Jimma University
School of Graduate Studies
College of Natural Sciences
Department of Biology

Indoor evaluation of anti-fertility effect of papaya (*Carica papaya L.*) leaf and seed in controlling prolific breeding of Nile tilapia (*Oreochromis niloticus*: Cichlidae)

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Approval sheet

Advisors	**Signature**	**date**
Mulugeta Wakjira (PhD)	_____	_____
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Declaration

I, the Undersigned, declare that this thesis entitled **Indoor evaluation of anti-fertility effect of papaya (*Carica papaya L.*) leaf and seed in controlling prolific breeding of Nile tilapia (*Oreochromis niloticus*: Cichlidae)** is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

Name	**Signature**	**Date**
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Abstract

Nile tilapia has been spread to all continents as an aquaculture fish due to its desirable qualities of a culture species. However, its precocious spawning is a global bottleneck with this species. A 90-days experiment was conducted to determine antifertility effect of Papaya seed powder (PSP) and papaya leaf powder (PLP) powders on Nile tilapia. The experiment was carried out in eight tanks grouped in to three treatments (T1, T2 & T3) and one control, each in duplicates. The treatments consisted of mixtures of PSP and PLP combined in different proportions as 1.5g PLP and 0.5g PSP in T1, 1.5g PSP and 0.5g PLP in T2 and 1g PSP and 1g PLP in T3 to 1 kg of basal diet. Fishes in the control tanks were provided only with the basal diet. The fishes were fed at 10 % of their body weight throughout the experimental period. One-way ANOVA was used to test the differences in the mean body weight, body length and gonad weight among the control and three treatment groups. For male highest gonad weight (0.22 ± 0.16 g) and GSI (0.79 ± 0.38) were recorded in control groups. Least values of gonad weight (0.03 ± 0.02 g) and GSI (0.11 ± 0.10) for male were recorded in T3. For the females, the highest gonad weight (0.27 ± 0.25g) and GSI (0.84 ± 0.80) were also recorded in control group, while the least values of gonad weight (0.17 ± 0.21g) and GSI (0.43 ± 0.54g) were recorded in T3 and thus maximum sterilizing effects, were recorded in T3 that consisted of equal proportions of the seed and leave powders. Gonad development was ranges II –IV in control groups and impaired in all treatments in both male and female fishes. Fishes in T3 had best food conversion rate (FCR) (0.91 to 1.80), whereas least food conversion rate (FCF) was recorded for fishes in control group (1.02 to 1.90). Maximum survival rate (55%) was recorded for fish in T3, whereas minimum survival rate (40%) recorded for fish in T2 and the survival rate of fish in control group was 45%.The growth performance (i.e. mean weight and length) of fish in all treatments and control group showed no significant differences (P>0.05).Reproductive parameters showed clearly that PSP and PLP induce sterility in Nile tilapia in both male and female. Both mixture of PSP and PLP at dosage of (1g of PSP and 1g of PLP) incorporated to 1kg of basal diet be used by farmers to control undesirable breeding of Nile tilapia.

Key words: Aquaculture, Nile tilapia, prolific breeding, undesirable breeding, papaya seed, papaya leaf

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List of Acronyms

ANOVA	Analysis of Variance
DO	Dissolved oxygen
FAO	Food and Agricultural Organization of the United Nations
FCR	Food conversion ratio
FTS	Flow through system
GSI	Gonadosomatic index
PSP	Papaya Seed Powder
PLP	Papaya leaf powder
SPSS	Statistical Package for Social Science
SR	Survival rate

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1. Introduction

1.1. Background of Study

Aquaculture is the breeding, rearing, and harvesting of fish, shellfish, algae, and other organisms in all types of water environments. It is currently the fastest-growing animal production sector in the world, expanding at an average annual rate of about 10.3% since 2010 (FAO, 2016). Apart from being one of the few suitable options for feeding the growing global population with cheap and beneficial animal proteins, aquaculture is also regarded as a way of preserving wild fish stock, providing employment and is an essential component of integrated rural development (Srinath *et al.*, 2000).

In freshwater aquaculture, tilapia species are widely acknowledged as one of the most important internationally traded fish (FAO, 2013), with a significant increase in production from 383,654 metric tons (mt) in 1990 to 3,500,000 mt in 2011 (Fitzsimmons *et al*, 2012). Tilapia are currently the second most farmed fish group in the world due to many beneficial characteristics, such as; reproducing easily in captivity, rapid growth, reaching their marketable size (in about six months), tolerance of a wide range of environmental conditions, resistance to stress and disease, occupying a low trophic level, with versatile feeding habits, acceptance of artificial feed immediately after yolk-sac absorption, adaptation to a variety of culture systems and marketability: nutritious and palatable(Teichert-Codding *et al*., 1992; Altun *et al.*, 2006; Fitzsimmons *et al*., 2011; Ghosa & Chakraboty, 2014). Among tilapia aquaculture species, Nile tilapia (*Oreochromis niloticus*) has long been responsible for the significant increase in global production from freshwater aquaculture (FAO, 2002)

Regardless of the widely reported global Nile tilapia aquaculture production and progress over the years, there are several challenges commonly associated with their reproductive ability that inhibit their full aquaculture potential. The most common setback in Nile tilapia aquaculture is their precocious maturity and frequent breeding behavior (Mires, 1995).Thus various techniques to control unwanted tilapia reproduction have been developed; which includes, biological control, manual separation of sex, hormonal sex reversal, hybridization, culture of fish in cages, culturing at very high densities, sterilization and temperature induced monosex tilapia production ((Lovshin *et al*., 1990; Mair and Little, 1991; Fortes, 2005; Mair and Little, 1991).

However, all these population control methods have their own limitations. To date biological control, making appropriate ratio is required; Manual separation of sex requires skilled man power to identify male from female especially at their immature stage, hormones are currently banned, because its residue may remain in the flesh and affect human health feeding on these fish. Hybridization also leads to certain problems; some of these constraints include limited fecundity of parent fish which restrict fry production and using temperature also lacks sufficient control and complete sex reversal. Therefore, it is important that alternative methods of controlling precocious maturation and prolific breeding in tilapia culture and other aquaculture species to be developed, to ensure a more cost effective and sustainable aquaculture industry. Hence, there is a need to examine less expensive means to control undesirable tilapia recruitment in aquaculture system using natural reproduction inhibitors found in plants.

Papaya (*Carica papaya L.*) is a common edible fruit available throughout the year in the tropics. It is referred to as the "medicine tree" or "melon of health", also papaya is rich with nutrients (Jackwheeler, 2003). It contains medicinal properties and the major active ingredients recorded include, carpine, chymopapain and papain, a bactericidal glycone of glucotropaeolin, the enzyme myrosin and carpasemine (Jackwheeler, 2003). Chinoy *et al.* (1997) reported that oleic, palmitic, stearic and linoleic acids are present in the seeds.

Many researchers used papaya seed powder (PSP) and Papaya leaf powder to some extent as a natural reproductive inhibitor in Nile tilapia (Ekanem and Bassey, 2003; Ekanem and Okoronkwo, 2003; Jegede and Fagbenro, 2008; Abbas and Abbas, 2011). On the other hand, some of results showed that adding of PSP with high dose in diets caused high percentage of mortality in Nile tilapia fingerling (Ekanem and Okoronkwo, 2003; Ayotunde and Ofem, 2008; Abbas and Abbas, 2011).Additionally there were no report on using combination of PSP and PLP as antifertility effect on Nile tilapia. Therefore, the present study aims to investigate the effectiveness and optimal sterility dose level of papaya seed powder (PSP) and papaya leaf powder (PLP) on Nile tilapia.

1.2. Statement of the problem

Nile tilapia has been spread to all continents as an aquaculture species due to its desirable qualities of a culture species and hardy nature including its tolerance to a wide range of salinity (Pullin, 1994; El-Sayed, 1999). However, a major drawback is its precocious spawning that can lead to uneven harvesting sizes and high stocking densities as potentially high amount of energy, which could otherwise be used for somatic growth, be channeled for reproduction.

Male Nile tilapias grow faster than females as they have better food conversion ratio and relatively high survival (Omasaki, 2017). Female Nile tilapias reach maturity in a range of 30 to 50 g weight under aquaculture conditions. Consequently, within a few months of culture the pond gets full with small fish resulting in overpopulation, stunt growth and the income of fish farmer gets very little or no profit (Guerrero, 1982). Generally, early maturation and prolific breeding of Nile tilapia in culture systems, especially earthen ponds is a major problem in tilapia farming, which is prevalent in developing countries. Though few farmers started Nile tilapia farming in Ethiopia, they have been facing the same problem (personal communication). Therefore, the proposed study was aimed to evaluate the efficiency of Papaya seed and leaf powders in inducing sterility as one of the techniques used to control prolific breeding of Nile tilapia.

