Evaluation of Bitter Melon (*Momordica charantia*) Seeds Powder as a Potential Infertility Inducer in Female Nile Tilapia (*Oreochromis niloticus)*

By

Muhammad Shahid

DEPARTMENT OF ZOOLOGY

FACULTY OF BIOLOGICAL SCIENCES

QUAID-I-AZAM UNIVERSITY

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MUHAMMAD SHAHID

Department of Zoology

Faculty of Biological Sciences

Quaid-I-Azam University

Islamabad

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“In the Name of ALLAH, the most beneficent, the most Merciful”

This thesis is dedicated to my parents' deepest gratitude whose love, & prayers have always been a source of strength for me.

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DECLARATION

I hereby declare that the work presented in the following thesis is my own effort and the material contained in the thesis is original work. I have not previously presented any part of this work elsewhere for any other degree.

MUHAMMAD SHAHID

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List of Abbreviations

POD	Peroxidase
SOD	Superoxidase dismutase
CAT	Catalase
EDTA	Ethylene diamine tetra acetic acid
H_2O_2	Hydrogen peroxide
PBS	Phosphate buffer saline
ROS	Reactive oxygen species
G	Gram
M	Molar
µmol	Micromole
mM	Millimole
µg	Microgram
Kg	Kilogram
Mg	Milligram
OD	Optical density
MT	17α-Methyl testosterone
FCR	Feed conversion ratio
SGR	Specific growth rate
NWG	Net weight gain
IBW	Initial body eight
FBW	Final body weight
HSI	Hepatosomatic index
GSI	Gonadosomatic index
FAO	Food and agricultural organization
GDP	Gross domestic product
kJ	Kilojoule
DO	Dissolve oxygen
Cm	Centimeter
MS-222	Tricaine methane sulfonate

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Abstract

The challenge of early maturation in Nile tilapia (*Oreochromis niloticus*), causing overpopulation, poor growth and productivity, has prompted a need of strategy to control its reproduction. Nowadays, medicinal plants are getting importance in aquaculture, as they are safe, effective, locally available and biodegradable. The herb *Momordica charantia* has been utilized as a nutritional and therapeutic supplement. This plant has a rich historical use across cultures for its impact on uterine functions, including promoting menstruation and inducing abortions. Therefore, this present study was carried out for 90 days to evaluate Bitter melon (*Momordica charantia*) seeds as a natural infertility inducer and to determine its effects on gonadal characteristics and histology of female Nile tilapia (*Oreochromis niloticus*). The experiment was carried-out in an indoor facility under semi-control condition, comprised of a Control group (C) fed only with basal diet, and treatment groups S2, S4, S6, S8, and S10, each supplemented with 2, 4, 6, 8, and 10g of *Momordica charantia* seeds/kg diet respectively, to provide 35% of crude protein required by tilapia. Uniform size, active and healthy fish with an average body weight of 10±1.6 grams, distributed across eighteen fiberglass tanks with triplicates for each treatment. The results revealed a significant effect on growth parameters (WG, SGR and FCR), hematological indices and ovarian antioxidant enzyme. Furthermore, all doses of *M.charantia* seed significantly decreased GSI and fecundity values ($p < 0.05$) with increasing dietary dosage of seeds. Histological examination showed dose-dependent structural alteration in ovary of all treatment groups. Consequently, the results showed that inclusion of *M. charantia* seed powder could be effective as infertility inducer in female *O. niloticus*. Therefore, it seems a possible way to control a vigorous spawning in tilapia thus help to prevent the issue of overcrowding in *O. niloticus* culture farms.

INTRODUCTION

The term "aquaculture" refers to the practice of raising aquatic organisms in controlled or semi-controlled conditions (Stickney & Gatlin, 2022). It is also defined as a controlled process of breeding, nurturing, feeding, collecting, and fostering the growth of aquatic organisms, including both finfish and shellfish. Commercial, recreational, or public goals are served by this technique. (Mizuta *et al*., 2022). According to Japanese resource council, Science and Technology Agency, aquaculture is an industrial process of raising aquatic organisms up to final commercial production within properly partitioned aquatic areas, controlling the environmental factors, and positively managing the life history of organisms, and it has to be considered as an independent industry from fisheries yet.

Over the past few decades, there has been a significant surge in the expansion of aquaculture, resulting in it now generating nearly equivalent amounts of fish and shellfish compared to traditional fisheries. Aquaculture represents the primary approach for augmenting our food production from aquatic environments in the future (FAO, 2014). The cultivation of aquatic plants and animals has a rich history spanning several thousand years. However, it is important to recognize that it emerged as a significant phenomenon primarily after World War II. In 1950, the worldwide production of farmed fish and shellfish stood at approximately 2 million metric tons (mmt), primarily concentrated in Asian regions. Over the past three decades, aquaculture production has experienced a remarkable annual growth rate of approximately 7-11%. By 2012, the production figures reached approximately 44 mmt for finfish, 6 mmt for crustaceans, 15 mmt for mollusks, and 23.8 mmt for aquatic plants (Troell *et al*., 2017).

According to bas-reliefs discovered in an Egyptian tomb that date back more than 4,000 years, the history of Nile tilapia (*Oreochromis niloticus*) farming can be linked to ancient Egypt. These bas-reliefs depicted the fish being held in ornamental ponds. Subsequently, Nile tilapia was introduced to China, which has emerged as the top producer of tilapia in the world. From 1992 to 2003, China consistently accounted for over half of the worldwide tilapia production. However, the unrestricted breeding of tilapia in ponds led to excessive recruitment, stunted growth, and a low percentage of marketable-sized fish, which at first tempered interest in tilapia as a food fish. The emergence of hormonal sex-reversal methods in the 1970s represented a significant advancement. This significant discovery made it possible to produce male monosex populations that could be raised to uniform sizes

suitable for the market. Several tilapia species are raised for commercial purposes, but the Nile tilapia is still the most widely raised species globally (Rakocy, 2009).

Tilapia ranks as the second most widely cultivated food fish globally, following carps. The remarkable growth in tilapia production can be attributed to its favorable aquacultural characteristics. These include a) ease to breed in captivity; (b) having a short production cycle due to rapid growth rate; (c) acceptability of artificial feeds after yolk-sac absorption; and (d) marketability (Abaho *et al*., 2021). Nile tilapia (*Oreochromis niloticus*) is highly valued as an aquaculture species due to its various desirable characteristics. One of these is their ease of breeding in captivity, allowing for efficient reproduction in controlled environments. Additionally, Nile tilapia exhibit adaptability to a wide range of water conditions, making them versatile in terms of growth potential. However, these advantageous traits can also give rise to certain challenges. For instance, the high survival rate of young tilapia can lead to overcrowding in grow-out ponds. This can result in stunted growth as the available food supply becomes insufficient to support the densely populated fish. To mitigate these issues, it becomes crucial to develop techniques that can control or limit the spawning process (Biswas *et al*., 2005). These techniques include slow-maturing tilapia species, sex reversal, cage/tank culture, predator use, high stocking density, sterilization, and monosex culture (Beardmore, 1996; Mair & Little, 1991). The most efficient and popular way for producing male tilapias in large numbers in industrial production systems is the sex reversal hormone method (El-Greisy & El-Gamal, 2012).

Exogenous steroids, specifically 17α-methyltestosterone (MT), are effective for hormonal sex reversal and produce high levels of masculinization (Homklin *et al*., 2011). This approach has a variety of drawbacks, including a potential risk to the environment, consumers, and workers' health. (Phelps & Popma, 2000). In tilapia farming, MT is the technique most frequently employed to produce all male fish. Public concerns persist regarding the potential carcinogenicity of MT (17α-Methyltestosterone) and its negative impacts on human health and aquatic environment (Abo-Al-Ela, 2018; Mlalila *et al*., 2015). Due to the aforementioned detrimental effects, many countries avoid the use of synthetic hormones in the processing of fish food (Chakraborty *et al*., 2013). Even in different countries where the practice of incorporating hormones to food fish is widely accepted, the aforementioned techniques have challenges, including labor intensiveness, contamination of the broodstock, the need for careful selection and regular maintenance of the broodstock, the need for a high level of control, and consumer acceptance of hormonally sex-reversed fish

(Biswas *et al*., 2005). As a result, research is required in order to develop synthetic steroid substitutes that are socially acceptable, commercially viable, and environmentally sustainable. The utilization of plants in aquaculture is favored due to their accessibility and comparatively lower impact on the environment and human health when compared to synthetic hormones (Chakraborty *et al*., 2013; Reverter *et al*., 2014). Consequently, there is a growing need to explore alternative approaches for effectively managing the issue of excessive tilapia reproduction in aquaculture systems. One emerging trend is the use of medicinal plants as natural inhibitors of reproduction, which offer a potential solution to this problem (Abdelhak, 2013).

Aquaculture production

Despite the global COVID-19 pandemic, aquaculture production continued to grow in 2020. A total of 87.5 million tonnes of aquatic animals were produced, primarily for human consumption. Of this production, 89 percent, equivalent to 157 million tonnes, was utilized as food for humans. The remaining 20 million tonnes were allocated for non-food purposes, primarily for the production of fishmeal and fish oil, accounting for 81 percent of the non-food use (FAO, 2022). Aquaculture serves as a key method for increasing food production from aquatic environments in the future. It has become the primary approach to meet the growing demand for seafood. Additionally, a significant portion of the global fish catch is utilized for the production of fishmeal and fish oil (FAO, 2014), an essential source of components for fatty acids and protein in the diets for many fish and shrimp species (Ling *et al*., 2015). Aquaculture plays a crucial role in meeting the rising global food demand and addressing nutritional deficiencies by providing essential nutrients. (Golden *et al*., 2017; Filipski & Belton, 2018).

