BACTERIOLOGICAL AND PHYSIOCHEMICAL PROPERTIES OF POULTRY FEEDS SOLD IN BENIN CITY

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UNIVERSITY OF BENIN

BENIN CITY

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CERTIFICATION

This is to certify that this project work was carried out by Ruth Richard ELEGBOGUN **(Miss)** in the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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MRS. A.S. OGBEBOR **DATE**

(Project Supervisor)

APPROVAL

This project work was carried out by Ruth Richard ELEGBOGUN (Miss) in partial fulfillment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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PROF. S.E. OMONIGHO

(Head of Department**)** **DATE**

DEDICATION

This project work is dedicated to God Almighty for his love, mercies, empowerment and enablement at every give up point in life and also to Mr. and Mrs. ELEGBOGUN for their love and utmost support.

ACKNOWLEDGEMENT

I want to first appreciate God for His mercies and grace that has brought me thus far; for helping me realize many of my dreams within this great citadel of learning. To him be all praise.

To my supervisor, MRS. A.S. OGBEBOR, for her patience, guidance, advice and corrections I say thank you. May the Almighty continue to pave way for you and your family. To all my lectures in the Department of Microbiology I say a huge thank you.

My sincere and undiluted appreciation goes to my amiable parents, Mr. and Mrs. ELEGBOGUN, for their undying love and believe in me. I pray God gives them the grace to enjoy the fruit of their labour. To my siblings Juliet, Evelyn, Franklin, Theresa, and Daniel I love you all. To my little mummy Miss Juliet Elegbogun for her continuous encouragement and support.

I am forever grateful for the friends that stood by through thick and thin Tobin, Success, Theophilus and John for their love, assistance and care.

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ABSTRACT

Despite the presence of different variety of chemical fungicides, the search for new antifungal substances against plant pathogens continues because of the negative effect these fungicides has on both the plants and the environment. This study was aimed at assessing the antifungal activities of *Moringa oleifera* and *Olea europaea* oil individually and their synergistic

combination against selected phytopathogenic fungi. Two fungi were isolated from agricultural soils obtained from different locations. Antifungal activities of *Moringa*, Olive oil individually and synergistically were performed using the food poisoning method. *Penicillium chrysogenum* and *Mucor circinelloides* used in this study were isolated from the agricultural soil. The phytochemical analysis of *Moringa* oil revealed a cocktail of phytochemicals while Olive oil showed only the presence of terpenoids. From the results, *Moringa* oil alone demonstrated strong activity against the pathogens with radial growth inhibition ranging from 2.83±0.04mm (*Penicillium chrysogenum*, 14%) to 5.83±0.10mm (*Mucor circinelloides,* 6%) this was significantly different from the controls (17.66±0.33mm for *Penicillium chrysogenum* and 39.16±0.05mm for *Mucor circinelloides*). For Olive oil alone the radial growth inhibition ranged from 4.90±0.05mm (*Penicillium chrysogenum*, 14%) to 24±0.30mm (*Mucor circinelloides,* 2%) this was significantly different from the controls (17.66±0.33mm for Penicillium *chrysogenum* and 39.16±0.05mm for *Mucor circinelloides*). The synergistic combination of *Moringa* oil and Olive oil gave a result ranging from 0.00±0.00mm (*Mucor circinelloides,* 14%) to 12.33±0.14mm (*Penicillium chrysogenum*, 14%) compared to their controls (17.66±0.33mm for *Penicillium chrysogenum* and 39.16±0.05mm for *Mucor circinelloides*). The highest percentage mycelial radial growth inhibiton for *Moringa* oil on day 7 was 89.79% (*Mucor circinelloides)* and the lowest was 71.70% (*Penicillium chrysogenum)*. The highest percentage mycelial radial growth inhibiton for Olive oil on day 7 was 82.55% (*Mucor circinelloides)* and the lowest was 16.98% (*Penicillium chrysogenum)*. The percentage mycelial radial growth inhibiton for the synergistic combination on day 7 gave a highest value of 100% (*Mucor circinelloides)* and the lowest was 30.19% for (*Penicillium chrysogenum)*. The results of this study reveal that *Moringa oleifera* and *Olea europaea* oil significantly reduced the mycelial radial growth of the tested

pathogens individually and synergistically. Further studies should however be conducted to ascertain the effectiveness of these natural fungicides *in vivo.*

CHAPTER 1

Poultry the domesticated of superorder Galloanserae (fowl), are domesticated birds kept by humans for their eggs, their meat or their feathers. These birds are most typically members of the superorder Galloanserae (fowl), especially the order Galliformes (which includes chickens, quails, and turkeys). The term also includes birds that are killed for their meat, such as the young of pigeons (known as squabs) but does not include similar wild birds hunted for sport or food known as game. The word "poultry" comes from the French/Norman word poule, itself derived from the Latin word pullus, which means small animal. The domestication of poultry took place around 5,400 years ago in Southeast Asia. This may have originally been as a result of people hatching and rearing young birds from eggs collected from the wild, but later involved keeping the birds permanently in captivity. Poultry provides nutritionally beneficial food containing high-quality protein accompanied by a low proportion of fat.

Poultry feed is food for farm poultry, including chickens, ducks, geese and other domestic birds. Feeds for poultry production are composed largely of grains such as corn, wheat or barley, oil seeds, cake meal (originating mainly from oil producing seeds such as soybeans), sunflower seeds, peanuts, cotton seed and protein products of animal origin such as fish meal, meat and bone meal, slaughter house offal's and feather meals (Bale et al., 2002). Since these feeds are expected to be the sole sources of nutrition of the birds, they usually contain essential mineral and vitamin additives (Dhand et al., 1998). However, there are variations in nutrient requirements for different farm animals, but the level of dietary energy and associated nutrient should be high enough to allow expression of animal potentials under certain environmental

circumstances within the economic limitations (Wilson, 1990). According to Cevger and Yalcin (2003), poultry feeds are essential source of energy needed to generate heat and to support the chemical reactions in which all physiological processes depended. Many of these reactions are catalysed by vitamins or some inorganic elements, hence must be provided in the diet (Uwaezuoke et al., 2000). In addition, is water, since virtually all cell mediated reactions take place in an aqueous medium.

Poultry feed is considered as one of the important sources of contamination to poultry as they are routinely subject to contamination from diverse sources including environmental pollution, activities of insects and microbes. Specifically some of the additives have been incriminated amongst the principal sources of bacteria of public health concern. Standard poultry feeds possess considerable percentage of ash, crude fat, crude fibre protein, moisture and carbohydrate contents which are vital for the poultry animal's health and development, these nutrients may as a matter of fact form nutrients for utilization by contaminating microorganisms. Both pre-harvest and post-harvest biological contaminants can be transmitted through feed ingredients to the mixed feed and finally to poultry animals. Different types of farm animal diseases such as dysentery, fowl cholera, samonellosis, staphylococcosis, colibacillosis, erysipelas, listeriosis have been associated with poultry feed. Microorganisms that can contaminate poultry feeds includes Escherichia coli, Staphylococcus aureus, Salmonella spp, Listeria spp, Streptococcus spp, Klebsiella spp, Pseudomonas spp, Aspergillus niger, Aspergillus flavus, Rhizopus spp, Penicillium spp, Fusarium spp. However, the number and types of microorganisms in poultry feeds vary depending on the function of materials, location of its origin, climate conditions encountered, harvesting, processing, storage, transport technologies employed and packaging

materials. Effects of microorganisms in poultry feeds may include degradation of nutrient value, change in smell and colour, caking of the feed and production of toxins, example of these toxins include mycotoxin which differ in their toxicological effect and are usually found in mixed form.

The occurrence of mycotoxigenic fungi is widespread in tropical countries due to favourable environmental conditions. Unsafe poultry feed may also lead to great economic losses in case of destroying an infected flock of birds. As it has become a popular industry for small scale holders and this has contributed sparingly to the economy of these countries. The quality of poultry feed is of public health importance because it affects the quality of poultry and the wholesomeness of meat consumed by man. Safety of feed is a fundamental requirement for all birds. Considering the health hazard, posed to poultry birds and the unsuspecting consumers of such contaminated feeds and its overwhelming socioeconomic impact.

1.1 AIMS AND OBJECTIVES

The aim of this research work was to evaluate bacteriological and physiochemical qualities of poultry feeds sold in Benin city.

The specific objectives were to:

1. Isolate, enumerate and identify bacteria from poultry feed.
2. Determine physiochemical properties of the feed samples (titratable acidity, pH and moisture content).