1.3. Research Questions

1. What are the proximate compositions of papaya seed and leaf powders?

2. What are the effects of papaya seed and leaf powders on Nile tilapia's gonad development?

3. What is the effective dose level of papaya seed and leaf powders in sterilizing Nile tilapia?

4. What are the side effects of papaya seed and leaf powders on Nile tilapia in terms of growth performance and survival rate?

1.4. Objectives of the Study

1.4.1. General Objective

- To evaluate the efficiency of Papaya seed and leaf powders as reproduction inhibitor for Nile tilapia culture in controlling undesirable breeding

1.4.2. Specific Objectives

- To evaluate the proximate compositions of papaya seed and leaf powders
- To compare the sterility effects of different dose levels of mixed papaya seed powder (PSP) and papaya leaf powder (PLP) on Nile tilapia in terms of gonad development
- To determine the optimal sterility dose level of papaya seed and leaf powders on Nile tilapia
- To compare the growth performance, feed conversion efficiency and survival rate of Nile tilapia exposed to varying PSP and PLP dose levels

1.5. Significance of the study

The present study will provide information on the effectiveness of combined use of PSP and PLP and optimal dose level to sterilize Nile tilapia. This study will also informs farmers and researchers about the effect of feed quality in final weight gain by comparing the growth performance of different fingerlings exposed to various dose level of mixed papaya seed powder and papaya leaf powder.

2. Literature Review

2.1. Aquaculture as an international food source

Aquaculture is the farming of aquatic animals and plant in controlled system. The farming of fish is believed to have originated in Asia, and particularly in China, as far back as 1100 BC, when common carp were raised in freshwater ponds for food. Aquaculture is internationally acknowledged as the fastest growing sector, with Asian countries alone contributing more than 90% to this production (Bondad-Reantaso *et al.,* 2005).

In the world contribution of aquaculture to human nutrition with aquaculture production of food fish estimated at 66.5 million tonnes in 2012 (Pullin & Neal, 1984; FAO, 2013). The increased demand for increased fish production through aquaculture is convincing, since the consumption of aquatic food is increasing whereas the catches from the wild stock is decreasing (De Silva, 2003). Food fish production from aquaculture increased from 59 million tonnes in 2009 to about 62.7 million tonnes (FAO) (2013).Data from the Fisheries and Aquaculture Department of FAO on the year 2011 aquaculture production shows that Asia remains the world leader in terms of total world production, producing 89% of all aquaculture products consumed.

In Sub-Saharan Africa the development of aquaculture is still lacking in meeting the increasing demands for food fish production (Brummett *et al*. 2008). Despite the slow pace of development of aquaculture in Sub-Saharan Africa, the sector have contributed through environmentally friendly and easily adaptable farming systems that enable the production of food fish by especially rural communities in Sub-Saharan Africa. In Africa, Egypt is perhaps the first country to venture into the culture of fish through using freshwater ponds for fish production activities (Bondad-Reantaso *et al*., 2005). According to the FAO (2010), Nigeria and Uganda is the second and third biggest producers, producing 16% and 7% respectively, of Africa's production. Together these three countries produce about 94% of the entire continent's production (FAO, 2014) In developing countries although, aquaculture practices are subsistence in nature and may not provide substantial employment to the crowded population, its impact in poverty alleviation cannot be ignored. Fish contributes over 25% of total animal protein intake worldwide especially in low income and developing

countries. It is a good source of vitamins especially A, D, E and B- complex vitamins and also omega-3 fatty acids (Bondad-Reantaso *et al*., 2005).

2.2. Tilapia as a food fish

Tilapia originates from Africa, and because of its easy adaptability to various environmental conditions, has been introduced to many countries. The low cost of production combined with the fact that tilapia is widely accepted by consumers as a food fish, promotes its culture worldwide (Rad *et al.*, 2006; Shalloof and Salama, 2008; El-Kashief *et al.*, 2013).

Tilapias are considered as a good source of food fish especially for the low income food deficit countries with three species in the genus Oreochromis (*O. niloticus, O. mossambicus, and O. aureus)*, two species in the genus Tilapia (T. rendalli and T. zilli), and one species in the genus Sarotheridon (*S. galilaeus*) being the most cultivated of the family (Siddiqui & Al-Harbi, 1995; El-Kashief *et al*., 2013).The genus Oreochromis exhibit maternal mouth brooding, the genus Sarotherodon is characterized according to the mouth brooding behaviour exhibited by both parents, and the Tilapia genus are classified as substrate spawners (Coward and Bromage, 2000; Specker and Kishida, 2000; Fishelson and Bresler, 2002). Members of the Tilapia family exhibit various degrees of parental care for their offspring. The genus Oreochromis orally incubates eggs and larvae, and the mouth brooding practice continues up to juvenile stage where the female at any threat or danger, takes the young into her mouth for safety (Tacon *et al*., 1996).

Oreochromis is the most diverse of the genera, and contain amongst others the species *O. niloticus, O. mossambicus,* and *O. aureus*. *O. niloticus* is one of the most important members of the tilapia species (Campos-Ramos *et al*., 2003). The species is extremely tolerant of high levels of salinity, which makes it a good candidate for culture in marine and brackish waters (Ron *et al.*, 1995; Kamal and Mair, 2005). The culture characteristics that make *Oreochromis niloticus* preferred species to farm with include their easy growth on natural grazing or formulated feeds, with no constraint for seed production, disease resistance and high consumer acceptability.

2.3. External factors affecting reproduction in tilapia

Reproductive processes such as gonadal maturation and spawning behavior are known to start in response to environmental stimuli such as temperature, photoperiod, and the amount of rainfall among other external factors. Water temperature plays a positive role in initiating gonadal maturation, egg and fry development in tilapia fish. According to Rana (1990), increasing temperatures accelerates while decreasing temperatures retard egg development. Poor environmental conditions such as unfavorable water temperature variations (Wang & Tsai, 2000) and low dissolved oxygen levels are known to cause regression of the gonads and interruption of reproductive cycles in fish (Madu, 1989). The optimum temperature for optimal growth and feed conversion ratio (FCR) for tilapia is between 26 – 30ºC (Azaza *et al.*, 2008)

Nutrition quality and quantity is essential for optimum reproductive performance in fish. A prolonged period of inadequate protein in the diet of brood fish will invariably lead to decline in the production output. The optimum level of protein for tilapia brood stock is 32% (Gunasekera *et al.,* 1995). Also the effect of dietary vitamin supplementation on the reproductive performance of tilapia has been extensively reported (Mohamed *et al.,* 2003; Alkobaby, 2008). Equally the quality and quantity of eggs spawned by brood fish are greatly improved with phosphorous and calcium in the diet (Mohamed, 2013). Lipids have also been shown to positively influence the reproductive performance of fish (Izquierdo *et al*., 2001).

The good culture potential of the tilapias as a food fish affected precocious maturation and indiscriminate breeding in mixed sex populations result in overcrowding of ponds and stunted growth of the fish (Toguyeni *et al.,* 2002).There is inverse relationship between stocking density and reproductive performance in fish. In tilapia culture a stocking density of 2 to 6 fish/ m2 is ideal for optimum performance (Bhujel, 2000) and exceeding this, will lead to a progressive decline in spawning.

2.4. Tilapia reproductive behaviors and physiology

Understanding reproductive behavior and physiology of animals is important in the development of sustainable food production, conservation of biodiversity, habitat protection, and establishing restoration initiatives. While some fishes change sex during their lifetime, tilapia species are gonochoristics, where by individuals sexually differentiate into males or females and remain the same sex throughout their life (Nakumura *et al*., 1998).There are differences and similarities in brooding behaviors of tilapia species. *Oreochromis* and *Sarotherodon* species are both mouth brooders, i.e. eggs are fertilized in the nest and then stored in their parents' mouth for incubation. Moreover, eggs and fry are held in the mouth for several days after hatching (Nandlal and Pickering, 2004). However, *Oreochromis* species possess a maternal mouth brooding nature, while *Sarotherodon* species exercise either paternal or bi-parental mouth brooding behavior (Nandlal and Pickering, 2004).

