THE EFFECTS of FEATHER PROTEIN HYDROLYSATE PRODUCED by *Bacillus safensis* and *Bacillus licheniformis* on THE GROWTH of *Telfaria* occidentalis and *Abelmoschus esculentus*

BY

USMAN, Abiola Mohammed
MTECH/SLS/2017/7445

DEPARTMENT OF MICROBIOLOGY,
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA.

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ABSTRACT

Keratins are proteins present in all life forms and are difficult to degrade. This research was carried out to investigate the biofertilization effect of protein hydrolysate produced by the biodegradation of keratin waste (chicken feather waste) on the growth of *Albemoschus esculentus* and *Telfaria occidentalis*. Feather degrading bacteria were isolated from feather dump site and identified using biochemical and molecular characterization. The isolates were used to produce protein hydrolysate in a submerged fermentation process. The isolates were confirmed to be *Bacillus safensis* and *Bacillus licheniformis*. The protein hydrolysate produced from *Bacillus safensis* and Bacillus *licheniformis* had high yield weights of 25.9±19.1 g and 88.7±3.12 g respectively. The protein hydrolysate combined with bacteria suspension also gave a high yield weight of 23.9±0.00 g and 95.3± 0.010 g respectively. There was a positive result when NPK was combined with feather hydrolysate resulting in a yield of 151.6±0.0011 g and 31.3± 0.00 g for *Albemoschus esculentus* and *Telfaria occidentalis* respectively. The untreated plants had the least yield weight of 47.5± 0.14 g and 14.8±0.00 g for *Albemoschus esculentus* and *Telfaria occidentalis* respectively. The result of this experiment shows that feather hydrolysate which is eco-friendly offers a promising prospect in agriculture as a biofertilizer.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Global population growth and the need to reduce the environmental effects of the chemicals used in agriculture have led to a variety of efforts to improve crop yields and quality, which includes the development of more economically and environmentally sustainable and rational methods (Ertani *et al.,* 2009; Kshetri *et al.,* 2019). Researchers have increasingly recommended the use of organic fertlizers, which include protein hydrolysates (PHs), as an alternative to chemical fertilizers for crop improvement (Colla and Rouphael, 2015; Popko *et al.,* 2015). Furthermore, organic fertlizers have been suggested to help reduce the need for chemical fertilizers and the pollution caused by their use on agricultural lands (Colla *et al.,* 2015). As the heavy use of inorganic fertilizers affects soil quality, it has led to a steady increase in the number of studies on the application of PHs, which include small-sized peptides, free amino acids, and some nutrient elements, due to their beneficial effects on crop cultivation (Schiavon *et al.,* 2008; Colla *et al.,* 2015). Studies have shown that low molecular weight peptides and free amino acids contained in PH compounds are the most beneficial properties of biofertilizer treatments, as they can be readily taken up by almost all plant tissues (Cerdán *et al.,* 2009; Bouhamed *et al.,* 2020).

Protein hydrolysates (PHs) may also increase plant productivity by influencing plant metabolism. Studies have shown that applying PHs to plant leaves may increase the efficiency of nutrient and water uptake from the soil (Ebru *et al.,* 2019; Halpern *et al.,* 2015), and upgrade some of the biochemicals associated with protein synthesis, photosynthesis,

lignification and resistance to abiotic stress (Ebru *et al.,* 2019), leading to increased crop growth, development and productivity (Colla *et al.,* 2015). PHs have also been found to increase tolerance to stressors such as drought, and act as a chelator of cationic metals in plants (De Vasconcelos, 2019). Some studies suggested that the ameliorative effects of PH treatment depended on the concentration, plant species, environmental conditions, phenological stage, time of application, and leaf permeability (Kunicki *et al.,* 2010; Ertani *et al.,* 2014). Enzymatic and chemical hydrolysis of proteins, which are not found in animals or plants, help develop PHs (Colla *et al.,* 2015; Colla and Rouphael, 2015).

Poultry feathers constitute a significant waste in the poultry industry, and their needless accumulation lead to environmental pollution (Sobucki *et al.,* 2019). According to some studies, chicken feathers may be a significant PH resource (Callegaro *et al.,* 2019). Chicken feathers are rich in glutamic acid, cysteine, glycine, arginine, and phenylalanine, as well as nutritional minerals and keratin (Nurdiawati *et al.,* 2019). Studies have shown the potential use of chicken feather hydrolysates (CFHs) as a general growth substrate for bacteria and fungal development, as an alternative organic fertilizer and as a biocontrol agent for the cultivation of crop plants (Gurav and Jadhav, 2013). Biological processing of feather waste into more available agricultural nitrogen products has been the subject of several studies (Joardar and Rahman, 2018; Mushtaq *et al.,* 2019).

1.2 Statements of the Research Problem

Feather wastes are one of the major wastes that are discharged into the environment due to the high consumption of chicken meat and their high industrial demand globally (Tesfaye *et al.,* 2017). The disposal of these chicken wastes poses a lot of ecological and economic challenges, most notably in the developing countries (Tesfaye *et al.,* 2017). Inorganic

fertlizers are widely applied to the soil in order to achieve high crop yields, but long-term application of these chemicals can cause environmental damage, such as increased soil acidity, reduced soil fertility, soil erosion, water pollution, lowering of groundwater levels, waterlogging in the soil, biodiversity depletion and greenhouse effect. Inorganic fertlizers also have a small but cumulative impact on the health of humans and animals that consume food crops produced with chemical fertlizers. These health effects include malnutrition, cancer and waterborne diseases.

1.3 Justification for the Study

Feathers are one of the most common waste products from the poultry industry and are rich in protein and amino acids. It has been shown that a mixture of peptides and amino acids from protein sources, called protein hydrolysate (PH), can promote plant growth (Nurdiawati *et al.*, 2019). However, traditional feather degradation methods consume large amount of energy and reduce the overall quality of the proteins, but degradation by keratinolytic bacteria may represent an alternative for the development of cheap, eco-friendly and easily available nitrogen (N) as a potential organic fertilizer and eliminate waste disposal problems.

1.4 Aim and Objectives of the Study

This research was aimed to study the bio-fertilization potential of feather protein hydrolysate produced by *Bacillus safensis* and *Bacillus licheniformis* on the growth of *Telfaria occidentalis* and *Abelmoschus esculentus* and the objectives were to:

I. isolate keratinolytic bacteria from the feather dumpsite

II. identify bacterial isolates using biochemical and molecular methods.

III. produce feather hydrolysate from feather using a submerged fermentation process.

IV. investigate the fertilizing effect of the feather hydrolysate on *Telfaria occidentalis* and *Albemoschus esculentus*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Keratin

Skins, claws, horns, hooves, furs and feathers all have structural proteins attached to epithelial cell fibers in the outer layers; this structural protein is called keratin. Keratin in the epithelium of vertebrates serves essential structural and protective functions, they have also been shown to control vital cellular functions, including cell growth and protein synthesis (Saarela *et al.*, 2017). Keratin is a fibrous protein that can be found all over the world, after cellulose and chitin, it is the third most abundant compound on earth. Soft keratin contains less than 10 % cysteine, while hard keratin contains 10-14 % cysteine (Saarela *et al.*, 2017).

Skins, furs, claws and hooves are examples of hard keratins, soft keratin is present in the epidermis of cells. Keratin is a rigid polymer that is insoluble in water and organic solvents and resistant to pepsin and trypsin degradation because of disulphide attachments, hydrogen bonds and hydrophobic interactions. The durability of keratins is a direct consequence of their complex architecture with extremely high molecular weight, and this keratin protein is not easily degraded by pepsin, trypsin, and papain because of disulphide bonds, hydrogen bonding and hydrophobic interactions (Wang *et al.*, 2019).

2.1.1 Types of keratin

Keratins are divided into two categories: Alpha keratin (α -Keratin) and Beta keratin (β -keratin). Every epithelial cell contains alpha keratin, the presence of α helix in alpha keratin makes it not vulnerable to microbial degradation, thus, causing environmental pollution (Brandelli *et al.*, 2015). Alpha keratins are rigid, durable, inelastic and insoluble proteins composed of hydrophobic amino acids such as methionine, phenylalanine, valine, isoleucine,

and alanine (Brandelli *et al.,* 2015). Beta-keratin is a structural protein present in mammals and birds that contains a high proportion of cysteine, a sulphur-containing amino acid that easily forms disulphide bonds, rigid, and resists degradation (Brandelli *et al.,* 2015). In the feathers, about 80 to 90 % keratin is present; keratin proteins have a molecular weight of about 10-14 kDa (Brandelli *et al.,* 2015).

2.1.2 Major sources of keratin protein

Keratin proteins are derived from living organisms or their body parts after death. The richest sources of keratin are feathers, wool, hair, hoof, scales, and stratum corneum (Figure 1.1). Keratin protein is present in the human hair and offers flexibility, strength, and durability to the hair in the form of different conformations (Schiavon *et al.,* 2008). Feather is made up of over 90 % of keratin protein and produced as waste by poultry-processing industries (Schiavon *et al.,* 2008). Keratin is a structural protein made up of α-helical coils joined together by disulphide linkages that accounts for approximately 90 % of the chicken feather (Tesfaye *et al.,* 2017). Feathers shield birds from cold, fog, heat, and injury, the feather's structural characteristics allow it to withstand environmental damage and proteases (Tesfaye *et al.,* 2017). The human hair is a natural filamentous biomaterial, and approximately 80 % keratin protein is present in human hair (Tesfaye *et al.,* 2017). The accumulation of hair causes many environmental problems and is considered waste protein.

The human nail is primarily made up of a tightly bound keratin chain and a scleroprotein containing a considerable amount of sulphur (3.8 %) and various disulphide bonds, resulting in a potent permeability membrane (Fang *et al.,* 2013). The beak of birds has an external shell of hard keratin, which consists almost entirely of proteins (Fang *et al.,* 2013). Structurally, hoof keratin contains α-helical conformation with an admixture of β-sheet and

possesses high thermal stability (Fang *et al.*, 2013). The horn is a tough animal tissue and has inflexible configuration due to the sulphur cross-linkages. Fundamental components of any horns are keratin, free amino acids, peptides, lipids, microelements: calcium, aluminum, chromium, copper, iron, manganese, and zinc (Alashwal *et al.*, 2019).

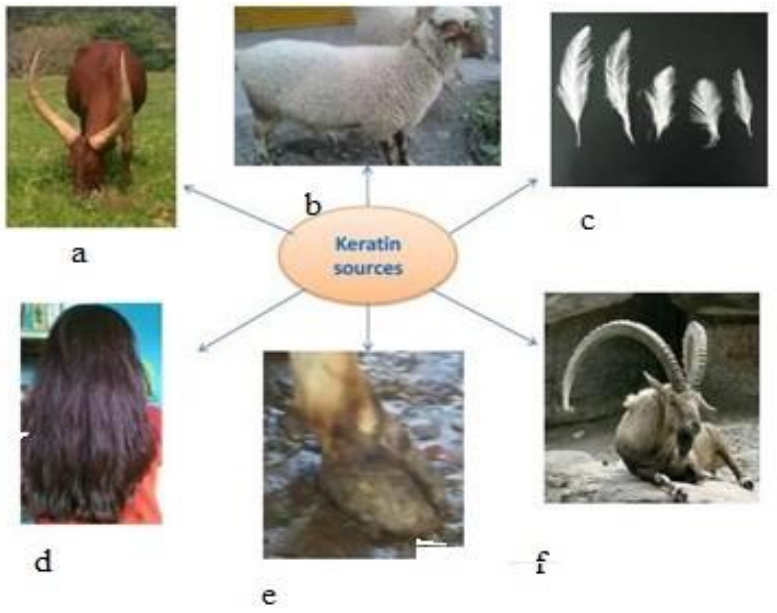


Figure 1.1 : Sources of Keratin Waste Brandelli *et al.*(2015).

Each year millions of tons of keratinous wastes are generated globally, especially in the wool textile industry and in poultry slaughterhouses (Ertani *et al.*, 2009). Keratinous wastes, generated mainly in the form of feathers, hairs, horns, hooves, and nails, are gradually accumulating in the environment, an enormous amount of urban wastes are accumulating in the form of sewage under the bottom sediments of rivers and canals, making it difficult for solid waste management, and it is important to recycle them (Ertani *et al.*, 2009).

Feather is the most keratinous waste material produced in modern civilization, accounting for approximately 9×10^5 tons (Popko *et al.*, 2015). The poultry processing industry is the leading source of feather waste, chicken is eaten in every country, as it is not considered a

taboo in any society or faith (Popko *et al.*, 2015). In poultry products, toxic microbial secretions like aflatoxins are popular, also, high levels of bacteria, parasites and yeasts; therefore, the treatment of stubborn keratin waste needs expert intervention (Colla and Rouphael, 2015).

2.2 Mechanism of Keratin Degradation

Adhesion, invasion, hydrolase processing, substrate disintegration and degradation are all processes in microbial keratin degradation (Popko *et al.*, 2015). The mechanism of keratin breakdown is still unknown since no comprehensive research on the differential expression of genes or proteins for feather degradation has been conducted. However, given keratin's complexity and biochemical properties, it's reasonable to assume that keratin degradation would entail two main events: sulphite breakdown (disulphide bond breaking) and keratinase proteolytic assault (Ertani *et al.*, 2009). Disulphide reductase, chemical redox, or living cells may help break down sulphates required for keratin degradation. In this regard, the earliest studies showed sulphite aggregation in *Streptomyces* sp. extracellular broth and suggested its role in reducing keratin disulphide bonds to facilitate feather degradation (Ramnani and Gupta, 2007).

The feathers can only partly degrade in the presence of chemical redox while they can only wholly degrade in the live cell fermentation phase (Ramnani and Gupta, 2007). It has also been discovered that even though redox is present, only keratinase can facilitate keratin degradation. Since the surface of the protein keratin is rich in hydrophobic amino acid residues that must be removed by keratinase, most traditional proteases cannot extract keratin without the aid of keratinase (Ramnani and Gupta, 2007).

2.2.1 Methods of Keratin digestion

Every year, approximately 58×10^9 chickens are slaughtered, resulting in vast quantities of manure waste produced in nature and the failure of microorganisms to break down keratin wastes contribute to environmental pollution (Ebru *et al.,* 2019). Poultry processing farms practise traditional methods of feather disposal, such as burial, incineration, and controlled landfilling, but these require a lot of water and electricity. Furthermore, pathogenic microorganisms in dead birds may cause severe public health problems when handled carelessly (Ebru *et al.,* 2019). Landfills take a long time to decompose keratin wastes, and incineration releases greenhouse gases. Also, the costs associated with disposing the feather wastes are high as the availability of landfill space is reduced (Ebru *et al.,* 2019). Recycling, reuse, reduction, handling, and treatment must also be seen as final options (Ebru *et al.,* 2019). As a result, keratin digestion has always been a significant concern in preserving a stable environment.

2.2.2 Hydrothermal method of keratin degradation

In the presence of acid or alkali, this process usually uses high vapour pressure (10-15 psi) and high temperature (80-140 ° C). Free amino acids, water-soluble polypeptides, and even oligopeptides are generated using this method. The most significant drawback of this approach is that hydrothermal keratin hydrolysis can result in partial or total degradation of specific amino acids. Furthermore, it causes lysine, methionine, and tryptophan to be destroyed. Also, this mechanism converts dietary amino acids, including casein and lysine, into lysinalanine and lanthionine, which are not valuable for animals as a protein source (Cerdán *et al.,* 2009).

Excessive steam and heat treatment will also inhibit the hydrolysis of certain amino acids (Cerdán *et al.,* 2009). Furthermore, the lanthionine content is proportional to the digestible amino acids, implying that lanthionine is found in feathers, indicating a high processing index. The racemization of amino acids, which occurs quickly after chemical hydrolysis, is another phenomenon that affects the consistency of proteins during this process (Halpern *et al.,* 2015). Compared to experiments without alkali or enzymes, autoclaving feather meals with sodium hydroxide improves amino acid digestion (Halpern *et al.,* 2015).

2.2.3 Chemical method of keratin degradation

The chemical hydrolysis process of keratin waste is based on the use chemicals (acids, bases and catalysts). Chemical hydrolysis requires more aggressive reaction conditions (higher temperature and pressure) and is more dangerous for the environment (Schiavon *et al.,* 2008). The chemical hydrolysis reaction is slow and efficient but results in losing some amino acids, e.g., tryptophan (Cerdán *et al.,* 2009). Chemical processes necessitate additional time and effort and the use of costly chemical and industrial manufacturing machinery. Since it contains a limited number of essential amino acids, this product has a low nutritional value. The degree of protein deficiency affects the solubility and stabilization of hydrolysates (Cerdán *et al.,* 2009). The chemical hydrolysis process causes lung illness, heart disease and cancer by increasing the release of gases such as CO_2 and SO_2 into the atmosphere (Cerdán *et al.,* 2009). As a result, biotechnology and environmentally sustainable solutions for keratin waste recycling are urgently required.

2.2.4 Microbial method of keratin degradation

Microbial degradation of keratin is reported in some bacteria, actinomycetes, keratinophilic fungi and larvae of the clothes moth "*Tineola bisselliella"* (Veerabadran *et al.,* 2012).