CHAPTER TWO

LITERATURE REVIEW

2.1 poultry terms and definition

Adulterant: any substance which if it is included in a poultry feed is likely to be deleterious to poultry when fed in proportions commonly used or as specified in the feeding instructions

Apparent metabolisable energy (ME): gross energy of the feed consumed minus the gross energy contained in the excreta. A correction for nitrogen retained in the body is usually

applied to yield a nitrogen-corrected ME, a MEn value. Typical dietary energy values are expressed in kcal MEn /kg diet

Breeder: female bird which is reared primarily for its fertile eggs

Broiler: bird which is reared for its meat

broiler breeder grower ration: poultry feed fed to breeder pullets 3 weeks to 23 weeks of age

Broiler breeder ration: poultry feed fed to breeder pullets over 23 weeks of age

Broiler breeder starter ration: poultry feed fed to breeder pullets 0 weeks to 3 weeks of age

Broiler finisher ration: poultry feed fed to broilers 6 week to 8 weeks of age

Broiler grower ration: poultry feed fed to broilers 4 and 5 weeks of age

Broiler ration: poultry feed fed to broilers from a day old to the time slaughter

Broiler starter ration: poultry feed fed to broilers 0 weeks to 3 weeks of age

Crumbles: feed particles which can be individually picked up and swallowed whole by poultry. Crumbles may be broken pellets.

Duck breeder developer: poultry feed typically fed to ducks until they commence to lay eggs

Duck breeder layer: poultry feed typically fed to ducks after the commencement of egg production

Duck grower ration: poultry feed typically fed to ducks 2 week to 7 weeks of age duck starter poultry feed typically fed to ducks 0 weeks to 2 weeks of age

feed additive: any intentionally added ingredient not normally consumed as feed by itself, whether or not it has a nutritional value, which affects the characteristics of feed or animal products

Feed ingredient: component part or constituent of any combination or mixture making up a feed, whether or not it has a nutritional value in the animal's diet, including feed additives. Ingredients are of plant, animal or aquatic origin, or other organic or inorganic substances.

Label: any mark, stamp, ticket or tag applied to, affixed to, printed on, accompanying, sold with or referring to poultry feed or any package or container containing poultry feed

Layer: bird that is reared principally for the laying of table eggs

Layer ration: poultry feed fed to layers over 20 weeks of age

medicated feed: any feed which contains veterinary drugs as defined in the CODEX Alimentarius Commission

Poultry: any domesticated bird including, but not limited to, chickens, turkeys, ducks geese, guinea-fowls and pigeons any single or multiple material whether processed, semi-processed or raw, which is intended to be fed directly to poultry animals

Pullet: young female bird which will later develop into a layer

pullet developer ration: poultry feed fed to pullets 14 weeks to 20 weeks of age

pullet grower ration: poultry feed fed to replacement pullets 6 weeks to 14 weeks of age

Pulet starter ration: poultry feed fed to replacement pullets 0 week to 6 weeks of age turkey

breeder ration poultry feed fed to turkeys over 20 weeks of age

Turkey grower ration: poultry feed fed to growing turkeys, 8 weeks to 20 weeks of age

Turkey pre-starter ration: poultry feed fed to turkey poults, 0 weeks to 4 weeks of age

turkey starter ration poultry feed fed to turkey poults, 4 weeks to 8 weeks of age

Undesirable substances: contaminants and other substances, which are present in and or on feed and feed ingredients and which constitute a risk to the health of consumers, including food safety related poultry health issues.

2.2 Poultry Feed Ingredients: Feed ingredients are broadly classified into cereal grains, protein meals, fats and oils, minerals, feed additives, and miscellaneous raw materials, such as roots and tubers. These will be discussed in separate headings below. More information on measuring the nutrient composition of ingredients and the process of formulating poultry feeds is available in the section on feed formulation.

2.2.1 Cereal Grains

The term "cereal gains" here includes cereal grains, cereal by-products and distillers dry grains with solubles (DDGS). Cereal grains are used mainly to satisfy the energy requirement of poultry. The dominant feed grain is corn, although different grains are used in various countries and regions of the world. For instance, in the US, Brazil and most Asian countries corn is by far the most important energy source for all poultry feed, whereas wheat is the predominant supplier of dietary energy for poultry diets in Europe, Canada, Australia, New Zealand and the Russian Federation. Of course, in reality, a feed manufacturer will use any grain in a poultry diet if it is available at a reasonable price. For instance, in some parts of the US and China wheat is often used in place of corn if its price

is below that of corn. In Australia, sorghum is a key grain during the summer season instead of wheat, while in the Scandinavian countries barley and rye are used when these grains are at the right price. Although the amounts and types of cereal grains included in poultry diets will depend largely on their current costs relative to their nutritive values, care must be taken to avoid making large changes to the cereal component of diets as sudden changes can cause digestive upsets that may reduce productivity and predispose the birds to disease. The quality of cereal grains will also depend on seasonal and storage conditions. Poor growing or storage conditions can lead to grains with a lower than expected energy content or contamination with mycotoxins or toxin-producing organisms such as fungi and ergots. Genetic and environmental factors also affect not only the content of nutrients in grains but also the nutritive value, which takes into account the digestibility of nutrients contained in an ingredient in the target animal. In addition to the cereals themselves, their by-products, such as wheat bran, rice bran and DDGS, are used widely in poultry feed. Cereal by-products are typically high in fibre, or non-starch polysaccharides (NSP), which are poorly utilised in poultry and are low in ME.

2.2.2 Protein Meals

Protein is provided from both vegetable and animal sources, such as oilseed meals, legumes and abattoir and fish processing by-products.

2.2.3 Vegetable Protein Sources

Vegetable protein sources usually come as meal or cake, the by-product of oilseed crops. The main oilseed crops include soybean, rapeseed/canola, sunflower, palm kernel, copra, linseed peanut and sesame seed. After the oil is extracted, the remaining residue is used as

feed ingredient. Oilseed meals make up 20-30% of a poultry diet. Inclusion levels do vary among formulations for different species and for the same species in different regions. The main vegetable protein sources used in Australian poultry diets are soybean and canola. Other sources like cottonseed, sunflower, peas and lupins may be included in poultry feed formulations if these are available at a reasonable price. Many oilseeds and legumes contain anti-nutritive factors. Some of these anti-nutritive factors can be destroyed by heat and are used in heat-treated meals. New cultivars of some oilseeds and legumes have been developed that are naturally low in anti-nutritive factors (ANF), permitting higher levels of the unprocessed grains to be included in poultry diets without ill-effect.

2.2.4 Animal Protein Source

The main animal protein sources used in poultry diets are meat meal, meat and bone meal, fish meal, poultry by-product meal, blood meal and feather meal. Although the production of animal protein for human consumption has been under continual pressure and marred by much controversy, the world-wide and domestic consumption of animal protein continues to grow and much of the future supply of meat protein will come from poultry. With increased animal protein production there will be increased demand for feed and, in particular, a demand for ingredients high in protein and energy. The animal industry evolved as a means of adding value (i.e. higher nutrient level and availability, flavour, variety, etc.) to ingredients that were of marginal food value for humans. These ingredients include grains that are of poor quality or damaged by harvest or storage conditions; as well as a means of recycling by-products of brewing, vegetable oil, meat, milk and egg production. Approximately 50% of the live market weight of ruminants and 30% of poultry is by-product. These by-products are rendered, ground and available as a feed

source. Animal protein meals are usually defined by inputs. Those specifically used in poultry diets include meat (no bone) or meat and bone meal from ruminants and/or swine; blood meal; poultry by-product meal; feather meal; and fish meal. There are specific limitations now assigned to these products with regards to inputs used and guarantees with respect to minimum nutrient levels. For example, meat and bone meal may be specifically from ruminants and must be free of hair, wool and hide trimmings, except where it is naturally adhering to heads and hoofs. The products are rendered, which is a biosecure process that evaporates water, extracts fat and yields a finished ground product high in protein (which has no resemblance to the raw product) and minerals. The products are marketed with guarantees as to minimum protein, phosphorus and calcium levels. There are some challenges associated with the use of animal protein sources. First, food safety is the most important concern people have about the recycling of animal protein meals back through animals as feed ingredients. This is based on the links between the prion disease bovine spongiform encephalopathy (BSE – mad cow disease) and a variant Creutzfeldt-Jakob disease in humans. Importantly for poultry production though, researchers have been unable to demonstrate the transfer of prions to poultry (Moore J et.al. (2011) BMC Res Notes. Vol.4, p.501) and no symptoms of disease have been observed in birds up to five years after direct challenges. The proteins (prions) associated with BSE are not destroyed by traditional methods of rendering and are capable of causing disease when BSE contaminated meat and bone meals are injected cerebrally into ruminants. As a consequence of the public's concerns about BSE, Australia does not allow the use of ruminant by-products in feed for ruminants; however, ruminant by-products are available for use in poultry feed. In addition to BSE contamination, there are concerns that animal

protein meals are responsible for food borne pathogen contamination, such as Salmonella. Typically these bacteria are destroyed by rendering and possible recontamination is often negated by pelleting of manufactured feeds. In most cases, if poultry acquire Salmonella it is likely to be from an environmental source other than feed. It is possible for animal protein meals to be contaminated with high levels of heavy metals, dioxins and PCBs (pesticides); however, meals are monitored and regulated to minimise this contamination. Animal protein meals have a long history in poultry nutrition. The utilisation of this valuable feed ingredient is important in minimising loss (nutrient and economic value) in the production of safe, high-quality poultry meat, eggs and bioproducts.