The major desirable characteristics of tilapias in various tropical and sub-tropical environments make them ideal aquaculture species. However, this judgment is challenged by their reproductive efficiency combined with precocious maturation (as early as 3 months) (Phelps and Popma, 2000). In tilapia species, the highest reproductive activity is associated with increasing photoperiod and warmer temperature, while low spawning rates are associated with lower temperatures and shorter photoperiod (Bairwa *et al*., 2013). One study concluded that a photoperiod of 12h light/dark cycle in Nile tilapia aquaculture ensured maximum fecundity, seed production, and spawning frequency (El-Sayed and Kawanna, 2007).A minimum temperature range of 20-23 ºC is reported to be suitable for breeding in most tilapia species (Bairwa *et al*., 2013). Under subtropical and temperate conditions, where temperature or photoperiods are more variable, a well-defined breeding season needs to be determined for most tilapia species (Bromage *et al*., 2001; Bairwa *et al*., 2013).

2.5. Methods to control undesirable tilapia prolific reproduction

Tilapias are one of the potential candidate species for aquaculture due to their major desirable characteristics. However, tilapias also have undesirable characteristics of precocious maturity and uncontrolled reproduction. This has necessitated the development of various methods to mitigate these behaviors. The main method used to control reproduction in tilapia is monosex culture of all male tilapias attained through manual separation of sexes, hybridization, and hormone and temperature induced sex reversal (Lovshin *et al*., 1990; Mair and Little, 1991; Fortes, 2005). Other methods include biological control and sterilization (Mair and Little, 1991).

2.5.1. Biological control

This is a means of controlling certain fish population by stocking predacious fish as fingerlings or adults in a pond. It needs an effective predatory fish that can control excessive reproduction. This method produces at least two different kinds of fish; the predatory fish and the prey species. In order to keep the predator-prey ratio at an advantageous level, there must be frequent stocking. Predators which could be used in controlling of tilapia reproduction is the African catfish (*Clarias gariepinus*). This species is known for its high growth rate, resistance to low level of dissolved oxygen (DO), poor water quality, handling stress and excellent meat quality (El-Naggar *et al*., 2006). However this technique require skilled man power in making good predator prey ratio, unless either the prey are not controlled or the predator can consume the prey beyond expected level. Then the fish culture is not sustainable.

2.5.2. Manual separation of sexes

Tilapia species possess sexual dimorphic characteristics, which make it easy to sort them into males and females. Manual separation of sexes as method of obtaining all male monosex tilapia populations is strictly based on separating males from females by visual inspection of external urogenital pores, often with the aid of dye applied (Fortes, 2005; Fuentes-Silva *et al*., 2013).The genital papilla of male is simple and smaller with two openings; the urogenital opening, where the milt and urine are excreted and the anus, for the discharge of fecal waste, whereas the female has a flatter and larger papilla with three openings; the anus, the urethra (for excretion of urine) and the oviduct, where the eggs pass through. With these methods, sex

separation is carried out before fish reach sexual maturity (Mair and Little, 1991), when they are large fingerlings (50- 80g), however the reliability of sexing depends on the skill of the workers, the species to be sorted and its size (Fortes, 2005). According to Dunham (2004), this technique is laborious and wasteful, as fingerlings must be grown to a size large enough to determine sex before manual separation and culling of the slower growing sex.

2.5.3. Hybridization

Is mating of genetically different individuals or groups, may involve crosses within a species (also known as line crossing or strain crossing) or crosses between species (Bartley *et al*., 2001). The rationale of this technique is to produce a hybrid or strain of superior quality than the parent species (Essa and Haroun, 1998). In aquaculture, hybridization is not only used to manipulate sex ratios or produce sterile fish, but also to increase growth rate, improve flesh quality, increase disease resistance, improve environmental tolerance and improve a variety of other traits to make fish production more profitable (Bartley *et al*., 2001).Despite the fact that hybridization is associated with the production of a high number of male progeny, this development is surrounded by numerous constraints, which make it unsustainable. Some of these constraints include limited fecundity of parent fish which restrict fry production, difficulty in producing sufficient number of hybrid fry due to spawning incompatibility between parent species (Mires, 1977; Varadaraj and Pandian, 1989).

2.5.4. Hormonal sex reversal

Hormonal sex reversal is the most efficient and commonly used method for mass production of all male tilapia in both small and large scale tilapia production (Pandian and Sheela, 1995; Phelps and Popma, 2000) and the success of global tilapia production is due to this technique. Tilapia larvae are believed to be sexually undifferentiated up to 2 weeks after hatching and at this time, larvae produce equal proportions of sex hormones; androgen (male), and estrogen (female) (Fuentes-Silva *et al*., 2013). Therefore, intervention or augmentation by exogenous steroid hormones such as androgen (male) or estrogen (female) during gonadal development or before sexual differentiation would influence the larvae to become either male or female depending on the hormone applied (Fortes, 2005; Fuentes-Silva *et al*., 2013). Two synthetic androgen hormones namely methyltestosterone(MT) and ethynyl testosterone have been

widely used for masculinizing genotypic female tilapia (Mair and Little, 1991; Phelps and Popma, 2000; Forbes, 2005).Production of monosex populations by direct hormonal treatment requires elucidation of the labile period of sexual differentiation during which the fish are susceptible to hormonal masculinization or feminization (Dunham, 2004).

Methods of producing a monosex (all-male) tilapia population involved technical limitations that make these methods inappropriate for small aquaculture farms. The main concerns regarding these methods (especially synthetic sex hormone application) in tilapia production include; potential health risks caused by improper implementation of this system by farm workers, detrimental impacts on the environment and social constraints (Mair and Little, 1991). To date, there is no substantial proof of any environmental damage or harm to humans caused by the synthetic hormone used for sex inversion (Mlalila *et al*., 2015), and this may lead to accumulation of this chemical in tilapia production systems. Therefore, the tilapia-farming sector is currently faced with a major challenge of finding sex control alternative methods, which are nonhazardous, cost effective, consumer and environmentally friendly.

2.5.5. Temperature induced monosex tilapia production

Studies have reported that water temperature can influence hormone biosynthesis and the gonadal sex differentiation process (Baroiller *et al*., 2009; Bairwa *et al*., 2013; Fuentes-Silva *et al*., 2013).Thus; it could provide alternative means of producing monosex fish populations in aquaculture, particularly in tilapia culture. Tilapia is a thermo-sensitive species, its male to female ratio increases with temperature and/or ovarian differentiation induced by low temperatures (Fuentes–Silva *et al*., 2013). Temperature is influential at a critical stage of sex differentiation in larval fish relatively similar to the hormone sensitive period. Inhibition of an enzyme called aromatase which catalyzes the conversion of androgen to estrogen during sex differentiation occurs at high temperatures, there by shifting larvae or fry sex ratio to male (Brodie *et al*., 1999; Baroiller and D'Cotta, 2001). Masculinization of tilapia was possible at temperatures above 32°C (Baroiller *et al.,* 1995). It has been suggested that this technique could be more effective when applied at least 10 days after fertilization.

The use of temperature to produce monosex tilapia populations may be environmentally friendly and does not pose a health hazard to humans. However, at present, this method lacks sufficient control and complete sex reversal that is required to ensure its commercial application has yet to be established (Fuentes-Silva *et al*., 2013).

2.5.6. Culture at very high densities

High density culture in ponds, cages, pens or raceways is considered a control measure for tilapia. It works on the principle that crowding reduces the urge to reproduce. When carried out in mesh cages that maintain free circulation of water, high density culture could attain significantly higher fish production. Cage culture of tilapia is considered a method of controlling tilapia population, because spawned eggs fall through the cage mesh and die. Cage culture offers several important advantages. The breeding cycle of tilapia is disrupted in cages, and therefore mixed-sex populations can be reared in cages without the problems of recruitment and stunting, which are major constraints in pond culture. In cage aquaculture, fish stocking density has great impact on growth, survival, health, water quality and production. For the maximization of monosex tilapia production, profitability and sustainability in cage culture system, it is essential to determine its optimum stocking density (Moniruzzaman *et al*., 2015)

However, it requires intensive feeding with a high quality ration, availability of good water supply, needs electric, gas or diesel aeration devices and skilled management (Fortes, 2005). Stocking density is an important parameter in fish culture operations, since it has direct effects on the growth and survival and hence on production. It is an established fact that growth rate of fishes progressively increase as the stocking densities decreases and vice-versa. This was because of relatively less number of fish in a pond of similar size could get more space, food and dissolved oxygen at the same time. To obtain maximum economic returns it would be necessary to stock the ponds at optimum stocking densities for optimum growth in relation to inputs and productivity of the water body (Hasan *et al*., 2010).Higher stocking densities usually result in higher fish yields, but individual fish growth is often sacrificed. Tilapia may require additional time to reach marketable size at high stocking densities. The longer tilapia remains in the culture pond, the greater the risk of disease and the probability that a few unwanted females will produce offspring that compete for food. Thus, the producer has to

find the economically optimum stocking rate providing highest yields with rapid fish growth to marketable size (Popma and Lovshin, 1995).

