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WATERMELON GROUP

BIOCONVERSION OF POULTRY FEATHER WASTE INTO FEATHER HYDROLYSATE: A SUSTAINABLE BIOFERTILIZER FOR WATERMELON CULTIVATION

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CERTIFICATION

This is to certify that, **OGUNJIMI Cecilia Ogundoyin**, matriculation number **192213**, a student of the Department of Pure and Applied Biology (Microbiology option). Faculty of Pure and Applied Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria under the supervision of Prof. A. Lateef.

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DEDICATION

I dedicate this project work to Almighty God and to my parents Mr and Mrs Robert Felicia Ogunjimi for their everyday encouragement towards the success of my academic.

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My sincere appreciation goes to God Almighty, the ultimate source of knowledge and wisdom, for seeing me through my studies, to my parents Mr and Mrs Robert Felicia Ogunjimi, thanks for your financial and parental support, thanks for being a good parent, and to my sibling thanks for your guidance and also for being a good example towards my academics and always supporting in the best ways you can.

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TABLE OF CONTENT

LIST OF TABLE

Tables **Page**

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

Billions of tons of keratinous waste in the form of feathers, antlers, bristles, claws, hair, hoofs, horns, and wool are generated by different industries and their demolition causes environmental deterioration. Chicken feathers have 92% keratin that can be a good source of peptides, amino acids, and minerals. This study is aimed at the bioconversion of poultry feather waste into a nutrient-rich feather hydrolysate and its application as a sustainable biofertilizer for watermelon (*Citrullus lanatus*) cultivation to develop an eco-friendly and efficient method to manage poultry feather waste while enhancing watermelon growth and yield through sustainable agricultural practices.

The bioconversion process involved microbial hydrolysis using keratinolytic bacteria (*Aqaumicrobium defluvii*) collected from the microbial bank in Laboratory of Industrial Microbiology and Nanobiotechnology, LAUTECH, Ogbomosho to break down feathers into a bioavailable form. The selected bacteria were isolated and cultured, and feather waste was treated under optimal conditions for keratin degradation, producing a feather hydrolysate rich in nitrogen, amino acids, and micronutrients essential for plant growth. The resulting feather hydrolysate was then applied to watermelon crops in a controlled field trial, comparing its effects to conventional chemical fertilizer(NPK) and untreated controls.

The results showed that the effectiveness of biofertilizers derived from feather and hoof hydrolysates on watermelon yield, while the biofertilizers did not significantly outperform conventional NPK fertilizer or water, they exhibited promising potential.

However, factors such as nutrient concentration, application timing, and environmental conditions may have influenced the results. Further research is needed to optimize biofertilizer formulations and application techniques to maximize their benefits for sustainable agriculture.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Agricultural practices are evolving towards more sustainable approaches in response to the global need for environmental conservation and resource efficiency. Among these advancements, the recycling and reuse of agricultural waste have gained significant attention (Liya and Umesh, 2023). The steady rise in the global population indicates that it would reach 9.7 billion people by 2050 (UN, 2022). This will require very huge staple and healthy foods for the survival of the people. Presently, agricultural sector is facing the challenge of how to increase food production to sustain the steadily rising world population in a cost-effective and environmentally benign manner (Adelere and Lateef, 2023). Agricultural food production currently depends largely on the use of fertilizer for the purpose of enhancing food production to feed the rapidly growing world population, and at the same time to boost the production of healthy foods like fruits and vegetables, whose demand is on the rise over the past two decades (Kyriacou *et al*., 2017). The use of chemical fertilizers is being discouraged in modern agricultural practices due to their high production cost and environmental problems associated with their production and application. Excessive use of chemical fertilizers is not only cost intensive but also leads to their gradual entry into water systems through rain water and seepage to contaminate groundwater, which can induce serious risks to human and animal health (Adelere and Lateef, 2023). However, the application of slow-release nitrogenous organic

fertilizers has proven to be a safer and better substitute to boost agricultural productivity (Chatzistathis *et al*., 2021) for the actualization of Sustainable Development Goal 2 (SDG 2; zero hunger) of the United Nations. Organic fertilizers stimulate physiological processes in plants and can enhance crop yield and quality and induce tolerance or recovery from abiotic stress (du Jardin, 2015).

Globally, about 8.5 million tons of chicken feather wastes are generated annually from agro-industrial processing (Da Silva, 2018), which constitutes a threat to the environment due to their recalcitrant keratin content (Lateef *et al*., 2010), but which can be degraded by some microorganisms that include fungi and bacteria (Lateef *et al*., 2015b). Keratins are insoluble, fibrous and structural proteins that are found in the epidermis and its appendages like feathers, hair, wool, nail, hoof, and horns (Hassan *et al*., 2020). They are resistant to degradation by common proteolytic enzymes like trypsin and pepsin due to their structural stabilization by tightly packed peptide chains and the presence of several cross-linkages by disulphide bonds, hydrogen bonding and hydrophobic interactions (Adelere and Lateef 2019). Microbial fermentation is an effective way to valorize feathers in a biotechnological manner to produce multi-applicable keratinolytic enzymes and feather hydrolysates (FHs) (Reddy *et al*. 2021).