2.2.5 Fats And Oils

Fats and oils, collectedly termed lipids, are regularly used in poultry feed to satisfy the energy need of the animal as lipids have more than twice the amount of ME compared with carbohydrates or proteins per kg weight. Lipids are also an important carrier for fat-soluble vitamins (A, D, E, and K) as wells for the provision of an essential fatty acid, linoleic acid, in the diet. A variety of fats and oils are used in feed, including lipids of animal origins (usually fats, i.e., tallow, lard, except fish oil) and lipids of vegetable origin (usually oils, i.e., soy oil, canola/rapeseed oil, sunflower oil, linseed oil, palm oil, cottonseed oil). In practical feed formulation, the level of lipids rarely exceeds 4% in compound feed. However, even a small decrease in digestibility can cost dearly in terms of dietary energy. Like any other nutrient, a varying proportion of lipids are undigested depending on their sources and the species and age of the animal to which they are fed. It is surprising that nearly a quarter of dietary lipids are lost in the excreta of chickens. The significance of this can be seen from the fact that even with a seemingly small amount of inclusion, say 2.5%

added fat in feed, it contributes as much as 7-9% of the dietary energy of a typical poultry diet. Thus, any improvement in digestibility, which may be achieved via the use of appropriate additives, such as enzymes, acidifiers and emulsifiers, will have a significant impact on the energy content of diets.

2.2.6 Minerals And Vitamins

Minerals are vital for normal growth and development in poultry, such as bone formation and body processes such as enzyme activation. Some minerals such calcium and phosphorus are required in large quantities. For example, laying hens require between 3.5-4% calcium, 0.3-0.4% available phosphorus and 0.2% sodium in their diets for egg production. Other minerals, such as copper, iron, manganese, zinc, selenium, cobalt, iodine and molybdenum, are required in milligram quantities but deficiency of these minerals will lead to serious health problems in mild cases and death in severe cases. Similarly, vitamins are essential for the body systems of poultry. Both fat soluble (A, D, E, K) and water soluble (biotin, choline, folic acid, niacin, riboflavin, thiamine, pyridoxine, pantothenic acid and B12) are needed in the diet to maintain proper health and wellbeing of poultry. Some vitamins and minerals are provided by most ingredients but the requirements for vitamins and minerals are generally met through premixes added to the diet. Diets may also contain additives for specific purposes.

2.3 Feed Additives

The diet of animals and humans contain a wide variety of additives. However, in poultry diets, these additives are primarily included to improve the efficiency of the bird's growth and/or laying capacity, prevent disease and improve feed utilisation. Any additives used in

feed must be approved for use and then used as directed with respect to inclusion levels and duration of feeding. They are also specific for the type and age of birds being fed. These guidelines are maintained by a government committee (Product Safety and Integrity; Australian Government Department of Agriculture, Fisheries and Forestry). Common feed additives used in poultry diets include antimicrobials, antioxidants, emulsifiers, binders, pH control agents and enzymes. Sometimes diets will also contain other additives used in diets for humans and pets such as flavour enhancers, artificial and nutritive sweeteners, colours, lubricants, etc. Within each one of these classes of additives, there can be dozens of specific additives manufactured and distributed by a wide variety of companies. Again, all ingredients and additives must be noted on the label and their use and inclusion levels meet the standards as defined by law. In some instances, additives are added to the animal's diet in order to enhance their value for human consumption, but mostly this is accomplished by use of natural ingredients containing significantly higher levels of these nutrients that can be deposited directly into meat and eggs. This fact sheet will highlight a few important feed additives and their use in the poultry industry

2.3.1Growth Promotion Additives

Growth promoting hormones are not used in the poultry industry. The efficient growth and egg productivity of commercial poultry has been achieved over the last 50 years through traditional animal breeding techniques (genetic selection – not genetic engineering) and improved nutrition and management (including health and housing) practices.

2.3.2 Antimicrobials

Antimicrobials have been used extensively in intensive poultry operations to minimise disease and improve growth and feed utilisation. However, the industry is currently evaluating alternatives to chemical therapeutics. It should be pointed out that antimicrobial practices do not extend to the production of commercial eggs (should a need for antimicrobials arise all eggs laid during the treatment and withdrawal period cannot be sold) and the meat industry must adhere to stringent guidelines with regard to drug withdrawal periods before marketing. There is much controversy in regard to the impact of antimicrobials in animal diets on the development of resistant strains of microbes that could directly impact human health and carry over into meat and bioproducts as well as the negative impacts associated with their excretion into the environment. The European Union has moved towards a complete ban of in-feed antimicrobials for these reasons since 2006. Development of alternatives to the present in-feed antimicrobials is an exciting area of current research worldwide. In all cases, it will be necessary to minimise disease challenges, strengthen the bird's natural defences (immune response, gut barrier/health) and optimise the diet to provide a balance of required nutrients for the bird's changing needs. All of these may be influenced by using feed additives. Alternatives to in-feed antibiotics mainly include acidifier, probiotics, prebiotics, herbal products, immune-modulators and also feed enzymes.

2.3.4 Feed enzymes

Enzymes are proteins that facilitate specific chemical reactions. After completion of the reaction, the enzyme disassociates and becomes available to assist in further reactions. Although animals and their associated gut microflora produce numerous enzymes, they are not necessarily able to produce sufficient quantities of specific enzymes or produce them at the right locations to facilitate absorption of all components in normal feedstuffs or to reduce anti-nutritional factors in feed that limit digestion. Some cereal grains (rye, barley, wheat, sorghum) have soluble long chains of sugar units (referred to as soluble non-starch polysaccharides – NSP) that can entrap large amounts of water during digestion and form very viscous (thick gel-like) gut contents. Enzymes that are harvested from microbial fermentation and added to feeds can break these bonds between sugar units of NSP and significantly reduce the gut content viscosity. Lower viscosity results in improved digestion as there is more interaction of the digestive enzymes with feeds and therefore more complete digestion; improved absorption as there is better contact between the digested feed nutrients and the absorptive surface of the gut; and improved health as the moisture and nutrient levels in the manure are reduced which reduces the nutrients available for harmful gut microflora to proliferate and challenge the birds (e.g. necrotic enteritis, a chronic intestinal disease caused by Clostridium perfringens, resulting in reduced performance, mortality and the main reason we currently use in-feed antimicrobials).

Commercial enzymes are also produced that significantly reduce the negative effects of phytates. Phytates are plant storage sources of phosphorus that also bind other minerals, amino acids (proteins) and energy and reduce their availability to the bird. Ongoing research will develop enzymes that are more effective in maintaining function under a

wider range of processing and digestive conditions. New enzymes may include those capable of reducing toxins produced during feed spoilage (mould growth in grains) and facilitating digestion of carbohydrates currently not available to simple-stomached animals (poultry, pigs, humans) such as cellulose, lignin and chitin. New feed additives are rapidly adopted by the poultry industry and have facilitated the development of significant new technology to advance the use and availability of in-feed enzymes.

2.3.5 Antioxidants

There are a variety of sources of reactive oxygen species (free radicals) in normal metabolism as well as those coming directly from feed ingredients. Oxidative stress can disrupt normal cellular function, damage tissues (also associated with the development of cancers) and reduce health status. Antioxidants bind these molecules and reduce their potential damage.

2.3.6 Acidifiers

Feed acidifiers are added to the feed to lower the pH of the feed and consequently the gut environment. A lower pH has the potential to inhibit or partly restrict the growth of pathogenic intestinal microbes. Acidifiers exist both as organic or inorganic acids or associated salts. They can exert their antimicrobial action both in the feed and throughout the gut. Health and performance promoting effects have been shown for a number of organic acids such as formic, fumaric, citric, propionic and lactic acids.However, the overall benefits of organic acids greatly depend upon the form of administered organic acid (protected or unprotected), uncontrolled variables such as buffering capacity of ingredients,

presence of any other microbial agent, cleanliness of production environment, and heterogeneity of microbes.