2.5.7. Periodic harvesting of fry and fingerlings

A mixed-sex population of tilapia is stocked in ponds and the normal pond culture procedures are carried out. However, instead of waiting to obtain the total yield at one harvest, collection of fry and fingerlings is done periodically every one to three weeks. One advantage of this method is that the fish are able to utilize natural foods. However, there is a need for a management scheme in order to maximize tilapia production. The extensive systems (using only organic or inorganic fertilizers) and the semi-intensive systems (using high-protein feed, aeration and water exchange).In the extensive system, the sexually mature fish are stocked in earthen ponds. Ponds are then fertilized and/or feeds are introduced to support brood fish and early growth of fry and fingerlings. Ponds are seined regularly then finally drained and harvested completely after a set period of three weeks to six months depending on desired efficiency. On the other hand, in the semi-intensive system, brood stocks are stocked into small, shallow ponds, typically 200-500 m^2 in area and 0.5-1m deep (Fortes, 2005). Brood stock replacement means all brood fish are replaced after a single spawning cycle in the cages. In a brood stock rotation strategy, brood fish are sexed after a spawning cycle and maintained separately for 10 to 14 days before rotation back to a spawning cage. The advantage of brood stock rotation is that the reproductive cycle of the brood females is more synchronized, permitting a higher percentage of females to spawn during the next cycle. Harvesting every 2 to 3 weeks without brood stock replacement, monthly production is 1 to 2 fry per g of brood female. Fry harvest per spawning cage may be double if brood fish are replaced each cycle, but this practice is considerably more labor-intensive (Popma and Lovshin, 1995).

2.5.8. Sterilization

Several plant materials had been reported to possess properties that prevent conception when administered orally amongst which are; *Carica papaya, Azadirachta indica, Psidium guajava,Curcumalonga, Gossypium herbaceium, Dioscoraesculenta, Mangifera indica* etc. most of these herbs were observed to have interfered with normal sperm production. Extract of *A. indica* leaf have been reported to cause sterility in rats (Khillare and Shrivastav, 2003), extract of *Mangifera indica* leaf is observed to reduce the number of litter in rats (Ibraheem *et al*., 2007). Extract of *Psidium guajava* leaf had also been reported to possess ant implantation substance in white mice (Retno *et al*., 2008).

Papaya (*C. papaya*) is a common human fruit; available throughout the year in the tropic. Seeds of papaya are accounted for about 16% of the fresh fruit weight (Passera and Spettoli, 1981).The seeds contain proteins, carbohydrates, fatty acids, an enzyme carpasemine, and a plant growth inhibitor caricacin (Casey, 1960). The fat content, on a dry weight basis, was 60% in papaya endosperm (Passera and Spettoli, 1981). Chinoy *et al.* (1997) reported that oleic, palmitic, stearic and linoleic acids are present in the seeds.

2.6. Antifertility effect of Pawpaw (*C. papaya*) on laboratory animals

The antifertility effect of pawpaw (*C. papaya*) seeds have been extensively studied in albino rats (Joshi and Chinoy, 1996; Udoh and Kehinde, 1999; Adebiyi *et al*., 2003; Manivannan *et al.*, 2004; Verma *et al*., 2006). Manivannan *et al.* (2004) included 10mg per day of chloroform extract of pawpaw seed in the diet of albino rats for 150 days and recorded decreased motility and sperm count. Chinoy *et al*. (1994) stated that the aqueous extract of pawpaw seed given 5mg/kg body weight per day intramuscularly and 20mg/kg body weight per day orally to male albino mice for 60 days did not have a toxic effect, and did not affect the reproductive organs and kidneys. They concluded that even though the aqueous extract is a potent male contraceptive agent, it has no harmful side effect. Lakshman and Changamma (2013) reported that pawpaw seed extract significantly decreased the cholesterol level in the testes leading to decreased steroidogenesis and ultimately decreased spermatogenesis in albino rats fed high levels of the extracts. They concluded that pawpaw seed contains oleanolic glycoside which causes sterility in male rats by inhibiting the steroidogenesis which

leads to anti spermatogenesis. Jaiswal and Singh (2008) were of the opinion that the efficacy and/or toxicity of the pawpaw seed depends on the dose and duration of the application. Agreeing on the dose dependent toxicity of pawpaw seed, Udoh and Kehinde (1999) reported that at a high dose of 100mg/kg body weight for 8 weeks, pawpaw seed induces degenerative lesions among other pathological conditions in the gonads of male albino rats while at a low dose milder effects occurred.

In a study on albino rats, Lohiya *et al*. (2005) investigated whether the ethyl acetate subfraction and methanol subfraction prepared from pawpaw seeds, differed in their ability to elicit an anti-fertility effect in albino rats. Their results indicated that the two sub-fractions were equally effective in disrupting spermatogenesis, and neither of the treatments had any toxic effect.

2.6.1. Antifertility effect of Pawpaw (*C. papaya*) on fish species

The effect of phytoestrogen exposure in fish includes impairment of the reproductive system in adults and outright sex reversal in larvae (Ampofo-Yeboah, 2013).The impairment of the reproductive activities manifests as infertility, reduced fecundity, ovo-testes in females and vitellogenin induction in males (Ribeiro *et al.*,2012).Compared to the literature on the anti-fertility effect of pawpaw seed on laboratory animals, there are relatively little information on the effect of pawpaw seed powder as part of fish diets on the reproductive potential of fish.

However, Hossam and Wafaa (2011) used 6g/kg/day to induce permanent sterility, and 3g/kg/day to induce reversible sterility in Nile tilapia (*O. niloticus*). It has been found that pawpaw seed meal fed to sexually undifferentiated fry of Mozambique tilapia (*O. mossambicus*) was able to skew the sex ratio in favour of males (Ampofo-Yeboah, 2013), however this experiment was confined to only one inclusion level (15g/kg of basal diet) of pawpaw seed meal, and therefore the optimum level could not be determined.

3. Materials and Methods

3.1. Experimental Design

3.1.1. Experimental fish and Experimental setup

The experiment was carried out at Jimma University, Department of Biology, Zoological Sciences Laboratory in a flow through system (FTS). The experiment used eight tanks each with a capacity of 40 L for three treatments and one control with their replicate (Plate 1; Table 1). Each tank was filled with a tap water and connected with aerator (Model Tetra APS 50, power 4W, frequency 50Hz). A total of 80 Nile tilapia fingerlings of mean initial body weight ranging from 20.11 ± 4.77g to 20.67 ± 6.39g and mean initial total length of 9.96 ± 0.81 cm to 10.52±1.01 cm were collected from Gilgel Gibe Reservoir. The fingerlings were collected with seine nets with the help of local fishermen. They were transported using plastic jars half filled with reservoir's water (plate 2). Upon arrival, the fingerlings were acclimatized to the water in a glass aquarium of 1.20 x 60 cm by slowly mixing water in the plastic jars holding fingerlings and water in glass aquarium in order to make temperature and other parameters in glass aquarium equivalent with those of reservoir's water. Fingerlings were fed with basal diet at 10% of their body weight during acclimatization period that lasted 17 days. After acclimatization, fingerlings of undifferentiated sex were randomly stocked with scoop net at a rate of 10 fishes/jar to both the treatment and control jars. Accumulated wastes and feed remnant were removed from each aquarium two times a day (morning and afternoon) by siphoning of 50% of the water volume per jar. Then, equal volume of water was replaced. Diet was provided to all fish at 10% of their body weight twice a day, i.e. morning at 9:00 and afternoon at 15:00 local time throughout the experimental period. The experiment was carried out for 90 days.

Plate 1: The experimental setup in the laboratory

(a)

(b)

(c)

(d)

(e)

(f)

Plate 2: showing fingerling collection, transportation and transferring to acclimatizing glass aquarium.

Table 1: Details of the Experimental Design

Treatments	**Detail**
C (2x)	0 g mixture of PLP and PSP /kg of basal diet
T1 (2x)	1.5g (75 %) mixture of PLP and 0.5g (25 %) PSP /kg of basal diet
T2 (2x)	0.5g (25%) mixture of PLP and 1.5g (75%) PSP /kg of basal diet
T3 (2x)	1 g (50 %) mixture of PLP and 1g (50 %) PSP /kg of basal diet

3.2. Preparation of Papaya seed and leave powder

Ripe fruits of papaya were procured from local market in Jimma Town. Fresh seeds were collected from the fruits and rinsed in water to remove the attached membrane of fruits. Whereas, papaya leaf were collected from Jimma University compound. Both papaya seeds and leaf were spread on newspaper and sun dried. The dried seeds and leaf were ground into powdery form using a laboratory grinder (blender 800ES, model BB90E), packed in polyethene bag and stored in a cool dry place until later use (plate 3).