Keratinolytic bacteria were mostly isolated from bird feathers and the plumage, composting, or feather waste processed by fermentation. The bacteria often belong to the genus *Bacillus* and order actinomycetes. Feather degrading abilities were mostly found in *Bacillus licheniformis* as well as in *Bacillus pumilis, Bacillus subtilis,* and *Bacillus cereus* and in some non-spore forming bacteria *Stenotrophomonas sp*, *Fervidobacterium pannavorans* and *F. islandicum* (Veerabadran *et al.,* 2012). Some species of actinomycetes intensively degrade keratins such as *Streptomyces*, which include *S. fradiae*, *S. pactum* S. *thermoviolaceus* or other actinomycetes such as *Thermoactinomyces* (Veerabadran *et al.,* 2012). The dietary keratinophilic fungus, which uses keratin as a nutrient, shows keratin degradation ability (Rubin *et al.,* 2017). These types of fungi, besides colonizing bird plumage and mammalian hair, also colonize natural habitats where keratin material is available such as places inhabited by birds, humans, and mammals (Rubin *et al.,* 2017). Fungi exhibiting high keratinolytic abilities include the following genera: *Aspergillus, Chrysosporium, Alternaria, Trichuris, Monodictys, Myrothecium, Paecilomyces, Stachybotrys, Urocladium, Scopulariopsis, Curvularia, Cladosporium, Fusarium, Geomyces, Gleomastis, Penicillium* and Doratomyces (Rubin *et al.,* 2017).

Based on the keratinolytic efficiencies, microbes can be divided into two types: True keratinolytic microbes with the ability to solubilize hard keratin structures or potentially keratinolytic microbes, which have strong proteolytic effects thereby solubilizing non-keratin proteins which are associated with hard keratins in keratin-rich material like feathers, nails amd horns. They can also degrade soft keratins as found in callus (Rubin *et al.,* 2017). The secretion of keratinase and proteolytic-keratinase enzymes by several microorganisms such as bacteria, actinomycetes and fungi has been shown to help degrade keratin wastes. Many

species of microorganisms have been isolated from keratin-rich habitats, degrading keratin-containing wastes from various outlets (Veerabadran *et al.,* 2012).

2.3 Microbial Keratinase

Proteolytic enzymes in microorganisms with keratinolytic ability help degrade the disulphide cross linking of keratin polypeptides and break down keratin (Schütz *et al.,* 2018). Bacteria, actinomycetes and fungi can easily remove keratin waste. Keratinases transform keratins into organic fertilizers and other beneficial feedstuffs. Keratinases are extracellular enzymes that work on keratin products. Phenylalanine methanosulphonyl fluoride (PMSF) and other serine protease inhibitors block microbial keratinolytic proteases, mainly serine proteins and sometimes metalloproteinases.The enzymes are either neutral or alkaline proteases requiring Ca^{2+}, as the activity is inhibited in the presence of Ca^{2+} chelating Ethylenediamine tetraacetic acid (EDTA) or ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) (Saarela *et al.,* 2017). Keratinases can hydrolyze both soluble proteins as well as insoluble fibrous proteins (Saarela *et al.,* 2017).

Although microorganisms capable of degrading keratinous substrates are generally isolated from soil and poultry wastes, these microorganisms are almost ubiquitous, thriving under diverse ecological and environmental conditions (Saarela *et al.,* 2017). Keratinase contributes to the valorization of the enormous keratin containing wastes in the form of hair, feathers, dead birds, and animals (Holkar *et al.,* 2018). These enzymes have gained increasing attention due to their biotechnological applications on the valorization of keratinous wastes especially byproducts of agro-industrial processes (Holkar *et al.,* 2018). These enzymes can also be employed for the degradation of prions and β-amyloid fibers (Holkar *et al.,* 2018).

2.4 Valorization of Keratinous Wastes

Microbial keratinases are gradually being recognised biotechnologically for their ability to add value to keratinous wastes. Microbial keratinases have been successfully used in degrading keratin into economically significant keratin protein hydrolysates, which can find potential applications as animal feed supplements, bio-fertilizers, biodegradable glues, films and foils (Holkar *et al.,* 2018). Also, valorization of keratinous waste by keratinolysis finds useful applications in various industries such as elimination of horny epithelial cells that adheres to textile fibers (Textile Industry), clearing obstructions in sewage systems (Waste Water Management Industry), conversion of poultry or agro-industrial wastes into valuable protein products such as amino acids for livestock feed, pharmaceutical, and cosmetic industries (Holkar *et al.,* 2018). The details of the valorization of keratinous wastes are described below.

2.4.1 Agricultural application

The use of chicken feathers as a biofertilizer, an organic fertilizer made from sulphur-containing amino acids found in keratin hydrolysates, has been confirmed (Gurung *et al.,* 2013). Feathers have a high amount of nitrogen (13 %) and can be used as compost or biofertilizer (Pati and Chaudhary, 2015). Nitrogen biofertilizers have been successfully produced from feathers using *Chryseobacterium* bacteria, demonstrating their use for bananas and other crops (Pati and Chaudhary, 2015). Feather hydrolysates, which were produced using thermophilic actinomycetes have been successfully used as a rye fertilizer (Wang *et al.,* 2019).

2.4.2 Biomedical applications

Keratin has biodegradability, biocompatibility, bioreabsorbality, functionality, self-assembly, and mechanical and thermal properties, making it ideal for biomedical applications (Wang *et al.,* 2019). The propensity of extracted keratin to self-aggregate and form 3D structures has enabled it to be used as scaffolds for tissue engineering (Brandelli *et al*., 2015). Nanoparticles prepared from feather keratin exhibited excellent biocompatibility and stability thereby opening up the possibility of controlled drug delivery (Brandelli *et al*., 2015). Also, keratin nanofibers developed by electrospinning is being applied in tissue engineering and regenerative medicine (Brandelli *et al*., 2015). Keratin biomaterials from chicken feathers are found to be capable of supporting cellular attachment as they possess cell-binding motifs, such as glutamic acid, aspartic acid, serine and leucine-aspartic acid-valine binding residues (Ertani *et al*., 2013).

2.4.3 Animal feeds industry

The ability of keratinases to hydrolyze keratins to produce soluble proteins and amino acids is of immense benefits in the development of high grade animal feeds. In a study, Tiwary and Gupta (2012) prepared feather meal by soaking native chicken feather in water and allowed to boil for few minutes before adding the dimeric bacterial keratinase. The feather meal produced was rich in nitrogen and amino acids and showed very remarkable in vitro digestibility. It was reported that the hydrolysate obtained after the fermentation of feather wastes by mixed microbial culture was rich in soluble proteins and essential amino acids including lysine, threonine, leucine, isoleucine, and valine (Vasileva- Tonkova *et al*., 2009). The authors therefore suggested that the hydrolysate could be used in the formulation of

animal feed because of its high nutritional value. Keratinases are essential commercially because they can turn feathers into animal feed (Gopinath *et al.,* 2015).

The production of proteinous hydrolysates of high antioxidative potential from wool waste degradation by a keratinolytic strain of *B. subtilis* was also reported (Fakhfakh *et al.*, 2013). Similarly, bioconversion of feather wastes by *Chryseobacterium* sp.kr6 and *Bacillus* sp. kr16 generated protein-rich hydrolyates that could be used as feed supplement in animal food production (Maciel *et al.,* 2017). The possibility of utilizing feather hydrolysate obtained after feather hydrolysis by nonpathogenic keratinolytic bacterial strains isolated from birds' nest for animal feed formulation due to its high amino acids composition was suggested (Saarela *et al.,* 2017). Similarly, the feather hydrolysate obtained after feather hydrolysis by *B. subtilis AMR* was applied in a blend with cornmeal to produce an extrudate food (Mazotto *et al.,* 2017). Therefore, biodegradation of keratins is an indispensable source of nutrient-rich feeds, nutraceuticals, and feed supplements with lots of promising applications in animal feeds industry. The enhancement of digestibility and nutritional improvement in the supplementation of feeds with keratinases may be an indication of its importance as a key component of animal feeds. It is envisaged that use of keratinases as additives to form essential components of feeds, particularly processed pellets would expand in the future (Mazotto *et al.,* 2017). In addition, the oligopeptides and amino acids obtained from the hydrolysates can veritably serve the purpose of nitrogen supplementation in the formulation and compounding of growth media for the cultivation of microorganisms. Therefore, keratin hydrolysates as complex media components could be cheap sources of nutrients, not only for the growth of microorganisms but also to produce novel bioproducts in fermentation processes (Mazotto *et al.,* 2017).

2.4.4 Cosmetic and pharmaceutical industry

In the pharmaceutical and cosmetic industries, non-collagenolytic keratinases are potential biocatalysts. One thing that gets mentioned very often when talking about hair removal methods is the depilatory ability of keratinases in hair removal solutions, skin lightening agents, and other depilatory substances (Yang *et al.*, 2012). It was found that some crude keratinases can boost hair qualities such as weight, flexibility, brightness, softness, and strength; thus, they could be added as hair care products (Cao *et al.*, 2012). Furthermore, keratinases have shown the ability to degrade hyperkeratosis, a thickened layer of dead skin found in the fingernails and toenails, which serves as a better alternative to the traditional approach of using salicylic acid (Gupta and Ramnani, 2006). Keratinases are capable of peeling skin to remove acne, which is caused by the obstruction of sebaceous glands by keratin (Selvam and Vishnupriya, 2012). Furthermore, the enzyme keratinase had been utilized for enhancing drug delivery through topical administration. It has been documented that the involvement of keratinase aids in improving drug penetration through the nail plate (Adelere and Lateef, 2016).

Because of their ability to degrade prion proteins, they may also be used to clean medical equipment and laboratory apparatus (Liang *et al.*, 2010; Sharma and Gupta, 2016). Keratin, along with other natural polymers including collagen, chitosan, and silk fiber, has been identified as a component of cosmetic blends. Keratin or keratin hydrolysates work with cosmetics, the stratum corneum, and hair cuticles to help the skin maintain moisture. Furthermore, keratin protects the cortex of human cells from heat and chemical damage regularly. The use of hydrolyzed keratin improves skin hydration and elasticity (Sharma and Gupta, 2016). Keratin is an essential part of shampoos and conditioners, hair loss correctors,

and other hair beauty products because of its moisture-retaining properties (Sharma and Gupta, 2016). Hair benefits from protein hydrolysates because they reinforce hair fibers and reduce fiber breakdown. Many plant and animal hydrolysates, such as wheat protein, fur, nails, and horn keratin, have been used as flax tanners to preserve hair colour and retain uniform colour. In the hair care processing industry, protein hydrolysates also serve as regenerators (Sharma and Gupta 2016).

2.4.5 Detergent industry

Due to their ability to degrade insoluble keratin, and properties such as stability at high temperatures and pH, activity over a broad temperature and pH range, stability in the presence of surfactants, oxidizing and bleaching agents, chelating agents, and compatibility with certain toxins, keratinolytic proteases can increase the value of proteolytic enzymes in detergent formulation (Gupta and Ramnani, 2006). A study found that keratinase produced from *Paenibacillus woosongensis* TKB2 got rid of stains like fruit juice and turmeric while safely removing blood and fruit stains from cotton (Paul *et al.*, 2013). It was also found that crude keratinase was safe for removing blood, egg yolk, and chocolate stains from fabrics, and also discovered that refined keratinase was safe for removing milk, soda, coffee, fruit juice, and urine stains from fabrics (Paul *et al.*, 2013).

The keratinase was shown to be relatively stable when treated with EDTA and had stayed in good shape over time; however, when utilizing enzyme beads manufactured from 1.5 percent CMC, a much lower percentage of CMC by weight, the formulation's storage stability was enhanced (Paul *et al.*, 2013). Upon investigating *B. safensis* LAU 13, researchers found significant desiccation of blood-stained cloth achieved in just two hours of incubation (Lateef *et al.*, 2015). The most notable of these is the significance of keratinases as enzymes that may

be used as additives in detergent formulations to remove keratinous by-products in an eco-friendly manner efficiently (Lateef *et al.*, 2015).

2.4.6 Leather and textile industry

In the textile industry, keratinous waste has a lot of potential. Chicken feathers are being researched as a possible replacement for natural and synthetic fibers. It helps to conserve the use of trees in the textile industry. Chicken feathers are durable, sturdy, have a wide surface area, a suitable diameter, and are long-lasting, making them a suitable replacement for natural and synthetic fibers, as well as wood pulp. However, before using the feathers in the textile industry, they must be pre-treated (Ningthoujam *et al.,* 2018). Since keratin can form film and bind, chicken feathers can also be used as a textile sizing agent in textile binding and printing. For the protective coating on the surface of the thread, sizing agents such as starch and polyvinyl has previously been used (Ningthoujam *et al.,* 2018). However, starch use is linked to social and economic issues, and polyvinyl alcohols are costly and non-biodegradable. Chicken feathers have keratin film production capabilities, which can be helpful in cloth binding and printing. In the leather industry, keratin hydrolysates are used for retaining and filling purposes; many leather tanning methods can cause serious health risks such as skin and respiratory problems (Ningthoujam *et al.,* 2018).

2.4.7 Construction industry

Composite materials made of thermoplastic and natural fibers are being investigated in the construction industry; natural fibres are often cellulosic and have enormous strength (Ho *et al.,* 2012). Natural fibers usage reduces the consumption of synthetic polymers, which reduces the consumption of petroleum products (Ho *et al.,* 2012). Feathers can be turned into plastic films, which can be used in packaging and other applications; grafting feather keratin

with acrylic monomers has been shown to enhance the thermoplastic properties of feather-based products (Ho *et al.*, 2012).

2.4.8 Environmental remediation

Keratins ubiquity and low cost have provided the opportunity to test whether keratin from feathers, wool, and other sources could be used as an absorbent to soak up different contaminants to help purify water. The efficacy of a colloidal keratin solution to extract Pb(II) from water was researched, there was a high degree of variability in how Pb(II) was removed from water by the colloidal keratin solution, depending on the conditions used. The removal efficiency ranged from 6.7 % to as high as 87 %. Pb absorption per gram of keratin ranged between 17 % and 43 % (Sekimoto *et al.*, 2013). Based on adsorption isotherms, it was proposed that the monolayer adsorption of Pb(II) on keratin occurred due to the presence of the thiol and amino groups on the surface of the particles. A relatively high amount of absorption was experienced by the keratin particles, around 43.3 mg/g. On the other hand, activated carbon and various biomaterials are all absorbed at about the same rate (Sekimoto *et al.*, 2013). While the Pb(II) removal from solutions containing binary or tertiary metal ions was lower than that of water, removing Pb(II) from solutions containing a mixture of binary or tertiary metal ions was more effective (Sekimoto *et al.*, 2013).

Since chicken feather barbs (fleshy protein) are great at grabbing onto ions, they are used to capture Zn^{2+} ions in both batch and fixed-bed columns (Aguayo-Villarreal, 2011). Feathers have been found to have a higher specific surface area (area under the micrograph) than any other substance used for metal absorption. Due to the lightweight of keratin, it was bonded with polyurethane to form a hybrid membrane. Acid and alkali were applied to the keratin, and the solution was dialyzed before being combined with polyurethane. Several membranes

ranging from 7 to >50 nm in pore size were obtained. Optimal removal of 38 % using alkali-treated feathers was found in the polyurethane membrane with a chromium content of the maximized feather. Chicken feather keratin was removed, and the resultant product was turned into a chromium-absorbing absorbent for use in manufacturing. After being saturated with Cr(VI) solution, the absorbent was left in Cr(VI) solution concentrations of 50-500 mg/L for up to 24 hours for complete absorption to occur. The mechanical strength of the keratin film was 6.2 MPa, and the porosity was 81 %. In contrast to when the initial keratin concentration was set at 50 mg/L, absorption increased from 3.7 to 20.3 mg/g when the initial keratin concentration was increased from 50 to 500 mg/L, however, rising pH above 6 decreased the capacity to pick up additional acid (Aguayo-Villarreal, 2011).

2.4.9 Energy sector

Keratinous wastes are currently being used to produce bio-hydrogen (Queiroga *et al.*, 2012). The initial step is to turn keratinous wastes into a fermentation product rich in amino acids and peptides. In the next step, minerals are added to the product to perform the function of bacto-peptone. To generate bio-hydrogen, the fermented enrichment product is placed in a container and placed in an anaerobic digester containing thermophilic archaea called *Thermococcus* sp. to ferment and produce bio-hydrogen (Queiroga *et al.*, 2012). In addition, chicken feathers contain the appropriate amount of fat; after extracting the fats from feather meal with a solvent, the remaining transesterification catalyst and other products can be used to make biodiesel (Queiroga *et al.*, 2012). Due to the vast supply of chicken feather waste in the world, it is estimated that hundreds of millions of liters of biodiesel can be produced from chicken feather waste globally. This will help decrease the demand for gasoline as well as cut carbon emissions.

2.5 Protein Hydrolysates

Plant growth stimulation and enhanced tolerance to biotic and abiotic stresses have been reported by the application of a variety of protein-based products. These plant stimulatory effects appear to be distinct from the nutritional effect of an additional nitrogen source (Ertani *et al*., 2009). Protein-based products can be divided into two major categories which are: protein hydrolysates and amino acids. Protein hydrolysates are a mixture of peptides and amino acids of animal or plant origin and individual amino acids such as glutamine, proline and glycine betaine. Protein hydrolysates are prepared by enzymatic, chemical or thermal hydrolysis of a variety of animal and plant residues, including animal epithelial or connective tissues (Ertani *et al.*, 2009), animal collagen and elastin (Cavani *et al*., 2006), carob germ protein (Parrado *et al*., 2008), alfalfa residue (Schiavon *et al*., 2008; Ertani *et al*., 2009), wheat-condensed distiller solubles (Ertani *et al*., 2009), Nicotiana cell wall glycoproteins (Schiavon *et al*., 2008), and algal protein (Ertani *et al*., 2009). Protein/peptide and free amino acid contents of the hydrolysates vary in these preparations in the range of 1–85 % (w/v) and 2–18 % (w/v), respectively.