Since 1961, there has been a considerable increase in worldwide consumption of aquatic foods, with an average annual growth rate of 3.0 percent. Per capita consumption has risen steadily, from 9.9 kg in the 1960s to a record high of 20.5 kg in 2019. While consumption dipped in 2020 due to reduced demand, preliminary estimates indicate a slight increase in 2021. High-quality proteins, vital amino acids, vitamins A, B, and D, minerals (iron, calcium, zinc, iodine, magnesium, potassium, selenium), and heart-healthy omega-3 fatty acids are all abundant in aquatic foods. Aquatic foods contribute around 17 percent of global animal protein consumption. For 3.3 billion people, aquatic foods make up at least 20 percent of their average per capita animal protein intake. Asia has long been the dominant region in aquaculture, accounting for 91.6 percent of global aquatic animal and algae

production in 2020. However, there remains an unequal distribution and difference in aquaculture development among regions and countries, with limited progress over the years. Developing countries, particularly those with low incomes, face significant challenges in achieving their goals of aquaculture development to support food production and job creation for their growing populations (FAO, 2022).

Aquaculture in Pakistan

Pakistan, being an agricultural country, possesses abundant natural water resources suitable for aquaculture. Freshwater bodies and marine water serve as the primary resources for aquaculture activities. The country has a vast expanse of approximately 8,563,820 km2 dedicated to rivers, lakes, ponds, and water reservoirs, providing ample opportunities for aquaculture development (Jarwar, 2008). Pakistan benefits from both coastal and inland water resources for aquaculture. It has a coastline stretching 1,120 km along the Arabian Sea, offering opportunities for marine aquaculture. Inland, the country has substantial water reserves covering approximately 3,102,408 hectares, which provide suitable conditions for freshwater aquaculture activities (Shah *et al*., 2018; Husain, 1992). These coastal and inland water resources in Pakistan are highly regarded as suitable environments for aquaculture and serve as the foundation for the development of aquaculture practices in the country (Laghari, 2018).

Aquaculture is a relatively new activity in Pakistan, and the private sector has played a significant role in its development. Dug-out fish farms have been constructed by private entities, contributing to the expansion of aquaculture in the country. It is estimated that there are around 3,300 fish farms in Pakistan, with a total area of approximately 0.06 million hectares (khan, 2014). The semi-intensive aquaculture system is widely used in Pakistan (Laghari, 2018). In Pakistan, inland fisheries and aquaculture began to advance in the 1970s (Akhter, 1995). In Pakistan, fish is regarded as a supplemental source of animal protein. In Pakistan, the average person consumes 1.9 kg of fish annually (FAO, 2017). A total of 179,900 metric tons are expected to be produced through aquaculture growing systems, while 600,000 metric tons come from natural catch (Minfal, 2012; Laghari, 2018). Pakistan is home to several major fish harbors, including Karachi Fisheries Harbor, Korangi Fish Harbor, Pasni Fish Harbor, and Gwadar Fish Harbor. Among them, Karachi Fisheries Harbor is the most prominent, handling approximately 90% of the fish and seafood catch in the country. Notably, around 95% of the fish products from Pakistan are exported through Karachi Fisheries Harbor, making it a vital hub for the seafood export industry (Shah *et al*., 2018).

Fisheries outputs from catch and aquaculture are vital resources for food, livelihoods, employment, and the economy. They provide nutritious food, support livelihoods, create jobs, and contribute to economic growth (Godfray *et al*., 2010). The economic growth of Pakistan's fisheries has enormous potential. According to estimates, Pakistan has around 50,000 individuals been employed in aquaculture (Mohsin *et al*., 2017). The fisheries sector in Pakistan plays a significant role in poverty alleviation, achieving food security, and making a modest contribution to the economy. In the fiscal year 2016-17, the sector contributed to the national Gross Domestic Product (GDP) with a growth rate of 5.3%. The fisheries sector's share in the overall agriculture sector was 2.12%, while its contribution to the GDP accounted for 0.41% (GoP, 2017). Approximately 30-35% of Pakistani seafood is imported by more than 50 countries in Europe (Ali *et al*., 2013). The fisheries sector in Pakistan provides direct employment to approximately 400,000 individuals, while indirectly supporting employment for around 600,000 people. This significant workforce in the fisheries sector accounts for nearly 1% of the national labor force (Ebrahim, 2014).

The fisheries sector in Pakistan requires reforms to address several challenges. Overfishing has put strain on marine fisheries, threatening their sustainability. The sector also lacks effective planning and management, which is essential for the growth of aquaculture. It is crucial for the government to allocate more resources and funding towards the fishing industry. Furthermore, improvements are still needed in the aquaculture sector, including better practices, technology adoption, and infrastructure development. These efforts are necessary to ensure the sustainability and growth of Pakistan's fisheries sector (Shahzad, 2022).

Tilapia Taxonomy

Tilapia is the common name now applied to three genera and species of fish in the family Cichlidae: Oreochromis, Sarotherodon, and Tilapia, native to Africa and the Middle East (Fitzsimmons, 2000). The species in the genus Oreochromis that are most vital for aquaculture are found in the Americas, including the Nile tilapia, *O. niloticus*, the Mozambique tilapia, *O. mossambicus*, the blue tilapia, *O. aureus*, and *O. urolepis hornorum* (Watanabe *et al*., 2002).

Culturing of tilapia

Tilapias are highly suitable for aquaculture due to their impressive range of attributes. They offer good-tasting flesh with a mild flavor, making them widely accepted as a food fish

(Watanabe *et al*., 2002). Tilapias rank as the second most extensively cultured group of fish worldwide, second only to carps (Stickney & Davis, 1981). The origins of tilapia farming can be traced back over 4,000 years ago to Egypt, where it is believed to have started in its crudest form. In Kenya in 1924, the first tilapia cultivation with a scientific focus emerged. It swiftly expanded across all of Africa (Gupta & Acosta,2004). 85 countries around the world currently cultivate the fish (FAO,2002) and over 98% of the tilapia produced in these nations is cultivated outside of its natural habitat (Shelton, 2002). China, Egypt, Indonesia, Philippines and Thailand produce the most Tilapia (FAO, 2013). Tilapia have a significant advantage in terms of their hardiness. They possess the ability to tolerate low levels of dissolved oxygen by skimming the water's surface, as well as high levels of ammonia and a wide range of salinity. Due to their capacity to thrive in degraded water conditions, tilapia can be reared at higher densities compared to other fish species. Tilapia are often referred to as "Superfish" due to their resilience, excellent flavor, and ability to fetch a good market price (Stickney & Davis, 1981). The Mozambique tilapia (*Oreochromis mossambicus*), which was introduced to Asia, marked the beginning of tilapia cultivation outside of Africa. Egypt is the eighth-largest aquaculture producer in the world, producing more than 1.6 million tonnes in 2019, of which 66 percent (or 1.6 million tonnes) are Nile tilapia (FAO, 2021). In tilapia populations, males exhibit faster growth rates and greater uniformity in size compared to females. This is why, monosex tilapia fish cultivation has been chosen as a solution. This is accomplished using a variety of techniques, such as manual sexing, hormone sex reversal directly, hybridization, or genetic modification. By producing monosex populations, the issue of early sexual maturation can be controlled, allowing for improved growth performance and more efficient aquaculture production (Gupta & Acosta, 2004).

Aim of Study

The control of sex in fish species is an important component for its effective marketing and development. It has important impacts on various aspects, including reproduction, growth, product quality, and economic considerations (Budd *et al*., 2015).

Researchers have identified that the uncontrolled reproduction of tilapia in grow-out ponds poses significant challenges to the promotion of its cultivation and hinders the path towards sustainable aquaculture development (Wang & Lu, 2016). Because of their early maturity and voracious breeding habits, tilapias in intensive production systems can have a negative impact on productivity metrics and eventually economic returns (Budd *et al*., 2015). To reduce undesired reproduction in tilapia production systems, many techniques are used,

including regular harvesting of fry and fingerlings, high-density culture, cage culture, polyculture with predator fish, sterilization by application of heat shock, and all-male culture (Fortes, 2005; Mair & Little, 1991). Exogenous steroids used for hormonal sex reversal, particularly 17α-methyl testosterone (MT), is a highly successful method for achieving all-male tilapia populations (Phelps & popma, 2000; Homklin *et al*., 2011). The use of 17α-methyl testosterone (MT) in tilapia seed production could be hazardous to the health of hatchery workers involved in the process (Megbowon & Mojekwu, 2014). Additionally, the fish are unable to consume 30% of the hormone-treated diet when being fed (Ramírez-Godínez *et al*., 2013; Vick & Hayton, 2001). Furthermore, only around 10% of the hormone present in the consumed diet is actually used for sex reversal purposes (Ong *et al*., 2012; De Ziegler & Fanchin, 2000). Given the limitations and concerns related with the practice of synthetic steroids in tilapia aquaculture, it is crucial to focus research efforts on identifying and developing alternative methods that are ecologically friendly, profitable, and socially acceptable.