Feather hydrolysates are rich in free amino acids, ammonia, peptides, and some biologically important products that can stimulate soil microbial activity which will in turn facilitate the assimilation of nutrients by plants for better growth by releasing essential and

non-essential amino acids (Bokveld *et al*., 2021), thereby resulting to the production of superior bio fertilizer (Bhari *et al*., 2021).

Watermelon (*Citrullus lanatus*) is a refreshing and popular fruit, especially enjoyed during summer due to its high water content, which makes it hydrating and cooling. Watermelon is low in calories and packed with vitamins, particularly vitamin C, vitamin A (from beta-carotene), and antioxidants like lycopene. Lycopene, which gives the watermelon its red color, is known for its potential health benefits, including reducing the risk of heart disease and certain cancers. Apart from being eaten fresh, watermelon can be used in salads, smoothies, sorbets, and even grilled. With over 90% water content, watermelon is one of the most hydrating fruits. It's an excellent choice for replenishing fluids on a hot day or after exercise (Liu *et al*., 2024).

Despite the huge benefits associated with the intake of fruits, their global consumption level is still below the WHO recommendation due to a gap in production. Hence, awareness was raised about the importance of fruits and vegetables to promote good health and well-being towards achieving the SDGs of the UN (FAO 2020). It has been estimated that by 2050, between 800 million and 1.9 billion people in sub-Saharan Africa will not have access to 400 g of fruits and vegetables per day as recommended by WHO (Adelere and Lateef, 2023). Part of the strategies for making fruits and vegetables available for human consumption is to increase their production at a low cost and in an environmentally sustainable manner. Thus, keratinous wastes can be valorized to produce organic fertilizer as one of the strategies to boost the growth of nutritious fruits.

1.2 Statement of the Research Problem

The problem of agricultural waste management and its environmental impacts has become a pressing issue in modern agriculture. In particular, the poultry industry generates a substantial volume of waste, with feathers constituting a significant portion. Annually, over 40 million tons of poultry feathers are produced worldwide as byproducts of commercial poultry farming. These feathers, which are composed primarily of keratin, pose a significant disposal challenge due to their recalcitrance to natural degradation processes. Most poultry feathers are incinerated or dumped into landfills, contributing to both environmental pollution and the loss of potentially valuable nutrients contained within the feathers. The improper disposal of this waste not only increases the environmental burden but also represents a missed opportunity to repurpose these organic materials for beneficial use in agriculture (Santos *et al*., 2024).

Keratin, the primary component of poultry feathers, is a fibrous and highly stable protein, resistant to microbial degradation due to the presence of strong disulfide bonds. This resistance complicates the biodegradation process, making traditional methods of waste management (such as composting) ineffective. Consequently, an innovative approach is required to break down keratin and convert poultry feathers into a useful product. Enzymatic hydrolysis has emerged as a viable solution to this problem, allowing for the controlled breakdown of keratin into simpler proteins, amino acids, and peptides, which can then be used as a nutrient source in bio fertilizers (Bhari *et al*., 2021).

1.3 Justification of the study

The rising global population and increasing demand for food necessitate more efficient and sustainable agricultural practices to ensure food security. Watermelon, as a widely cultivated fruit, plays a significant role in global horticulture. However, its cultivation often relies on high levels of chemical fertilizer inputs to achieve competitive yields, which can have negative environmental consequences. This study's exploration of feather hydrolysate as a bio fertilizer has the potential to contribute to more sustainable watermelon production systems that maintain high yields while minimizing the ecological impact.

Although this study focuses on watermelon cultivation, the findings may have broader applications in other crops and agricultural systems. Feather hydrolysate has the potential to be used as a bio fertilizer for a variety of crops, including grains, vegetables, and fruits, thus expanding its utility across different farming practices. The scalability of the bioconversion process also offers promising opportunities for large-scale agricultural operations, allowing farmers to reduce waste and improve crop yields simultaneously. If the bioconversion process can be optimized and made more efficient, feather hydrolysate could become a widely adopted biofertilizer in both conventional and organic farming systems. Its widespread use could contribute to the reduction of chemical fertilizer dependency, fostering more sustainable global agriculture.

1.4 Aim and Objectives

This study is aimed at achieving the bioconversion of poultry feather waste into feather hydrolysate and evaluating its potential as a sustainable bio fertilizer for watermelon cultivation. The Objectives of this study were:

i. to develop a bioconversion process for poultry feathers through enzymatic hydrolysis.

ii. to assess the impact of feather hydrolysate on the growth and yield of watermelon plants.

iii. to compare the effectiveness of feather hydrolysate with chemical fertilizers in terms of plant growth, soil health, and environmental impact.