2.3.7 Probiotics

Probiotics are defined as live mono or mixed culture of microorganisms which are non-pathogenic, resistant to gastric and bile acids, and when ingested can beneficially affect the host animal by improving the characteristics of intestinal microbiota. The main proposed modes of action of probiotics include 1) antagonistic action towards pathogenic bacteria by secretion of products which inhibit their development, such as bacteriocins, organic acids and hydrogen peroxide; 2) competitive exclusion which represents competition for locations to adhere to the intestinal mucous membranes and in this way pathogenic micro-organisms are prevented from inhabiting the digestive tract; 3) competition for nutritious substances. Probiotics have also been reported to exhibit immunomodulatory properties mostly through manipulation of gut microbiota composition and consequently affecting both innate and adaptive immunity. In this way, they create conditions in the intestine which favour useful bacteria and inhibit the development of pathogenic bacteria.

2.3.8 Prebiotics

Prebiotics are defined as indigestible food ingredients which stimulate the growth or activity of a selected number of bacteria in the gastrointestinal tract of the host animal. When entering the gut, prebiotics serve as a substrate for the endogenous beneficial bacteria thus can promote competitive exclusion of pathogenic microbes and selective colonization by beneficial microbes. Among the known prebiotics, mannan-oligosaccharide (MOS) fructo-oligosaccharide (FOS) and galacto-oligosaccharide (GOS) have extensively

been tested in poultry. Prebiotics proposed mechanism of actions include: 1) lowering gastrointestinal tract pH through lactic acid production; 2) inhibiting the colonization of pathogens and 3) producing a systemic effect on stimulation of immune responses.

2.4 Feed evaluation

is the testing of feed quality, providing information on the composition of feed or feed ingredients as well as their suitability for poultry. Poultry feed is made up of many ingredients, which are broadly grouped into providers of energy (fats, oils and carbohydrates), protein (amino acids), vitamins, minerals and product quality enhancement. Typically, cereals such as wheat, barley, sorghum and maize will provide energy while soybeans, lupins, canola and peanuts provide protein. These ingredients are then combined in such a way as to provide the energy, protein, vitamin and mineral requirements for poultry through the process of feed formulation. In order to know what amounts of these ingredients should be included in the diet, the ingredients are first evaluated, to see what nutrients they contain in what quantities. After the diet has been prepared, it may also be necessary to evaluate the complete product, to determine its suitability for the class of poultry that will be fed (such as egg layers, meat chickens or breeders). Feed evaluation is a key process in the poultry industry. Feed ingredients need to be tested in order to formulate the complete diet, and diets have to be evaluated to determine their suitability for poultry. Evaluation provides different types of information, as required by nutritionists and farmers. In general, the range of tests that can now be performed is wide and it is now possible to obtain results rapidly.

2.4.1 Measures Of Feed Quality

Feeds and feed ingredients can be evaluated physically as well as chemically. The physical evaluation of feed mostly provides preliminary information on the quality of the material. It involves assessing physical qualities such as weight, colour, smell and whether the material has suffered from any contamination by other materials. Chemically, feed is made up of water and dry matter. The dry matter contains organic and inorganic compounds. The organic part of feed is made of mainly carbohydrates, proteins, vitamins and fats and oils. The inorganic part is made of mineral elements, also known as ash. Feed or feed ingredients can be analysed to provide values of each of these components. Apart from obtaining values of chemical composition, the extent of utilisation of these components by the bird, termed nutritive value, is also measured.

2.4.2 How Feed Quality Is Measured

Feed quality is measured by chemically breaking up the food into the components mentioned above. In the industry, it is sometimes necessary to break down these large components into smaller analytical fractions. Thus, values of starch and the non-starch component (called fibre) of carbohydrates may be provided. Proteins are made of amino acids, 10 of which must be present in poultry diets, so their amounts should be indicated during feed evaluation. In the past, feed evaluation was a cumbersome process, requiring days to complete. However, newer equipment and procedures have been developed, which enable the rapid evaluation of most materials. For example, starch is determined using a ready-to-use kit and protein is rapidly determined on Leco® machines, which eliminate time-consuming digestion of feed with strong acids and reaction of material with acids and bases. Near-infrared reflectance spectroscopy (NIRS) is one of the latest techniques by which feed ingredients can be evaluated with the most minimal preparation of the sample.

NIRS provides the capability to rapidly measuring crude protein, fibre, fat, total and digestible amino acids, calcium, total and available phosphorus and also the energy value (ME) of individual ingredients. Of greater importance in feed evaluation is the response of poultry to particular feeds. This is regarded as the real nutritive value of the feed and must be measured as part of feed evaluation. Nutritive value does not necessarily entail animal growth or egg production. It gives information on how much of each of the fractions in feed, i.e. starch or energy, protein (amino acids), fats, minerals or vitamins, was used by the bird. When feed is given to poultry, they are able to break down only a fraction of the feed and absorb it into the body for growth and egg production. The rest is voided in faeces and urine, which are excreted together by poultry. The amount of nutrients retained by the bird is an indication of the nutritive value of the feed.

2.4.3 Importance Of Feed Evaluation

Feed evaluation is important because ingredients that belong to the same class contain different nutrients; for example, maize provides more energy than wheat while soybeans contain more proteins than lupins and canola. The same ingredient varies from one supplier to the other, and between years. In drought years, cereals fill poorly and are therefore lower in quality. Most importantly, if feeds are not evaluated, it is not possible to tell if the material will be suitable for feeding poultry. Feeding standards have already been set for different types of poultry, so the requirements for different nutrients must be met precisely. It is possible, with the current state of knowledge, to predict poultry growth or egg production by modelling feed quality, type of housing, class of poultry and duration of feeding. The central key issue in these models is feed quality, which can only be obtained through feed evaluation.

2.5 Microbial Evaluation of Poultry feed

2.5.1 Bacteriological quality of poultry feed

Most times poultry birds get infected through consumption of contaminated feeds, making the quality and safety of poultry feeds important part of poultry farming. All samples analyzed in this study showed the presence of microorganisms, which is an indication that poultry feeds serve as good growth medium for microorganisms owing to the nutritional quality. The result of this study may suggest that both bacteria and fungi might be implicated in health problems on the farm. D Mello attributed presence of microorganisms in poultry feeds to climatic conditions, harvesting of raw materials, feed formulation process, storage and transport technologies employed. Most of the microorganisms isolated in this study have been associated with diseases of the poultry farm. Salmonellosis is caused by bacterium of the genera Salmonella, this infection is common in two weeks old chicks and ducklings, Salmonella gastroenteritis of human have been associated with consumption of infected birds, hence the infection of birds with Salmonella has been attributed to contaminated feeds. Isolation of E. coli a coliform is an indication of feacal material contamination which can be associated with poor hygiene. Bacillus spp and Staphylococcus aureus have been implicated by the studies of Dhand et al. in the poultry farming microbial disease outbreak., Staphylococcus aureus was the most prevalent which is in agreement with the result of Arotupin et al. and Mahmudullah et al. The presence of Lactobacillus spp can be attributed to normal flora of raw material especially of plants origin, it cannot be said to be as a result of deliberate incorporation of lactic acid bacteria as probiotics since it

was not isolated from all the feed samples. Dhand et al. reported the beneficial effect of lactic acid bacteria on poultry feed.

2.5.2 Fungal quality of poultry feed

The isolation of fungi genera (Aspergillus, Mucor, Rhizopus and Penicillium) which could be mycotoxigenic from the poultry feeds can be linked to cereal raw materials used in feed formulation, mycotoxins are economically important toxins which are immunosuppressive and can result to low poultry production. Aspergillus spp can also cause aspergillosis in birds the presence of Aspergillus spp in food should be of a concern, Dhand et al. reported it to be leading in respect to mycotoxin production in poultry feeds. Rhizopus spp and Mucor spp were the predominant fungi in this study which might cause deterioration of the feeds ingredients making less nutrients available for the birds. Also some species of Rhizopus are mycotoxigenic. More so the use of agro wastes such as fish waste, cassava flour, bone meal, millet, lysine, maize, wheat offal, oyster shell, fish meal, groundnut cake, palm kernel cake, soya bean cake, brewery waste.

2.6 Storage of poultry feed

Proper poultry feed storage is essential to ensuring top quality for your poultry feed. It helps protect them from the pests, critters and the mold that may damage the feed, ruin its nutritional integrity or infect it with dangerous toxins that could be potentially harmful to birds and humans. Poor quality poultry feed will lead to a host of problems including poor growth, malnutrition, high rate of mortality as well as a host of health problems. All of these will have an impact on your poultry farm profitability. Here are some useful factors to keep in mind when it comes to poultry feed storage

2.6.1 Consider the Distance of the Silo from the Poultry House

The poultry storage facility should be kept as close to the poultry house as possible for practical reasons. Some poultry farms build the storage area for poultry feeds inside the poultry house. In many poultry farms, however, the feed silos are placed right next to the poultry house to provide for maximum convenience.