Use of plant material for controlling Tilapia reproduction

Plant extracts are increasingly being recognized and used in fish culture as substitutes to chemicals, drugs, and hormones. This shift is driven by the growing need to mitigate a negative effect on the health of people and the environment associated with aquaculture. Organic plant products offer several advantages, including their relative safety, affordability, and ease of preparation. As a result, they are viewed as a promising approach for achieving sustainable fish production while reducing the reliance on synthetic substances (Chakraborty *et al*., 2014).

Various governments have criticized the use of synthetic chemicals in food production due to the potential health and adverse effects on the environment. Synthetic sex reversal hormones have historically been utilized in tilapia farming to yield only male offspring. However, there is growing interest in exploring herbal extracts as safe alternatives to prevent excessive breeding and early tilapia maturity. It has been discovered that some plant extracts have estrogenic characteristics and can cause infertility, abortifacient effects, and sex inversion in animals. Therefore, herbal extracts hold promises as safer alternatives for managing tilapia reproduction in production systems, mitigating the need for synthetic hormones. (Xu *et al*., 2015; Yusuf *et al*., 2019). Plants are preferred for use in aquaculture, because they are more accessible and generally safer for the environment and human health than synthetic hormones (Reverter *et al*., 2014). Therefore, Efforts are indeed necessary to

explore and produce new plants-based products specifically for tilapia production, with the aim of replacing synthetic hormones and chemicals (Mehrim *et al*, 2019) Natural plant substances like flavonoids, tannins, terpenoids, alkaloids, and steroids stimulate digestion, hunger, and immunity in addition to androgenic and anabolic processes (Xu *et al.*, 2015; Emeka *et al*., 2014). The phytochemicals, such as steroidal saponins and flavonoids, reduce the production of estrogen by blocking the activity of aromatase. Additionally, phytochemicals may compete with endogenous estrogens for estrogen receptor binding sites, preventing the production of estrogen (Golan & Levavi-Sivan, 2014; Miyahara *et al*., 2003). Flavonoids in bitter kola, particularly apigenin, prevent the enzyme aromatase from functioning (Iwu, 1982). There is a significant number of alkaloids, saponins, steroids, tannins, and alkaloids in the leaf extracts of *M. indica* (Biu A. *et al*., 2009). The antifertility effects of *M. indica* extracts are thought to be caused by saponins, which are found in higher concentrations than the other bioactive components (Obaroh & Nzeh, 2013). The neem tree's extracts from various parts contain spermicidal, antibacterial, and anti-inflammatory qualities in addition to having abortifacient and antifertility effects (Jegede & Fagbenro, 2007; Priya *et al*., 2012). The primary bioactive component in pawpaw seeds that has the ability to prevent or cause sterility is the saponin, oleanolic acid 3-glucoside (Lohiya *et al*., 2005; Verma *et al*., 2006). Additionally, the tilapia testes and ovaries' gonadal cells can be damaged by the glucosides in pawpaw seed meal (Abbas & Abbas, 2011; Waweru *et al*., 2019). The consumption of 300 mg/kg of Q. Saponaria extract by sexually mature female Nile tilapia prevented them from spawning (Francis *et al*., 2005). The phytochemicals may reduce fertility by causing histological alterations in fish gonads. Testes and ovaries lose their ability to produce spermatids and oocytes due to gonadal injury (Abdelhak *et al*., 2013; Ampofo-Yeboah, 2013).

Momordica Charantia

Momordica charantia, commonly known as bitter melon, karela, bitter gourd, or balsam pear, is a medicinal plant from the Cucurbitaceae family; it is mostly grown in Africa, Asia, and South America (Cefalu *et al*., 2008). Momordicin, an extremely bitter ingredient, is present in all plant components, including the fruit, they all have a very bitter flavor (Fang *et al*., 2012; Gupta *et al*., 2011). Bitter melon is a therapeutic plant with various valuable properties (Joseph & Jini, 2013). *M.charantia* possess antidiabetic, antiviral, antitumor, antileukemic, antibacterial, anthelmintic, antimutagenic, anti-mycobacterial, antioxidant,

antiulcer, anti-inflammatory, hypocholesterolemia, hypotriglyceridemic, hypotensive, immunostimulant, and insecticidal properties (Grover & Yadav, 2004).

Bitter gourd has been utilized as folk medicine to treat toothache, diarrhea, furuncle, diabetes, dysmenorrhea, eczema, emmenagogue, galactagogue, gout, jaundice, kidney (stone), leprosy, leucorrhea, piles, pneumonia, psoriasis, rheumatism and scabies (Alam *et al*., 2015). The residue of alcoholic ether extract of *M. charantia* leaves, is reported to possess hypoglycemic activity (Kumar & Bhowmik, 2010). Other use of the plant includes to expel intestinal gas, for tumors, wound treatment, rheumatism, malaria, vaginal discharge and the seeds are used to induce abortion (Sofowora, 2006; Taylor, 2005).

The medicinal plant *Momordica charantia* contains sterols, terpenoids, phenolic compounds, proteins, peptides, amino acids, carbohydrates, fatty acids, alkaloids, sterols, flavonoids, vitamins, and metals, which are distributed throughout the plant (Rohajatien *et al*., 2018). Additionally, it contains substances such as vicine, zeatin, lutein, lycopene, pipecolic acid, cryptoxanthin, momorcharasides, momorcharins, momordenol, momordicilin, momordicin, momordicin, momordicin, momordicin, momordin, and momordolo (Dhalla *et al*., 1961). The oil content of *M.charantia* seeds has been observed to range from 18.1-37.6%, with the majority coming from the conjugated triene cis-9, trans-11, and trans-13 isomer of linolenic acid, also known as -elearic acid (-ESA) (Chan *et al.,* 2018) and protein (28–30%) (Anjum *et al*., 2013). *M. charantia's* fruits and leaves displayed a high level of antioxidant activity and were rich in phenolics. According to research, the edible fruit and leaves are both excellent providers of the B vitamins, and the leaves are a good source of calcium, magnesium, potassium, phosphorus, and iron. (Dhalla *et al*., 1961). It has been demonstrated that the fruits of *M.charantia* contain a number of amino acids, including alanine, aspartic acid, butyric acid, g-amino, glutamic acid, isoleucine, leucine, luteolin, methionine, phenylalanine, pipecolic acid, serine, threonine, and valine (Paul & Raychaudhuri, 2010). Fresh bitter melon has a composition of 93.8 percent water, 0.9% protein, 0.1% fat, 3.3% dietary fiber, 20 kJ of calories per 100 g, 0.6% ash, and 0.05% vitamin C (Zafar *et al*., 2016).

Hypothesis

We assume that supplementation of Bitter Melon (*Momordica charantia*) seed powder in the feed of female Nile tilapia (*O. niloticus*) positively induces infertility. So, to prove our hypothesis, our research will focus on the following aim and objectives;

Aim and objectives

To evaluate the potential antifertility impact of Bitter Melon (*Momordica charantia*) seed powder on female Nile Tilapia (*Oreochromis niloticus*). For these 90 days feeding trail was executed and studied the effect of dietary supplementation of *M. charantia* seed powder on the following indices:

- Health status by analysing Haematological indices (RBCs, WBCs, HCT, Hb etc.).
- Growth performance by determining weight gain (%), SGR.
- Gonadosomatic index (GSI).
- Fecundity.
- Antioxidant status of ovarian tissue by examination of reactive oxygen species (ROS) and enzymes catalase (CAT), superoxidase dismutase (SOD), peroxidase (POD).
- Histological Examination of ovary tissues of Nile tilapia.

MATERIAL AND METHODS

2.1 Fish collection and Transportation:

About 220 healthy female tilapia fish mean weight 10±1.6 grams (*Oreochromis niloticus*) were collected from NARC Islamabad and transported to the Fisheries and Aquaculture Research Centre in Quaid-i-Azam University, Islamabad. The transportation was conducted using tight, closed oxygen-filled plastic bags to ensure proper oxygen supply during the journey.

Upon arrival at the research center, they were carefully shifted to concrete raceways, and acclimatized there for about 10 days. Throughout the acclimatization period, a 35% crude protein basal diet was given to the fish to meet their nutritional requirements and promote their overall health. Prior to shifting the fish to the concrete raceway, thorough cleaning and washing of the raceway were conducted using lime. This process aimed to eliminate any potential bacteria or contaminants that could pose a risk to the fish health. Additionally, as a preventive measure against diseases or parasites, the fish were treated with a 0.2% $KMNO_4$ (potassium permanganate) solution. This treatment helps to minimize the presence of pathogens and reduce the likelihood of disease outbreaks among the fish population.

2.2 Preparation of Experimental feed

Basal feed containing 35% crude protein was prepared to meet the nutritional requirements of *O. niloticus* (see Table 1). Dry feed ingredients, including fish meal, wheat bran, rice polish, sunflower oil, soybean meal, corn gluten, and vitamin and mineral premixes, were combined. *M. charantia* seeds, obtained from a local shop, were finely ground using a grinder and added to the basal diet in graded levels. All the ingredients were thoroughly mixed with water to ensure uniform distribution and create a dough-like consistency. The dough was passed through a meat grinder to obtain pellets of desired size and shape. The pellets were placed in an oven and dried for 24 hours at 60 °C to remove moisture and ensure long-term storage stability. After drying, the pellets were crushed into smaller pieces or crumbles to match the size of the fish's mouth for ease of consumption. Both the experimental feed, containing *M. charantia* seed powder, and the control feed (without *M. charantia* seed powder) were prepared. To maintain feed quality, both types of feed were packed separately in airtight and Ziplock plastic bags. These bags were stored in a cool place to prevent moisture and minimize the risk of fungal or microbial contamination.