CHAPTER TWO

LITERATURE REVIEW

2.1 Feather

Feathers are unique to Birds. They are a defining characteristic of the group, meaning simply that if an animal has feathers, then it is a bird (Benton *et al*., 2021). Feathers serve many function in birds but most notable is the critical role feathers play in enabling birds to fly. Feathers are the most complex integumentary appendages found in vertebrates, and they are formed in tiny follicles that contain keratin proteins in the epidermis or outer skin layer. The β-keratins in feathers, claws and beaks - and the claws, shells, and scales of reptiles - are composed of protein strands hydrogen-bonded into the β-pleated sheets, then, that are much stronger than -keratins of mammalian horns, hooves, and hair Disulfide bridges twist and crosslink the -keratins of mammalian horns, hooves, and hair into structures (Ehrlich *et al*., 2020). The exact signals that cause the feather to grow on the skin are unclear, but transcription factor cDermo-1 has been discovered to cause the feather to grow on the skin and scales on the leg. Feathers are made up of Keratin, an insoluble protein that is also found in mammalian hair and reptilian scales. In general feathers consist of the following structures:

i.Calamus (quill) - the hollow shaft of the feather that attaches it to the bird's skin.

ii. Rachis - the central shaft of the feather to which the vanes are attached.

iii. Vane - the flattened part of the feather that is attached on either side of the rachis (each feather has two vanes).

iv. Barbs - the numerous branches off the rachis that form the vanes.

v. Barbules - tiny extensions from barbs that are held together by barbicels.

vi. Barbicels - tiny hooks that interlock to hold the barbules together.

Feathers are versatile, and their unique properties make them useful across various industries. They are popularly used in fashion accessories like hats, jewelry, and costumes. Feathers are increasingly used as a sustainable alternative in agriculture. They can be processed into feather meal, a protein-rich supplement for animal feed, or used as a natural fertilizer. Keratin extracted from feathers is being explored for tissue engineering and wound dressings due to its biocompatibility and structural properties (Ehrlich *et al*., 2020).