The primary amino acids include alanine, arginine, glycine, proline, glutamate, glutamine, valine, and leucine. Siapton contains a high proportion of proline and glycine, while in carob germ hydrolysate, glutamine, and arginine predominate (Parrado *et al*., 2008). Non-protein components present in these hydrolysates may also contribute to the stimulatory effects on plants. For example, in addition to proteins, peptides and free amino acids, Carob germ extract hydrolysate, a type of protein hydrolysate, contains fats, carbohydrates, macro and micronutrient elements and at least six phytohormones, while an animal-based product, Siapton, another protein hydrolysate has a similar profile of proteins, amino acids, fats, and

macro and micronutrients, but lacked carbohydrates and phytohormones (Parrado *et al*., 2008). The alfalfa hydrolysate contains more free amino acids (at 1.9 %), vitaminutes, macro and micronutrient (Schiavon *et al.,* 2008). It was found that triacontanol and indole acetic acid (IAA) were present in the Alfalfa hydrolysate product and IAA was also present in Meat hydrolysate, Alfalfa hydrolysate may be employed as a source of IAA in food (Ertani *et al.,* 2013) . According to, the lipid-soluble fraction of the product, it was found to produce 2 % plant-available nitrogen, 21.3 % free amino acids, peptides, nucleotides, and fatty acids, and 14.8 % unknown organic matter (Kauffman *et al.,* 2007).

2.5.1 Effects of protein hydrolysates on plant nutrient uptake and yield

Early studies outlined by Maini (2006) include the use of the first protein hydrolysate derived from animal epithelial tissues, which was called "Siapton." Maini (2006) described that maize grains (which were grown under poor conditions and treated with Siapton and Mg) exhibited higher N, P, K, and Mg content than those observed in grains treated with Mg alone. Increased plant height and the number of flowers per plant were recorded for tomato plants fertilized with Siapton and tomato plants fertilized with Carob germ hydrolysate. However, while the plants' heights and the number of flowers were more significant for the Siapton and Carob germ hydrolysate-fertilized plants, the number of fruits per plant was increased only in the case of the Carob germ hydrolysate-fertilized plants after 18 weeks in a greenhouse (Parrado *et al.,* 2008).

Applying Amino16R, a protein hydrolysate made up of eleven-percent L-amino acids, to greenhouse tomatoes led to an increased yield (Koukounaras *et al.,* 2013). There was no effect of Aminoplant (Siapton) on Spinach (*Spinacia oleracea*) or Endive (*Chicorium premium*) yield in any study (Gajc-Wolska *et al.*, 2012). In one year out of three years of

field trials, only one variety had an effect on carrot (*Daucus carota*) yield, and that soluble sugars and carotenoids increased while nitrate content decreased in amino plant-treated carrot roots compared to the control (Schiavon *et al.,* 2008). Hydroponically-grown maize plants with an alfalfa hydrolysate had increased leaf growth, foliar sugar content and lower nitrate levels (Schiavon *et al.,* 2008). However, studies on the effects of alfalfa hydrolysates on the short-term growth of hydroponically-cultivated maize in the absence or presence of salt stress (NaCl) found they significantly increased growth in both the absence and presence of NaCl (Ertani *et al.,* 2013).

In another instance of hydroponically-grown maize seedlings, which were treated with a meat hydrolysate derived from tanning residues, which caused an increase in short-term growth and micro-element content and a decrease in nitrate, phosphate and sulphate contents, tanning residues treated seedlings had higher initial growth and micronutrient content and lower nitrate, phosphate and sulphate contents (Ertani *et al.,* 2013). Amino acids play a significant role in regulating nitrogen acquisition by roots. The exogenously-applied glutamine, in particular, decreased the nitrate and ammonium influx as well as the transporter transcript in barley roots (Sharma and Gupta 2016). Two biofertilizers, one of which was made from alfalfa plants (AH) and the other from red grapes (RG), were analyzed using enzyme-linked immunosorbent assays, Fourier transforms infrared (FT-IR), and Raman spectroscopies. Two applications (50 and 100 mL of "biofertilizer" for RG and 25 and 50 mL of "biofertilizer" for AH) of the biofertilizers mentioned above were applied to *Capsicum chinensis L*. vegetables that are grown in indoor garden pots inside a tunnel. The two different biofertilizers used different concentrations of indole acetic acid and isopentenyl adenosine, which resulted in two different AH spectra and one RG spectrum. In this case, the different

spectra revealed differences in peptidic structure, with amino acid functional groups in the biofertilizer and polyphenols in the plant fertilizer (Ertani *et al.*, 2014).

These findings showed that at flowering, RG and AH increased the fresh weight of leaves and fruits and the number of green fruits. However, the biofertilizers affected leaves and fruits only by increasing their new weight and red fruits at maturity. While the biofertilizer-treated plants were still at the early stages of development, they had significant levels of epicatechin, ascorbic acid, quercetin, and dihydrocapsaicin present. At maturity, the plants displayed considerable levels of fructose, glucose, chlorogenic, and ferulic acids. Green fruits contain higher levels of chlorogenic acid, hydroxybenzoic acid, p-coumaric acid, and antioxidant activity. In contrast, red fruits, which were treated with AH and RG, were highly enriched in capsaicin. To investigate whether both fructo-oligosaccharides (FOS) and high molecular weight compounds (HMWCs) were produced in red fruits, HRMAS-NMR experiments were ran on them. The results show that both FOS and HMWCs have significant amounts of $NADP^+$, and also FOS and HMWC increase fumarate, ascorbate, glucose, thymidine, and other high molecular weight compounds. In these findings, AH and RG are shown to lead to increased plant growth and the development of secondary metabolites, such as phenols.

2.5.2 Hormone-like activity of protein hydrolysate

Alfalfa protein hydrolysate with gibberellin and auxin-like activity might help increase plant N nutrition in maize (*Zea mays*) treatment with 0.01 or 0.1 mg/l protein hydrolysate for 48 hours (Schiavon *et al.*, 2008). The study indicated that plants experienced more significant growth and an increase in leaf sugar accumulation in response to the treatment. As

hydrolyzed proteins supplied to plants were made available, the operation of a variety of enzymes involved in carbon metabolism (malate dehydrogenase, MDH; isocitrate dehydrogenase, IDH; citrate synthase, CS) and N reduction and assimilation (nitrate reductase, NR; nitrite reductase, NiR; glutamine synthetase, GS; glutamate synthase, GOGAT; aspartate aminotransferase, AspAT) increased significantly. In a rooting experiment with tomato cuttings, the protein hydrolysate was found to have an auxin-like effect. Tomato plants treated with protein hydrolysate at 6mg/L had significantly higher shoot, dry root weight, and root length than untreated plants by 21, 35, 24, and 26 % respectively (Colla *et al.,* 2014).

2.5.3 Influence of protein hydrolysate on plant physiology and metabolism

Proteins that have been broken down into their peptide and amino acid components have been shown to improve the use of carbon and nitrogen and help nitrogen assimilation. Maini (2006) found that Siapton increased the activity of NAD-dependent glutamate dehydrogenase, nitrate reductase, and malate dehydrogenase in maize. An alfalfa protein hydrolysate applied to hydroponically-grown maize increased the activity of three tricarboxylic acid cycle enzymes (malate dehydrogenase, isocitrate dehydrogenase and citrate synthase) and five N reduction and assimilation enzymes (nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase and aspartate aminotransferase) (Schiavon *et al.*, 2008).

In a study comparing the effects of alfalfa (*Medicago sativa*) hydrolysates and meat flour on maize seedling development, it was discovered that increased glutamine synthase (GS) and nitrate reductase (NR) activities in the leaves and roots when compared to the control. GS1 isoforms, which are involved in the assimilation of ammonia from nitrate reduction, rose

higher, while GS2 isoforms which are essential for nitrogen assimilation increased. This confirmed the stimulating effect of the hydrolysates on nitrogen assimilation (Ertani *et al.,* 2009). Hydrolyzed proteins could increase nitrogen assimilation in plants through coordinated C and N metabolism regulation. A prominent case is the protein hydrolysate obtained from alfalfa plants, which resulted in significant increases in biomass yield, increased sugar accumulation, and nitrogen assimilation in hydroponically-grown maize plants (Schiavon *et al.,* 2008). This research revealed that the same biofertilizer was beneficial to maize plants cultivated under salinity stress by increasing the ratio of sodium and potassium in leaves and the synthesis of flavonoids (Ertani *et al.,* 2013).

2.5.4 Protein hydrolysates and stress tolerance

Protein hydrolysates and specific amino acids such as proline, betaine, their derivatives, and precursors have been shown to induce plant defence responses and increase plant resistance to a range of abiotic stresses such as salinity, drought, temperature, and oxidative stress (Ertani *et al.,* 2013). It was found that perennial ryegrass plants treated with with a product-based protein and subjected to sustained high temperatures had increased photochemical efficiency and membrane thermostability compared to untreated plants. These findings were consistent, demonstrating that foliar applications of protein hydrolysates would improve plant tolerance to heat stress (Ho *et al.,* 2012). An amino acid/peptide/sugar mixture extracted from plant cell walls increased the resistance of cucumber plants to oxidative stress by inducing the expression of three stress marker genes and two genes involved in the oxidative stress response in *Arabidopsis* plants (Apone *et al.,* 2010). It was found out that an alfalfa hydrolysate applied to maize grown hydroponically under increasing salt stress increased plant biomass reduced antioxidant enzyme activity but increased leaf proline and flavonoid content (Ertani *et al.,* 2014).

Proline and glycine betaine, an N-methyl-substituted glycine derivative, serve as osmolytes, protecting proteins, enzymes, and membranes from denaturing effects of high salt concentrations and non-physiological temperatures (Ashraf and Foolad, 2007). Glycine betaine and proline accumulation have been linked to improved stress tolerance in various higher plants. Exogenous application of these compounds has been shown to improve tolerance to abiotic stresses in these plants, including maize, barley, soybean, alfalfa, and rice (Chen and Murata, 2008).

Glycine-betaine, and proline have been shown to scavenge reactive oxygen species and induce the expression of salt stress-responsive genes as well as genes involved in transcription factors, membrane trafficking, and reactive oxygen species, in addition to their functions in protein and membrane stabilization (Kinnersley and Turano, 2000). Other amino acids affect the ability to withstand biotic stresses since glutamate and ornithine, which are precursors to proline, aid in salt tolerance (Da Rocha *et al.,* 2012). Arginine is involved in nitrogen assimilation and transport, but a study found that arginine levels are significantly higher in response to biotic and abiotic stress (Kinnersley and Turano, 2000). Plants exposed to heavy metals, such as lead, for a prolonged period are likely to develop resistance to those metals. Proline accumulation is typical in plants subjected to heavy metal stress. Some metal-tolerant plants have an elevated constitutive proline content even when metal concentrations are below the saturation limit (Sharma and Dietz, 2006).

2.5.5 Effects of protein hydrolysate on plant microbiome

Since plant-associated microbes are being increasingly recognized for their ability to enhance plant fitness by altering physiological and developmental processes, they have become widespread in agriculture (Philippot *et al.,* 2013). Most of these interactions occur in the

rhizosphere, a microscale area of soil influenced by plant roots. Additionally, interactions happen in the phyllosphere, a macroscale area of the earth that encompasses plant leaves. These ecosystems are perfect environments for the organic molecules found in protein hydrolsates (PHs) to serve as carbon, nitrogen, and energy sources for the microorganisms. As a result, alterations in the composition and behavior of plant microbiomes caused by PHs may be yet another mechanism by which the increased crop production by these products can be attributed.

Often, microorganisms, such as bacteria, appear to be the most prevalent organisms in phyllosphere communities. Particularly highlighted in this regard are the Proteobacteria, Firmicutes and Actinobacteria which commonly dominate the plant phyllosphere, with *Methylobacterium, Pseudomonas* and *Sphingomonas* among the most common bacterial genera at the leaf level in *Arabidopsis thaliana*, soybean and grapevine (Delmotte *et al.,* 2009). It was also observed that *Methylobacterium*, *Pseudomonas*, and *Sphingomonas* are among the most prominent bacterial genera in soybean and grapevine (Delmotte *et al.,* 2009). While the beneficial or detrimental effects of root-associated microbes have received considerable attention (Berendsen *et al.,* 2012; Bulgarelli *et al.,* 2013; Mendes *et al.,* 2013; Philippot *et al.,* 2013; Berg *et al.,* 2016), little is known about the effects of epiphytes on plant health and productivity. Root-associated microbes are the other group of epiphytic microbes that have been shown to promote plant growth by the production of hormones (Wu *et al.,* 2009) or volatile organic compounds (VOCs) (Ruzzi and Aroca, 2015) or by biotransformation or de novo biosynthesis (Schulz and Dickschat, 2007). The functions above also mean that certain epiphytic microbes may inhibit plant pathogens, either by inducing pathogen resistance or producing antimicrobial compounds.

When a specific strain of *Pseudomonas* and a *Bacillus* strain are added to the root zone of the pepper plant, then the amount of VOCs emissions and the number of phenolic compounds synthesized increased (Del Rosario Cappellari *et al.,* 2017). On the other hand, a reduction in phyllosphere microbial communities resulted in a decrease in a variety of metabolites, including citraconic acid, acetyl-CoA, isoleucine, as well as secondary compounds, including terpenes and phenols (Gargallo-Garriga *et al.,* 2016).

In conclusion, effective plant productivity gains can be attained when beneficial plant-microbiome connections are developed and maintained in the rhizosphere and phyllosphere. Nevertheless, a better understanding of the various environmental factors regulating these critical plant-associated ecosystems must be understood for this to be a possibility. Since amino acids make up a large part of PHs, it may be possible to alter their composition and thus the structure and behavior of rhizosphere microbial communities. Individual microbial taxa have been shown to have different preferences for particular amino acids (Moe, 2013), adding to the idea that PHs may be specifically formulated to help specific microbial taxa. As opposed to the rhizosphere, which is thought to be an excellent incubator for copiotrophic species capable of using labile carbon compounds released from plant roots, the phyllosphere is believed to be oligotrophic, with a minimal amount of available nutrients, mainly carbon (Vorholt, 2012).

The phyllosphere is expected to be a more temporary or short-lived ecosystem compared to the rhizosphere, with the microbes here subjected to more stressful environmental conditions. When plants shed their leaves, water within the plant cannot evaporate or escape; and when exposed to UV light or arid conditions, microbes that live on the leaves are exposed to radiation that can harm them (Vorholt, 2012). Additionally, during intense rainfall events,

phyllosphere microbes are exposed to the microorganisms that inhabit the soil. Although phylloplankton diversity in the phyllosphere is good, the diversity relative to the rhizosphere and bulk soil is poor, with 70 % of phylum representation attributed to Alphaproteobacteria, the remainder is shared primarily between Gammaproteobacteria, Bacteroides, and Actinobacteria (Vorholt, 2012).

Since microbial communities are more in the rhizosphere, it may be challenging to regulate the microbiome of the rhizosphere using PHs, which means it may be more feasible to attempt to monitor the microbiome of the phyllosphere using PHs. However, as in the rhizosphere, there is a wide range of microbial distributions throughout the phyllosphere, and several of the microbes seem to cluster around spots where plant nutrients leak out, for example, at the stomata and base of trichomes (Lindow and Brandl, 2003; Vorholt, 2012). Researchers have used DNA sequencing to analyze bacterial diversity on a leaf to detect and determine how much variation exists. It was discovered that the microbial composition also varies based on plant types, genetic characteristics, developmental stage, and location on the leaf, possibly due to variations in surface appendages and plant leachates (Lindow and Brandl, 2003; Vorholt, 2012; Ortega *et al.*, 2016).

Chemotaxis and the ability to create biofilms, as well as pigments that help in UV tolerance, are expected to be key traits for supporting microbial life in the phyllosphere (Lindow and Brandl, 2003; Vorholt, 2012). The ability of rhizosphere microbes to produce antagonistic compounds is also expected to be important to the capability of taxa to compete for nutrients and space. Due to this, it was found that 58 % of the microbes collected from the phyllosphere were capable of producing VOCs and interfering with pathogen development (Ortega *et al.*, 2016). The capability for microbes to synthesize IAA appears to be widely dispersed

throughout the phyllosphere. It may be an essential component in promoting microbial colonization and helping microbes overcome water stress (Lindow and Brandl, 2003). Once the synthesis of IAA has taken place, there is evidence that microbial synthesis of IAA can increase the availability of nutrients by loosening cell walls, thus releasing saccharides from plants (Lindow and Brandl, 2003; Vorholt, 2012). Finally, the ability of phyllosphere microbes to produce surfactants appears to be a critical trait for them to withstand drought stress (Lindow and Brandl, 2003; Vorholt, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the Federal University of Technology, Minna, Niger State. The state is located in the North Central geopolitical zone of Nigeria and covers a landmass of 76,363 square kilometers. It lies between Latitude 8°.00-11°.30'N and Longitude 4º.00-8.00'E (Figure 3.1).