The fish were fed twice a day, with each feeding amounting to 5% of their body weight. For the experimental feed, five different batches were prepared, each containing a graded level of *M. charantia* seed powder as a supplement. The control diet consisted solely of the basal diet without the inclusion of *M. charantia* seed powder.

2.3 Experimental design

Following the acclimatization period, a completely randomized 90 days feeding experiment was designed using active and healthy female tilapia fish of uniform size. The experiment was conducted in triplicate. Approximately 220 fish with an initial body weight of 10±1.6 grams and a length of 7.7±1.6 centimeters were distributed among 18 fiberglass tanks. The stocking density was set at 1.5 grams per liter. The tanks were randomly divided into six groups. The first three tanks were designated as the control group, while the remaining tanks were assigned as treatment groups, named S2, S4, S6, S8, and S10.

During the 90-day experimental duration, fish were fed with respective diet containing 35% crude protein plus supplement. The feeding rate was set at 5% of the fish's body weight and adjusted fortnightly based on their body mass. The 35% crude protein diet with different supplement dose was formulated as follows:

Control group (C): Fish diet without any supplement.

S2: 0.2% of seed powder added to the fish feed.

S4: 0.4% of seed powder added to the fish feed.

S6: 0.6% of seed powder added to the fish feed.

S8: 0.8% of seed powder added to the fish feed.

S10: 1% of seed powder added to the fish feed.

Throughout the experimental period, regular monitoring of water parameters such as temperature, dissolved oxygen (DO) levels, and pH were conducted. The water quality and turbidity were checked, and partial water changes were performed every 3 to 4 days based on the monitoring results.

To assess the ammonia concentration in the water, an ammonia test kit specifically designed for freshwater (API freshwater test kit) was used. This allowed for the measurement of total ammonia levels, which is an important indicator of water quality. The water temperature during experimental period ranged between 26-28 °C. All tanks were in the same

vicinity and in indoor facility, so no noticeable difference in temperature among tanks was observed.

2.4 Fish sampling

The fish were fasted for 24 hours before sample collection. Following this, the fish were immediately anesthetized using MS-222 at a concentration of 150mg/L (El-Erian *et al.,* 2023). After anesthesia, measurements of fish length and weight were taken. A 3ml pre-heparinized syringe was used to take blood samples from the caudal vein of fish. The blood samples were stored in EDTA tubes for hematological parameter analysis.

For further analysis, serum blood samples from nine fish were collected using serum separation SST tubes. The tubes were then centrifuged at 5000xg for 20 minutes at room temperature to obtain the serum. The fish were placed on icebox for dissection and various organs including muscle, gills, liver, brain, and intestine, were carefully separated and collected in Ziplock bags. Ovarian tissue samples (from three fish per tank) were obtained, weighed, and kept for histological investigation in 10% formalin. The Gonadosomatic Index (GSI) was determined by weighing the ovaries of each female Nile tilapia. For further analysis, targeted samples were frozen in liquid nitrogen (-196℃) and stored in a refrigerator at 20 ℃ until they could be processed for subsequent analyses and experiments.

2.5 Growth Performance

Before dissection, each fish weight and length were noted and the total number of fish in each fiber glass tank were calculated to find the average body weight

For growth performance the following formula were used.

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

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

Where *ln*=natural log

2.6 Hematological studies

By using heparinized syringes, blood samples were obtained from both control and experimental groups in the VACUETTE® EDTA tubes. The blood samples were used to determine hematological indices like white blood cells (WBCs), red blood cells (RBCs), Hemoglobin (Hb), hematocrit (HCT), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) by using automatic

hematology analyzer (Sysmex hematology analyzer). All the samples were run in duplicate with 15 second interval time and mean values were calculated.

2.7 Histology of ovarian tissues

To examine the changes in gonadal tissues of female Nile tilapia**,** (3 fish/ group) were sampled from six group and fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleaned in xylene, embedded in paraffin and sectioned in 5um thickness. The sectioned were subjected to staining with hematoxylin and eosin (Spencer *et al.,* 2013).

2.8 Gonadal somatic index (GSI)

At the end of experiment, fish (n=5) from each group were weighed and their gonadal mass was measured. The following formula was used to calculate the gonadal somatic index.

$$\text{GSI} = \frac{\text{weight of gonads in gram}}{\text{weight of fish in gram}} \times 100$$

2.9 Fecundity

The gravimetric subsampling approach was used to count the number of eggs in the ovaries of fish (n=6) from each experimental group in order to estimate the gonads' fecundity (Bagenal, 1978). The process involved weighting of the entire gonads. From the posterior, middle, and anterior lobes of the ovary, a tiny portion of eggs were removed, weighed and the number of eggs were counted. The following formula was used to determine the total number of eggs present in a single fish's gonad:

$$\text{Fecundity} = \frac{\text{number of eggs in a sample(n)} \times \text{weight of ovary(g)}}{\textit{weight of eggs in egg sample}(g)}$$

2.10 Antioxidant enzymes determination

The ovarian tissues were homogenized using a Dounce manual homogenizer (Sigma, Aldrich) in 100 mmol potassium phosphate buffer (PBS) containing 1 mmol Ethylenediaminetetraacetic acid (EDTA) and the centrifuge at 4℃ for 30 minutes at 12000×g. An Eppendorf tube was used for separating the supernatant, the pellet was discarded and aliquots were made and stored at -20℃ until antioxidant enzymes were determined.

2.10.1 Superoxide Dismutase (SOD)

To determine SOD activity in ovarian tissue a modified Kakkar *et al.* (1984) method was used. In this reaction 0.3 mL supernatant and 0.1 mL of 186 µM phenazine methosulphate solution were mixed along with 1.2 mL of 0.052 mM sodium pyro-phosphate buffer (pH=7.0). To initiate reaction 0.2 mL of 780 µM NADH+ solution was added and then after 1 mint, 1mL of glacial acetic acid was used to stop the reaction. A spectrophotometer was used to observe the chromogen's absorbance wavelength of 560nm. One SOD unit is the number of enzymes per mg of protein that prevent the quercetin oxidation by 50%. The activity of SOD was represented in moles/min/mg protein by using a molar coefficient of 6.22×10^3/M cm.

2.10.2 Catalase (CAT) Assay

The catalase activity was determined by the Chance & Maehly (1955) method. 0.1mL (100 µl) of supernatant, 2.5mL of 50 mM phosphate buffer (pH=5.0), and 0.4mL (400 µl) of 5.9 mM hydrogen peroxide (H_2O_2) were mixed. Absorbance in reaction mixture was measured at 240 nm wavelength via spectrophotometry. The activity of CAT was expressed as µmol/min/mg protein with a molar coefficient of 43.6/ M cm.

2.10.3 Peroxidase (POD) assay

Previously report (Chance & Maehly, (1955) and (Bibi, (2012) technique was used to determinede POD activity in ovarian tissues. To carry out the reaction, 2.5 ml of 50mM PBS (pH 5.0), 0.3ml of 40 mM H_2O_2, 0.1 ml 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).

2.10.4 Reactive oxygen species

The method used by Hayashi et al. (2007) to determine ROS production was used. distilled water and 1 mg/10 mL N′N-Diethyl para phenylenediamine sulfate (DEPPD) were the components of Reagent 1. Reagent 2 was made up of 100 mL of sodium acetate buffer and 50 L of FeSO4 stock solution (50 mg ferrous sulfate in 10 mL of sodium acetate buffer, pH 4.8). In a 1:25 mixture, reagents 1 and 2 were held in the dark for two minutes. 1680 liters of the aforesaid reagent mixture and 1200 liters of sodium acetate buffer were added to 60 liters of tissue homogenate that had been drawn into a cuvette. A UV spectrophotometer (Agilent 8453, California, USA) was used to detect absorbance at 505 nm. Three readings were obtained for each at a 15-second interval.

RESULTS

3.1 Growth performance:

Momordica charantia seed powder supplemented diets showed a significant effect on the growth performance of female *Oreochromis niloticus* as shown in Table 3. Statistical analysis of results by adopting One-way ANOVA indicated a significant effect of *M.charantia* seed supplemented diets at different dosage level on final body weight (n=3, $F_{5,30}$= 8798 ; P<0.01), %WG (n=3 $F_{5,30}$= 11015; P<0.007), and %SGR (n=3, $F_{5,30}$= 1012; P<0.001) among all experimental groups of fish. Pair-wise comparison of results indicated significantly highest %WG in S_{10} supplemented group of fish followed by S_8, S_6, S_4 and S_2 while lowest in control group of fish.

Furthermore, the statistical analysis using one-way ANOVA showed a significant difference on FCR of *M.charantia* seed supplemented fish (n=3, $F_{5,30}$ = 5.88; P<0.001) among all experimental groups. The pair-wise comparison of results indicated significantly lowest and best FCR in S_{10} supplemented group of fish followed by S_8, S_6, S_4 and S_2 while lowest in control group of fish.