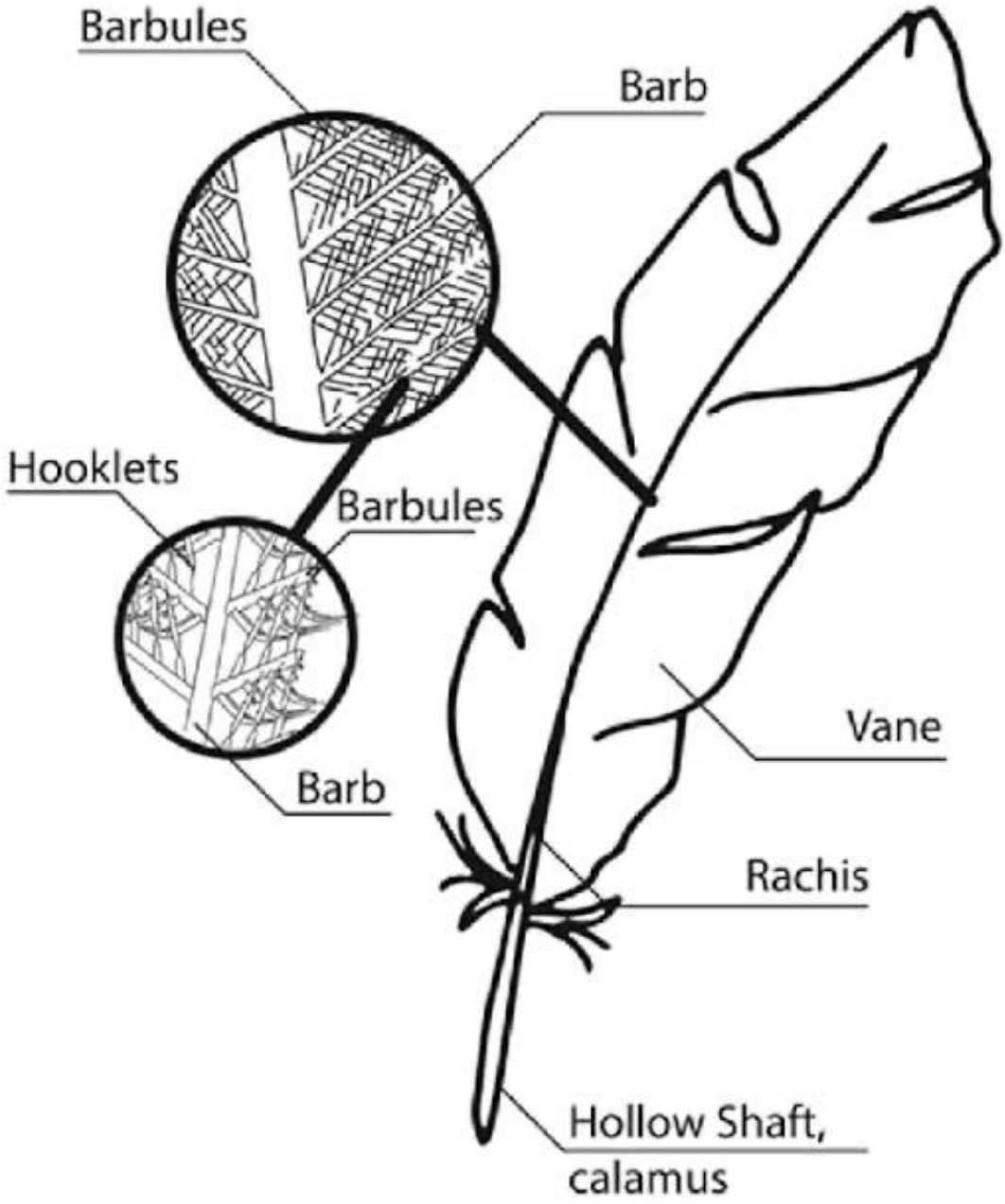

Fig 2.1 Modern detail of a Chicken Feather (Ehrlich *et al*., 2020).

2.2 Keratin

Keratin belongs to the family of fibrous structural proteins called scleroproteins. It is the most abundant structural protein found in hair, nails, feathers, horns, claws of animals; along with collagen, it is the most important biopolymer encountered in animals. Its characteristic feature is its high cysteine content as compared to other fibrous proteins like elastin, collagen and myofibrillar protein (Feroz *et al*., 2020). Over the years, keratin has been extracted from chicken feathers, beaks, claws, nails, horns, hooves, human hair and toenails. The other significant source of keratin is wool. Wool with up to 95% keratin by weight is considered to be a pure source of intermediate filament proteins, which have gained importance in cosmetic and biomedical fields. Keratin biomaterials prepared from wool and human hair possess cell-binding motifs which have hemostatic and cell-binding potential. Keratin has an intrinsic ability to self-assemble and form polymers. These biomaterials are exceptionally biocompatible and have cellular proliferation abilities, making them a great candidate for drug delivery systems and tissue engineering (Idrees *et al*., 2020). They have also found potential roles in energy sectors, agricultural fields, pharmaceutical and cosmetic industries, leather and textile industries (Donato and Mija, 2020).

Keratin is one of the most important structural proteins found in certain epithelial cells of vertebrates, belonging to the intermediate filament protein's superfamily. Keratin proteins are fibrous with long polypeptide chains and cross-linking fibre (Murray *et al*., 2017). Structurally keratins can be classified as α- and β-keratins, with β-keratin being tougher than the α-form.

The α-form is found in mammals and is the primary constituent of wool, hair, claws, hooves, horns and stratum corneum. In contrast, the β-form is mainly found in hard avian and reptilian tissues, such as feathers, beaks and claws of birds, and scales and claws of reptiles.

Keratins are robust in structure, highly stable and remain insoluble in most of the organic solvents. They are also resistant to enzymatic degradation by proteolytic enzymes. The high cysteine content in keratin confers mechanical and chemical resistance. Keratin protein also has a high thermal resistance, and according to Takahashi *et al*., it can be denatured at a temperature higher than 100°C (Feroz *et al*., 2020).