Plate 3: Papaya seed powder (PSP) and Papaya leaf powder (PLP)

Similar procedure were followed for the collection of papaya seeds and leaf for proximate analysis of the treatment feed (papaya seeds and leaf).The proximate composition of both the seed and leaf powders were analyzed following analytical protocols of Association of Official Analytical Chemists (AOAC, 1990).

Moisture content was measured by taking the weight of fresh sample of papaya seed and leave and exposed to sun dried until the sample attained constant weight. The weight loss from the sample was considered as the moisture content and the remaining weight as dry matter. The moisture % was calculated as follow:

$$\textbf{Moisture content (\%)} = \frac{WF-WD}{WF} * 100$$

Where, WF = Weight of fresh sample, WD = Weight of dry sample

Ash content was measured by placing 3 g powder of dried PSP and PLP in an empty pre-weighed crucible and placed in a muffle furnace of 550 °C for 6 hrs. Then furnace was turned off to cool to 250 °C before sample removal. Sample was placed in desiccators and cooled prior to weighing. The ash content was calculated as follows:

$$\textbf{Ash (\%)} = \frac{\mathbf{WA}}{\mathbf{WS}} * \mathbf{100}$$ where, WA- weight of ash; WS- weight of sample

Protein content of a sample of PSP and PLP was determined using Kjeldhal method (AOAC, 2005). Crude fat was determined by subjecting the samples to a continuous extraction with petroleum ether method using soxhlet apparatus as described by AOAC (1990).

$$\textbf{Crude fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} * 100$$

Estimate of carbohydrate was made by calculating the difference as:

CHO (%) = 100 – (% crude protein +% crude fat + % ash + % moisture)

3. 3. Basal feed processing and formulation

The basal diet consisted of fish meal, wheat bran and barley bran. The wheat and barley brans were prepared from flour residues obtained from open market. Fish meal was prepared by collecting whole body of Nile tilapia from fish production marketing enterprise found at Gilgel Gibe reservoir. The collected whole bodies of Nile tilapia were cooked for 1 hour in order to allow the release of water, avoid pathogenic microorganism and excess oil from protein. The heated product was exposed to sun drying for 3-4 days. The dried fish meal was ground to powder and packed in polyethene bag.

The basal diet was formulated based on the preferable percentage of crude protein requirement to fish. The percentage of crude protein in formulated feeds that leads to better growth performance of Nile tilapia is 35% (Admasu *et al*., 2017). Therefore food ingredient including fish meal, wheat bran and barley bran were prepared and mixed in proportion that they form 35% crude protein. The proportions of the experimental feed ingredients in the formulated feeds were determined using the Pearson square method (box method); the most commonly used methods for balancing crude protein levels. The crude protein of fish meal prepared from Nile tilapia is 50 % (Degebassa *et al.*,2004) and wheat and barley brans contain 13% and 10% (Admasu *et al*., 2017), respectively.

To formulate 35% crude protein using Pearson square method, first combination for wheat and barley bran was carried out by taking the average of their crude protein. The average crude protein of wheat and barley brans was 11.5% CP. Thus, fish feed having 35% crude protein was formed from fish meal having 50% CP and combination of wheat and barley bran having 11.5% crude protein. Percent proportion (%) of each feed ingredient and percent contribution of each ingredient to crude protein (35%CP) were prepared in Table: 2

Table 2: Proportion (%) and percent contribution of feed ingredient to crude protein(35%)

Ingredients	**CP**	**Proportions (%)**	**Contribution to CP (35%)**
Fish meal	50	61	30.5
Wheat bran	13	19.48	2.53
Barley bran	10	19.48	1.95
Total		**100%**	**34.98**

Then, the prepared basal diet was hand mixed with water in order to mix the entire contents of ingredients , exposed to sun drying, grinded and packed in polyethene bag until later use (Plate 4).

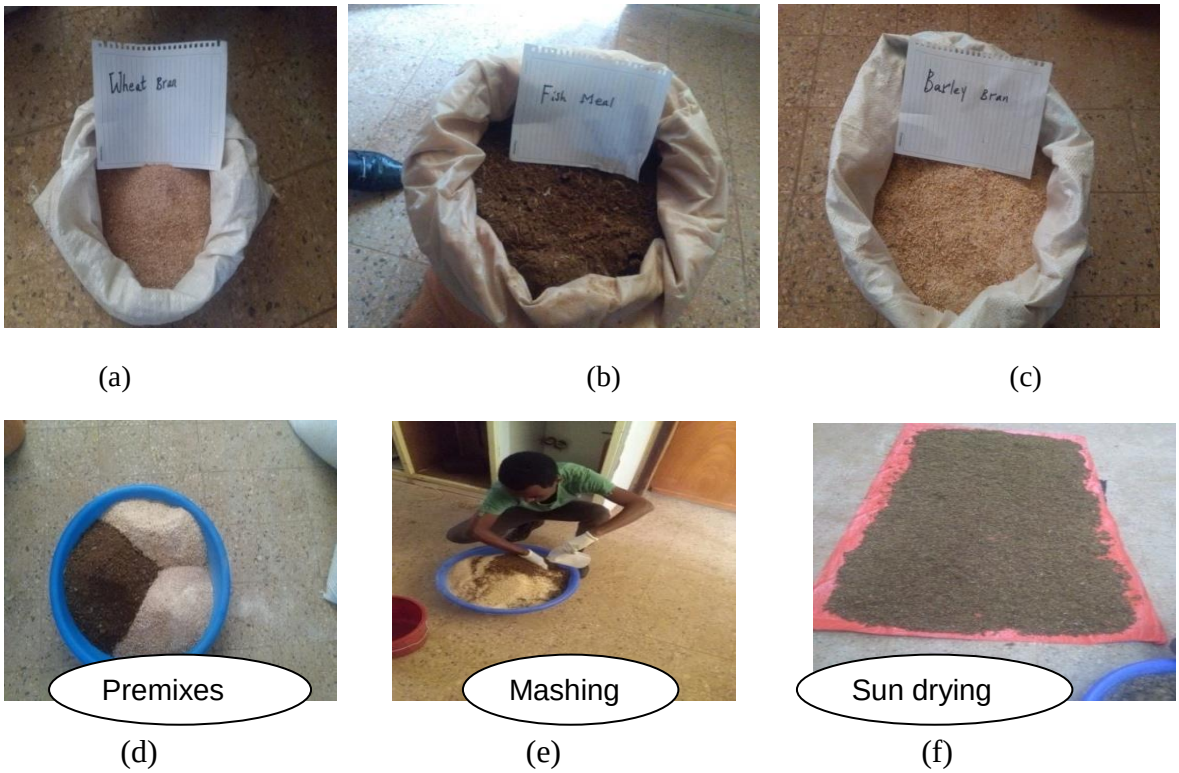

Plate 4: The process of feed formulation from local ingredients

3.4. Data Collection

3.4.1. Growth performance, Feed utilization and Survival rates

Initial length, weight and number of stocked fish were recorded for each of the eight tanks to evaluate the fish growth performance, feed utilization and survival rate under different experimental setups (Pechsiri *et al*., 2005; Hassanin, 2009; Salem, 2010). The size measurements were also used for adjusting the quantity of feed provided to the fishes. Total length (TL) and weights (W) were measured using measuring board and electronic weighing balance, respectively, every two weeks. Growth performance was evaluated using the relative growth rate (RGR) as:

- Mean relative growth rate (RGR, %/day) = (Wf-Wi)/Wi*100, where, Wf = mean final weight, Wi = mean initial weight.

The RGR sets growth in relation to the initial size. It is also a reasonable way for growth comparison studies, e.g. when different individuals of the same initial size are studied with different treatments (Lugert *et al*., 2014).

- Feed conversion rate (FCR) was computed using the equation: FCR = Feed weight, kg/ Weight gain, kg
- Survival rate (SR) was computed as: (SR, %) = Final number of fish/Number of fish stocked *100

3.4.2. Measurement of reproductive parameters

At the end of 90 days experimental period, all fish from each jar were taken out with scoop net and anesthetized in diluted formalin one by one. Then, following weight and length measurements fish specimens were dissected to determine maturity stage of the gonads and gonad weight. Any effects of the treatments on gonad i.e. physical deviation from the normal gonad (gonad of fish in control tanks) was visually observed and gonad maturity stage was determined following standard protocol (Oldorf *et al*, 1989). Moreover, gonadosomatic index (GSI) was calculated as follows: $\mathbf{GSI\ (\%) = \frac{Gonad\ weight}{Body\ weight} * 100}$

3.5. Physicochemical parameters

Water quality was monitored during the experiment. Water temperature, dissolved oxygen, pH and conductivity of each jar's water were measured once per week throughout experimental period using a relevant multiprobe meter (code-HQd40). As large number of helminthic parasites were observed during fish dissection, the number and weight of the parasites were also recorded.