3.2 Haematological results:

Dietary supplementation of *Momordica charantia* seed powder showed a significant effect on the haematological indices of female *Oreochromis niloticus* as shown in Table 4. Statistical analysis of results by adopting One-way ANOVA indicated a significant effect of *M.charantia* seed supplemented diets at different dosage level on RBC_s (n=9, $F_{5,48}$= 17.6 ; P<0.001), WBC_s (n=9 $F_{5,48}$= 412; P<0.003), Haemoglobin (n=9 $F_{5,48}$= 19.1; P<0.00), PCV (n=9 $F_{5,48}$= 6707; P<0.001), MCV (n=9 $F_{5,48}$= 4508; P<0.001), MCH (n=9 $F_{5,48}$= 16892; P<0.001), and MCHC (n=6, $F_{5,30}$= 1550 ; P<0.001) among all experimental groups of fish. Pair-wise comparison of results indicated that inclusion of 10 g seed showed a significant effect among all groups while lowest in S2 group. Furthermore, it was observed that there was a little but significant effect on RBCs level.

3.4 Gonadal somatic index:

Study for the gonadic somatic index (GSI) for female Nile tilapia (*Oreochromis niloticus)* revealed a significant alteration between the treatment groups as compared to control, shown in Table 5. Statistical analysis of results by adopting One-way ANOVA indicated a significant effect (n=3 $F_{5,12}$= 200; P<0.001), among all experimental groups of fish. However, Multiple pairwise comparison Tuckey HSD (using SPSS) GSI was

decrease significantly($P<0.05$) in S10 among all treatment group followed by S8>S6, S4> and S2 respectively, while that lowest was recorded in control group.

3.5 Fecundity:

The administration *Momordica charantia* seed powder showed a significant effect on the absolute fecundity of female *Oreochromis niloticus* as shown in Table 5. Statistical analysis of results by adopting One-way ANOVA indicated a significant effect ($n=3$ $F_{5,30}= 416$; $P<0.00$), among all experimental groups of fish. Multiple Pair-wise comparison Tuckey HSD (using SPSS) revealed a significant reduction in fecundity of S_{10} treated group of fish followed by S_8, S_6, S_4 and S_2 as compared to control group of fish which shows highest value of fecundity.

3.6 Antioxidant activity:

A significant effect of antioxidant enzyme in the serum of female *Oreochromis niloticus* was observed fed with *Momordica charantia* seed powder as shown in Table 6. Statistical analysis of results by adopting One-way ANOVA indicated a significant effect of *M.charantia* seed supplemented diets at different dosage level on catalase ($n=3$, $F_{5,12}= 2.66$; $P<0.0764$), superoxide dimutase ($n=3$ $F_{5,12}= 1.18$; $P<0.3752$), and peroxidase ($n=3$ $F_{5,12}= 1.38$; $P<0.2975$), among all experimental groups of fish. Pair-wise comparison of results indicated that inclusion of 10 g seed increase antioxidant activity among all treated groups while lowest in control group. Moreover, by adopting One-way ANOVA the treated groups revealed a significant effect ($n=3$ $F_{5,12}= 7.36$; $P<0.05$) on ROS levels in serum of Nile Tilapia. Multiple pairwise comparison Tuckey HSD (using SPSS) shows that ROS activity was decrease with increasing treated dosage level i.e., the lowest activity was observed in S10 treatment group.

3.7 Histology

Our current study revealed that in *O.niloticus* fed only with basal diet i.e., Control group have typical bilateral lobes of ovaries, olive green in color and showed normal ovary structure. Furthermore, ovaries also have no pathological lesions. While in fish supplemented with seed have ruptured follicles and altered ovary structure. Moreover, increased atretic follicles and necrosis was also noticed in high dose treatment fish i.e., S10 and S8.

3.8 Hepatosomatic index

Study for the hepato somatic index (HSI) for female Nile tilapia (*Oreochromis niloticus)* revealed a non-significant difference between the treatment groups and control, shown in Table 7. Statistical analysis of results by adopting One-way ANOVA indicated a non-significant effect (n=3 $F_{5,12}$= 2.02; P<0.1050), among all experimental groups of fish. However, Multiple pairwise comparison LSD HSD (using SPSS) indicated highest and lowest HSI for S10 and S2 treatment group respectively.

Table 01: Formulation of 35% CP diet for *O.niloticus* with Bitter melon (*Momordica charantia*) seeds powder supplement 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
Sun flower (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 Vitamin 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 diet.

Ingredient %	Control	S2	S4	S6	S8	S10
Moisture	10.08	10.26	10.25	10.31	10.32	10.29
Crude protein	33.80	34.21	34.24	34.53	35.29	35.67
Crude lipids	12.21	12.32	12.50	12.52	12.97	12.98
Crude fiber	7.09	8.01	8.03	8.04	8.06	8.13
Total ash	12.14	12.21	12.12	12.32	12.21	12.14
Bitter melon seeds powder	0	2	4	6	8	10

Mineral mix (El Bardeny Company), each 0.25 kg contains: Iron 30,000 mg; Manganese 60,000 mg; Zinc 50,000 mg; 1) Copper 4,000 mg; Cobalt 100 mg; Iodine 300 mg and Selenium 100 mg. 2) Vitamin mix., each 0.25 kg contains: (A) 10,000,000 IU; (D3) 2,200,000 IU; (E) 10,000 mg; (K3) 1,000 mg; (B1) 1,000 mg; (B2) 5,000 mg; (B6)1,500 mg; (B12) 10,000 mg; Panthotenic acid 10,000 mg; Niacin 30,000 mg; Folic acid 1,000 mg; Biotin 50,000 mg; Colinechloride 600,000 mg.

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

Parameter	Control	S2	S4	S6	S8	S10	F-value	P
IBW(g)	10.1± 0.3^{a}	9.98 ± 0.1^{a}	10.4± 0.2^{a}	9.88 ±0.3^{a}	10.1 ±0.6^{a}	9.95 ±0.4^{a}	1253	0.71
FWG(g)	19.3± 0.8^{f}	21.4± 0.7^{e}	22.2± 0.6^{d}	26.1 ±0.5^{c}	30.1 ±0.7^{b}	33.5 ±0.4^{a}	8798	P<0.001
NWG(g)	9.2± 0.1^{f}	10.8± 0.3^{e}	11.8± 0.2^{d}	15.6 ±0.4^{c}	20.5 ±0.3^{b}	23.5 ±0.5^{a}	4126	P<0.001
SGR (%body weight/day)	0.39± 9.8^{e}	0.40± 7.2^{e}	0.56± 6.2^{d}	0.63 ±8.2^{c}	0.88 ±4.2^{b}	0.93 ±2.2^{a}	1012	P<0.001
FCR	2.3± 0.05^{a}	2.1± 0.03^{b}	1.9± 0.10^{c}	1.8 ±0.04^{d}	1.6 ±0.1^{e}	1.5 ±0.03^{f}	5.88	P<0.001
%WG	91.30± 0.7^{f}	96.5± 0.5^{e}	124.1± 0.3^{d}	147.8 ±0.6^{c}	213.3 ±0.7^{b}	229.0 ±0.5^{a}	1101	P<0.001

Data in Table (3) 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).

Table 04: Hematological indices of Nile tilapia feeding with Bitter melon (*M.charantia*) seeds powder for 90 days trail.

Group	Control	S2	S4	S6	S8	S10	F-value	P
RBCs(10^6/u)	1.81 ±0.02^{d}	1.85 ±0.04^{cd}	1.88±0.06^{c}	1.93±0.05^{b}	1.96 ±0.03^{b}	2.00 ±0.08^{a}	17.6	0.001
WBCs(10^3/l)	3.30± 0.004^{f}	3.50±0.003^{e}	3.70±0.007^{d}	4.10±0.005^{c}	4.24±0.003^{b}	4.34±0.004^{a}	412	0.003
Hemoglobin(g/)	4.71±0.005^{d}	4.75±0.009^{cd}	4.77±0.006^{bc}	4.78±0.008^{bc}	4.80±0.012^{b}	4.90±0.007^{a}	19.1	0.001
PCV	27.61±0.007^{f}	28.60±0.009e	29.84±0.008^{d}	30.84±0.013^{c}	31.78±0.014^{b}	32.27±0.017^{a}	6707	0.001
MCV (um^3)	110.2±0.002^{f}	115.5±0.003^{e}	122.2±0.002^{d}	127.4±0.001^{c}	132.7±0.013^{b}	134.0±0.012^{a}	4508	0.001
MCH (pg.)	51.7±0.008^{f}	56.4±0.006^{e}	60.2±0.004^{d}	64.5±0.001^{c}	68.1±0.009^{b}	69.2±0.013^{a}	16892	0.001
MCHC(g/dl)	33.5±0.003^{f}	34.7±0.006^{e}	36.5±0.003^{d}	38.1±0.001^{c}	40.1±0.002^{b}	41.6±0.003^{a}	1550	0.001

Data in Table (4) is expressed as Mean ± SE (n=9). ANOVA and post-hoc LSD tests were utilized to illustrate pairwise comparisons of RBCs, WBCs, Hemoglobin, PCV, MCV, MCH, MHCH among 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).

Table 05: Gonadosomatic index (GSI) and Fecundity of Nile tilapia feeding with Bitter melon (*M.charantia*) seeds powder for 90 days.

Parameter	C	S2	S4	S6	S8	S10	F- value	P
GSI	2.88 ± 1^{f}	2.59 ± 0.8^{e}	2.37 ± 1^{d}	2.10 ± 1.1^{c}	1.85 ± 0.9^{b}	1.51 ± 1^{a}	200	P<0.001
Fecundity	548 ± 1.0^{f}	507 ± 0.8^{e}	493 ± 1.0^{d}	462 ± 0.9^{c}	378 ± 1.0^{b}	296 ± 0.8^{a}	416	P<0.001

Data in Table (5) is expressed as Mean ± SE (n=9). ANOVA and post-hoc LSD tests were utilized to illustrate pairwise comparisons of GSI and Fecundity between different 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).