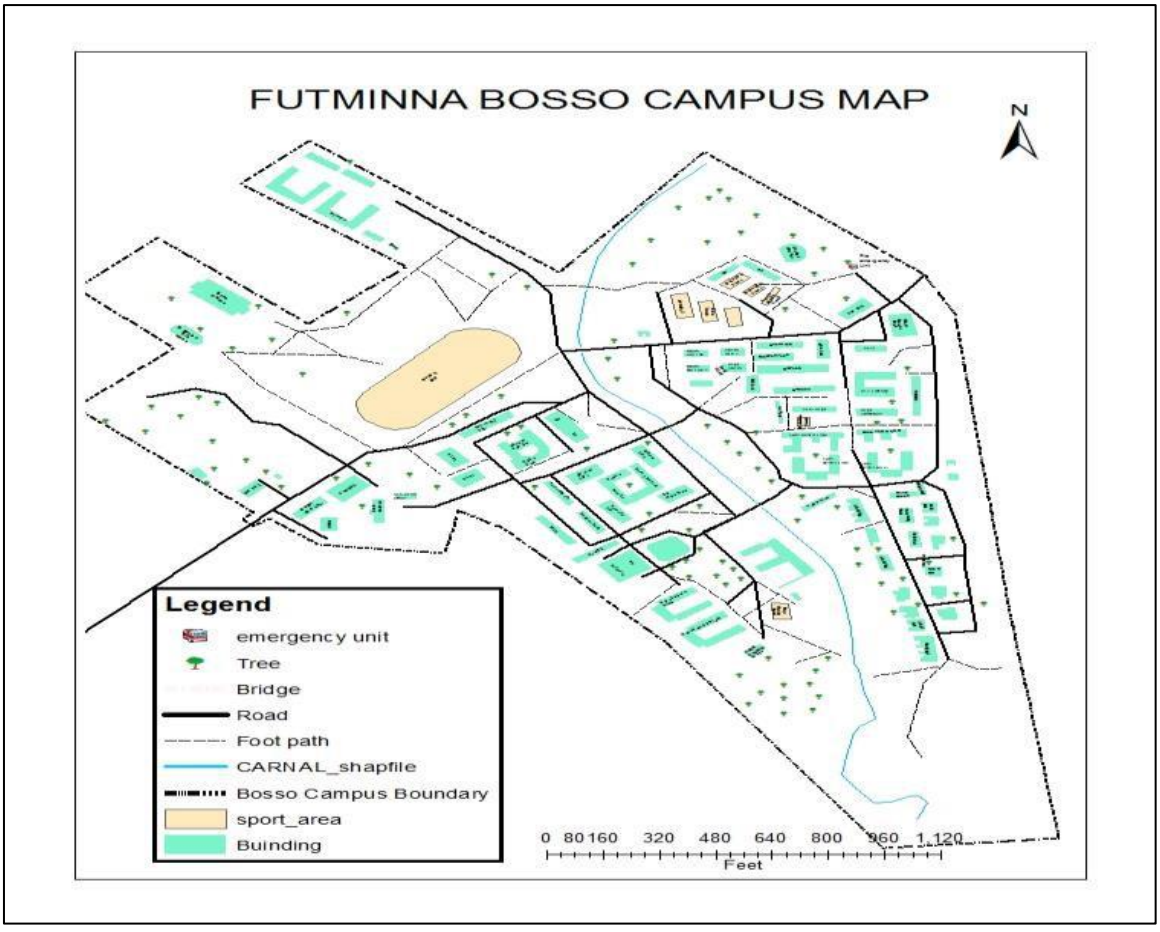


Figure 3.1 Map of the Study Area Odekunle *et al.* (2018)

3.2 Feather Sample Collection

The chicken feathers were collected from the feather dumpsite in Kure market, Minna, Niger State. The feathers were washed repeatedly with water and then dried for a period of 2 days and were processed to 60-mesh particle sized powders, as described by (Lateef *et al.*, 2015). The powders were kept at ambient temperature and used for further studies.

3.3 Sources of the Seeds

Abelmoschus esculentus and *Telfaria occidentalis* were purchased from local dealers in Kure Market for the study.

3.4 Isolation of Keratin Degrading Bacteria

To isolate feather-degrading bacteria, 1 g of soil sample was dissolved in 250 mL of sterile saline water and the solution was thoroughly mixed by shaking. Then, 1 mL of the solution was serially diluted up to 10^{-10}. The diluted samples were individually spread on yeast extract agar plates for the selective isolation of spore forming bacteria, nystatin was added to prevent the growth of fungi. The plates were incubated at 37° C for up to 48 hours. The plates were then examined for the appearance of colonies. Colonies with a comparatively larger size were selected for further study. The pure isolates were then grown in keratin agar medium of 0.05 g of feather, 0.03 g of K_2HPO_4, 0.04 g of $MgS0_4$, 0.05 g of Nacl, 0.01 g of $CaC0_3$, 1.5 g of agar and incubated at 37°C. The pure cultures were stored on yeast extract agar slants at 4 °C until needed (Lateef *et al.*, 2015).

3.5 Identification of the Selected Bacterial Isolates

Bacteria isolates were identified presumptively on the basis of the following features: colonial morphology, Gram-staining and biochemical tests. The biochemical test carried out were: urease, motility, starch hydrolysis, catalase and citrate. The bacterial isolates were

identified by comparing their characteristics with those of known taxa using the Bergey's manual of systematic microbiology (Brenner *et al*., 2005).

3.5.1 Gram staining

A smear of the pure isolate and distilled water was made on a clean and sterile glass slide, and allowed to air dry. After it was heat-fixed, the smeared organism remained permanently on the glass slide. The fixed smear was stained with crystal violet for 60 seconds. It was washed with distilled water, iodine was applied on the slide as a mordant for 1 minute, then washed with 95 % alcohol and washed with distilled water. Lastly, safranin was applied on the slides and allowed to stay for 30 seconds, then washed with distilled water and air dried. The dried stained slides were viewed under the microscope for the bacterial morphology using the oil immersion lens (Anubrata and Rajendra, 2014).

3.5.2 Motility test

This test was to identify isolates that were motile and non-motile. A semi-solid nutrient medium was prepared in test tubes and autoclaved at 121 ℃ for 15 minutes and allowed to cool. A straight sterile needle was used to pick a colony of a young 24 hour culture growing on agar medium. The semi-solid medium was stabbed with the needle to a depth of only 1/3 inch in the middle of the tube. Extra caution was taken to keep the needle in the same line it entered as it was removed from the medium. The test tubes were incubated at 37 °C and examined daily for up to 7 days. The test tubes were observed for non- diffused (Non-motile) and diffused zones of growth (Motile) flaring out from the line of inoculation.

3.5.3 Catalase test

This test was carried out to differentiate between organisms that are capable of catalase production and organisms those that can not. A sterile inoculating loop was used to remove some colonies from the 24 hour test organism and smeared on a drop of 3 % hydrogen

peroxide (H_2O_2) solution. Formation of bubbles indicate a positive catalase result, but no bubbles indicate a negative result. This procedure was carried out on all isolates (Cheesbrough, 2006)

3.5.4 Starch hydrolysis

This test was to determine the ability of the bacterial isolates to hydrolyse starch and differentiate the isolates based on their α- amylase enzyme activity. The starch medium was sterilised in an autoclave at 121 ℃ for 15 minutes, and allowed to cool. After cooling, it was poured into petri dishes and allowed to solidify. Each plate was inoculated aseptically with pure cultures and incubated at 37 ℃ for 24 hours. After incubation, the surface of the plates were flooded with iodine solution with a dropper for 30 seconds and the excess iodine was poured off. A clear zone was formed around the line of growth after the addition of iodine solution, this indicated that the organism had hydrolysed starch.

3.5.5 Urease test

This test was to determine the presence of urease enzyme activity in the isolates. Urea solution and Urea broth were sterilised in an autoclave at 121 ℃ for 15 minutes. The medium and solution were allowed to cool to 40 ℃. At 40 ℃ the urea solution was turned into the urea broth and dispensed into test tubes. When cooled, each test tubes were inoculated with the isolates and incubated at 37 °C for 24 hours. The change of colour of the broth from yellow-orange to bright pink indicates a positive result, no colour change indicates a negative result (Cheesbrough, 2006).

3.5.6 Citrate test

This test was conducted to identify the presence of enterobacteria and the ability of the organism to use citrate as a source of carbon. Simmons' citrate agar medium was sterilised

at 121 ℃ for 15 minutes in an autoclave. The slants were inoculated and incubated at 37 °C for 24-hours. A positive slant was indicated by a change in colour from green to blue while a negative slant had no colour change (Cheesbrough, 2006).

3.5.7 Indole test

This is a biochemical test employed to distinguish between closely related bacteria species. It aids in the identification of bacteria species capable of hydrolysing the amino acid tryptophan *via* the expression of the enzyme trytophanase, releasing the indole ring of the amino acid. The organisms capable of expressing this trytophanase are classified as positive to indole (characterized by the formation of a pink to red ring on the surface of the inoculated medium (tryptophan broth) after the addition of Kovac's reagent), while the ones that are not capable are said to be negative to indole.

In this study, the bacteria isolates for test were aseptically inoculated into 5 mL of sterile peptone broth and aerobically incubated for 48 hours at 37 °C. The Kovac's reagent was prepared by the dissolution of 5 g of dimethyl amino-benzaldehyde in 75 mL of amyl-alcohol and 25 mL of concentrated hydrochloric acid (HCl). After the incubation period, the Kovac's reagent was added in a drop-wise manner to each cultured broth and shaken gently. Observations were made on the cultures and the findings recorded (Cheesbrough, 2006).

3.5.8 Oxidase test

This test is employed to identify and differentiate microorganism containing a protein called cytochrome c, which is functionally involved in the electron transport chain of the mitochondria during aerobic respiration, through the production of an intracellular enzyme called cytochrome c oxidase. In this study, 3 drops of the oxidase reagent was aseptically placed on a sterile filter paper, the test bacteria isolates were smeared on the wet filter paper

using sterile glass rod. Observations were made and the findings were recorded (Cheesbrough, 2006).

3.5.9 Carbohydrate utilization test (production of acid and gas from carbohydrate)

This test is carried out to separately test for the ability of a bacterium to aid the breakdown of sugars like glucose, sucrose, mannose, maltose, lactose, and fructose, and also their pattern of fermentation so as to differentiate it from other species. Gas and/or acid production is an indication of a fermentation reaction; acid production is indicated by a colour change (from red to yellow) of the phenol red (serving as the indicator) in the test medium, while gas production is indicated by the gaseous accumulation in the Durham tube used.

In this study, bacteria isolates were inoculated aseptically into the test tubes containing sterile phenol red-peptone-sugar broth (phenol red peptone broth containing sucrose, fructose, lactose, D-glucose, D-mannitol, arabinose, sorbitol, D-mannose) and Durham tubes, except the tubes that served as control for all the different peptone-sugar broth. The inoculated broths were then aerobically incubated at 37 °C for a period 24-48 hours. After incubation, observations were made of broth and Durham tubes and the findings recorded (Cheesbrough, 2006).

3.5.10 Methyl red and Voges-Proskauer test

This test is carried out for the identification of bacteria species based on their ability to produce sufficient acid during glucose fermentation. This reaction involves the conversion of glucose into pyruvate and a further metabolic reaction through the mixed acid pathway for the production of a stable acid (which is dependent on the microbial species involved), lowering the pH of the test medium (methyl red-Voges-Proskauer broth), and changing the colour from yellow to red upon the addition of methyl red. The Voges-Proskauer test is

employed for the determination of an organism's ability to produce acetoin during the fermentation of glucose. This is carried out by the addition of alpha-naphthol reagent which functions to convert the acetoin into diacetyl when in the presence of atmospheric pressure and then condenses with quinidine, giving the test broth a pinkish red colouration.

In this study, the isolates were aseptically inoculated into 2 test tubes containing 2 ml of sterile glucose-phosphate-peptone broth, and incubated 37 °C. Four (4) drops of methyl red was then added to the cultured broth using Pasteur's pipette, and was gently shaken to allow proper mixing. Observations were then made and the findings recorded. In addition, in the 2 other cultured VP broth, 1 milliliter of 40 % potassium hydroxide (KOH) solution and 3ml of 5 % alcoholic alpha-naphthol was aseptically added and was shaken properly, it was left to stand for 240 seconds. Observations were made and findings were recorded (Cheesbrough, 2006).

3.6 DNA Extraction Protocol

Pure colonies grown on nutrient agar plate were transferred to 1.5 mL of nutrient broth and cultures were grown on a shaker for 48 hours at 28 ºC. After which the cultures were spun using a centrifuge at 4600 xg for 5 minutes. The resulting pellets were re-suspended in 520 µL of Tris Acetate-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Fifteen microliters of 20 % Sodium Dodecyl Sulphate (SDS) and three microlitres of Proteinase K (20 mg/mL) were added. The mixture was incubated for 1 hour at 37 ºC, and then 100 mL of 5 M NaCl and 80 µL of a 10 % CTAB solution in 0.7 M NaCl were added and mixed. The suspension was incubated for 10 minutes at 65 ºC. After which, it was immediately removed and kept in ice bucket for 15 minutes. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 minutes and centrifugation at 7200 xg for 20 minutes. The aqueous phase was transferred to a new tube, isopropanol (1: 0.6) was added

and DNA was precipitated at –20 ºC for 16 hours. The DNA was collected by centrifugation using a centrifuge at 7200 rpm for 15 minutes, then washed with 500 µL of 70 % ethanol, air-dried at ambient temperature for three hours and finally dissolved in 50 µL of Tris Acetate-EDTA buffer (Frank *et al.,* 2008).

3.6.1 Polymerase chain reaction (PCR)

The cocktail of preparation for PCR sequencing comprised of 10 µL of 5x GoTaq colourless reaction, 3 µL of 25mM $MgCl_2$, 1 µL of 10mM of dNTPs mixture, 1 µL of 10pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and -1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water and 8 µL DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile comprising of an initial denaturation for 5minutes at 94 °C, followed by a 30 cycles consisting of 94 °C for 30 seconds, 50 °C for 60 seconds and 72 °C for 90 seconds; and a final termination at 72 °C for 10 minutes. The mixture was allowed to cool to 4°C (Frank *et al.,* 2008).

3.6.2 Integrity check of amplified gene

The integrity of the amplified gene fragment was checked of 1.5 Mb gene fragment was checked on a 1 % Agrose gel. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5 % agarose gel. The suspension was heated for 5 minutes in a microwave. The molten agarose was then allowed to cool to 60 °C and stained with 3 µL of 0.05 g/mL ethidium bromide (which absorbed invisible UV light and transmited the energy as visible orange light). A comb was placed into the casting tray slots and the molten agarose was transfered into the tray. To form the wells, the gel was allowed to stand for 20 minutes in order to solidify. The 1XTAE buffer was poured into the gel tank to barely cover the gel. Two microliter (2 µL) of 10X blue gel loading dye (which gave colour and density to the

samples to make it easy to load into the wells and keep tab on the progress of the gel) was added to 4 µL of each of the PCR product and loaded into the wells after the 100 bp DNA ladder was loaded into well 1. The gel was electrophoresed for 45 minutes at 120 V, visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were determined by comparison with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel (Frank *et al.,* 2008).

3.6.3 Purification of amplified product

The purification of the amplified fragments with ethanol in order to remove the PCR reagents, debris and other reagent are described as follows; 7.6 µL of sodium 3M acetate and 240 µL 95 % ethanol was added to each fragment of the PCR amplified products in a new sterile Eppendorf tube. The mixture was vortexed for 5 seconds and kept at – 20 °C for 30 minutes and thereafter centrifuged for 10 minutes at 13000 xg and kept at 4 °C. This was followed by removal of the supernatant (by inverting the tube on trash once). The pellet was washed by adding 150 µL of 70 % ethanol, mixed and then centrifuged in a centrifuge for 15 minutes at 7500 rpm at 4 °C. The supernatant was decanted and the tubes were inverted on blotting paper and was allowed to dry in the fume hood at room temperature for 15 minutes. The supernatant was then resuspended in 20 µL of distilled water and kept in a refrigerator at -20 °C prior to sequencing. The purified fragment was checked on a 1.5 % Agarose gel, ran on a voltage of 110 V for 1 hour to confirm the presence of the purified product before sequencing using a nanodrop of model 2000 (Frank *et al.,* 2008).

3.6.4 Sequencing and blasting

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer (Applied Biosystems, USA), while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis.

3.7 Physicochemical Properties of the Experimental Soil

The soil samples were air-dried for 72 hours. The air-dried samples were sieved using a 2 mm mesh. Parameters such as pH, Electrical conductivity, Organic Carbon, Phosphorus, Sodium (Na), Potassium (K), Calcium (Ca) and Magnesium (Mg) present in the soil were determined. The estimation of these parameters is explained below.

3.7.1 pH

The pH of the soil was determined using Soil pH in H_2O (1:1) and Soil pH in 1 M $CaCl_2$ (1:1) method. This experiment was carried out by weighing 10 g of the soil into an extraction cup dispensed with 10mL of distilled water. It stood for 15 minutes before mixing on a mechanical shaker for 30minutes at 150rpm**.** After standing for 10 minutes, the pH meter was standardized using buffers 7.0 and 4.0. After recording the pH in H_2O_2 a drop of 1M $CaCl_2$ solution was added to the soil water suspension, stirred for 15 minutes and stood for 25 minutes. The pH value of the soil was standardized on the pH meter using buffers 7.0 and 4.0, then the pH was read using the pH meter (Boraste *et al.*, 2009).

3.7.2 Organic carbon

Determination of organic carbon is a complete Oxidation method. The soil sample was grounded further to pass through a 0.05 mm sieve before weighing. 500 mg of the soil was weighed into a 50 mL digestion tube, and a standard sucrose solution was prepared with 1

mL added into 5 digest tubes. 5 mL of Potassium dichromate ($K_2Cr_2O_7$) solution and 10 mL of concentrated H_2SO_4 was added to the soil and standard tubes, closed with a rubber stopper, and swirled on a vortex mixer until the soil sample was completely dispersed. The tubes were placed in a digestion block preheated to 150 ^oC for 30 minutes and allowed to cool, diluted to 50 mL, mixed, and let to stand overnight. The readings of the standards and sample were taken on a spectrophotometer with a 1cm cell at 600 nm and using a wavelength of 600 nm. According to the Heanes (1984) method, the standards comprised 0, 2.50, 5.00, 7.50, and 10.00 mg of carbon. The percentage of organic carbon present in the soil and the amount of organic carbon present in the soil are computed from the acquired result using the formula below, which is then used to calculate the quantity of organic carbon present in the soil.

$$\%\, OC \;=\; mg\; C \div mg\; of\; soil\; X\; 100$$

3.7.3 Nitrogen analysis

The nitrogen analysis was conducted by three process methods, Digestion, Neutralisation and Titration. To digest the soil, 200 mg of the soil sample was measured into a 50 mL digestion flask, 5 mL H_2SO_4 (Tetraoxosulphate VI Acid) and 5 mL of H_2O_2 were added and kept in a digestion chamber for 24 hours. After 24 hours, the digested sample was transferred to a 100 mL volumetric flask which was filled up to the mark with distilled water. 10 mL of 40% NaOH (Sodium Hydroxide) was added to the volumetric flask to neutralise the solution to alkaline, a receiving flask containing 10 mL boric acid (As an indicator) was attached to the volumetric flask. After the addition of NaOH, nitrogen was released in the form of ammonia and collected in the receiving flask turning boric acid green. The ammonium borate solution was titrated against H_2SO_4. The percentage of nitrogen in the soil was calculated using the formula below (Boraste *et al.*, 2009) : -

$$\%\ nitrogen\ =\ Burette\ reading\ x\ 0.02\ x\ 0.014\ x\frac{100}{10}x\frac{100}{0.02}$$

3.7.4 Phosphorous

Stock standard solution (20 ppm Phosphors) - Reagent grade potassium di-hydrogen phosphate (KH_2PO_4) was dried at 105 ^oC for 2 hours and cooled in a desiccator. 0.0879 g of KH_2PO_4 was weighed and dissolved in distilled water and diluted to 1000 mL. It was mixed by shaking.