3.6. Statistical analysis

Growth performance (weight and length gain), proximate composition of papaya leaf and seed, gonadosomatic parameters (gonad weight) and water physicochemical parameters were expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was used to determine if there was a significant difference among and between the groups at the significance level of 0.05%. All the tests were performed using the statistical package for social sciences (SPSS) computer software (version 23).

4. Results

4.1. Proximate composition of papaya seed and leaf

The percentage of moisture content, crude protein, crude fat, ash content and carbohydrate of papaya seed and papaya leaf were analyzed and presented in Table 3. The result shows that papaya leaf has higher moisture content, crude protein and carbohydrate than papaya seed, where as papaya seed has higher crude fat and ash than papaya leaf.

Table 3: The mean and standard deviation (M ± SD) of proximate composition of papaya seed and papaya leaf (n = 2).

Samples	**Papaya Seed Powder (%)**	**Papaya Leaf Powder (%)**
Moisture content	11.01±0.15	20.16±0.66
Crude protein	24.01±0.19	25.92±0.74
Crude fat	24.03±0.80	5.14±0.23
Ash	8.29 ± 0.97	7.92±0.16
CHO	33.180 ± 0.70	40.45 ± 0.80

4.2. Growth performance

The mean initial and final weight and length and relative growth rate of the fish specimens in the different setups are summarized in Table 4 while, trend of weight gain over time is provided in Fig.1. The mean initial length and weight of experimental fish ranged from 9.96 ± 0.81 to 10.52±1.01 cm and 20.11 ± 4.77 to 20.67 ± 6.39 g, respectively. However, there was no significant statistical difference in the mean initial length and weight among the treatments and control groups ($p > 0.05$) (Appendix 2A). Similarly, there was no significant difference in the final weight of the fish ($p > 0.05$) (Appendix 2B).

Table 4: Final mean lengths, mean weights and relative growth rate (RGR) of Nile tilapia during experimental period; T = treatment

Groups	Parameter	Sampling weeks						
		W_o	**W2**	**W4**	**W6**	**W8**	**W10**	**W12**
Control	W (fish, g) ± SD	20.11 ± 4.77	22.31±6.99	23.61± 7.16	25.32± 6.46	26.03± 6.52	26.37±8.37	27.92±6.87
	RGR (%/day)		10.93	5.82	7.24	2.80	1.30	5.87
	TL (cm)	10.04 ± 1.04	10.50±1.09	10.62± 1.21	10.76 ±1.10	10.86 ±0.99	11.25± 1.21	11.40±1.02
	W (feed, kg)	0.60	0.028	0.032	0.025	0.049	0.027	0.028
	FCR		1.29	1.40	1.02	1.90	1.05	1.04
T1	W (fish, g) ± SD	20.21±4.82	22.35±7.12	23.1±7.12	24.22± 4.51	25.79± 5.05	26.69±6.02	27.43±6.27
	RGR (%/day)		10.58	3.35	4.84	6.48	3.49	2.77
	TL (cm)	9.96±0.81	10.27±1.07	10.43± 1.06	10.55 ±0.86	10.55 ±0.86	10.99± 0.98	11.37±0.87
	W (feed, kg)	0.60	0.027	0.023	0.024	0.031	0.043	0.027
	FCR		1.25	1.00	0.94	1.27	1.67	1.01
T2	W (fish, g) ± SD	20.21±4.82	21.1±4.75	22.01± 5.61	22.62± 4.90	22.92± 4.90	22.98±6.21	25.34±5.67
	RGR (%/day)		4.40	4.31	2.77	1.33	0.26	10.27
	TL (cm)	9.96±0.81	10.08±0.78	10.24± 1.02	10.27 ±1.01	10.27 ±1.01	10.64± 0.97	11.10±1.26
	W (feed, kg)	0.60	0.035	0.021	0.02	0.027	0.04	0.03
	FCR		1.67	0.99	0.95	1.23	1.83	1.22
T3	W (fish, g) ± SD	20.67±6.39	22.68 ±7.94	23.07± 6.93	24.36± 6.43	25.04± 6.92	25.1±8.41	27.05±9.28
	RGR (%/day)		9.72	1.71	5.59	2.79	0.23	7.77
	TL (cm)	10.52±1.01	10.48±0.90	10.70± 0.90	10.73 ±0.83	10.73 ±0.83	10.98± 1.17	11.20±1.14
	W (feed, kg)	0.62	0.022	0.029	0.032	0.022	0.04	0.31
	FCR		1.02	1.29	1.36	0.91	1.80	1.18

Relative growth rate (RGR), Total length (TL), Food conversion rate (FCR)

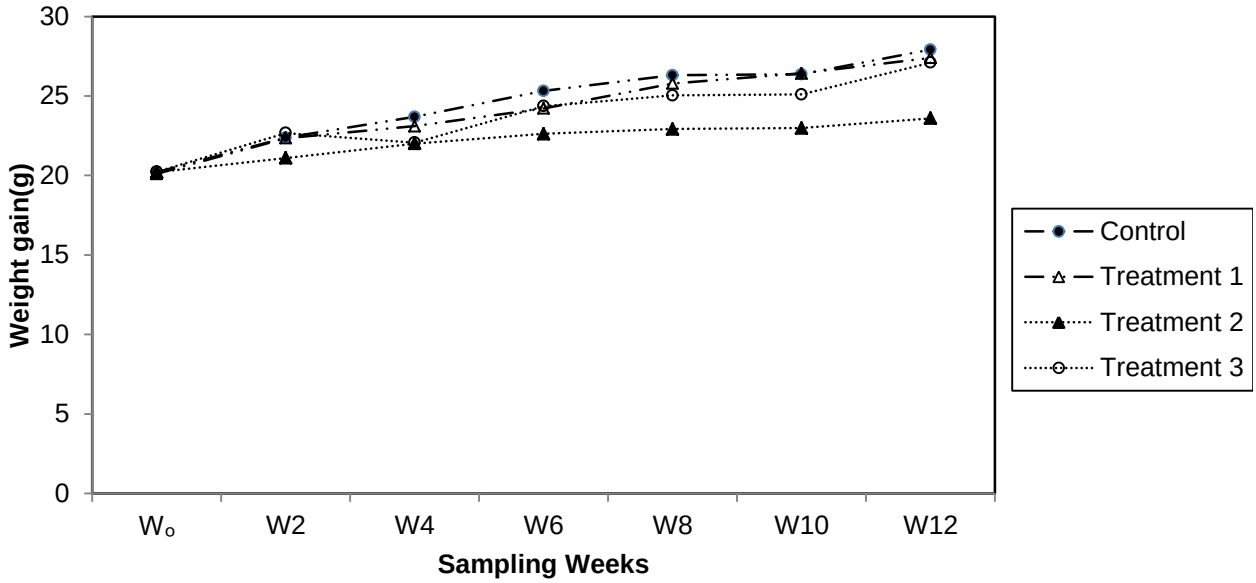

Figure 1: Growth trend (Weight) measured during sampling weeks of the experimental period

4.3. Feed conversion rate

The results of food intake and feed conversion rate (FCR) are shown in Table 4. The FCR values ranged between 0.94 and 1.67 for T1, 0.99 and 1.83 for T2 and 0.91 and 1.80 for T3 and 1.02 and 1.90 for control.

4.4. Survival rate

The survival rate for experimental fish ranged from 40% to 55%. These are 55%, 50%, 45% and 40% for T3, T1, Control and T2 respectively (Table 5). Maximum death (high mortality) was registered in week two in all treatments and control group.

Table 5: Survival rate of experimental fish throughout the experimental period.

Groups.	Number of stocked fish	Final number of fish	Survival rate (%)
Control	20	9	45
T1	20	10	50
T2	20	8	40
T3	20	11	55

4.5. Reproductive parameters

Data for mean gonad weight, gonadosomatic index (GSI) and gonad developmental stage for Nile tilapia exposed to different inclusion levels of mixed PSP and PLP are presented in Table 6. The highest GSI for males (0.79 ± 0.38) was recorded for the control group, followed by T1 (0.25 ± 0.18) and T2 (0.20 ± 0.10), while the least values (0.11 ± 0.10) was recorded in T3 that contained equal proportions of PSP and PLP. For the females, the highest GSI (0.84 ± 0.80) was also recorded in control group, followed by T2 (0.83 ± 0.28) and T1 (0.66 ± 0.02), while the least values (0.43 ± 0.54) was recorded in T3 (that contained equal proportion of PSP and PLP). However, there was no significant difference in GSI of both males and females between treatments and control groups ($P>0.05$) (Appendix 3B).The effect size between control and treatment group of GSI for male was 0.69, whereas the effect size of GSI for female was 0.12 (Appendix 4B).