Table 06: Antioxidant activity of Nile tilapia feeding with supplemented Bitter melon (*M.charantia*) seeds powder for 90 days.

Parameter	C	S2	S4	S6	S8	S10	F-value	P
SOD	56.707±3.1^{e}	57.093±3.4^{d}	57.730±3.6^{d}	59.400±3.9^{c}	61.440±4.0^{b}	62.803±4.1^{a}	1.18	0.3752
POD	68.520±4.2^{d}	68.860±4.4^{d}	70.033±4.7^{c}	72.350±4.9^{b}	72.760±5.0^{b}	74.200±5.2^{a}	1.38	0.2975
CAT	64.667±1.41^{c}	65.323±1.43^{bc}	68.189±1.52^{abc}	68.422±1.58^{abc}	71.389±1.60^{ab}	72.8817±1.61^{a}	2.66	0.0764
ROS	10.50±0.05^{a}	9.84±0.04^{ab}	9.68±0.07^{ab}	9.34±0.04^{b}	8.20±0.06^{c}	8.12±0.02^{c}	7.36	0.0003

Data in Table (6) 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 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).

Table 07: Hepatosomatic index (HSI) of Nile tilapia feeding with Bitter melon (*M.charantia*) seeds powder for 90 days.

Parameter	Control	S2	S4	S6	S8	S10	F-value	P
HSI	0.6667± 0.02^{b}	0.6817± 0.05^{ab}	0.6850± 0.09^{ab}	0.6950± 1.0^{ab}	0.7017± 1.01^{a}	0.7083± 1.02^{a}	2.02	0.1050

Data in Table (7) is expressed as Mean ± SE (n=9). ANOVA and post-hoc LSD tests were utilized to illustrate pairwise comparison of HSI between the groups. Lower-case superscripts were used to 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).

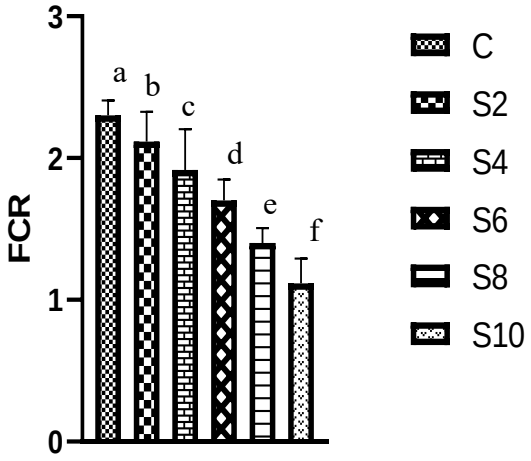

Fig.1: The graph illustrates the FCR value of *O. niloticus* when exposed to dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

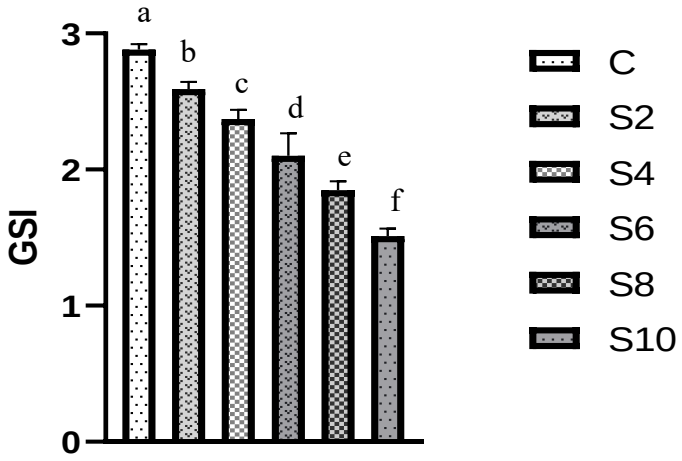

Fig.2: The graph illustrates the GSI value of *O. niloticus* when exposed to dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

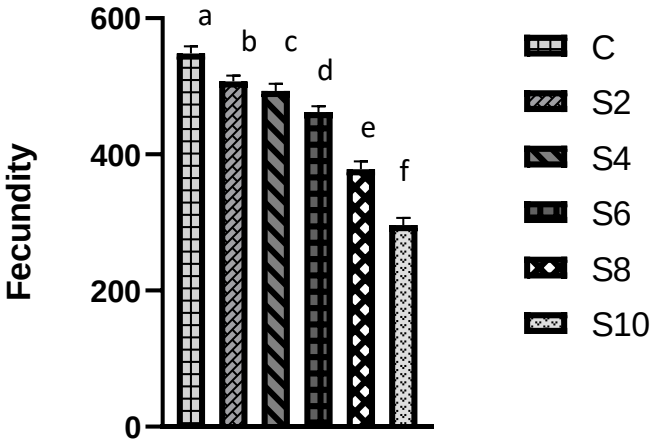

Fig.3: The graph illustrates the Fecundity value of *O. niloticus* when exposed to dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

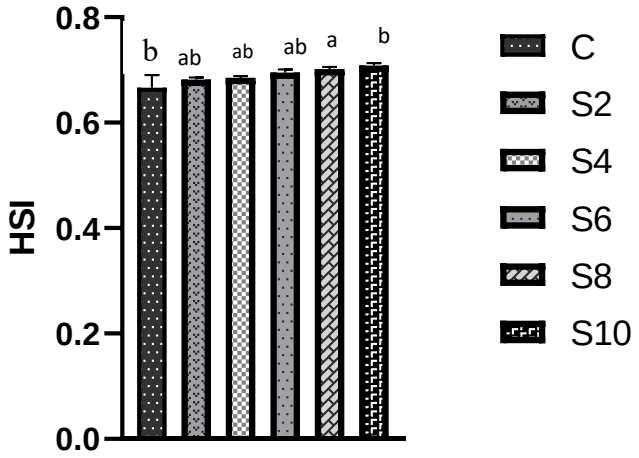

Fig.4: The graph illustrates the HIS value of *O. niloticus* when exposed to dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

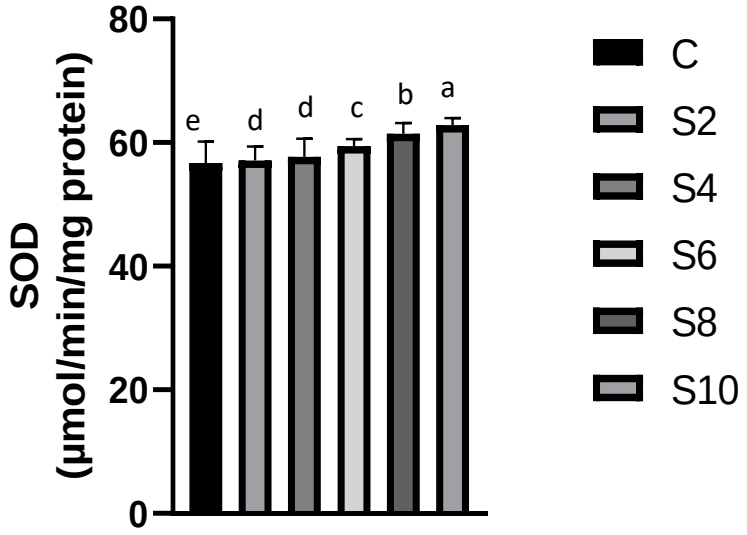

Fig.5: The graph illustrates the superoxide dismutase activity of *O. niloticus* in ovaries when exposed to dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

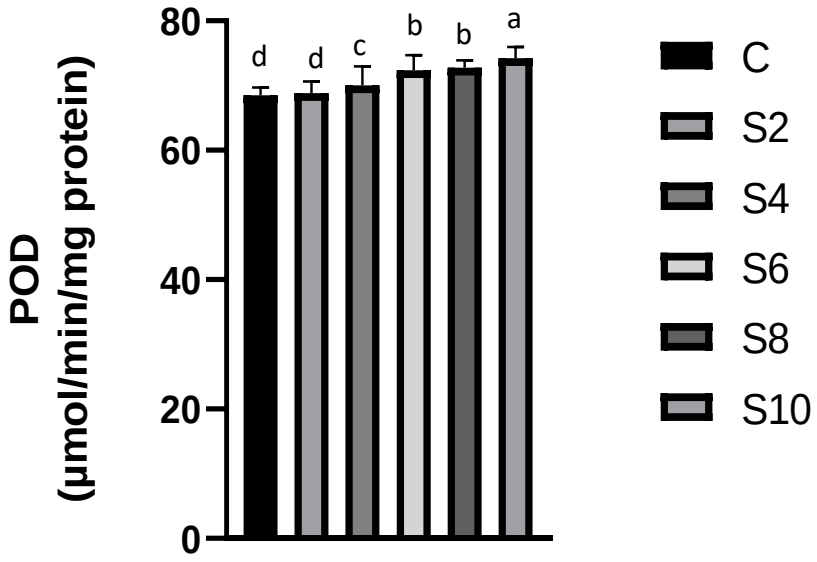

Fig.6: The graph illustrates the peroxidase activity of *O. niloticus* in ovaries when exposed to dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