2.6.2 The Storage Facility

The poultry feed storage facility should meet certain minimum requirements. It should provide shelter from direct sunshine; must be cool and well ventilated; should be able to keep the feed dry and ensure low humidity; must protect the poultry feed from vermin such as mice and rats and insect pests; must keep the poultry feed off the ground to protect it from mold spoilage and ground condensation and finally, it should protect the poultry feed from drugs and chemicals.

2.6.3 The Storage Conditions

Poor storage conditions for your poultry feeds will reduce their shelf life and may lead to the loss of important nutrients like the anti-oxidants, some essential fatty acids along with vitamins. This will in turn hinder the growth of your poultry and lead to higher mortalities in your flock. If poultry feed is stored in wet area, it will get moldy and stale and will pose a serious health risk for your birds. The worst are the molds that grow quickly on the poultry feed and produce mycotoxins. This mostly occurs during warm and humid weather. The use of moldy poultry feeds inevitably leads to illnesses, performance losses as well as possible mass mortality in your flock. It is hard to estimate the shelf life of poultry feed in storage facilities, even under the best of conditions. The longevity will depend on the

conditions in the storage as well as the composition of the poultry feed. The local climate will also play a key role in determining the life-span of your poultry feed. This is especially so when it comes to the temperature and the relative humidity of the local area and the housing. Even with an excellent food storage system, food should only be stored for a short duration of time. The ideal timeframe should be two months after the date of manufacture. By reducing the storage time, you can ensure a faster turnover thereby cutting down on the inventory costs while giving your flock fresh and high quality poultry feed.

2.6.4 The temperature

When storing the poultry feed, you should be wary of the exposure to the sunshine. Radiation from the sun can degrade the quality of the feed through the effect of the greenhouse gases. Overheating may also negatively impact the nutritional content of the food leading to the breaking down and degradation of the proteins or the fats being rancid. Use housing material such as iron sheets that will reflect back most of the heat and radiation thus helping preserve a cool and dry environment inside the poultry feed storage area. In very hot climates, it is advisable to use insulated bins for poultry feed storage so as to help keep cool and stable temperatures inside the poultry feed storage bin. Another advantage of insulated bins is that they can help reduce or entirely prevent condensation at night when the temperatures fall. Condensation creates humid conditions and is likely to lead to the growth of mold on top of the layers of poultry feed

2.7 Poultry waste disposal methods

The disposal of poultry carcasses is a serious trouble for poultry industry, as it has environmental, biological, and financial concern (Cai et al., 1994). The carcasses may not

be disposed of by dumping on any public road or right-of-way left where they may be consumed by animals (Olexa and Goldfarb,2008). Worldwide, there are several ways of disposing of poultry waste including; burial, rendering, incineration, compositing, feed for livestock, fertilizer or source of energy. Each disposal option has advantages and disadvantages.

2.7.1 Burial

Besides burning and rendering, the carcasses of dead domestic animals may be disposed of by burial. According to (Malone, 2005), on- farm burial has been the predominant disposal option for many catastrophic mortality events such as avian influenza outbreaks. It was suggested (Anon, 2005) that for mass disposal

of animals (poultry, swine, and calves) burial pits can be used if they are designed, constructed, maintained, and used in a manner to prevent the spread of diseases. Burial is one of the simplest and most cost-effective methods employed to deal with many types of mass mortality losses. However, burial of dead birds in a pit can lead to ground water contamination (Cai et al., 1994) and public perception concerns if not properly managed. Payne mentioned that when proper guidelines

2.7.2 Burning

This is one of the common methods of disposing, especially among small-scale farmers. In this disposal method, mortalities are fully burned at relatively high temperatures using fuels such as wood, tyres or diesel. However, this waste disposal method may lead to

atmospheric pollution in the event of catastrophic mortalities resulting from outbreaks of highly infectious diseases such as Newcastle disease and avian influenza. Anon (2005) argued that burning is not a preferred method of disposal because of the resulting air pollutants.

2.7.3 Incineration

Incineration is recognized as one of the biologically safest methods of disposal, eliminating the threat of disease (Blake et al.,2008). Incineration refers to process of thermal destruction, apparently among the most effective methods for destroying potentially infectious agents (Ritter and Chinside, 1995). The major concern during incineration is, the air emission, process conditions, and the disposal of solid and liquid residues need to be strictly controlled. The residue from properly incinerated mortality is largely harmless and does not attract rodents or insects. Payne stated that incineration eliminates all pathogens but has high operational costs and if not properly conducted it can contribute to air pollution that decreases its usefulness for widespread use as a mortality carcass disposal option.

2.7.4 Compositing

Composting is a natural, biological process by which organic material is broken down and decomposed (Malone, 2004). This process is carried out by successive microbial populations which function under increasing temperatures to break down organic materials into carbon dioxide, water, minerals, and stabilized organic matter (Evanylo et al.,2009). However, wastes having high moisture with low fibre content need higher amounts of moisture-sorbing and structural support to compost well (Tritt and Schuchardt,

1992). It is a biological process in which organic wastes are converted into products which can be potentially used as soil conditioner and organic fertilizer (Brake, 1992). According to Malone (2005), microorganisms will rapidly compost carcasses in the presence of oxygen (>5%), moisture (40-60%), and a proper carbon to nitrogen ratio (20:1 to 35:1). This process produces carbon dioxide, water vapour, heat and compost. It takes 2 to 6 months for the animal to decompose (Anon, 2002) Furthermore, compositing reduces the pathogenic organisms due to the high heat produced during the process of compositing. Das et al., (2002) reported that hatchery waste compositing reduces E. coli and salmonella by 99.9% and 100%, respectively. The disadvantages of compositing are loss of some nutrients including nitrogen, the land area required for the compositing and odour problems (Glatz et al., 2011). A potential problem with compositing is the emission of greenhouse gases such as methane and nitrous oxide, which are efficient in absorbing infra-red radiation resulting in global warming and acid rain. Animal production contributes 7% of greenhouse emissions worldwide through the decomposition and degradation of manure(Hao et al., 2004).

2.7.5 Rendering

Rendering is a process of application of heat to remove fat from meat (Swan, 1992). It is much suited for high-risk material disposal. Rendering products can be used in animal feed, as fertiliser or further processed via anaerobic digestion or composting. Materials are exposed to 133°C temperature for a minimum of 20 min at 3 bars or an alternative heat treatment, to make it suitable for fertilizing and feeding purposes. Heat treatment also increases the storage time of resultant products by removing moisture and killing microorganisms (NABC, 2004 Carcass Disposal). Rendered feed product can be used for

chemical industry or energy source in the form of fuel. Slaughterhouse by-products are preserved with formic acid as it has a good source of proteins and vitamins and is used as animal feed (Pulsa, 1996).

CHAPTER THREE

MATERIALS AND METHODS

3.1 COLLECTION OF SAMPLES

Three (3) forms of feeds (mash, starter and finisher) and two (2) types (one local and one foreign) were sampled in five (5) stores in Benin City. Feed samples were collected aseptically using sterile spatula in sterile polyethylene bags and labelled accordingly and were transported immediately to the laboratory for analysis

3.2 PREPARATION OF FEED SAMPLES

The wings and coats of *Moringa oleifera* seeds were removed and were dried. Fine powder was prepared by using mortar and pestle.

3.3 COLLECTION OF AGRICULTURAL SOIL SAMPLE

Soil samples collected from three different agricultural farmlands. At each location, soil sample was taken at a depth of 2 – 15cm and homogenized. The samples were then taken to the laboratory using sterile polyethylene bags (Aina *et al*., 2011).

3.4 STERILIZATION OF MATERIALS

Glasswares such as test tubes, glass rod, measuring cylinder, beakers and conical flasks required for this research work were soaked and washed in detergent and rinsed with distilled water. They were wrapped with aluminum foil paper and dried in the oven in an inverted position at 160^{O}-170^{O}C for 45-60 minutes. All the glasswares used were manufactured in England by Pyrex.

3.5 PREPARATION OF CULTURE MEDIA

Culture media were prepared according to manufacturer instruction. The medium used was Potato dextrose agar. The medium composition is shown in the appendix.

3.5.1 Potato Dextrose Agar:

In the preparation, 39.0g of potato dextrose agar powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. This was stirred and autoclaved at 121^{o}C for 15 minutes and then cooled to 45-50^{o}C. A portion of the medium (20ml) was poured into a sterile Petri dish and allowed to solidify.