The highest gonad weight for males (0.22 ± 0.16 g) was recorded for the control group followed by T1(0.08 ± 0.06g) and T2(0.04 ± 0.01g),while the least values (0.03 ± 0.02 g) was recorded in T3.For the females, the highest gonad weight (0.27 ± 0.25g) was also recorded in control group followed by T2 (0.19 ± 0.02g) and T1 (0.18 ± 0.08), while the least values (0.17 ± 0.21) were recorded in T3.However, there was no significant difference in gonad weight of both males and females between treatments and control groups ($P>0.05$) (Appendix -3A). The effect size calculated as partial eta squared between control and treatment groups for gonad weight of male was 0.51, whereas the effect size of gonad weight for female was 0.08 (Appendix- 4A).

Percentage gonad weight of male's fish in T1, T2 and T3 were 36%, 18% and 13% respectively of control groups, whereas percentage gonad weight of female's fish in T1, T2 and T3 were 66%, 70% and 62% respectively of control groups. Percentage GSI of male fish in T1, T2 and T3 were 31%, 25% and 13% respectively of GSI of male fish in control groups, whereas percentage GSI of females fish in T1, T2 and T3 were 78%, 98% and 51% respectively of GSI of female's fish in control groups. Fishes in the control group had gonad maturity stage ranging II to IV while development of fish gonads in the treatment groups was delayed as the gonads appeared physically retarded in development (plate 5)

(C) (T1)

(T2) (T3)

Plate 5: showing gonads of fish from control and treatment groups

Additionally, the physico-chemical parameters of water in the experimental jars measured during the experimental period are provided in (Appendix 1).

Table 6: Mean ± *S*D reproductive parameters of Nile tilapia fed different inclusion level of mixed PSP and PLP after a 90-days experiment. SD = Standard deviation.

Groups	**Gonad weight**		**Gonadosomatic index**		**Gonad developmental stage**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Control	0.22 ± 0.16	0.27 ± 0.25	0.79 ± 0.38	0.84 ± 0.80	II,III	III, IV
T1	0.08 ± 0.06	0.18 ± 0.08	0.25 ± 0.18	0.66 ± 0.02	-	-
T2	0.04 ± 0.01	0.19 ± 0.02	0.20 ± 0.10	0.83 ± 0.28	-	-
T3	0.03± 0.02	0.17 ± 0.21	0.11 ± 0.10	0.43 ± 0.54	-	-

4.6. Parasite load

At the end of experimental period the fish in all treatments were dissected and the number of parasites counted and its weight recorded. All harvested fish from each experimental tank were infected with parasite. The number and weight of parasite were recorded from all fish harvested from each experimental tank and prepared in (Table 7).

Table 7: The number, weight and percentage of parasite recorded from harvested fish in each experimental tank.

Parasite group		No. of parasite	Parasites weight(g)
Nematodes	Control	83	0.64 ± 0.47
	T1	142	0.82 ± 0.20
Trematodes	T2	35	0.56 ± 0.21
	T3	107	0.71 ± 0.23
	Total	**367**	

5. Discussions

The proximate composition of papaya seeds and leaf used in the present study were as shown in Table 3. In present study the moisture content of seed was (11.01±0.15%) while that of leaf was (20.16±0.66%) indicating high moisture content found in leaf. The moisture content of PSP was in agreement with the work of (Oke and Afolabi (1986) who reported (10.81± 0.12%) moisture content of papaya seed.

The protein content for both papaya seeds and leaf was 24.01±0.19 % and 25.92±0.74% respectively indicating a higher protein presence in papaya leaf. Protein is an essential component of diet needed for the survival of both animal and human of which basic function is to supply adequate amount required (Pugalenthi *et al*, 2014). The protein content of leaf was higher than (24.34±0.09 %) reported by (Makanjule, 2018). However protein contents of PSP were in agreement with (24.34±0.09 %) in similar work.

According to (Bello, 2008), ash of food samples gives an idea of the organic content from where the mineral contents could be obtained. Papaya seed exhibited high amount of ash (8.29 ± 0.97 %) than that of leaf (7.92±0.16 %).The ash content of papaya seed obtained in this work is similar with (8.27 ± 0.01%) reported by Abdulkarim and Ghazali (2005). The ash content of papaya leaf obtained in this work is lower than that of (18.3± 0.26 %) reported by Rita and Mithu (2016).

Fat contents of (24.03±0.80 %) and (5.14±0.23%) were obtained for the seeds and leaf indicating papaya seeds as a good source of fat which can serve as energy source. The fat content of papaya seed obtained in this work is higher than that of reported by Karune and Vijaya (2014) (2.57±0.06 %) and fat content of papaya leaf is in agreement with (5.57±0.49 %) in similar work.

The carbohydrate contents of both the seeds and leaf were (33.18 ± 0.70%) and (40.45 ± 0.80%) respectively. The value of carbohydrate of papaya seed obtained in this study is in agreement with (30.51%) reported by (puangsri, 2005) and higher than (15.5±0.03%) reported by (Maisarah, 2014). The value of carbohydrate of papaya leaf obtained in present study is lower than that of (63.5±0.09%) reported by Ritha and Mithu (2016) and in agreement with

(38.4 ±0.04%) reported by (Maisarah, 2014). Higher carbohydrate content of papaya leaf suggesting that papaya leaf can be considered as a potential of carbohydrate for energy.

In present study a maximum mean final weight (27.92±6.87g) was recorded in control group and minimum mean final weight (25.34±5.67g) recorded in T2. The morphometric characteristics of the control and papaya seed powder and papaya leaf powder exposed Nile tilapia obtained from the present study indicated that there was no significant difference ($P > 0.05$) in the total length and weight among all the treatment and control groups. The insignificant difference in weight of experimental fish in both control and papaya treated fish may be due to uniform infestation of parasite that hinders all fish from growing more and also may be due to small sample size. According to Stevens (1996), when the sample size is large (e.g. 100 or more subjects), 'power is not an issue'. However, when study conducted with small sample size (e.g. n=20), it needs to be aware of the possibility that a non-significant result may be due to insufficient power. Therefore the insignificant difference in weight and length between treatment and control may be due to small sample size used in present study. A mean weight of the different treatment groups calculated during sampling occasion of between fifteen days throughout experimental period as shown in table 4 indicates a uniform increases in the weight of the treated fish and the control group. This is in contrary to the work of Ekanem and Okoronkwo (2003) who reported decrease in weight of *O. niloticus* fed 9.8 g of PSM/kg of standard tilapia diet.

Feed conversion ratio is a valuable and powerful tool in aquaculture that allows fish farmers or nutritionist to make wise choices in selecting or estimating the amount of feed that will be required in the growing cycles (Anderson and Silva, 2003) and aids them in using of feed efficiently to maximize profitability. A low feed conversion ratio is a good indication of high quality feed and it means that fish utilized the feed better. FAO (2015) reported that the optimum feed conversion ratio level for tilapia species is 1.5. De Long *et al.* (2009) stated that the feed conversion ratio for Nile tilapia generally ranges from 1.4 – 1.8. In this study the food conversion ratio ranged between 1.02 to 1.90, 0.94 to 1.67, 0.99 to 1.83 and 0.91 to 1.80 in Control, T1, T2 and T3 respectively. Therefore the FCR in all treatment and control are in line with (FAO, 2015) and found in acceptable range.

Maximum survival rate was recorded in T3 (55%) and minimum was recorded in T2 (40%). One stressor influencing fish health is parasites and temperature. All experimental fish were infected with nematode parasite that locates between fish's gill and liver. This result demonstrated that the mortalities of experimental fish may be due to parasite load those cause disease. Although, the temperature recorded in present study was at acceptable level it was not at optimum level, so this may be one of a factor that increase mortality of fish used in present study. High mortality of fish was observed in T2 followed by control, T1 and T3.

Gonad weight and gonadosomatic index (GSI) are indicators of gonad maturation in fish. GSI represents the relationship between the gonad weight and body weight (Horstegen-Schwark and Langholz, 1998) and it is an important tool in science as it is used to indicate the gonadal maturation in fish (Omeje, 2016).