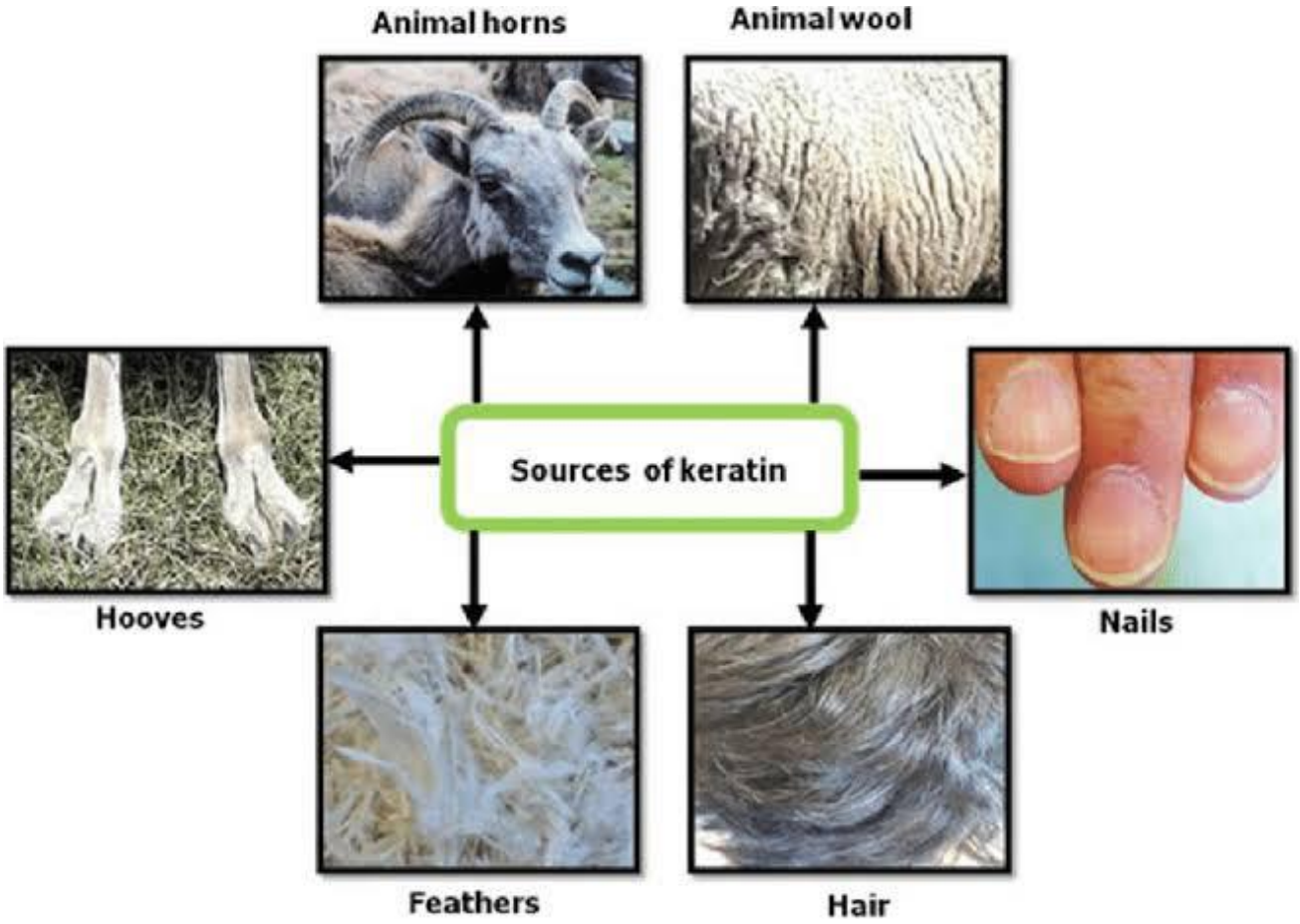

Fig 2.2 Major Sources of Keratin (Feroz *et al*., 2020)

2.3 Microbial degradation of Keratin

The degradation of keratinous material is potentially important both medically and bio technologically. As a durable structural protein found in hair, feathers, nails, and animal horns, keratin resists breakdown due to its tightly packed, disulfide-bonded structure. However, certain microorganisms, known as keratinolytic microbes, have evolved the ability to degrade keratin (Allure *et al*., 2015). Worldwide poultry processing plants produce millions of tons of feathers as a waste product annually, which consists of approximately 90% keratin and is largely responsible for their high degree of recalcitrance. The keratinous wastes are increasingly accumulating in the environment mainly in the form of feathers, hair, horns, hooves and nails generated from various industries. The sewage and bottom sediments of rivers and canals contains an enormous amount of hidden keratinous waste because of daily shaving habits in metros. Today, it is also becoming a part of solid waste management and it is difficult to degrade and recycling of such wastes is increasing attention. Recent findings suggest that, several proteases may have keratinolytic activity but such activity only leads to full keratin decomposition if several different keratinolytic enzymes act together (Lange *et al*., 2014). A vast group of Bacteria, Actinomycetes and Fungi are known to be keratin degraders. There are several species of keratinolytic bacteria but members of *Bacillus* are the most predominant (Lange *et al*., 2016). *Streptomyces* have also been found to be one of the prominent keratinolytic actinomycetes (Allure *et al*., 2015), while the common keratinolytic fungi are distributed in the genera *Aspergillus*, *Penicillium*, and *Fusarium.* It has been established that biodegradation of keratins through the activities of keratinolytic microorganisms is an efficient

alternative means to manage keratin wastes and way of improving their biotechnological value (Jayalakshmi *et al.*, 2012). These microorganisms have been isolated from diverse habitats such as keratin dumping sites, keratin wastes, hot spring, livestock processing plants, ponds among others (Adelere and Lateef, 2018).

2.3.1 Degradation by Bacteria

The keratinolytic bacteria are commonly Gram-positive, but a few strains of keratinolytic Gram-negative bacteria are also known. Keratinolytic bacteria that belonged to vast varieties of genera, namely, *Bacillus*, *Stenotrophomonas*, *Pseudomonas, Chryseobacterium*, *Keratinibaculum*, *Paenibacillus, Meiothermus*, *Rhodococcus*, *Achromobacter*, *Exiguobacterium*, *Rummeliibacillus*, *Sporosarcina*, *Brevibacillus*, and *Aeromonas* have been isolated from soils, hot spring, bird's nests, and keratin wastes. Species of *Bacillus*are the predominant keratinolytic bacteria with very high efficiency to degrade keratins. Several authors have documented very remarkable keratinolytic activities of their strains, namely *B. licheniformis,B. pumilus,B. cereus,* and *B. subtilis* (Lateef *et al*., 2010).