3.6 ISOLATION OF FUNGI

Serial dilution plate method was used for the isolation process of plant pathogenic fungi according to the method described by Waksman (1994). Soil dilutions were made by suspending 1g of soil each sample in 10ml of sterile water. Dilutions of 10^{-3}, 10^{-4} and 10^{-5} were used to isolate fungi in order to avoid over-crowding of the fungal colonies. 1ml of the suspension of each concentration was added to sterile Potato dextrose agar media and was performed in triplicates. Chloramphenicol (250mg) was added to the medium to prevent bacterial growth before pouring into the Petri dishes. The plates were then incubated at 28 ± 2ºC for 7 days.

3.7 IDENTIFICATION OF FUNGI ISOLATES

The fungi isolated were identified using the morphological characteristics of the colony and microscopic examination according to the method described by Barnett and Hunter (1972). The colony length which includes the length and width of the colonies, the presence or absence of aerial mycelium, the colour, wrinkles, furrows and any other pigment and the macro morphological characters were evaluated (Diba *et al.,* 2007).

3.8 EXTRACTION OF *Moringa* Oil

After fine powder was obtained from dried *Moringa oleifera* seeds, the seed powder was soaked in hot water and allowed to stand for 4 – 5 days. After 5 days, the oil was collected from the surface and boiled until clear oil was observed. After boiling, the oil was filtered with a fine sieve to remove any impurities.

3.9 CONCENTRATION OF *Moringa* Oil

Different concentrations of *Moringa* oil were made by dilution of the appropriate amount of the crude extract with the molten Potato Dextrose Agar medium followed by the addition of Tween 80 to disperse the oil in the medium to give concentrations of 2%, 6%, 10% and 14% (El-Mohamedy and Abdallah, 2014).

3.10 PHYTOCHEMICAL ANALYSIS

Phytochemical analysis was carried out for *Moringa* oil and Olive oil as described by Ezeonu and Ejikeme, (2016). The following procedures were used:

1. Test for Anthraquinones: benzene was added to the oil extract in a conical flask and left form 10 minutes. 10% ammonia solution was added and shaken vigorously for 30 seconds and pink, violet, or red colour indicates the presence of anthraquinones in the ammonia phase.
2. Test for Tannins: a few drops of 0.1% ferric chloride was added to the oil. A brownish green or a blue black colouration shows a positive test.
3. Test for Terpenoids: a mixture of chloroform and concentrated tetraoxosulphate (VI) acid was added to the oil. The presence of reddish brown colouration shows a positive result for the presence of terpenoids.
4. Test for Flavonoids: 10M dilute of ammonia solution was added to the oil, followed by the addition of concentrated tetraoxosulphate (IV) acid. Appearance of a yellow colouration which disappears on standing shows the presence of flavonoids.
5. Test for Alkaloids: chloroform was added to the oil in a test tube. Appearance of pink colour indicates the absence of alkaloids in the oil. Appearance of brown colour indicates the presence of alkaloids.

6. Test for Steroids: acetic anhydride was added to the oil followed by the addition of concentrated tetraoxosulphate (VI) acid. A violet to blue or green colour change indicates the presence of steroids.
7. Test for Phenols: a few drops of dilute ferric chloride solution were added to the oil. The formation of red, blue, green, or purple colouration indicates the presence of phenols.
8. Test for Saponins: the oil was placed in a test tube and shake vigorously, if no persistent foam is formed it indicates the absence of saponins.

3.11 ANTIFUNGAL ASSAY

3.11.1 DETERMINATION OF MYCELIAL RADIAL GROWTH INHIBITION

Antifungal activities of *Moringa* oil and Olive oil were performed using the food poisoning method. About 20ml of the medium was poured into Petri dishes. A 9-mm diameter agar disk bearing the hyphae of the fungi from 7-day old colonies was transferred to the centre of each Petri dish. This process was repeated for all the fungus and was done in triplicates. The controls used in this experiment were a standard fungicide, Mancozeb and Tween 80. The plates containing fungi without the plant extract served as negative control. Plates were incubated at 28ºC for 7 days and the mycelial radial growth inhibition were recorded daily (El-Mohamedy and Abdallah 2014). .

3.11.2 DETERMINATION OF SYNERGISTIC ACTIVITIES OF BOTH OILS

Equal volume of 100% solution of *Moringa* oil and Olive oil were mixed. Appropriate amount of the combined oils were diluted with the molten potato dextrose agar media followed by the

addition of Tween 80 to disperse the oil in the medium to give concentrations of 2%, 6%, 10% and 14% (El-Mohamedy and Abdallah 2014).

3.11.3 DETERMINATION OF THE PERCENTAGE MYCELIAL RADIAL GROWTH INHIBITION

The percentage mycelial inhibition was determined on the 7th day for both *Moringa* and Olive oils according to the equation:

Percentage growth inhibition= R- r / R × 100

Where;

R- Linear growth of fungus on control Petri dishes

r- Linear growth of fungus on Petri dishes with *Moringa* and Olive oils

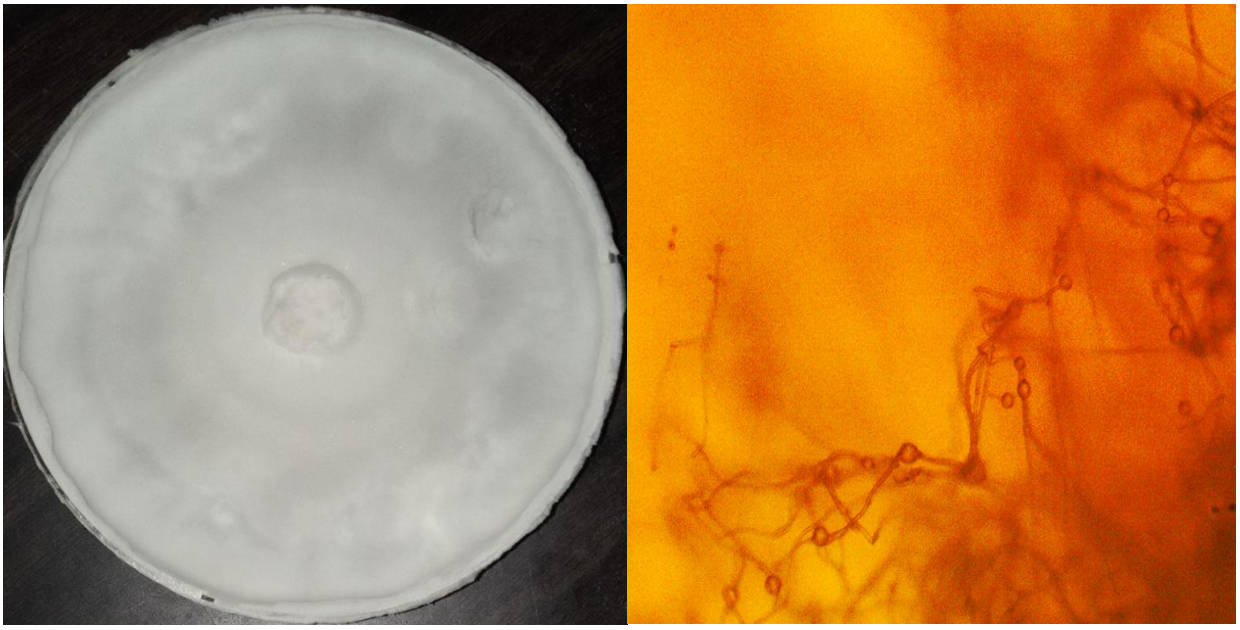
(Elgorban *et al*., 2015).

CHAPTER FOUR

RESULTS

The phytopathogenic fungi isolated and identified were *Penicillium chrysogenum* and *Mucor circinelloides*.

The cultural and microscopic characteristics of the isolates used in this study are shown in plates 1A, 1B, 2A and 2B.

