... & Sagi, A. (2020). Prawn monosex populations as biocontrol agents for snail vectors of fish parasites. *Aquaculture*, *520*, 735016.

Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current biology*, *24*(10), R453-R462.

Shah, M. R., Lutzu, G. A., Alam, A., Sarker, P., Kabir Chowdhury, M. A., Parsaeimehr, A., ... & Daroch, M. (2018). Microalgae in aquafeeds for a sustainable aquaculture industry. *Journal of applied phycology*, *30*, 197-213.

Sharma, A., Verma, P. K., & Dixit, V. P. (2003). Effect of Semecarpus anacardium fruits on reproductive function of male albino rats. *Asian journal of andrology*, *5*(2), 121-124.

Sharpe, R. M. (1994). Regulation of spermatogenesis. *The physiology of reproduction*.

Sogbesan, A. O., & Ugwumba, A. A. A. (2008). Nutritional evaluation of termite (Macrotermes subhyalinus) meal as animal protein supplements in the diets of Heterobranchus longifilis (Valenciennes, 1840) fingerlings. *Turkish journal of fisheries and aquatic Sciences*, *8*(1), 149-158.

Stankus, A. (2021). State of world aquaculture 2020 and regional reviews: FAO webinar series. *FAO aquaculture newsletter*, (63), 17-18.

Stickney, R. R., & Gatlin III, D. M. (2022). *Aquaculture: An introductory text*. Cabi.

Tadese, D. A., Song, C., Sun, C., Liu, B., Liu, B., Zhou, Q., ... & Kevin, N. T. (2022). The role of currently used medicinal plants in aquaculture and their action mechanisms: A review. *Reviews in Aquaculture*, *14*(2), 816-847.

Takihara, H., Cosentino, M. J., Sakatoku, J., & Cockett, A. T. (1987). Significance of testicular size measurement in andrology: II. Correlation of testicular size with testicular function. *The Journal of urology*, *137*(3), 416-419.

Teichert- Coddington, D., Manning, B., Eya, J., & Brock, D. (2000). Concentration of 17α- Methyltestosterone in HormoneTreated Feed: Effects of Analytical Technique, Fabrication, and Storage Temperature. *Journal of the World Aquaculture Society*, *31*(1), 42-50.

Tsakiridis, A., O'Donoghue, C., Hynes, S., & Kilcline, K. (2020). A comparison of environmental and economic sustainability across seafood and livestock product value chains. *Marine Policy*, *117*, 103968.

Tumkiratiwong, P., & Pongakkasira, K. (2020). Effect of ethanol extracts of Momordica charantia seeds on testicular antioxidant enzyme activity and protein patterns in male Wistar rats. *Agriculture and Natural Resources*, *54*(5), 515-520.

Tumkiratiwong, P., & Pongakkasira, K. (2020). Effect of ethanol extracts of Momordica charantia seeds on testicular antioxidant enzyme activity and protein patterns in male Wistar rats. *Agriculture and Natural Resources*, *54*(5), 515-520.

Tumkiratiwong, P., Ploypattarapinyo, R., Pongchairerk, U., & Thong-Asa, W. (2014). Reproductive toxicity of Momordica charantia ethanol seed extracts in male rats. *Iranian journal of reproductive medicine*, *12*(10), 695.

Tumkiratiwong, P., Ploypattarapinyo, R., Pongchairerk, U., & Thong-Asa, W. (2014). Reproductive toxicity of Momordica charantia ethanol seed extracts in male rats. *Iranian journal of reproductive medicine*, *12*(10), 695.

Turan, C., Yalçin, S., Turan, F., Okur, E., & Akyurt, I. (2005). Morphometric comparisons of African catfish, Clarias gariepinus, populations in Turkey. *Folia Zoologica*, *54*(1/2), 165.

Waite, R., Beveridge, M., Brummett, R., Castine, S., Chaiyawannakarn, N., Kaushik, S., ... & Phillips, M. I. C. H. A. E. L. (2014). *Improving productivity and environmental performance of aquaculture*. WorldFish.

Wright, J. R., Colby, H. D., & Miles, P. R. (1981). Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. *Archives of biochemistry and biophysics*, *206*(2), 296-304.

Yama, O. E., Duru, F. I., Oremosu, A. A., Noronha, C. C., & Okanlawon, A. (2011). Suppressive effects of Momordica charantia on pituitary-testicular axis and sperm production in male Sprague-Dawley rats. *Int J Med Med Sci*, *3*(12), 354-350.

Yama, O. E., Duru, F. I., Oremosu, A. A., Osinubi, A. A., Noronha, C. C., & Okanlawon, A. O. (2011). Sperm quotient in Sprague–Dawley rats fed graded doses of seed extract of Momordica charantia. *Middle East Fertility Society Journal*, *16*(2), 154-158.

Zahra, S., Shah, S. A. R., & Badeeb, R. A. (2022). A sustainable green–blue revolution in Pakistan: a new perspective from quantile regression analysis. *Environment, Development and Sustainability*, 1-28.

Zelko, I. N., Mariani, T. J., & Folz, R. J. (2002). Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radical Biology and Medicine*, *33*(3), 337-349.

Zirkin, B. R., Santulli, R., Awoniyi, C. A., & Ewing, L. L. (1989). Maintenance of advanced spermatogenic cells in the adult rat testis: quantitative relationship to testosterone concentration within the testis. *Endocrinology*, *124*(6), 3043-3049.