2.4 Mechanism of Keratin degradation by Microorganism

The degradation of keratin by microorganisms involves two steps, sulfitolysis, i.e., the reduction of disulfide bonds and proteolysis which is hydrolysis of peptide bonds. It is assumed that sulfitolysis requires the presence of disulfide reductases which act in collaboration with keratinases to ensure complete degradation of keratin. Some authors agreed that keratinases act synergistically with other microbial enzymes like disulfide reductases and cysteine

dioxygenase for the effective degradation of keratinous wastes (Adelere and Lateef, 2018). Disulfide reductase and cysteine dioxygenase initiate denaturation of keratin structure by breaking the cross-linkages formed by disulfide bonds to make the peptide bonds accessible for hydrolysis by keratinase enzyme. Efficient degradation of keratin by microorganisms not only relies on enzymes, but on fermentation conditions like pH, temperature, rate of agitation, and sources of carbon, energy and nitrogen (Adelere and Lateef, 2018).

2.5 Valorization of keratinous wastes

Microbial keratinases are gradually getting importance biotechnologically for valorization of keratinous wastes. Microbial keratinases are being used successfully in degrading keratin into economically important keratin protein hydrolysates which can find potential applications as animal feed supplements, bio- fertilizers, biodegradable glues, films, and foils. 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 (Ningthoujam *et al*., 2018). The details of valorization of keratinous wastes are described below

Fig 2.3 Different Applications of Keratin (Ningthoujam *et al.*, 2018).

2.6 Significance of Biofertilizer over Chemical Fertilizer

The use of chemical fertilizers (e.g. urea, calcium nitrate, ammonium sulphate, diammonium phosphate etc.) have a great importance for the world's food production as it works as a fast food for plants causing them to grow more rapidly and efficiently (Suhag, 2016). While adverse effects are being noticed due to the excessive and imbalanced use of these synthetic inputs. Moreover, persistent use of conventional chemical fertilizers subverts the soil ecology, disrupt environment, degrade soil fertility and consequently shows harmful effects on human health and contaminates ground water. For these reasons, biofertilizers, the organic substances, which make use of microorganisms to increase the fertility of soil, has been identified as harmless input help in safeguarding the soil health and also the quality of crop products (Suhag, 2016). Biofertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth promoting substances. They are also environment friendly and responsible for continuous availability of nutrients from natural sources.

Biofertilizers most commonly referred to as the fertilizer that contains living soil micro-organisms to increase the availability and uptake of mineral nutrients for plants. It is expected that their activities will influence the soil ecosystem and produce supplementary substance for the plants (Ossai *et al*., 2022). Bio fertilizers also include organic fertilizers (manure, etc.), which are rendered in an available form due to the interaction of micro-organisms or due to their association with plants When bio fertilizers are applied as seed or soil inoculants, they

multiply and participate in nutrient cycling and benefit crop productivity. Bio fertilizers keep the soil environment rich in all kinds of micro and macro-nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil providing better nutrient uptake and increased tolerance towards drought and moisture stress. Bio fertilizers differ from chemical and organic fertilizers in the sense that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi, relatively simple and having low installation cost. Biofertilizer overall produced higher growth rates, yield development of rice production compared with Chemical fertilizer. Therefore, biofertilizers can solve the problem of feeding an increasing global population at a time when agriculture is facing various environmental stresses and change (Suhag, 2016).

2.7 Watermelon

Watermelon (*Citrullus lanatus*) botanically considered as the fruit is belonging to the family *Cucurbitaceae* (Liu *et al*., 2024). *Cucurbitaceae* family ranks among the highest of plant families for number and percentage of species used as human food. Watermelon is originated from Kalahari Desert of Africa but nowadays cultivated abundantly in tropical regions of the world. It has great economic importance with 29.6 million tonnes estimated production worldwide. It is a large, sprawling annual plant with coarse, hairy pinnately-lobed leaves and yellow flowers. It is grown for its edible fruit, which is a special kind of berry botanically called a pepo. The watermelon fruit has deep green smooth thick exterior rind with grey or light green

vertical stripes. Inside the fruit is red in color with small black seeds embedded in the middle third of the flesh (Wehner *et al*., 2008).

Watermelon is a warm season crop grown mainly in sub-tropical and hot-arid regions. Temperature range of 24-27°C is considered as optimum for the growth of the vines. Cool nights and warm days are ideal for accumulation of sugars in the fruits. The seed germinates best when temperatures are higher than 20°C. High humidity at the time of vegetative growth renders the crop susceptible to various fungal diseases. Depending upon the season about 2-3 weeding operations is required. (Wehner *et al*., 2008). The first weeding should be done 20-25 days after sowing while subsequent weeding is done at an interval of one month. The biggest watermelon pest is the leaf-eating beetles, they damage the flowers. The other main problem with growing watermelons is mildew, a fungus that makes the leaves look as if they were coated with white powder. Watermelon is one of the commonly consumed fruits in many countries. Watermelon contains more than 91% water and up to 7% of carbohydrates. It is a rich source of lycopene and citrulline. Watermelon rind contains more amounts of citrulline than flesh. Additionally, watermelon has a number of essential micronutrients and vitamins. Watermelon contains high levels of lycopene that is very effective in protect cells from damage and lower the risk of heart disease. Watermelon extracts help to reduce hypertension and lower blood pressure in obese adults. Watermelon fruit is also a good source of potassium. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure. Thus, it prevents against stroke and coronary heart diseases (Wehner *et al*., 2008).