Standard X Curve- 0, 1, 5, 10, 15 & 20 mL was pipetted out of the stock solution and each was kept in 50 mL volumetric flask. 10 mL of vanado-molybdate reagent was added to make up the volume to the mark with distilled water. This resulted to 0, 5, 10, 15 & 20 ppm of phosphorus. The intensity was read using a colorimeter at 470 nm wavelength

Procedure - The estimation of phosphorous content in the soil began with digestion of the soil. The soil was digested in similar procedure as in nitrogen analysis. After 24 hours of digestion, the digested soil was transferred to a 100 mL volumetric flask which was filled up to the mark with distilled water. 10 mL of the sample aliquot and vanando-molybdate reagent was pipetted into a 50 mL volumetric flask and diluted to 50 mL volume with distilled water. It was further observed at 470 nm wavelength using a colorimeter. After using the colorimeter to measure, the parts per million (ppm) of the phosphorus of the soil from the standard X curves were worked out. Calculation of Phosphorus is illustrated in the formula below (Boraste *et al.*, 2009) : -

$$\%\ phosphorous\ =\frac{X}{1000{,}000}x\ 50\ x\frac{100}{10}x\frac{100}{0.2}$$

3.7.5 Potassium

Preparation of standard curve: - 0, 1, 2, 4, 6, 8 and 10 mL of stock solution was pipetted into 100 mL volumetric flask. The volume was marked up to the mark with addition of distilled water, this resulted to 0, 10, 20, 40, 60, 80, and 100 parts per million (ppm) of potassium respectively.

Procedure: The intensity of potassium at flame photometer was observed. An aliquot of the soil sample was subjected directly to a flame photometer. The ppm of potassium from the standard curve run and the blank reading were worked out. The percentage of potassium by Flame photometer method is calculated with the formula below (Boraste *et al.*, 2009) : -

$$\%\ phosphorous\ = X/1000{,}000\ x\ 50\ x\ 100/10\ x\ 100/0.2$$

3.7.6 Exchangeable acidity

After 3 g of the soil was weighed (grind to pass a 2 mm sieve) into a folded filter paper and placed on an extraction cup, 50 mL of 1.0 N KCL (Potassium chloride) solution was poured gently into the soil on the filter paper while the leachate was collected in the extraction cup. 5 drops of phenolphtalein indicator were added to the leachate and titrated with 0.05 N NaOH to pink end point. The volume (mL) of NaOH used was recorded. The reaction was further calculated using the following formula (Boraste *et al.*, 2009) : -

Exchangeable acidity (meg/100 g) =

$$\frac{v \times 0.05 \times 100}{w} = v \times 1 \cdot 67$$

Where,
V = Titre volume of NaOH used (mL)
W = weight of soil sample used (3 g)

3.7.7 Moisture

Moisture was determined by measuring 4 g of soil sample on a dry petri-dish. It was heated in an oven for 5 hours at 65 ℃, constant weighing was done. Cooling was done in a desiccator and weighed. Percentage loss in weight was determined as moisture content of the soil in the calculation using the formula (Boraste *et al.,* 2009) : -

$$Moisture\ content\ = \frac{(B - C)}{(B - A)} \times 100$$

3.7.8 Physical properties

The colour of the soil sample was determined using munsel colour chart. Soil texture was determined by feel method (Boraste *et al.,* 2009).

3.8 Determination of Proximate Composition of Experimental Crops

Proximate composition of *Telfaria occidentalis* and *Albemoschus esculentus* were determined: The crops were dried and pulverized into powder using an electric blender. The powdered samples were sieved to obtain uniform particle size samples for the analysis of moisture, protein, ash, fibre, carbon, nitrogen and phosphorus content by the methods described by Association of Analytical Chemists (Parikh *et al.,* 2005).

3.9 Inoculum Development

A loopful of pure culture was inoculated into an inoculum medium consisting of 1 % feather meal and 0.2 % yeast extract (pH 7.5). The culture was incubated at 37 °C and 100 r/min for 24 hours (Lateef *et al.,* 2015).

3.10 Production of Protein Hydrolysate (PH)

Production of Protein hydrolysate was carried out by inoculating 1 mL of the inoculum into 19 mL of the fermentation medium in 100 mL flasks. The fermentation medium consisted of 0.05 g feather, 0.03 g K_2HPO_4, 0.04 g $MgS0_4$, 0.05 g Nacl, 0.01 g $CaC0_3$ and 250 mL distilled water. The flasks were incubated at 37 °C at 100 r/min for 120 hours. At 24-hour intervals, whole flasks were taken out ,the broth was centrifuged at 5000 rpm at 10° C for 20 minutes and the supernatants served as crude hydrolysates, which were used without further purification. When not used immediately, the crude hydrolysates were stored at 4 °C (Lateef *et al.,* 2015).

3.11 Experimental Layout and Treatments

The field experiment was carried out at the Department of Biology Farmland, Federal University of Technology, Minna. The experiment was arranged in a randomized complete block design with five treatments. The five treatments were as follows: Feather Hydrolysate only (FH), Feather hydrolysate and bacteria suspension (FH+ bacteria suspension), Feather hydrolysate and NPK (FH+NPK), NPK only (NPK) and NT (no treatment). There were two replicates of each treatments. Plastic pots were filled with non-sterilized soil and the different treatments were applied. NPK and NT served as positive and negative controls.

Twenty seeds were sown in each pot, the plants were pruned one week after seed germination by keeping ten healthy plants in each pot. Inorganic fertilizer treatment was applied five times every 2 weeks. Plants were irrigated manually once a day in the morning.

After harvest, the total yield weight (g) and proximate analysis (moisture, fibre, ash, lipid, carbohydrate and protein) of the experimental crops *Albemoschus esculentus* and *Telfaria occidentalis* were determined (Adejumo and Adetunji, 2018).

3.12 Data Analysis

In all the experiments, one way ANOVA test was conducted. The software package Minitab version 14 for Windows was used. Tukey's HSD was employed at $P = 0.05$ to test the level of significance and find out which of the treatments are significantly different.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Identification of keratinolytic bacteria

The biochemical characteristics of the possible keratinolytic bacteria isolated from the feather dump site are shown in Figure 4.1. Two organisms showed highest potential keratinolytic ability. The keratinolytic bacteria were suspected to be *Bacillus safensis* and *Bacillus licheniformis.*

Table 4.1: Biochemical characteristics of the bacterial isolates

Test	**(A)**	**Suspected Organism**	**(B)**	**Suspected organism**
Gram's reaction	+	*Bacillus safensis*	+	*Bacillus licheniformis*
Shape		Rod		Rod
Motility		+		+
Catalase		+		+
Citrate		-		+
Starch hdrolysis		-		+
Urease		+		+
indole		-		-
Oxidase		-		-
Voges Proskauer		-		-
H_2S		-		-
Glucose		+		+
Sucrose		+		+
Lactose		-		-
Methyl Red		-		-

Key: (+): Positive, (-): Negative

4.1.2 Molecular identity of isolates

Gel electrophoresis indicating a positive amplification of the 16S region of the bacterial isolates using 16S ribosomal universal primer showed that the isolates had a band size of approximately 1500bp, as seen in Plate I. The rRNA sequence showed that the sequences were 100 % and 96.45 % similar to *Bacillus safensis* and *Bacillus licheniformis* respectively, (Table 4.2 and Table 4.3).

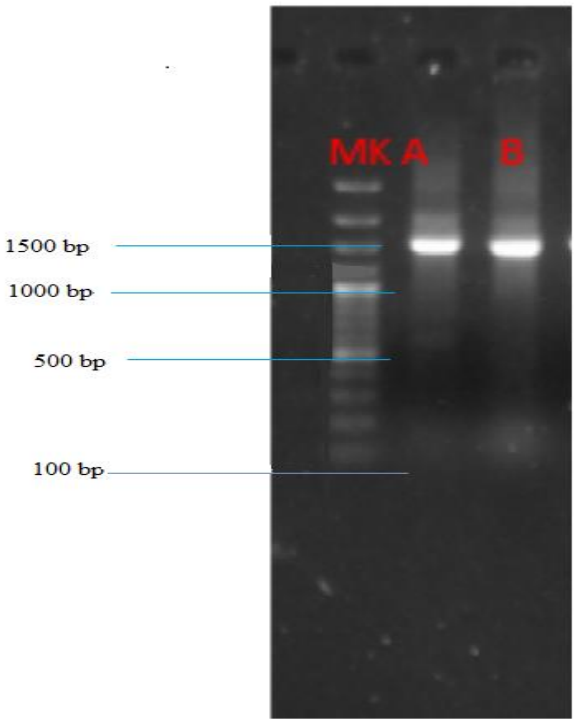


Plate I: Agarose gel image of the 16s rRNA of the bacterial isolates indicating approximately 1500bp

Table 4.2: Sequence Similarity of *Bacillus safensis* with known isolates

Description	Max Score	Total Score	Query cover (%)	E value	Accession number
Bacillus safensis Strain LAU 13 16s ribosomal RNA Gene, partial sequence	1458	1458	100	0.00	KJ461434.1
Bacillus safensis strain CBN-8 16s ribosomal RNA gene, partial sequence	1458	1458	100	0.00	JQ353775.1
Bacillus *safensis* Strain MUGA 168 16s ribosomal RNA gene, Partial sequence	1452	1452	100	0.00	KJ672350.1
Bacillus *safensis* Strain MUGA 102 16s ribosomal RNA gene, Partial Sequenc	1452	1452	100	0.00	KJ67203.1

The sequence alignment of the isolates is shown below:

ACACGGGTTCGAATCCCGTACGGGTCATTTTTAAAAGAGATCTTGTCAAAGATC

TCTTTTTTATTTTCTCCAAAACGAAAGACCGCATCATGGCGGTCTTTTTTTACAT

ATTCATGGAATAGATCGTATAAGACAAGTATGTGGCAAAAAAGCCCCATAAGA

CATAAGGAATCAGTAAAATTGGAACGACATTGCTTAGTTTACGTGAGACGATG

ACAAGTGCAAGTGCTGTCAGTGCGACGAGCAGGCAGTCGATTGTAGCTGCAAA

CAAATCCTTTTGTGTAAATTGAAAAAAGCTAAAGGCTTGATTGAAAACATAATT

TAAAAGGAAAAGAATCCAAAATGGTAAAGTGATTTTTTGGAACCCATATTTTC

CATAAATGATCGCTGCGGACAATGAGATGAGTGCAAATAAGACAGCCCACACA

GCAGCAATGACACCGCCACCAGGCGTCCATTCTGGTTTATTCAATGCGTCATAC

CATTCTCTGTCGATGGGGAACAAGAATCCGGCAATAGAGAATAGTGCGTATGT

AATAAAGAAGACGACAATCGCCCAAATGATACTTTTTCTAGCCATCAAGAATC

TCCTCCAATCAAATTCACTCTTAAAATGATTTCCCAATAATTACATGATATAAA

CATAAAGCCCCCAATATGAGGGGACTAATGATTTTCTTCTGTATAAGGTTCTTC

CTCGTACGGTTTCAAGTCACCAAATACCGTCATGACACCTTCTTCGTCTAGTGC

TTCTTCATAGGCCTCGTGTGCTTTGGTCGGATACACCTTGCTGGCTTTTCCTTCA

ATATCAGCAGCTGCGAATCCTTCGTAGTCCTCCACATAATCTTCATCTTCT

Table 4.3: Sequence similarity of *Bacillus licheniformis* with known isolate

Description	Max Score	Total Score	Query cover (%)	E value	Percentage Identity (%)	Accession number
Bacillus haynesii strain NRRL B-41327 16s ribosomal RNA partial sequence	1415	1415	99	0	96.45	NR157609.1
Bacillus paralichenformis strain KJ-16 16s ribosomal RNA partial sequence	1415	1415	99	0	96.45	NR137421.1
Bacillus lichenformis DSM 13 16s ribosomal RNA partial sequence	1415	1415	99	0	96.45	NR118996.1
Bacillus aerius strain 24k 16s ribosomal RNA partial sequence	1415	1415	99	0	96.45	NR042338.1
Bacillus lichenformis strain ATCC 14580 16s ribosomal partial sequence	1410	1410	99	0	96.33	NR074923.1

The sequence alignment of the isolates is shown below:

GTCCTATACGACTTCACCCCAATCATCTGTCCCACCTTCGGCGGCTGGCTCCAA

AAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGT

GTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTA

GCGATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACA

GATTTGTGGGATTGGCTTAGCCTCGCGGCTTCGCTGCCCTTTGTTCTGCCCATTG

TAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCC

ACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTG

GCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACG

ACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCCCCCGAAGGGGAA

GCCCTATCTCTAGGGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGT

TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCT

TTGAGTTTCAGTCTTGCGACCGTACTCCCCAAGGCGGAGTGCTTAATGCGTTTG

CTGCAGCACTAAAAGGGCGGAAAACCCTCTAACACTTAGCACTCATCGTTTAC

GGCGTGACTACCAGGTATCTAATCTGTCGCTCCCCCACGCTTCGCGCTCAGCGT

CAGTTACAGACAGAAGAGTCGCTCGCCACTGGTGTCCTCAAATCTCTACGCATA

CCGCTACCGTGAAATCCATCTCTCTTTCGCATCAAGTCCCAGTTCCATG

4.1.3 Shoot height of the crops

The effect of feather hydrolysate treatment on the shoot height of *Albemoschus esuclentus* and *Telfaria occidentalis* during the harvest period is shown in figure 4.1. A bar chart was used to compare the heights of the crops across the various treatments. The treatment combining Feather Hydrolysate (FH) and chemical fertilizer (NPK) produced the longest shoots in both *Albemoschus esculentus* (25 cm) and *Telfaria occidentalis* (28 cm). There was no significant difference in the shoot height of crops treated with feather hydrolysate

only (FH), chemical fertilizer (NPK) and feather hydrolysate combined with bacteria suspension (FH + susp).

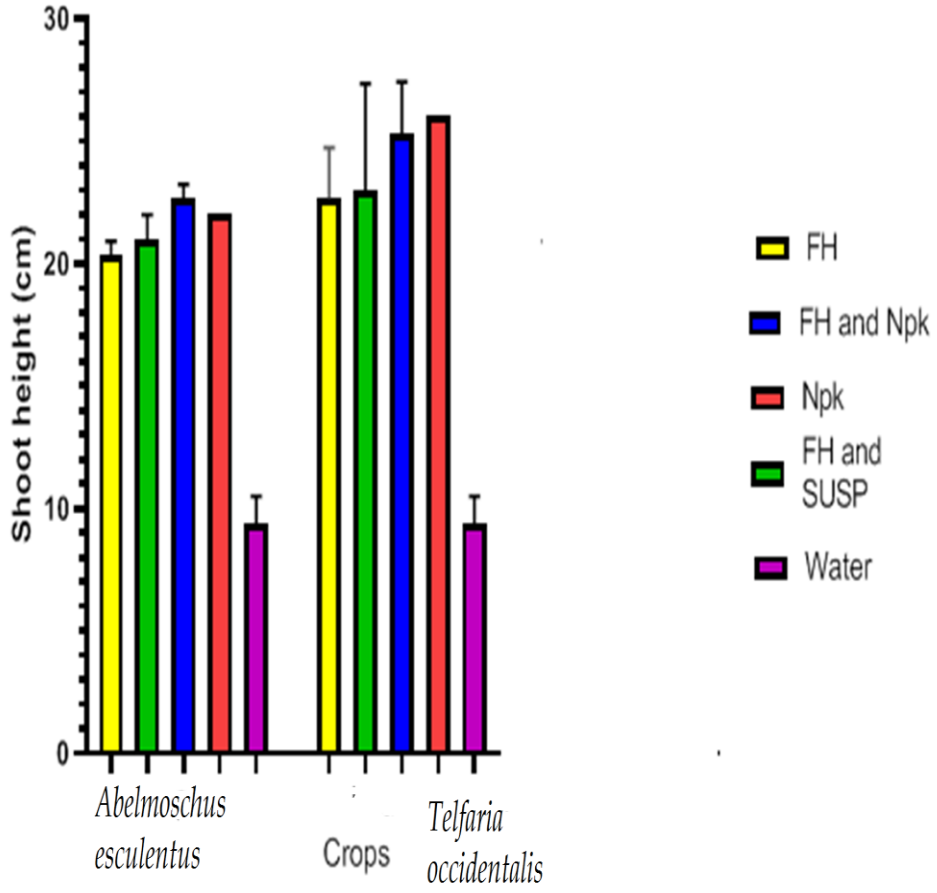
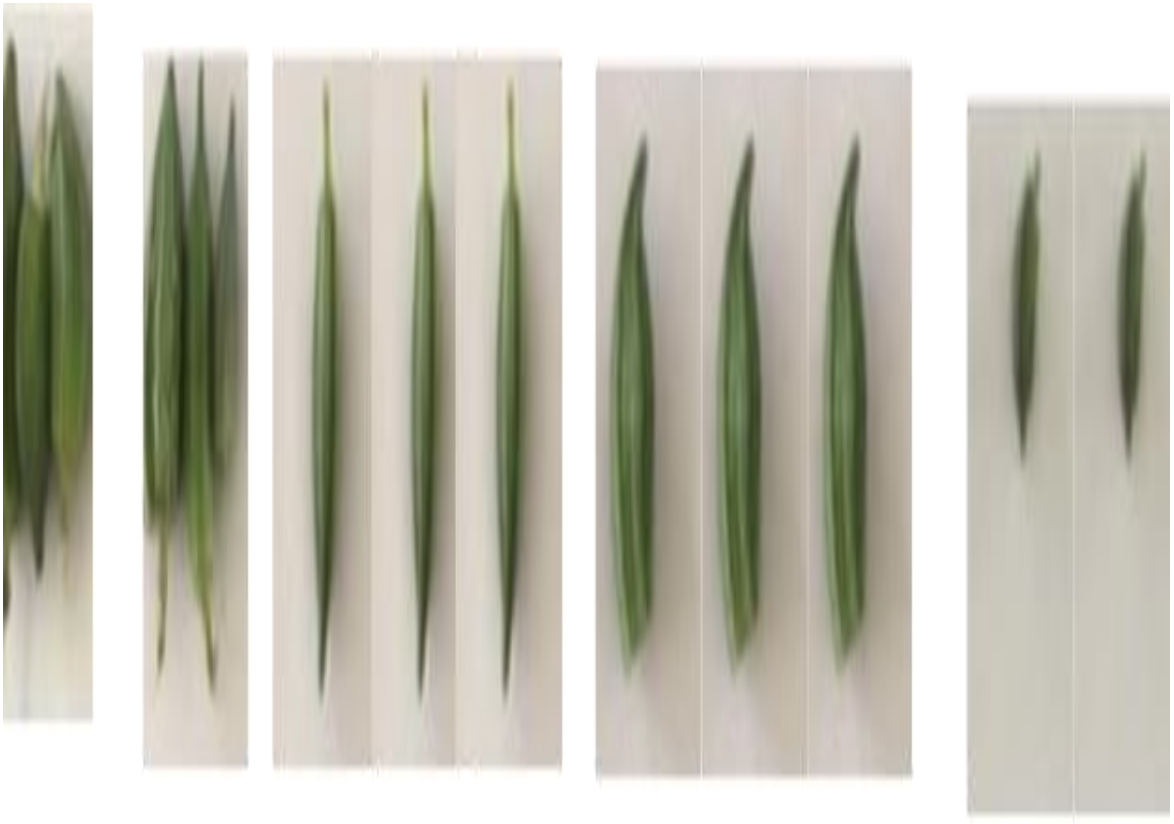