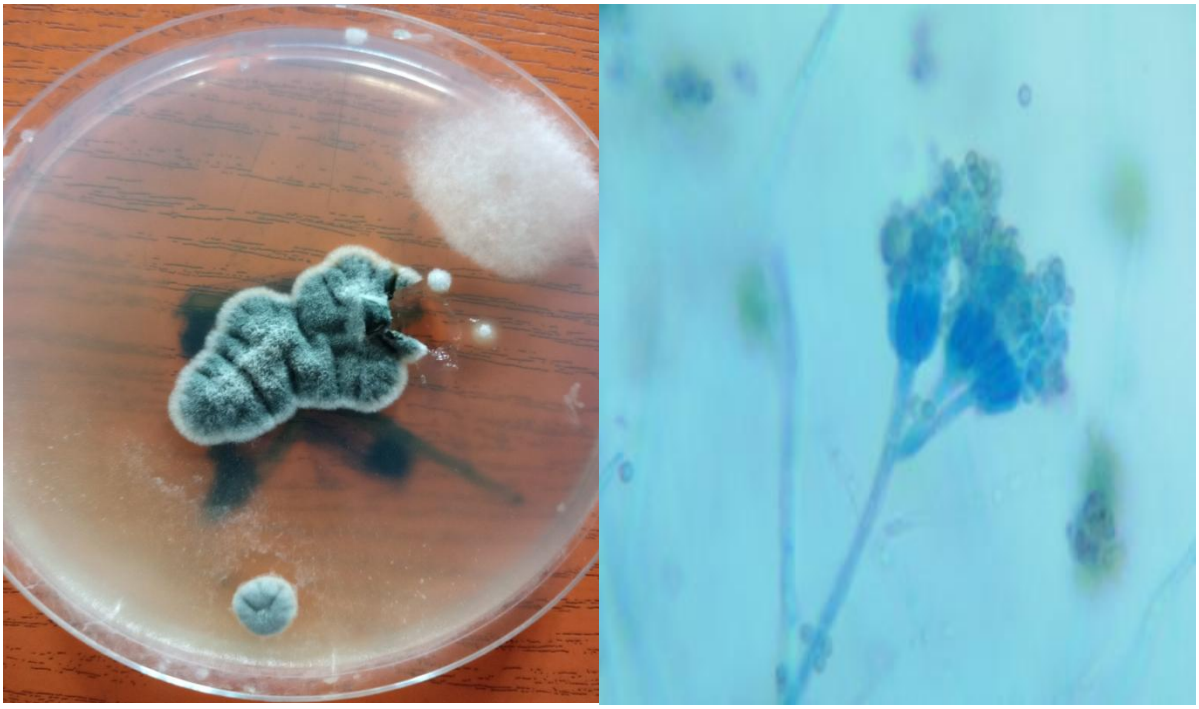


1A 1B

Plate 1: cultural and microscopic characteristics of *Mucor circinelloides*

A. 7-day old pure culture of *Mucor circinelloides*

B. Photomicrograph of *Mucor circinelloides*



2A **2B**

Plate 2: cultural and microscopic characteristics of *Penicillium chrysogenum*

A. 7-day old culture of *Penicillium chrysogenum*

B. Photomicrograph of *Penicillium chrysogenum*

Table 6 shows the cultural and morphological characteristics of the fungal isolates. After the isolation of fungi from an agricultural soil, pure cultures where made as shown in plates 1A and 2A. These pure cultures where used to culturally characterize the fungal isolates. *Penicilliun chrysogenum* displayed a greenish colony with white growing edges while *Mucor circinelloides* displayed white cotton – like colony on Potato Dextrose Agar plates.

Further identification of the isolates was carried out using microscopy. Colonies of *Penicillium chrysogenum* showed septate hyphae, shape of the conidia is subglobules having a spherical to ellipsoidal smooth texture as shown in plate 1B. Colonies of *Mucor circinelloides* showed non-septate hyphae, the shape of the conidia is globules having an ellipsoidal texture as shown in plate 2B.

Table 6: Cultural and morphological characteristics of the fungal isolates.

ISOLATE	**A**	**B**
MORPHOLOGY	Greenish with white growing	White cotton-like growth
Description on PDA	edges	
MICROSCOPIC		
DESCRIPTION		
Shape of the conidia	Subglobules	Globulus
Texture of the conidia	Spherical to ellipsoidal	Ellipsoidal
	smooth	
Hyphae	Septate	Non-septate
Fungi isolate	*Penicillium chrysogenum*	*Mucor circinelloides*

Table 7 shows the *In-vitro* antifungal activity of *Moringa* oil alone (Day 7) against *Penicillium chrysogenum* and *Mucor circinelloides* and the controls as represented by their mycelial radial growth inhibition (mm). It was observed that *Moringa* oil displayed a higher inhibitory effect on *Penicillium chrysogenum* (2.83±0.04mm) than *Mucor circinelloides* (4.00±0.07mm) at the highest concentration of 14%. The pathogens used where sensitive to *Moringa* oil even at the lowest concentration. *Penicillium chrysogenum* (4.5±0.05mm) and *Mucor circinelloides* (5.0±0.10mm) where sensitive at the lowest concentration of 2% compared to the negative controls (*Penicillium chrysogenum*: 17.66±0.33mm and *Mucor circinelloides*: 39.16±0.05mm). Mancozeb gives a mycelial radial growth of 0.66±0.10mm for *Penicillium chrysogenum* and 0.33±0.03mm for *Mucor circinelloides*.

Table 7: Antifungal activity of *Moringa* oil against the selected fungal isolates represented by the mycelial radial growth inhibition (mm).

TEST	CONCENTRATIONS (%)				CONTROLS		
ORGANISMS	**2**	**6**	**10**	**14**	**Water**	**Mancozeb**	**Tween 80**
Penicillium chrysogenum	5.00±0.10	4.50±0.05	3.0±0.05	2.83±0.04	17.66±0.33	0.66±0.10	19.66±0.29
Mucor circinelloides	4.50±0.05	5.83±0.10	5.33±0.07	4.00±0.07	39.16±0.05	0.33±0.03	60.50±0.02

***values are mean ± standard error of triplicates.**

Table 8 shows the In-vitro antifungal activity of Olive oil alone (Day 7) against *Penicillium chrysogenum* and *Mucor circinelloides* and the controls as represented by their mycelial radial growth inhibition (mm). It was observed that Olive oil displayed a higher inhibitory effect on *Penicillium chrysogenum* (4.90±0.05mm) than *Mucor circinelloides* (6.83±0.19mm) at the highest concentration of 14%. The pathogens used where sensitive to Olive oil even at the lowest concentration and the organism's reaction to Olive oil is concentration dependent (i.e.: the highest concentration which is 14% has the highest inhibitory effect on the organism and the lowest concentration which is 2% has the lowest inhibitory effect on the organisms). *Penicillium chrysogenum* (14.66±0.13mm) and *Mucor circinelloides* (24.00±0.30mm) where sensitive at the lowest concentration of 2% compared to the negative controls (*Penicillium chrysogenum*: 17.66±0.33mm and *Mucor circinelloides*: 39.16±0.05mm).

Table 8: Antifungal activity of Olive oil against the selected fungal isolates represented by the mycelial radial growth inhibition (mm).

TEST	CONCENTRATIONS (%)				CONTROLS		
ORGANISMS	**2**	**6**	**10**	**14**	**Water**	**Mancozeb**	**Tween 80**
Penicilliun chrysogenum	14.66±0.13	9.33±0.49	7.20±0.25	4.90±0.05	17.66±0.33	0.66±0.10	19.66±0.29
Mucor circinelloides	24±0.30	15.66±0.31	7.33±0.07	6.83±0.19	39.16±0.03	0.33±0.05	60.5±0.02

***values are mean ± standard error of triplicates.**

Table 9 shows the synergistic combination of *Moringa* oil and olive oil against *Penicillium chrysogenum* and *Mucor circinelloides* and the controls showing their mycelial radial growth inhibition measured in mm showed a good antifungal activity. It was observed that the combination had a higher percentage mycelial radial growth inhibition for *Mucor circinelloides* than for *Penicillium chrysogenum*. There was a total growth inhibition of 0.00mm for *Mucor circinelloides* at 14% of the combination and *Penicillium chrysogenum* had a mycelial radial growth inhibition of 5.20±0.11mm at a concentration of 14%, this shows that this combination is a better antifungal agent of *Mucor circinelloides*. This combination had a moderate inhibition on *Penicillium chrysogenum* compared to the results of *Moringa* oil alone and Olive oil alone. *Penicillium chrysogenum* (12.33±0.14mm) and *Mucor circinelloides* (5.33±0.13mm) where sensitive at the lowest concentration of 2% compared to the negative controls (*Penicillium chrysogenum*: 17.66±0.33mm and *Mucor circinelloides*: 39.16±0.05mm).

Table 9: Antifungal activity of the synergistic combination of *Moringa* oil and Olive oil against the selected fungal isolates represented by the mycelial radial growth inhibition (mm).

TEST	CONCENTRATIONS (%)					CONTROLS	
ORGANISMS	**2**	**6**	**10**	**14**	**Water**	**Mancozeb**	**Tween 80**
Penicillium chrysogenum	12.33±0.14	9.5±0.37	5.2±0.13	5.2±0.11	17.66±0.33	0.66±0.10	19.66±0.29
Mucor circinelloides	5.33±0.13	5.00±0.01	4.5±0.15	0.00±0.00	39.16±0.03	0.33±0.05	60.50±0.03

***values are mean ± standard error of triplicates.**

Table 10: The phytochemical analysis of Olive oil

Phytochemicals	Inference
Phenol	-
Flavonoids	-
Alkaloids	-
Saponins	-
Terpenoids	+
Tannins	-
Steroids	-
Anthraquinone	-

+ = Present

- = Absent

Table 11: Phytochemical analysis of *Moringa* oil

Phytochemicals	Inference
Phenols	+
Flavonoids	+
Alkaloids	+
Saponins	+
Terpenoids	+
Tannins	-
Anthraquinone	-
Steroids	-

+ = Present

- = Absent

Table 12 shows the percentage mycelial radial growth inhibition at day 7 of *Moringa* oil on *Penicillium chrysogenum* and *Mucor circinelloides* at different concentrations. The highest percentage growth inhibition was 83.963 % for *Penicillium chrysogenum* and 89.79% for *Mucor circinelloides* (*Moringa* oil 14%) while the lowest was 71.70% *Penicillium chrysogenum* and 85.11% for *Mucor circinelloides*.