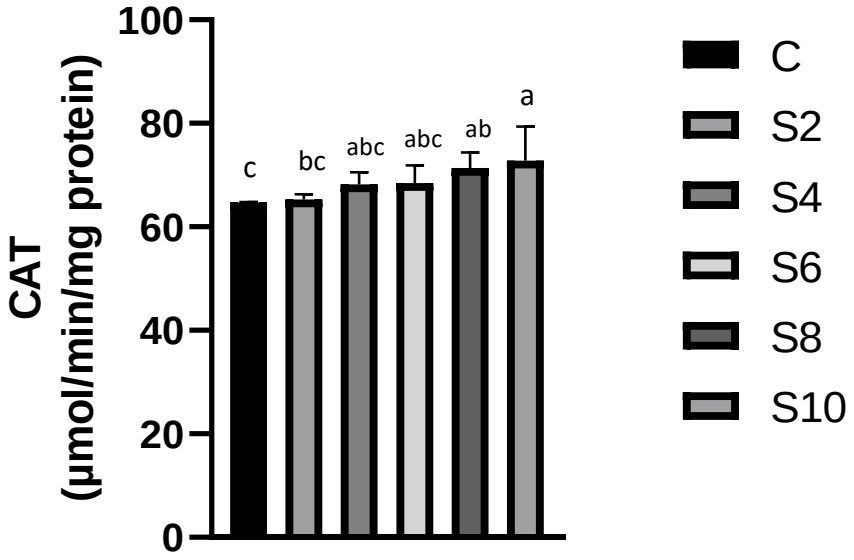

Fig.7: The graph illustrates the catalase activity of *O. niloticus* in ovaries when exposed to dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

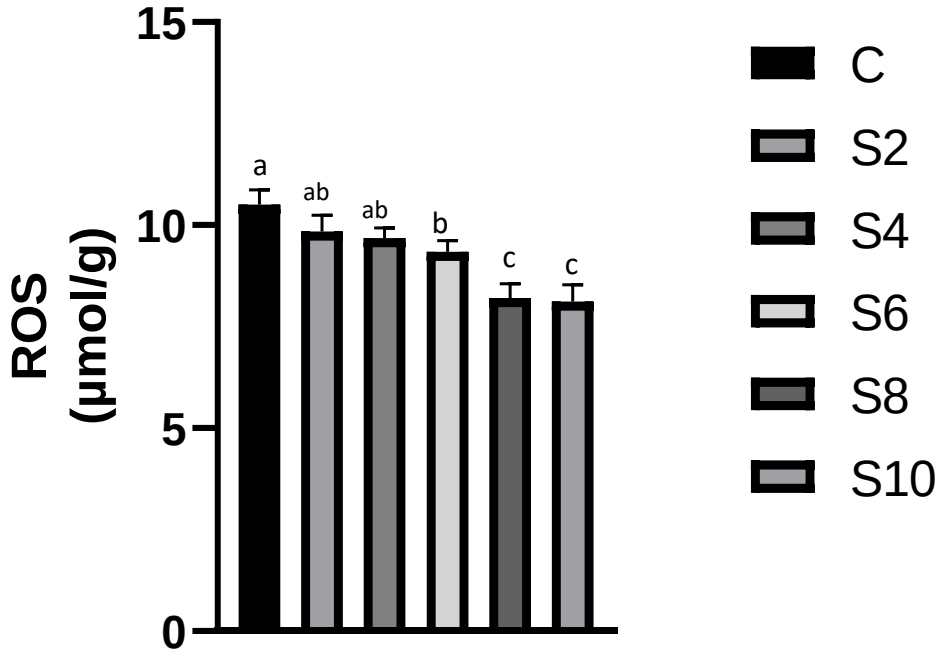

Fig.8: The graph illustrates the ROS activity of *O. niloticus* in ovaries when exposed to dietary powdered seeds of *M. charantia* at various concentrations. Each bar on the graph represents the Mean±S.E with a sample size (n) of 9.

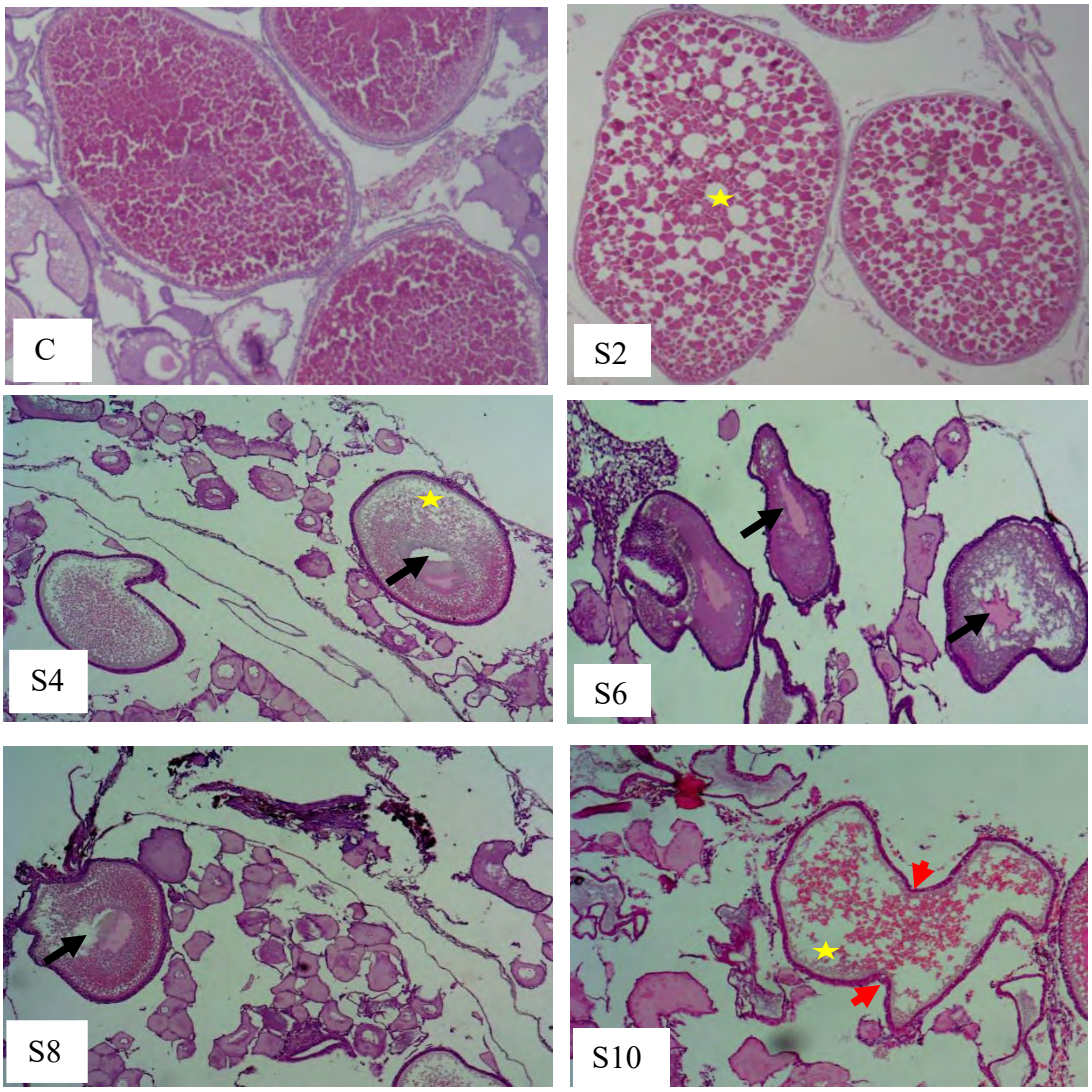

Fig 09: Histological examination of ovarian tissues. The groups include: Control (C) (diet without bitter melon seed), S2 (2g of seed/kg of diet), S4 (4g of seed/kg of diet), S6 (6g of seed/kg of diet), S8 (8g of seed/kg of diet), and S10 (10g of seed/kg of diet).
S2, S4 and S10 showing (Yellow stars) degeneration or autolysis of oocytes. S4, S6, and S8 (black arrows) showing absent of nucleus wall, elongation of nucleus and swell nucleolus. In S10 (arrow head) showing buckling of oocyte wall.

DISCUSSION

Numerous studies have emphasized the advantages of medicinal herbs as potential alternatives to chemicals and pharmaceutical drugs (Green & Kelly, 2009; Bai *et al.,* 2012). Medicinal herbs have also been found to have antifertility and abortifacient properties when given to animals through oral administration (Obaroh & Achionye-Nzeh, 2011). Early research has shown that certain phytochemicals are present in medicinal herbs (phytoestrogens or phytoandrogens), which have a similar structure to steroid hormones like 17-β estradiol (E2) in animals (Glazier & Bowman, 2001). In recent times, these plant-based compounds have been found to trigger effects such as inducing masculinity, femininity, or impair fertility in tilapia species (Gabriel *et al.,* 2017; Jegede, 2010). Phytoestrogen, which includes isoflavonoids, flavonoids, lignans, and coumestans among others, is thought to mimic or behave as sex hormones and can decrease the production of estrogen by competing with nuclear estrogen receptors in gonad germ cells and acting as aromatase inhibitors (Das *et al.,* 2012) and consequently, these compounds might have the potential to cause sex reversal or even delay the maturity of fish. Incorporating medicinal herbs at all levels of aquaculture could not only enhance production but also boost the safety and quality of aquatic products. This could lead to increased global consumption of these products. The use of easily available medicinal plants offers advantages such as simple application, no need for specialized labor, cost-effectiveness, and reversible effects. This approach has proven effective in addressing challenges related to controlling tilapia population (Priya *et al.,* 2012).