Figure 4.1: Shoot height of *Albemoschus esculentus* and *Terratia occidentalis*

4.1.4 Harvested Crops

The sizes of the harvested crops after different treatments are illustrated in Plates II and III. The effects of the treatments on the sizes of *Albemoschus esculentus* show that FH + susppension > FH + NPK > NPK only > FH only > Water (Plate II). The effects of the treatments on the sizes of *Telfaria occidentalis* show that the treatment with NPK only > FH only > FH + NPK > FH + susp suspension > water only (Plate II).



NPK FH and NPK FH only FH + suspension Water only

Plate II: *Abelmoschus esculentus*

FH only | Water | FH + sus | NPK | FH + NPK

Plate III: *Telfaria occidentalis*

4.1.5 Yield weight of *Telfaria occdentalis* and *Albemoschus esculentus*

The the mean yield weight and standard deviation of *Albemoschus esculentus* and *Telfaria occidentalis* in line with the various treaments in the study ia shown in Table 4.4. There was a significant difference in the yield weight of *Albemoschus esculentus* treated with Feather hydrolysate + bacteria suspension and the treatment Feather hydrolysate only (FH). Treatment with the chemical fertilizer (NPK) gave the highest yield for *Albemoschus esculentus* (154.5 g) followed by the combined treatment of FH + NPK (151.6 g). There was a significant difference between the yield weights of the protein hydrolysate treated *Albemoschus esculentu*s and the negative contol. The yield weight of the FH treatment was significantly different from the yield weight of FH + susp. The FH + NPK treatment had a higher yield weight than FH + sus treatment. The untreated *Telfaria* occidentalis (14.8 g) had the least weight while the NPK treatment had the highest yield weight (47.3 g).

Table 4.4: Mean and Standard deviation of Yield weight of *Telfaria occidentalis* and *Albemoschus esculentus* (g)

Treatments	*Abelmoschus esculentus* (g)	*Telfaria occidentalis* (g)
FH only	88.70^{c} ±3.12	25.9^{c} ±19.1
FH + susp	95.30^{b} ±0.010	23.90^{d}±0.00
FH + NPK	151.6^{a} ±0.011	31.30^{b}±0.00
NPK	154.5^{a} ±0.20	47.30^{a}±0.00
No treatment	47.50^{d}±0.14	14.80^{e}±0.00

N. B *Means carrying the same letter are not different from each other at P <0.05

4.1.6: Physicochemical properties of the experimental soil

The soil was Grey in colour and moderately coarse in texture. The pH of the soil was acidic and the nitrogen content was low. The organic matter content of the soil was 5 %. The organic matter of the soil is composed of macro elements such as organic carbon, nitrogen, phosphorus, sodium, potassium, calcium, and magnesium. However, Nitrogen and Potassium contents (0.052 % and 0.69 C molkg^{-1}) had low values in the soil (Table 4.5).

Table 4.5: Physicochemical Properties of the Experimental Soil

PARAMETERS	VALUES
pH %	5.86
Nitrogen (N) (%)	0.052
Organic Carbon (%)	7.77
Organic Matter (%)	5.96
Phosphorous (P) (Mg/kg)	30.29
Sodium (Na) ($Cmolkg^{-1}$)	1.450
Potassium (K) ($Cmolkg^{-1}$)	0.69
Calcium (Ca) ($Cmolkg^{-1}$)	6.85
Magnesium ($Cmolkg^{-1}$)	5.69
Exchangeable acidity ($Cmolkg^{-1}$)	1.80
Electrical Conductivity	56
Sand (%)	55.34
Clay (%)	26.60
Silt (%)	13.19
Soil Texture	Moderately coarse
Soil Type	Sandy Loam
Soil Colour	Grey

4.1.7 Proximate composition of *Abelmoschus esculentus*

Table 4.6 show the proximate composition of *Albemoschus esculentus* resulting from different treatments. The Feather hydrolysate combination with NPK treatment (FH + NPK) had the highest moisture content of 18.61 % followed by the NPK only treatment with a mean moisture content of 15.61 %. The Feather hydrolysate combined with a bcateria suspension treatment (FH + susp) had a higher moisture content (14.21 %) than FH only (12.3 %). The untreated *Albemoschus esculentus* had the least moisture content (8.41 %). Although the FH + NPK and the NPK treatments both had high fibre contents of 21.2 % and 20.31 %, they were not statistitically significant different from each other. Also, there was no significant difference between the fibre contents of FH + FH + susp treatments. FH + NPK treatment had the highest ash content of 8.1 % followed by NPK only treatment (4.1 %). The ash contents of FH + susp treatment and FH only were not significantly different fro each other, although the ash content of FH& NPK treatment was significantly different from the two. The untreated crop had the least ash content of 2.12 %. There was no statisticical significant difference between the lipid contents of FH only and FH + susp, also there was no statistical difference between the lipid contents of NPK only treatment and FH + NPK. The FH only and FH + susp treatments had high percentages of carbohydrate (58.41 %) and (58. 42 %). There was no significant difference between the carbohydrate contents of both treatments. The FH + NPK treatment had a higher carbohydrate content (58.34 %) than NPK only (53.32 %), there was a statistical significant difference between the carbohydrate contents of these two treatments at $P < 0.05$. The FH treatment had the highest protein content (59.31 %) followed by FH + sus (58.31 %). There was a significant difference between the protein contents of FH treatment and NPK treatment. The untreated plant had the least protein content of 11.31 %.

Table 4.6 proximate composition of *Abelmoschus esculentus*

Treatments	Moisture (%)	Fibre (%)	Ash (%)	Lipid (%)	Carbohydrate (%)	Protein (%)
FH only	12.3000^{ab} ±0.0100	2.06000^{c} ±0.0100	3.45000^{c} ±0.0100	3.25000^{a} ±0.0100	58.4100^{a}± 0.0100	59.3400^{a} ±0.0100
FH + susp	14.2100^{ab} ±0.0100	2.06000^{c} ±0.0100	3.58000^{c} ±0.0100	3.24000^{a} ±0.0100	58.4200^{a}± 0.0100	58.3100^{b} ±0.0100
FH + NPK	18.6100^{a} ±0.0100	21.1200^{a} ±0.0100	8.10000^{a} ±0.0100	8.97000^{b} ±0.0100	58.3400^{b}± 0.0100	55.3100^{c} ±0.0100
NPK only	15.6100^{ab} ±0.0100	20.310^{a}± 0.0100	4.10000^{b} ±0.0100	8.97000^{b} ±0.0100	53.3200^{c} ±0.0100	50.3200^{d} ±0.0100
No treatment	8.41^{b} ±4.92	2.08000^{d} ±0.0100	2.128000^{d} ±0.010	2.19000^{c} ±0.0100	10.3300^{d} ±0.0100	11.3300^{e} ±0.0100

N. B *Means carrying the same letter are not statistically different from each other at $P < 0.05$

4.1.8 Proximate composition of *Telfaria occidentalis*

The proximate composition of *Telfaria occidentalis* resuling from different treatments is shown in Table 4.7. The Feather hydrolysate treatment (FH) had the highest moisture content (55.74 %) followed by the FH + susp treatment with a mean moisture content of 50.057 %. The Feather hydrolysate combined with NPK (FH + NPK) had a higher moisture content (48.37 %) than NPK only (43.3 %). The untrated *Telfaria occidentalis* had the least moisture content (42.97 %). The FH treatment had the highest fibre contents (27.05 %) and was statistitically significant different from FH + suspp treatment at $P < 0.05$ (25.07 %). Also, there was significant difference between the fibre contents of FH + NPK and NPK only treatments. FH treatment had the highest ash content (7.43 %) followed by NPK only treatment (6.76 %). The ash contents of FH + susp treatment and FH only were significantly different from each other, also the ash content of FH& NPK treatment was significantly different from NPK only. The untreated crop had the least ash content of 4.07 %. The FH + NPK treatment had the highest lipid content (4.35 %), there was a significant difference between the lipid content of FH + susp and NPK treatments.

The FH + NPK treatment had highest percentages of carbohydrate (48.47 %) followed by FH (30.8 %). There was a significant difference between the carbohydrate contents of both treatments. The carbohydrate content of FH was statitstcally significant different from that of FH + susp. The FH + FH + NPK treatments both had similar high protein content (17.13 %) followed by FH + sus (15.07 %). The untreated plant had the least protein content of 9.24 %.

Table 4. 7 Proximate composition of *Telfaria occidentalis*

Treatments	Moisture	Fiber	Ash	Lipid	Carbohydrate	Protein
FH only	55.7400^{a}± 0.0361	27.0533^{a}± 0.0551	7.43333^{a}±0.01155	3.15333^{c}±0.01528	30.800^{b}±0.778	17.13^{a} ±0.00
FH + susp	50.05767^{b}± 0.1124	25.0733^{b}± 0.0252	5.883^{d}±0.01155	2.7500^{e}±0.0200	25.425^{c}±0.247	15.0767^{b} ±0.055
FH + NPK	48.3733^{c} ±0.0153	19.9900^{d}± 0.1212	6.0833^{c}±5.883	4.3500^{a}±0.0200	48.470^{a}±0.693	17.13^{a}± 0.00
NPK	43.33000^{d}± 0.0964	21.0400^{c}± 0.0300	6.7600^{b}± 0.0173	4.000^{b}±00.0000	21.30^{d}±1.48	11.3833^{d}± 0.035
No treatment	42.976^{e}± 0.0306	17.0133^{e}± 0.0231	6.0833^{e}±0.1021	2.9267^{d}±0.1358	4.6350^{e}±0.41	9.2433^{e} ±0.0252

N. B *Means carrying the same letter are not statistically different from each other at $P < 0.05$

4.2 Discussion

Keratinolytic enzymes could be used to enhance the digestibility of feather keratin in the degradation of feathers and their utilization as a feedstuff (Adejumo and Adetunji, 2018). The biofertilization potential of protein hydrolysates produced from feather wastes *via* submerged fermentation on the growth of *Abelmoschus esculentus* and *Telfaria occidentalis* was investigated in this study. The isolated bacteria showed good growth with distinct characteristics on yeast extract agar. Yeast extract agar is selective for spore forming bacteria which is an indication that the isolated organisms are spore formers. Morphological and physiological characteristics of the bacteria were compared with the Bergey's Manual of Systemic Bacteriology. The isolates were Gram-positive and rod shaped. The analysis based on 16S rRNA sequence showed that the bacteria had 100 % sequence homology with *B. safensis* CBN-8 (JQ353775) and *Bacillus lichenformis* strain ATCC 14580. The organisms isolated were confirmed to be *Bacillus licheniformis* and *Bacillus safensis.*

In agreement with this study, degradation of keratin has been reported to be mostly confined to Gram-positive bacteria, including *Bacillus*, *Streptomyces* and a few strains of Gram-negative bacteria (Veerabadran *et al.,* 2012). The isolation of keratinase producing strains of *B. licheniformis* and *B. safensis* had been previously reported (Veerabadran *et al.,* 2012; Lateef *et al.,* 2015). The bacteria were able to degrade feather due to the ability of *Bacillu*s species to secrete keratinase enzymes. Many *Bacillus* species have been reported to possess keratinolytic ability (Rubin *et al.,* 2017). *Bacillus licheniformis* had previously been reported as the bacteria with the highest keratin degrading ability in the *Bacillus* family on both powdered feather and whole feather (Saarela *et al.,* 2017; Gurung *et al*., 2013). *B. safensis* was first identified in 2006 as a contaminant from spacecraft–assembly facilities

in USA from which it derived its specific epithet ‘safensis’(Lateef *et al.,* 2015). Lateef *et al.,* 2015 was the first to report *Bacillus safensis* as a keratinase producer.

The production of protein hydrolysates from by-products of agroindustry provides an environmental and economically friendly solution for disposing of waste (Colla *et al.,* 2015). These products do not only contain amino acids and proteins/peptides but also consist of other non-protein components, which also contribute to their stimulating effect on plants (Yakhin *et al.,* 2017). From this study’s assessment of the experimental soil, the pH of the soil was acidic and the nitrogen content was low which makes it unsuitable for plant growth. The bacteria suspensions in the treatment are microbial inocula of the *Bacillus* species.

The treatment combining Feather Hydrolysate (FH) and chemical fertilizer (NPK) produced the longest shoots in both *Albemoschus esculentus* (25 cm) and *Telfaria occidentalis* (28 cm). There was a significant difference in the shoot height of crops treated with feather hydrolysate only (FH) and feather hydrolysate combined with bacteria suspension (FH + susp) compared to the negative control at $P < 0.05$ also, the effects of the treatments on the sizes of *Albemoschus esculentus* and *Telfaria occidentalis* show that treatments with FH only and FH+ susp produced bigger fruits than the negative control. Furthermore, there was a significant difference in the yield weight of *Albemoschus esculentus* treated with Feather hydrolysate + susp and the negative control, also the yield weight of the treatment with Feather hydrolysate only (FH) was highly significant compared to the negative control at $P < 0.05$. This may be because protein hydrolysates play a major role in the assimilation and modulation of N uptake (Caruso *et al.,* 2020). This is achieved through regulating enzymes that aid in the assimilation of N and their structural genes and by acting on the signaling pathway of N acquisition in roots (Colla *et al.,* 2015). These products further regulate

enzymes of the tricarboxylic acid cycle (TCA), which plays a significant role in the cross-talk between carbon and nitrogen metabolism (Du Jardin, 2015). They also influence soil chemical and physical properties. In soil, they increase the respiration together with microbial biomass and activity (Du Jardin, 2015). They further improve the solubility and mobility of micronutrients, especially Fe, Zn, Mn, and Cu (Abbas, 2013). The hydrolysates may also modify the morphology of plant roots in a similar way to IAA, suggesting that they induce a "nutrient acquisition response" that favours the uptake of nutrients via an increase in the absorptive surface area (Ertani *et al.*, 2012). Phytohormone content in protein hydrolysates may also contribute to their stimulating effect. For instance, cytokinins regulate vascular development and promote flower development while auxins promote cell elongation in the coleoptile and rooting (Farooq and Al-Sanoussi, 2019).

Furthermore, auxins enhance the production of adventitious roots, overall cell division and formation of meristem (Farooq and Al-Sanoussi, 2019). The positive effect the FH + susp treatment had on plant height, and yield weight may also be attributed to the presence of microbial inoculant of the *Bacillus* species. Microbial inoculants stimulate plant growth through the production of volatile organic compounds, sequestering of iron by the production of siderophores, asymbiotic nitrogen fixation, and solubilization of nutrients (Caruso *et al.*, 2020). Several plant growth promoting rhizobacteria (PGPR) also produce volatile organic compounds (VOCs) which promote plant growth (Gowtham *et al.*, 2018). Volatile organic compounds produced by biocontrol strains can induce systematic resistance against pathogens and inhibit nematodes, fungal, and bacteria pathogens; and can further promote leaf surface area, biomass, lateral root number and yield (Asghari *et al.*, 2020). Siderophores

are molecules that bind and transports iron under iron-limiting conditions, and enhance iron (Fe) uptake capacity in microorganisms (Gouda *et al.*, 2018).