Table 12: Percentage mycelial radial growth inhibition at day 7 of *Moringa* oil against the selected fungal isolates.

TEST ORGANISMS	CONCENTRATIONS (%) 2	6	10	14
Penicillium chrysogenum	71.70	74.50	83.02	83.96
Mucor circinelloides	88.50	85.11	86.38	89.79

Table 13 shows the percentage mycelial radial growth inhibition of Olive oil on *Penicillium chrysogenum* and *Mucor circinelloides* at different concentrations. The highest percentage growth inhibition was 72.27% for *Penicillium chrysogenum* and 82.55% for *Mucor circinelloides* (Olive oil 14%) while the lowest was 16.98% *Penicillium chrysogenum* and 38.72% for *Mucor circinelloides* (Olive oil 2%).

Table 13: Percentage mycelial radial growth inhibition at day 7 of Olive oil against the selected fungal isolates.

TEST ORGANISMS	CONCENTRATIONS 2	6	10	14
Pencillium chrysogenum	16.98	47.17	59.24	72.26
Mucor circinelloides	38.72	60.00	81.28	82.55

Table 14 shows the percentage mycelial radial growth inhibition of the synergistic combination of both *Moringa* and Olive oil on *Penicillium chrysogenum* and *Mucor circinelloides* at different concentrations. The highest percentage growth inhibition was 70.56% for *Penicillium chrysogenum* and 100% for *Mucor circinelloides* while the lowest was 30.19% *Penicillium chrysogenum* and 86.38% for *Mucor circinelloides*.

Table 14: Percentage mycelial radial growth inhibition at day 7 of the synergistic combination of *Moringa* oil and Olive oil against the selected fungal isolates.

TEST ORGANISMS	CONCENTRATIONS (%)			
	2	**6**	**10**	**14**
Penicillium chrysogenum	30.19	46.22	70.56	70.56
Mucor circinelloides	86.38	87.23	88.51	100

CHAPTER FIVE

DISCUSSION AND CONCLUSION

Synthetic fungicides are right now utilized as the essential methods for the control of plant diseases. However, alternative control methods are required due to the negative public perceptions about the use of synthetic chemicals, resistance to fungicides among fungal pathogens and high development cost of new chemicals. The uses of plant derived products as diseases control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance (Seema *et al*., 2011; Moyo *et al*., 2012). To develop environment-friendly alternatives to synthetic fungicides for the control of fungal plant diseases, the interest on essential oils and plant extracts has been increased (Gnanamanickam, 2002). In this study, we investigated the antifungal activities of *Moringa oleifera* seed oil and Olive oil against *Penicillium chrysogenum* and *Mucor circinelloides*.

Most published literatures in this field lay emphasis on the effect of *Moringa* and Olive oil separately against plant pathogenic fungi but not on their synergistic combination.

Moringa oil displayed a very strong activity against *Mucor circinelloides* and *Penicillium chrysogenum* compared to Olive oil. The results demonstrated that *Moringa* oil at all concentrations had considerable effect on the mycelial growth rate of the selected phytopathogenic organisms. *Moringa* oil proved to be a better antifungal agent to *Penicillium chrysogenum* than Olive oil and the synergistic combination, however, the combination proved to be a better antifungal against *M. circinelloides* compared to the oils when used singly. Many essential oils and plant extracts have been found to be potent fungitoxic agents against many plant pathogens (Siripornvisal and Ngamchawee, 2010; El-Mohamedy *et al*., 2013; Tabassum and Vidyasagar, 2013; Abd el-kader *et al*., 2013) due to the presence of potent phytochemicals. Several *in vitro* studies have been published confirming the effect of essential oil and their major compounds on plant and human pathogenic fungi (Lee *et al.,* 2007; Chuang *et al*., 2007; Tabassum and Vidyasagar, 2013; Hadi and Kashefi, 2013). The effects that *Moringa* oil has *Penicillium chrysogenum* and *Mucor circinelloides* can be attributed to the presence of phytochemicals which are naturally present in *Moringa* oil. The antifungal activity of *Moringa* oil might be caused by the property of terpenoids due to their highly lipophilic nature and low molecular weight. They are capable of disrupting the cell membrane, causing cell death or inhibiting the sporulation of fungi. There are several *in-vitro* tests indicates that terpenoids shows ineffective antimicrobial activity when used as a singular compound compared to the whole essential oil (Bajpai *et al*., 2011). This indicates the important nature of other phytochemicals present in *Moringa* oil like Alkaloids, Saponins, Phenols and Flavonoids also have their separate use and antimicrobial effect (Tabassum and Vidyasagar, 2013).

In line with this study, El–Mohamedy and Abdallah (2014) reported that *Moringa* oil had a percentage mycelial radial growth inhibition of 100% against *Fusarium oxysporum* and

Fusarium solani at a concentration of 2%. This same study also reported a percentage mycelial radial growth inhibition of 55.8% and 54% for *Fusarium oxysporum* and *Fusarium solani* respectively at a concentration as low as 0.5% using *Moringa* oil. In general *Moringa* oil had a higher inhibitory effect than olive oil.

Olive oil showed moderate activity against *Penicillium chrysogenum* and *Mucor circinelloides* compared to *Moringa* oil. The results demonstrated that Olive oil at all concentrations had considerable effect on the mycelial growth rate of the selected phytopathogenic organisms. Boskou (1996) reported that some minor constituents of olive oil are present only in the crude oil. Refining removes phospholipids and phenols, and causes significant quantitative and qualitative changes in the other classes. Most of the minor constituents of olive oil are present in the 0.5–1.5% of unsaponifiable matter. Extra virgin olive oil which was used in this study is a refined form of crude olive oil, so most of these phytochemicals has been removed in the process of processing. The phytochemical analysis of Olive oil shows the presence of terpenoids which are capable of disrupting the cell membrane, causing cell death or inhibiting the sporulation of fungi. As stated earlier, terpenoids can perform these effects on fungi more effectively with the help of other phytochemicals present in olive oil. Compounds that are naturally found in olives has a possibility of been used as biopesticides, so more research is needed to disclose the best method to extract and apply the antimicrobial compounds present in olive fruits.

Varol *et al.,* 2017 reported that Olive oil had a significant antifungal activity, with minimal inhibitory concentration of 50% against *Candida albicans* at a concentration of 2% which agrees with the results of this study.

The synergistic combination of *Moringa* oil and olive oil against *Penicillium chrysogenum* and *Mucor circinelloides* showed a good antifungal activity. This is the first study so far reported where this combination was used as an antifungal agent against these phytopathogens. When *Moringa* and Olive oil was used separately on *Penicillium chrysogenum* and *Mucor circinelloides*, *Mucor circinelloides* had the highest percentage mycelial radial growth inhibition for both oils. When these oils where combined it gave a total growth inhibition of 100% for *Mucor circinelloides* at 14% of the combination. This means that the synergistic combination is a better antifungal agent for *Mucor circinelloides*. This combination had a moderate inhibition on *Penicillium chrysogenum*, having a percentage mycelial radial growth inhibition of 70.56% at 14% concentration.

5.1 CONCLUSION

Antimicrobial agents from plants, which retard the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies with special reference to the management of plant diseases. The results obtained from this study indicate that *Moringa oleifera* oil, Olive oil and their synergistic combination had antifungal activity against *Penicillium chrysogenum* and *Mucor circinelloides in vitro*. It is proposed that we adopt *Moringa* oil, Olive oil and their combination as an eco-friendly alternative to combact the growing resistant development in pest. Further studies can however be conducted to ascertain the effectiveness of these natural fungicides *in vivo.*

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APPENDIX

COMPOSITION OF CULTURE MEDIUM

Potato Dextrose Agar

Potato Extract	4.0g
Dextrose	20.0g
Agar No. 1	15.0g

Preparation:

In the preparation, 39.0g of potato dextrose agar powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminum foil paper. This was stirred and autoclaved at 121^{o}C for 15 minutes and then cooled to 45-50^{o}C. A portion of the medium (20ml) was poured into a sterile Petri dish and allowed to solidify.