2.8 Nanotechnology

Nanotechnology is the manipulation of matter with at least one dimension sized from 1 to 100 nanometers (nm). At this scale, commonly known as the nanoscale, surface area and quantum mechanical effects become important in describing properties of matter (Sahu *et al*., 2023). This definition of nanotechnology includes all types of research and technologies that deal with these special properties. It is common to see the plural form "nanotechnologies" as well as "nanoscale technologies" to refer to research and applications whose common trait is scale. An earlier understanding of nanotechnology referred to the particular technological goal of precisely manipulating atoms and molecules for fabricating macro-scale products, now referred to as molecular nanotechnology. Nanotechnology may be able to create new materials and devices with diverse applications, such as in nanomedicine, nanoelectronics, biomaterials energy production, and consumer products (Sahu *et al*., 2023). However, nanotechnology raises issues, including concerns about the toxicity and environmental impact of nanomaterials, and their potential effects on global economics, as well as various doomsday scenarios. These concerns have led to a debate among advocacy groups and governments on whether special regulation of nanotechnology is warranted (Patel *et al.*, 2021).

2.8.1 Nanoparticles

Nanoparticles are the particles of matters with the diameter usually ranging from 1 to 100 nanometres (nm), and possess distinctly different physicochemical properties, for example, colloidal properties, compared to that of microparticles or macroparticles (Nahar and Sarker,

2017; Khan *et al*., 2019). Simply, nanoparticles are particles with dimensions and tolerances of 1–100 nanometres (nm). However, the term 'nanoparticles' sometimes is used to describe larger particles (100–500 nm), fibres and tubes with 100 nm in two directions only. Depending on the overall shape, nanoparticles can be 0D, 1D, 2D or 3D, and can be classified into the following categories: carbon-based nanoparticles (e.g., fullerenes and carbon nanotubes), organic nanoparticles (e.g., dendrimers, metal nanoparticles [e.g., copper (Cu), silver (Ag), zinc (Zn) and gold (Au) nanoparticles], ceramic nanoparticles (e.g., inorganic nanometallic solids), semiconductor nanoparticles (e.g., nanoparticles possessing metal and nonmetal properties), polymeric nanoparticles (e.g., nanospheres or nanocapsular shaped polymeric particles), hybrid nanoparticles (e.g., hydrogels), magnetic nanoparticles (e.g., superparamagnetic/poly(methyl methacrylate) nanoparticles), and lipid-based nanoparticles (e.g., nanoparticles containing lipid moieties) (Khan *et al*., 2019). Nanoparticles can be synthesized applying either a top-down approach, where larger particles are broken down to smaller particles using grinding and other degradation/decomposition techniques, or bottom-up approach (also known as building up approach), where nanoparticles are produced from relatively simpler substances by integration of smaller particles into active nanoparticles using sedimentation and reduction techniques.

In recent years, nanoparticles have been used in medicine, particularly in targeted and controlled-release drug delivery (Nahar and Sarker, 2017). As nanoparticles penetrate cells more efficiently than larger microparticles, they can be used as effective transport and delivery systems, for example, for medicinal purposes, drugs can either be incorporated into the matrix of the particle or attached to the particle surface. Because of the small particle size,

nanoparticles have larger surface area and can increase solubility and stability of drug molecules, facilitate absorption of drugs, increase permeation and retention of drugs in target tissues, improve bioavailability, protect them from premature degradation in the body, exhibit high differential uptake efficiency in the target cells over normal cells and prolong their circulation time. Nanoparticles and nanomaterials have also been utilized in dentistry and oral health, and paved the way for the development of a new avenue in dentistry, called 'nanodentistry'.

Nanoparticles can be prepared in different shapes, sizes, compositions, and functionalized, and modified physicochemically to achieve specific properties depending on the characteristics of both the drug molecule and the targeted organ (Nahar and Sarker, 2017). However, before considering specific applications of nanoparticles, it is essential to properly characterize nanoparticles including morphological characterization, structural characterization, particle size and surface area characterization, chemical characterization, and optical characterization (Khan *et al*., 2019). As nanoparticles, in most cases, are considerably smaller than the wavelengths of visible lights (400–700 nm), they cannot be observed under optical microscopes, and thus, electron microscopes, e.g., Transmission Electron Microscope (TEM) or Scanning Electron Microscope (SEM), are needed for characterization of nanoparticles, especially their shapes and sizes. Apart from electron microscopic methods, several other methods, such as X-ray Diffraction (XRD), X-ray Absorption (XAS), X-ray Photoelectron Spectroscopy (XPS), Dynamic Light Scattering (DLS), Brunauer–Emmett–Teller (BET), Nuclear Magnetic

Resonance (NMR) Spectroscopy and Fourier-Transform Infrared (FTIR) Spectroscopy, are now routinely used.