In diverse animal models, the anti-fertility properties of numerous tropical plants have been investigated and validated (Kumar *et al.,* 2012). Bitter melon (*Momordica charantia*) fruit and leaves been proven to have a mammalian in vivo anti-fertility effect (Girini *et al.,* 2005). Amah *et al.,* (2011) conducted research on the impact of *M. charantia* on the estrous cycle of Sprague-Dawley rats. Their findings revealed sporadic alterations in the phases of the estrous cycle among all the treated rats they observed. Seeds of *Momordica charantia* have shown the ability in mice and rats to induce abortion (Kumar *et al.,* 2010). Sharma *et al.,* (1960) also determined that fruit extract of *Momordica charantia* induced abortion in rabbits. Yeung *et al.,* (1986) found that two proteins with abortifacient properties, categorized as "a" and "β" momorcharins, were separated from the seeds of the bitter gourd, *Momordica charantia*. This isolation process involved acetone fractionation. Both of these proteins were found to have nearly equal potency in inducing mid-term abortions in mice. The present study was intended to assess the effect of *Momordica charanatia* seed powder as a potential

infertility inducer in female Nile tilapia (*Oreochromis niloticus*). The results from this study support the hypothesis that inclusion of *M. charantia* seed powder in basal diet can be effective as infertility inducer in female *O.niloticus* .Therefore , it seems a possible way to control a vigorous spawning in tilapia thus help to prevent the issue of overcrowding in *O. niloticus* culture farms.

In our present study the results showed that the incorporation of *M. charantia* seed powder yielded pronounced effects on growth performance, as well as on Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR). Notably, significant improvements were observed in these parameters among the treatment groups supplemented with *M. charantia* seed powder. In the S10 treatment group which is provided with high dose of seed powder, showed a notable increase in growth rate, SGR and lower value for FCR. No mortality was recorded in all diet treatment groups. This suggests the potential application of *M. charantia* seed powder as a source of protein as well. Similarly, Crude extracts from *Aloe vera* (Gabriel *et al.,* 2015), *Cinnamomum camphora*, *Euphorbia hirta*, *Azadirachta indica*, and *Carica papaya* (Kareem *et al.,* 2016), *Allium sativum* (Shalaby *et al.,* 2006) were shown to have greatly improved Nile tilapia weight gain (WG), specific growth rate (SGR), feed intake (FI), and feed conversion ratio (FCR).

The Gonadosomatic index (GSI) has proven to be a helpful indicator for observing the development of gametogenesis in teleost fish. (Guerrero *et al.,* 2009). In the current study, female GSI decreased with increasing level of *Momordica charantia* seed powder compared with the control group. GSI values of treatment groups S10 and S8 was significantly different as compared to control group which suggested that inclusion of seed powder in feed at higher dose i.e., 10g/kg were more effective. Similar results were obtained by Jegede & Fagbenro (2008), who observed a significant reduction in the GSI of female O. niloticus treated with neem (*Azadirachta indica*). Furthermore, Temitope (2010) also determined significant decrease in GSI of *O.niloticus* female when *Hibiscus rosa sinensis* leaf meal (HLM) were included in a basal diet for 60 days.

The fecundity of *O.niloticus* fed with *M.charantia* seed powder was significantly different from the control group.. The lowest fecundity value was obtained from S10 group among all treatment group. In our present study it was recorded that increasing the dose of seed powder decreases the absolute fecundity. Sharma *et al.,* (2011) reported that pregnant rats fed the juice of *Momordica charantia* were able to cause uterine hemorrhage, which could lead to abortions, and that pregnant rabbits were also able to induce uterine bleeding.

The results of fecundity was supported by Temitope (2011) who observed a decrease in fecundity of O.*niloticus* supplemented with various doses of *Aloe vera* latex. Udoh *et al.,* (2005) also observed female rats do not become pregnant when administered with *Carica papaya* seed extract for 3 days. The results of our study also similar to Kapinga *et al.,* (2018) fed juvenile nile tilapia with leaf powder of Aspilia plant, *Aspilia mossambicensis* and Neem tree, *Azadirachta indica* for 90 days resulting in significant decrease in AF and GSI ($p < 0.05$). Furthermore, Akin-Obasola (2021) also observed that using *Abrus precatorius* root bark meal can effectively reduce ovary weight, fecundity, and gonadosomatic index with increase in the concentration of treatments in female *Coptodon zillii* fish. Moreover, Abdelhak *et al.,* (2013) also identified that reproductive parameters such as gonadic somatic index and fecundity of female *O.niloticus* were significantly decreased when fed with *C.papaya* seed meal while the GSI of male did not show significant difference. So, *Momordica charantia* is the most effective, specially its seeds are highly to alter gonadal characteristics in *O.niloticus* without any harm to tilapia`s health condition.

In vertebrates, including fish, blood is the most commonly analyzed to determine their health or physiological condition. Essential hematological indicators, such as red blood cell (RBC) count, hemoglobin concentration (Hb), the proportion of blood volume occupied by red cells, and hematocrit (Hct), are used to directly establish the health status, particularly in terms of oxygen transportation (Houston, 1997). In our present study hematological indices such Hb, HCt and MCH were significantly improved in fish while that on RBCs had little effect that were fed with *M.charantia* seed, compared to control group. Numerous studies in aquaculture have effectively shown that various medicinal plants can improve the mentioned hematological parameters. Such as reported by Gabriel *et al.,* (2015) in tilapia culture, adding *Aloe vera* to the diet led to notable increases in blood parameters like RBC, Hct, Hb, WBC, and specific leukocyte counts in Nile tilapia (GIFT-strain) observed before and after a challenge with Streptococcus iniae. Moreover, administration of *Rosmarinus officinalis*, *Trigonella foenum graecum*, *Thymus vulgaris* in *O. mossambicus* (Gültepe *et al.,* 2014), *Camellia sinensis* in *O. niloticus* (Abdel-Tawwab *et al.,* 2010), and ginseng in *O. niloticus* (Goda, 2008) reportedly enhanced certain hematological parameters. The observed enhancement of hematological indices is likely due to the rich nutritional properties of plant such as polysaccharides, essential vitamins and non-essential amino acid. These components are primarily required for the synthesis of hemoglobin (Latona *et al.,* 2012; Hamman, 2008).

Various chemical compounds present in plants that have antioxidant properties. These properties aid organisms in managing oxidative stress caused by damage from free radicals. Consequently, these compounds can enhance the overall status of fish's physiology (Ali *et al.,* 2008; Chakraborty & Hancz, 2011). In the present study antioxidant enzymes such as SOD, POD, and CAT activity was increased in ovarian tissues of Nile tilapia in all treatment groups as compared to control groups. Moreover, high antioxidant activity was recorded at high dose (10g) of seed powder. Finding of this study are similar to those reported by Gabriel *et al.,* (2015) who observed that *aloe vera* crude extracts can boost antioxidant enzymes (CAT, SOD, GSH-Px) and reduce stress in GIFT- tilapia. Metwally (2009), also reported the same results in *O. niloticus* after a dietary supplementation with *Allium sativum* extracts. The n-hexane extract from Momordica seeds has been shown to contain conjugated octadecatrienoic fatty acids and ά-eleostearic acid. These acids have been investigated for their antioxidant properties and have demonstrated success in an in vitro study (Sharma *et al.,* 2011). Enhancing fish antioxidants with herbal extracts is often linked to specific phytochemicals such as phenols/polyphenol (gallic acid, tannins, and ellagic acid), enzymes (SOD, CAT, GSH-Px), vitamins (C, E, carotenoids), and flavonoids such as flavones, isoflavones, anthocyanins (Gupta & Sharma ,2014).

Although gonadal development is a continuous process, different histological traits can be used to classify several stages of gonadal development over the course of the reproductive cycle (Bucholtz *et al.,* 2008). Results of this study revealed structural alteration such as separation of follicular layer, follicular rupture, atresia, and necrosis in ovaries of treatment groups of female O. *niloticus*, compared to control group which showed normal histological structure as expected. Compared to the present study similar results were reported in *O. niloticus* supplemented with *carica papaya* (Abdelhak *et al.,* 2013), *Hibiscus rosa-sinensis* (Jegede, 2010), and *Aloe vera* (Jegede, 2011) as well as in *O. mossambicus* fed dietary *Carica papaya* and *Moringa oleifera* respectively (Ampofo-Yeboah, 2013). Kumar *et al.,* (2010) elucidated that *Momordica charantia* seeds have exhibited the potential to cause abortions in both rats and mice. These abortifacient properties of the bitter gourd (*Momordica charantia*) seeds are due to two proteins, categorized as "a" and "β" momorcharins, were separated from their seeds (Sharma *et al.,* 1960).

Conclusion

In recent times, *Momordica charantia* has emerged as a potent antifertility medicinal plant. The global recognition of the value of such traditional medicinal plants has grown, as their efficacy holds potential benefits for humanity. Conducting comprehensive scientific research on their properties could prove highly advantageous. The results from this study shows that *Momordica Charantia* seed can control Nile tilapia population and act as good infertility inducer, which is cheap, safe, biodegradable and easy to obtain. Farmers can incorporate Bitter Melon seed into fish feeds with adjustable amount to control tilapia breeding. Thus, help to prevent the issue of overcrowding in *O. niloticus* culture farms.

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