Plant growth promoting rhizobacteria produce and utilize the siderophores produced by other microbes present in the rhizosphere for fulfilling their iron requirement (Gouda *et al.*, 2018). Plant growth promoting rhizobacteria can increase the concentration and accessibility of nutrients by either locking or fixing their supply for plant growth and productivity (Gouda *et al.*, 2018). Plant growth promoting rhizobacteria can fix nitrogen either through symbiotic or non-symbiotic interactions between plants and microbes (Farooq and Al-Sanoussi, 2019). Inoculation with PGPR can also enhance phosphorus availability in plants through solubilization and mineralization of phosphorus by phosphate solubilizing bacteria. Furthermore, PGPR can increase the availability of potassium by solubilizing potassium rock through the production of organic acids that can release inaccessible potassium (Farooq and Al-Sanoussi, 2019). Microbial inoculants can also modify plant hormone status through synthesis, localization, and signalling of phytohormones (Gowtham *et al.*, 2018). Plant growth promoting rhizobacteria can alter the localization, signalling and concentration of phytohormones including gibberellins, cytokinins, abscisic acid, ethylene, brassinosteroids, and auxins, which are responsible for various actions including root and shoot invigoration (Asghari *et al.*, 2020). Microbial inoculants alter root architecture via the degradation or production of major groups of plant hormones (Asghari *et al.*, 2020). Microbial inoculants increase root biomass and nutrient uptake capacity (Asghari *et al.*, 2020). PGPR promote plant growth through the synthesis of plant growth regulators, promoting symbiotic N2 fixation and solubilisation of mineral phosphate and other nutrients. The efficacy of PGPR is dependent on environmental factors such as composition of microbial flora and soil

characteristics. Although several authors have reported that microbial inoculants containing *Bacillus* species improve the growth and yield of plants (Chaudhary *et al.,* 2021; Rahman *et al.,* 2018; Beibei *et al.,* 2016), to the best of our knowledge this is the first report combining microbial inoculants with protein hydrolysates to examine their effects on crop yield.

The result of this study is in agreement with the findings by Wang *et al.* (2020) who obserserved that the plant height of *Malus hupehensis Rehd* was significantly enhanced by the application of brown seaweed extracts hydrolysate relative to the control and Koukounaras *et al.* (2013), who found that using Amino16R, a protein hydrolysate, increased tomato fruit yields.

The results of this experiment are in contrast with the reports of Cerdán *et al.* (2009) who reported that foliar applications of commercial protein hydrolysate products from animal origin can cause phytotoxicity and plant growth depression. In some situations, wrong product concentrations or environmental aspects like field conditions may contribute to no-response to hydrolysates. For instance, application of hydrolysates in excess might induce no-response or negative responses in plants. Also, Asli and Neumann (2010) reported that multiple applications of humic acid inhibited the shoot growth of maize grown hydroponically. No-positive effects were also reported by Kirn *et al.,* (2010) in a trial with okra (*Abelmoschus esculentus*) grown in field soil experiments, as no significant increases in fruits per plant were observed when the recommended dose was not applied.

Concerning the proximate composition of the crops, the FH treatment and FH + susp had higher protein, lipid, carbohydrate, ash and moisture content compared to the negative control. This may be attributed to the fact that protein hydrolysates and plant growth

promoting bacteria inoculants are major contributors to plant immunity and nutrition (Du Jardin, 2015) and they can also increase nutrient uptake capacity (Asghari *et al.*, 2020). This is in accordance with the report of Schiavon *et al.*, (2008) who reported that a hydroponically-grown maize plants had an increase in soluble sugar accumulation when treated with alfafa protein hydrolysate.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Bacillus lichenformis and *Bacillus safensis* were isolated and identified as feather degrading bacteria from this study. The isolated bacteria were able to produce a protein hydrolysate through the process of submerged fermentation. The protein hydrolysate produced was subsequently used as biofertilizer and its effects were investigated on the growth of *Telfaria occidentalis* and. *Albemoschus esculentus*.

The result of this study showed that the treatment with Feather hydrolysate gave a high yield weight of *Albemoschus esculentus* (88.70 g) and *Telfaria occidentalis* (25.9 g). Also, the treatment with FH+suspension gave a high yield weight of (95.30 g) for *Albemoschus esculentus* and (23.9 g) for *Telfaria occidentalis*

Furthermore, there was a positive effect between the Feather hydrolysate and the chemical fertilizer (NPK) in which the growth of the plants were enhanced in *Telfaria occidentalis* (151.6 g), this may help reduce the use of chemical fertilizers in situations where it can not be avoided. From this study, the untreated vegetables had the lowest yields for both *Albemoschus esculentus* (47.5 g) and *Telfaria occidentalis* (14.8 g).

The proximate composition of the protein hydrolysates (FH + FH+sus) were significantly different from the negative control at $P < 0.05$.

5.2 Recommendations

1. A microbial consorta should be investigated in the degradation of keratin, instead of individual organisms. A combination of two or more microorganisms may help reduce the time employed to completely degrade keratin.

2. Further investigation should be carried out to find out the exact mechanism by which protein hydrolysates help to improve plant growth and what effects they have on the soil microbial population.
3. Other PH dosage which the protein hydrolysates are effective should be investigated.
4. The protein hydrolysates produced from feather should be used to replace chemical fertilizers after their mechanism of action is fully understood.

REFERENCES

Abbas, S. M. (2013). The influence of biostimulants on the growth and on the biochemical composition of *Vicia faba* CV. Giza 3 beans. *Romanian Biotechnological Letters*, 18(2), 8061-8068.

Adejumo, I. O., & Adetunji, C. O. (2018). Production and evaluation of biodegraded feather meal using immobilised and crude enzyme from *Bacillus subtilis* on broiler chickens. *Brazilian Journal of Biological Sciences*, 5(10), 405-416.

Adelere, I. A., & Lateef, A. (2016). Keratinases: emerging trends in production and applications as novel multifunctional biocatalysts. *Kuwait Journal of Science*, 43(3), 1-3.

Aguayo-Villarreal, I. A., Bonilla-Petriciolet, A., Hernández-Montoya, V., Montes-Morán, M. A., & Reynel-Avila, H. E. (2011). Batch and column studies of Zn^{2+} removal from aqueous solution using chicken feathers as sorbents. *Chemical Engineering Journal*, 167(1), 67-76.

Alashwal, B. Y., Gupta, A., & Husain, M. S. B. (2019). Characterization of dehydrated keratin protein extracted from chicken feather. *IOP Conference Series: Materials Science and Engineering*, 702(1), 012033.

Anubrata, P., & Rajendra, D. (2014). Isolation, Characterization, Production of Biofertilizer, and Its Effect on Vegetable Plants with and without Carrier Materials. *International Journal of Current Research*, 6(1), 7986-7995.

Apone, F., Tito, A., Carola, A., Arciello, S., Tortora, A., Filippini, L., & Colucci, G. (2010). A mixture of peptides and sugars derived from plant cell walls increases plant defense responses to stress and attenuates ageing-associated molecular changes in cultured skin cells. *Journal of Biotechnology*, 145(4), 367-376.

Asghari, B., Khademian, R., & Sedaghati, B. (2020). Plant growth promoting rhizobacteria (PGPR) confer drought resistance and stimulate biosynthesis of secondary metabolites in pennyroyal (*Mentha pulegium* L.) under water shortage condition. *Scientia Horticulturae*, 263(1), 109132.

Ashraf, M., & Foolad, M. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, 59(2), 206-216.

Asli, S., & Neumann, P. M. (2010). Rhizosphere humic acid interacts with root cell walls to reduce hydraulic conductivity and plant development. *Plant and Soil*, 336(1), 313-322.

Bai, Y., Müller, D. B., Srinivas, G., Garrido-Oter, R., Potthoff, E., Rott, M., & Hüttel, B. (2015). Functional overlap of the *Arabidopsis* leaf and root microbiota. *Nature*, 528(7582), 364-369.

Beibei, W. A. N. G., Zongzhuan, S. H. E. N., Zhang, F., Waseem, R. A. Z. A., Jim, Y. U. A. N., Huang, R., ... & Qirong, S. H. E. N. (2016). *Bacillus amyloliquefaciens* strain W19 can promote growth and yield and suppress *Fusarium* wilt in banana under greenhouse and field conditions. *Pedosphere*, 26(5), 733-744.

Berendsen, R. L., Pieterse, C. M., & Bakker, P. A. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, 17(8), 478-486.

Berg, G., Rybakova, D., Grube, M., & Köberl, M. (2016). The plant microbiome explored: implications for experimental botany. *Journal of Experimental Botany*, 67(4), 995-1002.

Boraste, A., Vamsi, K. K., Jhadav, A., Khairnar, Y., Gupta, N., Trivedi, S., ... & Joshi, B. (2009). Biofertilizers: A novel tool for agriculture. *International Journal of Microbiology Research*, 1(2), 23-25

Bouhamed, S. B. H., Krichen, F., & Kechaou, N. (2020). Feather protein hydrolysates: a study of physicochemical, functional properties and antioxidant activity. *Waste and Biomass Valorization*, 11(1), 51-62.

Brandelli, A., Daroit, D. J., & Corrêa, A. P. F. (2015). Whey as a source of peptides with remarkable biological activities. *Food Research International*, 73(1), 149-161.

Brenner, D. J., Krieg, N. R., Staley, J. T., & Garrity, G. M. (Eds.). (2005). *Bergey's Manual of Systematic Bacteriology: Volume Two: The Proteobacteria, Part A Introductory Essays*. *Springer*, 46(3), 476.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Van Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, 64(2), 807-838.

Callegaro, K., Brandelli, A., & Daroit, D. J. (2019). Beyond plucking: Feathers bioprocessing into valuable protein hydrolysates. *Waste Management*, 95(1), 399-415.

Cao, Y., Yao, Y., Li, Y., Yang, X., Cao, Z., & Yang, G. (2012). Tunable keratin hydrogel based on disulfide shuffling strategy for drug delivery and tissue engineering. *Journal of Colloid and Interface Science*, 544(1), 121-129.

Caruso, G., El-Nakhel, C., Rouphael, Y., Comite, E., Lombardi, N., Cuciniello, A., & Woo, S. L. (2020). *Diplotaxis tenuifolia* (L.) DC. yield and quality as influenced by cropping season, protein hydrolysates, and *Trichoderma* applications. *Plants*, 9(6), 697-698.

Cavani, L., Ter Halle, A., Richard, C., & Ciavatta, C. (2006). Photosensitizing properties of protein hydrolysate-based fertilizers. *Journal of Agricultural and Food Chemistry*, 54(24), 9160-9167.

Cerdán, M., Sánchez-Sánchez, A., Oliver, M., Juárez, M., & Sánchez-Andreu, J. J. (2009). Effect of foliar and root applications of amino acids on iron uptake by tomato plants. *Acta Horticulturae*, 8(30), 481-488.

Chaudhary, P., Khati, P., Chaudhary, A., Gangola, S., Kumar, R., & Sharma, A. (2021). Bioinoculation using indigenous *Bacillus* spp. improves growth and yield of *Zea mays* under the influence of nanozeolite. *3 Biotech*, 11(1), 1-12.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries* Part 2 Cambridge University Press. *New York*, 300-301.

Chen, T. H., & Murata, N. (2008). Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends in Plant Science*, 13(9), 499-505.

Coleman-Derr, D., Desgarennes, D., Fonseca-Garcia, C., Gross, S., Clingenpeel, S., Woyke, T., & Tringe, S. G. (2016). Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *New Phytologist*, 209(2), 798-811.

Colla, G., & Rouphael, Y. (2015). Biostimulants in horticulture. *Scientia Horticulturae*, 196(1), 1-2.

Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., & Rouphael, Y. (2017). Biofertilizer action of protein hydrolysates: unraveling their effects on plant physiology and microbiome. *Frontiers in Plant Science*, 8(4), 2202-203.

Colla, G., Rouphael, Y., Canaguier, R., Svecova, E., & Cardarelli, M. (2014). Biofertilizer action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. *Frontiers in Plant Science*, 5(1), 448-450.

Colla, G., Rouphael, Y., Lucini, L., Canaguier, R., Stefanoni, W., Fiorillo, A., & Cardarelli, M. (2015). Protein hydrolysate-based biostimulants: Origin, biological activity and application methods. *World Congress on the Use of Biostimulants in Agriculture*, 1148(1), 27-34.

Da Rocha, I. M. A., Vitorello, V. A., Silva, J. S., Ferreira-Silva, S. L., Viégas, R. A., Silva, E. N., & Silveira, J. A. G. (2012). Exogenous ornithine is an effective precursor and the δ-ornithine amino transferase pathway contributes to proline accumulation under high N recycling in salt-stressed cashew leaves. *Journal of Plant Physiology*, 169(1), 41-49.

De Vasconcelos, A. C. F. (2019). Effect of biofertilizers on the nutrition of maize and soybean plants. *International Journal of Environment, Agriculture and Biotechnology*, 4(1), 2-3.

Del Rosario Cappellari, L., Chiappero, J., Santoro, M. V., Giordano, W., & Banchio, E. (2017). Inducing phenolic production and volatile organic compounds emission by inoculating Mentha piperita with plant growth-promoting rhizobacteria. *Scientia*

Horticulturae, 220(1), 193-198.

Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., & Vorholt, J. A. (2009). Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences*, 106(38), 16428-16433.

Du Jardin, P. (2015). Plant biostimulants: definition, concept, main categories and regulation. *Scientia Horticulturae*, 196(1), 3-14.

Ebru, G. E. N. Ç., & ATICI, Ö. (2019). Chicken feather protein hydrolysate as a biofertilizer improves the growth of wheat seedlings by affecting biochemical and physiological parameters. *Turkish Journal of Botany*, 43(1), 67-79.

Ertani, A., Cavani, L., Pizzeghello, D., Brandellero, E., Altissimo, A., Ciavatta, C., & Nardi, S. (2009). Biofertilizer activity of two protein hydrolyzates in the growth and nitrogen metabolism of maize seedlings. *Journal of Plant Nutrition and Soil Science*, 172(2), 237-244.

Ertani, A., Pizzeghello, D., Baglieri, A., Cadili, V., Tambone, F., Gennari, M., & Nardi, S. (2012). Agro-industrial residues and their biological activity on maize (*Zea mays* L.) metabolism. *Journal of Geochemical Exploration*, 129(1), 103-111.

Ertani, A., Pizzeghello, D., Francioso, O., Sambo, P., Sanchez-Cortes, S., & Nardi, S. (2014). *Capsicum chinensis* L. growth and nutraceutical properties are enhanced by biofertilizers in a long-term period: chemical and metabolomic approaches. *Frontiers in Plant Science*, 5(1), 375-376.

Ertani, A., Schiavon, M., Muscolo, A., & Nardi, S. (2013). Alfalfa plant-derived biofertilizer stimulate short-term growth of salt stressed *Zea mays* L. plants. *Plant and Soil*, 364(12), 145-158.

Fakhfakh, N., Ktari, N., Siala, R., & Nasri, M. (2013). Wool-waste valorization: production of protein hydrolysate with high antioxidative potential by fermentation with a new keratinolytic bacterium, *Bacillus Pumilus* A1. *Journal of Applied Microbiology*, 115(2), 424-433.

Fang, Z., Zhang, J., Liu, B., Du, G., & Chen, J. (2013). Biochemical characterization of three keratinolytic enzymes from *Stenotrophomonas maltophilia* BBE11-1 for biodegrading keratin wastes. *International Biodeterioration & Biodegradation*, 82(2), 166-172.

Farouk, S., & Al-Sanoussi, A. J. (2019). The Role of Biostimulants in Increasing Barley Plant Growth and Yield under Newly Cultivated Sandy Soil. *Cercetari Agronomice in Moldova*, 52(2), 116-127.

Farré-Armengol, G., Filella, I., Llusia, J., & Peñuelas, J. (2016). Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions. *Trends in Plant*

Science, 21(10), 854-860.

Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A., & Olsen, G. J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and Environmental Microbiology*, 74(8), 2461-2470.

Gajc-Wolska, J., Kowalczyk, K., Nowecka, M., Mazur, K., & Metera, A. (2012). Effect of organic-mineral fertilizers on the yield and quality of Endive (*Cichorium endivia* L.). *Acta Scientiarum Polonorum*, 11(1), 189-200.

Gargallo-Garriga, A., Sardans, J., Pérez-Trujillo, M., Guenther, A., Llusià, J., Rico, L., ... & Peñuelas, J. (2016). Shifts in plant foliar and floral metabolomes in response to the suppression of the associated microbiota. *BMC Plant Biology*, 16(1), 78-79.

Gopinath, S. C., Anbu, P., Lakshmipriya, T., Tang, T. H., Chen, Y., Hashim, U., & Arshad, M. K. (2015). Biotechnological aspects and perspective of microbial keratinase production. *BioMed Research International*, 20(1),15-16.

Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., & Patra, J. K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiological Research*, 206(1), 131-140.

Gowtham, H. G., Murali, M., Singh, S. B., Lakshmeesha, T. R., Murthy, K. N., Amruthesh, K. N., & Niranjana, S. R. (2018). Plant growth promoting rhizobacteria-*Bacillus amyloliquefaciens* improves plant growth and induces resistance in chilli against anthracnose disease. *Biological Control*, 126(1), 209-217.

Grabowska, A., Kunicki, E., Sękara, A., Kalisz, A. and Wojciechowska, R., 2012. The Effect of Cultivar and Biostimulant Treatment on the Carrot Yield and its Quality. *Vegetable Crops Research Bulletin*, 77(1), 37-48.

Gupta, R., & Ramnani, P. (2006). Microbial keratinases and their prospective applications: an overview. *Applied Microbiology and Biotechnology*, 70(1), 21-33.

Gurav, R. G., & Jadhav, J. P. (2013). A novel source of biofertilizer from feather biomass for banana cultivation. *Environmental Science and Pollution Research*, 20(7), 4532-4539.