For males maximum mean GSI (0.79 ± 0.38) was recorded in control group and minimum value (0.11 ± 0.10) was recorded in T3. For females maximum mean GSI (0.84 ± 0.80) was recorded in control groups and minimum value (0.43 ± 0.36) was recorded in T3. Maximum GSI recorded in control group shows that as gonad weight to body weight ratio of fish was high as compared to GSI of fish in treatment groups. Minimum value recorded in T3 (treated with mixture of 50% PSP and 50 PLP) shows that as gonad of fish treated with equal proportion of PSP and PLP for both male and female were greatly retarded. However there was no significant difference in GSI between control and treatment groups ($P>0.05$). The effect size calculated as partial eta squared between control and treatment groups for GSI of male was 0.69, whereas the effect size of gonad weight for female was 0.12. Based on guidelines developed by Cohen (1988), effect size (0.69) of GSI of males between treatment groups and control is large. This indicates that even though there was no significant difference observed papaya meal induce large effect on gonads of male fish in treatment group as compared to control groups. Similarly the effect size (0.12) of GSI for females indicates treatment feed induce small effect. The present result shows that mean gonad weight of both male and female decreased in papaya meal treated groups.

For males maximum mean gonad weight (0.22 ± 0.16) was recorded in control group and minimum value (0.03 ± 0.02) was recorded in T3. For females maximum mean gonad weight (0.27 ± 0.25) was recorded in control groups and minimum value (0.17 ± 0.14) was recorded in T3. Maximum gonad weight recorded in control group shows that as gonads of fish develop in well manner and filled with mass of egg and milt as compared to papaya treated fish. Minimum value recorded in T3 (treated with mixture of 50% PSP and 50 PLP) shows that as gonad of fish treated with equal proportion of PSP and PLP for both male and female were greatly retarded as compared to other treatments and control groups. However there was no significant difference in mean gonad weight between control and treatment groups ($P>0.05$).

Percentage gonad weight was calculated by considering the gonad of fish in control groups as well developed and gonad of fish in all treatments as retarded because of treatment feed. Then the mean gonad weight of fish in control group used as total and percentage of gonad weight of fish in treatment groups were calculated from gonad weight of fish in control group. In present study percentage gonad weight of male's fish in T1, T2 and T3 were 36%, 18% and 13% respectively of control groups. This means that gonad of male fish in T1,T2 and T3 grow only up to 36%,18% and 13% of gonad weight of male fish in control groups. whereas percentage gonad weight of female's fish in T1, T2 and T3 were 66%, 70% and 62% respectively of female's fish in control group. This shows that gonad of female's fish in T1, T2, T3 grow only 66%, 70% and 62% respectively of females fish in control group. Percentage GSI of male fish in T1, T2 and T3 were 31%, 25% and 13% respectively of GSI of male fish in control groups. This indicates that gonad weight to body weight ratio of male fish in T1, T2 and T3 were 31%, 25% and 13% respectively of GSI of male fish in control group. Whereas percentage GSI of female's fish in T1, T2 and T3 were 78%, 98% and 51% respectively of GSI of female's fish in control groups. This also shows that gonad weight to body weight ratio of female's fish in T1, T2 and T3 were 78%, 98% and 51% respectively of GSI of female's fish in control group.

6. Conclusions and Recommendations

6.1. Conclusion

In present study growth performance of both control group and fish treated with different dose of the mixture of PSP and PLP shows no significant difference. Even though there was no significant difference between treatment groups in gonad weight and GSI, maximum mean gonad weight and GSI was observed in control group. From treatment groups those treated with different inclusion level of PSP and PLP mixture, minimum Gonad weight and GSI was recorded in T3(1g of PSP and 1g of PLP) per 1kg of basal diet for both male and female. The present study shows that PSP and PLP could be used as antifertility agent than growth promoting agent. In general it could be concluded that 1g of PSP and 1g of PLP incorporated to 1kg of basal diet is optimum dose to control undesirable breeding. The experimental fish was infected with nematode parasite and other trematodes those disturbs the well being of Nile tilapia.

6.2. Recommendation

- Future study

- Would focus on bioaccumulation of both PSP and PLP content in order to determine the effects of phytochemicals on the liver and other organs architectural integrity.
- Need to isolate active ingredient from papaya and determine which one is responsible for promoting sterility in Nile tilapia rather than using all powder.
- Fingerling used in present study was collected from Gilgel Gibe reserviour and infected with parasite that hinders their growth. So future study need to be conducted on the treatment of Nile tilapia in Gilgel Gibe reservior infected with this mass of nematode parasite

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Appendixes

Appendix 1. Physicochemical parameters of water in fish tank

Physicochemical parameters of water in fish tank were recorded once per week throughout experimental period and prepared in Table 8. Temperature in all treatment tanks ranged in value from 19.88 ± 0.50 recorded in treatment two to 19.94 ± 0.50 recorded in treatment one. Dissolved oxygen in treatment tanks ranged in value from 3.08 ± 1.53 recorded in treatment one to 3.97 ± 1.46 recorded in treatment three. pH in treatment tanks ranged in value from 6.38 ± 0.07 recorded in control tank to 6.49 ± 0.09 recorded in treatment three. Electric conductivity in treatment tanks ranged in value from 129.618 ±18.64 recorded in treatment three to 134.98 ±18.63 recorded in treatment one.

Table 8: The mean and standard deviation (M ± SD) of physicochemical parameters

Treatments	**Parameters**			
	Tº(ºC)	**DO(mg/l)**	**pH**	**EC(µs/cm)**
Control	19.92 ± 0.52	3.42 ± 1.37	6.38 ± 0.07	130.80 ±19.75
T1	19.94 ± 0.50	3.08 ± 1.53	6.46 ±0.11	134.98 ±18.63
T2	19.88 ± 0.50	3.74 ± 1.61	6.47 ± 0.09	129.69 ± 23.56
T3	19.89 ± 0.48	3.97 ± 1.46	6.49 ± 0.09	129.618 ±18.64

Appendix 2A: One way ANOVA output of initial weight and length of fishes in control group and treatment groups.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
weight	Between Groups	3.746	3	1.249	.045	.987
	Within Groups	2096.464	76	27.585		
	Total	2100.210	79			
length	Between Groups	4.336	3	1.445	1.679	.179
	Within Groups	65.417	76	.861		
	Total	69.754	79			

Appendix 2B: One way ANOVA output of final weight and length of fishes in control group and treatment groups.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Weight	Between Groups	32.221	3	10.740	.198	.897
	Within Groups	1517.832	28	54.208		
	Total	1550.053	31			
Length	Between Groups	.389	3	.130	.119	.948
	Within Groups	30.561	28	1.091		
	Total	30.950	31			

Appendix 3A: One way ANOVA output of gonad weight of fishes in control group and treatment groups by sex.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Male	Between Groups	.061	3	.020	1.769	.269
	Within Groups	.058	5	.012		
	Total	.119	8			
Female	Between Groups	.017	3	.006	.151	.925
	Within Groups	.184	5	.037		
	Total	.200	8			

Appendix 3B: One way ANOVA output of GSI of fishes in control group and treatment groups by sex

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
male	Between Groups	.746	3	.249	3.738	.095
	Within Groups	.332	5	.066		
	Total	1.078	8			
female	Between Groups	.240	3	.080	.240	.865
	Within Groups	1.668	5	.334		
	Total	1.908	8			

Appendix 4A-One way ANOVA output of gonad weight showing effect size between control and treatment groups by sex.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Male	.061[a]	3	.020	1.769	.269	.515
	Female	.017[b]	3	.006	.151	.925	.083
Intercept	Male	.082	1	.082	7.124	.044	.588
	Female	.371	1	.371	10.113	.025	.669
Groups	Male	.061	3	.020	1.769	.269	.515
	Female	.017	3	.006	.151	.925	.083
Error	Male	.058	5	.012			
	Female	.184	5	.037			
Total	Male	.230	9				
	Female	.612	9				
Corrected Total	Male	.119	8				
	Female	.200	8				

a. R Squared = .515 (Adjusted R Squared = .224)
b. R Squared = .083 (Adjusted R Squared = -.467)

Appendix 4B: One way ANOVA output of GSI showing effect size between control and treatment groups by sex.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	Male	.746[a]	3	.249	3.738	.095	.692
	Female	.240[b]	3	.080	.240	.865	.126
Intercept	male	.986	1	.986	14.826	.012	.748
	female	4.211	1	4.211	12.623	.016	.716
group	male	.746	3	.249	3.738	.095	.692
	female	.240	3	.080	.240	.865	.126
Error	male	.332	5	.066			
	female	1.668	5	.334			
Total	male	2.421	9				
	female	6.460	9				
Corrected Total	male	1.078	8				
	female	1.908	8				

a. R Squared = .692 (Adjusted R Squared = .507)
b. R Squared = .126 (Adjusted R Squared = -.399)