Gurung, N., Ray, S., Bose, S., & Rai, V. (2013). A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. *BioMed Research International*, 20(13),1-2.

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., & Yermiyahu, U. (2015). The use of biostimulants for enhancing nutrient uptake. *Advances in Agronomy*, 130(1), 141-174.

Ho, M. P., Wang, H., Lee, J. H., Ho, C. K., Lau, K. T., Leng, J., & Hui, D. (2012). Critical factors on manufacturing processes of natural fibre composites. *Composites Part B:*

Engineering, 43(8), 3549-3562

Holkar, C. R., Jain, S. S., Jadhav, A. J., & Pinjari, D. V. (2018). Valorization of keratin based waste. *Process Safety and Environmental Protection*, 115(1), 85-98.

Joardar, J. C., & Rahman, M. M. (2018). Poultry feather waste management and effects on plant growth. *International Journal of Recycling of Organic Waste in Agriculture*, 7(3), 183-188.

Kauffman, G. L., Kneivel, D. P., & Watschke, T. L. (2007). Effects of a biofertilizer on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol production of perennial ryegrass. *Crop Science*, 47(1), 261-267.

Kinnersley, A. M., & Turano, F. J. (2000). Gamma aminobutyric acid (GABA) and plant responses to stress. *Critical Reviews in Plant Sciences*, 19(6), 479-509.

Koukounaras, A., Tsouvaltzis, P., & Siomos, A. S. (2013). Effect of root and foliar application of amino acids on the growth and yield of greenhouse tomato in different fertilization levels. *Journal of Food, Agriculture & Environment*, 11(2), 644-648.

Kshetri, P., Roy, S. S., Sharma, S. K., Singh, T. S., Ansari, M. A., Prakash, N., & Ngachan, S. V. (2019). Transforming chicken feather waste into feather protein hydrolysate using a newly isolated multifaceted keratinolytic bacterium Chryseobacterium sediminis RCM-SSR-7. *Waste and Biomass Valorization*, 10(1), 1-11.

Kunicki, E., Grabowska, A., Sękara, A., & Wojciechowska, R. (2010). The effect of cultivar type, time of cultivation, and biofertilizer treatment on the yield of spinach (*Spinacia oleracea* L.). *Folia Horticulturae*, 22(2), 9-13.

Lateef, A., Adelere, I. A., & Gueguim-Kana, E. B. (2015). *Bacillus safensis* LAU 13: a new source of keratinase and its multi-functional biocatalytic applications. *Biotechnology & Biotechnological Equipment*, 29(1), 54-63.

Liang, X., Bian, Y., Tang, X. F., Xiao, G., & Tang, B. (2010). Enhancement of keratinolytic activity of a thermophilic subtilase by improving its autolysis resistance and thermostability under reducing conditions. *Applied Microbiology and Biotechnology*, 87(3), 999-1006.

Lindow, S. E., & Brandl, M. T. (2003). Microbiology of the phyllosphere. *Applied and Environmental Microbiology*, 69(4), 1875-1883.

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., & Edgar, R. C. (2012). Defining the core Arabidopsis thaliana root microbiome. *Nature*, *488*(7409), 86-90.

Maciel, J. L., Werlang, P. O., Daroit, D. J., & Brandelli, A. (2017). Characterization of protein-rich hydrolysates produced through microbial conversion of waste feathers. *Waste and Biomass Valorization*, 8(4), 1177-1186.

Maini, P. (2006). The experience of the first biofertilizer, based on amino acids and peptides: a short retrospective review on the laboratory researches and the practical results. *Fertilitas Agrorum*, 1(1), 29-43.

Mazotto, A. M., Ascheri, J. L. R., de Oliveira Godoy, R. L., Damaso, M. C. T., Couri, S., & Vermelho, A. B. (2017). Production of feather protein hydrolyzed by *B. subtilis* AMR and its application in a blend with cornmeal by extrusion. *Lebensmittel-Wissenschaft & Technologie*, 84(1), 701-709.

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, 5(5), 634-663.

Moe, L. A. (2013). Amino acids in the rhizosphere: from plants to microbes. *American Journal of Botany*, 100(9), 1692-1705.

Mushtaq, S., Ahmad, Z., Hoskins, C., Shahpal, A., & Choudhary, M. A. (2019). A facile synthesis of palladium encased Ag nanowires and its effect on fluorescence and catalysis. *Materials Research Express*, 6(5), 055015.

Ningthoujam, D. S., Tamreihao, K., Mukherjee, S., Khunjamayum, R., Devi, L. J., & Asem, R. S. (2018). Keratinaceous Wastes and Their Valorization through Keratinolytic , B., Gonzales, H. B., & Yoshikawa, K. (2019). Nitrogen mineralization dynamics of liquid feather hydrolysates obtained by hydrothermal treatment. *Applied Soil Ecology*, 134 (1), 98-104.

Odekunle, M. O., Odo, E. K., Sule, I., … Adenle, A. A. (2018). Application of Geographic Information Systems in creating smart campus map of Federal University of Technology, Minna Bosso campus. *I-manager's Journal on Digital Signal Processing*, 6(3), 10-11.

Ortega, R. A., Mahnert, A., Berg, C., Müller, H., & Berg, G. (2016). The plant is crucial: specific composition and function of the phyllosphere microbiome of indoor ornamentals. *FEMS Microbiology Ecology*, 92(12), fiw173.

Parikh, J., Channiwala, S. A., & Ghosal, G. K. (2005). A correlation for calculating HHV from proximate analysis of solid fuels. *Fuel*, 84(5), 487-494.

Parrado, J., Bautista, J., Romero, E. J., García-Martínez, A. M., Friaza, V., & Tejada, M. (2008). Production of a carob enzymatic extract: potential use as a biofertilizer. *Bioresource Technology*, 99(7), 2312-2318.

Pati, A., & Chaudhary, R. (2015). Soybean plant growth study conducted using purified protein hydrolysate-based fertilizer made from chrome-tanned leather waste. *Environmental Science and Pollution Research*, 22(24), 20316-20321.

Paul, T., Halder, S. K., Das, A., Bera, S., Maity, C., Mandal, A., ... & Mondal, K. C. (2013). Exploitation of chicken feather waste as a plant growth promoting agent using

keratinase producing novel isolate *Paenibacillus woosongensis* TKB2. *Biocatalysis and Agricultural Biotechnology*, *2*(1), 50-57.

Philippot, L., Raaijmakers, J. M., Lemanceau, P., & Van Der Putten, W. H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology*, 11(11), 789-799.

Popko, M., Wilk, R., Górecka, H., Chojnacka, K., & Górecki, H. (2015). Assessment of New NKSMg Fertilizer Based on Protein Hydrolysate of Keratin in Pot Experiments. *Polish Journal of Environmental Studies*, 24(4),1-3.

Queiroga, A. C., Pintado, M. E., & Malcata, F. X. (2012). Potential use of wool-associated *Bacillus* species for biodegradation of keratinous materials. *International Biodeterioration & Biodegradation*, 70(1)), 60-65.

Rahman, M., Sabir, A. A., Mukta, J. A., Khan, M. M. A., Mohi-Ud-Din, M., Miah, M. G., ... & Islam, M. T. (2018). Plant probiotic bacteria *Bacillus* and *Paraburkholderia* improve growth, yield and content of antioxidants in strawberry fruit. *Scientific Reports*, 8(1), 1-11.

Ramnani, P., & Gupta, R. (2007). Keratinases vis-à-vis conventional proteases and feather degradation. *World Journal of Microbiology and Biotechnology*, 23(11), 1537-1540.

Rubin, R. L., van Groenigen, K. J., & Hungate, B. A. (2017). Plant growth promoting rhizobacteria are more effective under drought: a meta-analysis. *Plant and Soil*, 416(1-2), 309-323.

Ruzzi, M., & Aroca, R. (2015). Plant growth-promoting rhizobacteria act as biofertilizers in horticulture. *Scientia Horticulturae*, 196, 124-134.

Saarela, M., Berlin, M., Nygren, H., Lahtinen, P., Honkapää, K., Lantto, R., & Maukonen, J. (2017). Characterization of feather-degrading bacterial populations from birds' nests–Potential strains for biomass production for animal feed. *International Biodeterioration & Biodegradation*, 123, 262-268.

Schiavon, M., Ertani, A., & Nardi, S. (2008). Effects of an alfalfa protein hydrolysate on the gene expression and activity of enzymes of the tricarboxylic acid (TCA) cycle and nitrogen metabolism in *Zea mays* L. *Journal of Agricultural and Food Chemistry*, 56(24), 11800-11808.

Schulz, S., & Dickschat, J. S. (2007). Bacterial volatiles: the smell of small organisms. *Natural Product Reports*, 24(4), 814-842.

Schütz, L., Gattinger, A., Meier, M., Müller, A., Boller, T., Mäder, P., & Mathimaran, N. (2018). Improving crop yield and nutrient use efficiency via biofertilization—A global meta-analysis. *Frontiers in Plant Science*, 8(1), 2204-22043.

Sekimoto, Y., Okiharu, T., Nakajima, H., Fujii, T., Shirai, K., & Moriwaki, H. (2013). Removal of Pb (II) from water using keratin colloidal solution obtained from wool. *Environmental Science and Pollution Research*, 20(9), 6531-6538.

Selvam, K., & Vishnupriya, B. (2012). Biochemical and molecular characterization of microbial keratinase and its remarkable applications. *International Journal of Pharmaceutical Biology Archives*, 3(2), 267-275.

Shakya, M., Gottel, N., Castro, H., Yang, Z. K., Gunter, L., Labbé, J., & Podar, M. (2013). A multifactor analysis of fungal and bacterial community structure in the root microbiome of mature Populus deltoides trees. *PloS One*, 8(10),1-3.

Sharma, S. S., & Dietz, K. J. (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany*, 57(4), 711-726.

Sharma, S., & Gupta, A. (2016). Sustainable management of keratin waste biomass: applications and future perspectives. *Brazilian Archives of Biology and Technology*, 59(1),1-2.

Sobucki, L., Ramos, R. F., Gubiani, E., Brunetto, G., Kaiser, D. R., & Daroit, D. J. (2019). Feather hydrolysate as a promising nitrogen-rich fertilizer for greenhouse lettuce cultivation. *International Journal of Recycling of Organic Waste in Agriculture*,1(1), 1-7.

Tesfaye, T., Sithole, B., & Ramjugernath, D. (2017). Valorisation of chicken feathers: a review on recycling and recovery route—current status and future prospects. *Clean Technologies and Environmental Policy*, 19(10), 2363-2378.

Tiwary, E., & Gupta, R. (2012). Rapid conversion of chicken feather to feather meal using dimeric keratinase from *Bacillus licheniformis* ER-15. *J Bioprocess Biotech*, 2(4), 1000123-1000124.

Vasileva-Tonkova, E., Gousterova, A., & Neshev, G. (2009). Ecologically safe method for improved feather wastes biodegradation. *International Biodeterioration & Biodegradation*, 63(8), 1008-1012.

Veerabadran, V., Balasundari, S. N., Devi, D. M., & Kumar, D. M. (2012). Optimization and production of proteinacious chicken feather fertilizer by proteolytic activity of Bacillus sp. MPTK 6. *Indian Journal of Innovations and Developments*, 1(3), 193-198.

Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology*, 10(12), 828-840.

Wang, B., Yang, W., McKittrick, J., & Meyers, M. A. (2019). Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration. *Progress in Materials Science*, 76(1), 229-318.

Wang, C., Zhao, D., Qi, G., Mao, Z., Hu, X., Du, B., ... & Ding, Y. (2020). Effects of *Bacillus velezensis* FKM10 for Promoting the Growth of *Malus hupehensis Rehd.* and Inhibiting *Fusarium verticillioides*. *Frontiers in Microbiology*, 10(1), 2889.-28890.

Wu, C. H., Bernard, S. M., Andersen, G. L., & Chen, W. (2009). Developing microbe–plant interactions for applications in plant-growth promotion and disease control, production of useful compounds, remediation and carbon sequestration. *Microbial biotechnology*, 2(4), 428-440.

Yakhin, O. I., Lubyanov, A. A., Yakhin, I. A., & Brown, P. H. (2017). Biostimulants in plant science: a global perspective. *Frontiers in Plant Science*, 7(1), 2049-2050.

Yang, L., Fan, X., Cui, T., Dang, E., & Wang, G. (2012). Nrf2 promotes keratinocyte proliferation in psoriasis through up-regulation of keratin 6, keratin 16, and keratin 17. *Journal of Investigative Dermatology*, 137(10), 2168-2176.

APPENDIX

Determination of proximate Composition of the Test Crops

Proximate composition of test crops (*Telfaria occidentalis* and *Albemoschus esculentus* was detrrmined. The crops were grounded to powdered form by using an electric blender. The powdered samples were sieved to obtain uniform size that were analyzed for moisture, protein, fat, ash, fiber and nitrogen free extract by the methods of Association of Analytical Chemist (Parikh *et al.*, 2005).

Determination of Moisture Content of the Crops

The moisture was determined by oven drying method. One point five gram (1.5g) of well-mixed sample was accurately weighed into a clean dried crucible (W1). The crucible was transferred to an oven at 105 ^{o}C for 6 hours until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 minutes to cool. After cooling, it was weighed again (W2). The percentage moisture content was calculated using the relation below:

$$\% \, Moisture = \frac{W1 - W2 \; x \; 100}{Wt}$$

Where:

W1 = Initial weight of crucible + Sample

W2 = Final weight of crucible + Sample

Wt = weight of the sample

Determination of ash content of the Crops

For the determination of ash content of the samples, clean empty crucible was placed in a muffle furnace at 600 ^{o}C for an hour, cooled in desiccator and then weight of empty crucible was noted (W_1). One gram of each of sample was taken in crucible (W_2). The sample was

ignited over a burner with the help of blowpipe, until it charred. Then the crucible was placed in muffle furnace at 550 ^{o}C for 2 hours. The appearance of gray white ash indicated complete oxidation of all organic matter in the sample and thereafter the ashing furnace was switch off. The crucible was cooled, percentage ash content was calculated using the relation below: Difference in weight of Ash= W_3 -W_1 and weighed (W_3), sample weight (W_2).

$$\% \, Ash \; = \; \frac{Difference \, in \, Wt. of \; Ash}{Weight \; of \; sample} \, x \, 100$$

Determination of crude protein content of the Crops

Protein in the sample was determined by Kjeldahl's method. One gram (1g) of dried samples was taken in digestion flask. Fifteen millilitre (15mL) of concentrated H_2SO_4 and eight gram (8g) of digestion mixture i.e. $K2SO_4$: $CuSO_4$ (8:1) was added. The flask was then swirled in order to mix the contents thoroughly then placed on heater to start digestion till the mixture become clear (blue green in colour). It was left to stand for 2 hours. The digest was cooled and transferred to 100 mL volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markam Still Distillation Apparatus. Ten millilitres (10 mL) of digest was introduced in the distillation tube then 10 mL of 0.05 N NaOH was gradually added through the same way. Distillation was continued for at least 10 minutes and NH_3 produced was collected as NH_4OH in a conical flask containing 20 mL of 4 % boric acid solution with few drops of modified methyl red indicator. During distillation, yellowish colour appeared due to NH_4OH. The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink colour. A blank was also run through all steps above. Percentage crude protein content of the sample was calculated using the relation below:

% Crude Protein = 6.25* x %N (*. Correction factor)

$$\%N = \frac{(S - B)\ x\ N\ x\ 0.014\ x\ D\ x\ 100}{Weight\ of\ the\ sample\ x\ V}$$

Where:

S = Sample titration reading

B = Blank titration reading

N = Normality of HCl

D = Dilution of sample after digestion

V = Volume taken for distillation

0.014 = Milli equivalent weight of Nitrogen

Determination of Crude Fat Content of the Crops

One gram (1g) of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. Weighed, cleaned and dried receiving beaker was filled with petroleum ether and fitted into the apparatus. Water and heater were turned on to start the extraction. After six siphoning, petroleum ether was allowed to evaporate and then beaker was disconnected. The extract was transferred into clean glass dish with petroleum ether washed and evaporated on water bath. The dish was placed in an oven at 105 ^{o}C for 2 hours and was cooled in a desiccator. The percent crude fat was determined using the formula below:

$$\%\ Crude\ Fat = \frac{Weight\ of\ petroleum\ ether\ extract\ x\ 100}{Weight\ of\ sample}$$

Determination of Crude Fibre Content of the Crops

Aliquots of 0.15g of the sample was weighed (W0) and transferred to porous crucible and the crucible was placed into the Dosi-fiber unit and the valve was kept in "OFF" position. Thereafter, 150 mL of preheated H_2SO_4 solution and some drops of foam-suppresser were added to each column. The cooling circuit was opened and the heating elements (power at 90 %) turned on. On boiling, the power was reduced to 30 % and left for 30 minutes. Valves were opened for drainage of acid and rinsed with distilled water thrice to ensure the complete removal of acid from the sample. The same procedure was used for alkali digestion by using KOH instead of H_2SO_4. The sample was dried in an oven at 150 °C for 1 hour. Then it was allowed to cool in a desiccator and weighed (W_1). The samples in crucibles were then kept in muffle furnace at 55 °C for 4 hours. The samples were then cooled in a desiccator and weighed again (W_2). Calculations were done by using the formula:

$$\% \, Crude \; Fiber \; = \; W1 - \; W2 \; x \; 100$$

$$W0$$

Where: W_1 = initial weight, W_2 = final weight, W_0 = weight of sample

Determination of Nitrogen Free Extracts Content of the Crops

Nitrogen Free Extract (NFE) was calculated by difference after analysis of all the other items. NFE = (100-% moisture + % crude protein + % crude fat + % crude fiber + % ash) (Parikh *et al.,* 2003).