2.8.2 Significance of Nanotechnology in Agriculture

Importance of agriculture to all human societies is characterized more than ever with increasing world population. The first and most important need of every human is needs to food, and food supply for humans associated with agriculture directly and indirectly (Usman *et al.*, 2020). Growth of the agricultural sector as a context for development objectives is seen as essential in developing countries. Now, after years of green revolution and decline in the agricultural products ratio to world population growth, it is obvious the necessity of employing new technologies in the agriculture industry more than ever. Modern technologies such as bio and nanotechnologies can play an important role in increasing production and improving the quality of food produced by farmers. Many believe that modern technologies will secure growing world food needs as well as deliver a huge range of environmental, health and economic advantages (Chhipa, 2019). Food security has always been the biggest concern of the mankind. Nations, communities and governments have been struggling with the issue since long. Recent decades have seen even bigger challenges on this front. The future looks even bleaker with food shortage issue looming large. The challenge is how to feed the growing population by producing more on a stagnant or shrinking landscape; with lesser input costs and with lesser hazards to the eco-system (Usman *et al.*, 2020). In between, nanotechnology has proved its place in agricultural sciences and related industries, as an interdisciplinary

technology and a pioneer in solve problems and lacks. Nanotechnology has many applications in all stages of production, processing, storing, packaging and transport of agricultural products. The use of nanotechnology in agriculture and forestry will likely have environmental benefits. Farm applications of nanotechnology are also commanding attention. Nano materials are being developed that offer the opportunity to more efficiently and safely administer pesticides, herbicides, and fertilizers by controlling precisely when and where they are release. Nanotechnology as a new powerful technology has the ability to create massive changes in food and agricultural systems (Pramanik *et al*., 2020). Nanotechnology is able to introduce new tools for use in cellular and molecular biology and new materials to identify plant pathogens. Hitherto numerous applications of nanotechnology in agriculture, food and animal sciences, has been proposed. Use of nanotechnology in agriculture and food industry can revolutionize the sector with new tools for disease detection, targeted treatment, enhancing the ability of plants to absorb nutrients, fight diseases and withstand environmental pressures and effective systems for processing, storage and packaging. Nanotechnology has provided new solutions to problems in plants and food science (post-harvest products) and offers new approaches to the rational selection of raw materials, or the processing of such materials to enhance the quality of plant products. In the agricultural sector, nanotechnology research and development is likely to facilitate and frame the next stage of development of genetically modified crops, animal production inputs, chemical pesticides and precision farming techniques. Precision agriculture means that there is a system controller for each growth factor such as nutrition, light, temperature, etc. Available Information for planting and harvest time are controlled by satellite

systems. This system allows the farmer to know, when is the best time for planting and harvesting to avoid of encountering bad weather conditions (Usman *et al*., 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection and preparation of substrate

Aqaumicrobium defluvii culture was collected from the microbial bank in Laboratory of Industrial Microbiology and Nanobiotechnology, LAUTECH, Ogbomosho. The culture was sub-cultured for use and stored in agar slant for future use.

Sample acquisition was executed at the feather waste site of LAUTECH Teaching and Research poultry farm, Ogbomoso (8° 10` 7 N, 4° 16` 15 E), focusing on an area designated as a repository for discarding poultry feathers. The soil from the dump site was carefully gathered in a clean polythene bag, brought to the laboratory, ambient conditions, for further investigations. Chicken feathers were brought to the laboratory also, cleaned by washing thoroughly with tap water to remove dirt and then dried in hot air oven at 75°C for 8 h. The dried feather waste was chopped into pieces, milled and then sieved using 60 mesh particle size, to obtain the feather powder which was preserved in airtight container and kept under ambient condition to safeguard its compositional integrity for subsequent utilization as keratin powder.

Cow hoof were collected from cow hoof waste dump site at Atenda abattoir Waso Ogbomoso. The hooves were collected randomly and taken to the laboratory. The hooves were thoroughly washed with soap and water and further rinsed to remove impurity. The hooves

were then dried in the oven at 70°C for 72 h. Some of the hooves were milled into powder while some were cut into pieces and stored 70°under ambient condition.

Plate 3.1 Pictorial representation of processed hoof and feather substrate

3.2 Inoculum development and preparation of fermentation media

Inoculum development involved inoculating loopful pure cultures of *Aquamicrobium defluvii* into a sterilized media (sterilized for 121°C for 15 minutes) which contains 4g of nutrient broth and 0.05g feather powder (2 g in the case of hoof powder) added to 40ml distilled water. The culture was incubated for 24-48 h at 37°C and placed on a shaker at 100rpm

The fermentation medium for the degradation of keratin substrate was compounded with keratin powder as follows (g/l); $NaN0_3$: 2, NaCl: 2, KH_2PO_4: 2, $MgSO_4$: 0.05, $FeSO_4.7H_2O$: 0.1, $CaCO_3$: 0.1, keratin powder: 20. The medium was sterilized at 121°C for 15 minutes. Aliquots of this mixture was made in glass bottle occupying only 75% of the bottle space, giving room for agitation, then incubated at 37°C at 100 rpm for 7 days.

3.3 Determination of keratinolytic activity of *Aqaumicrobium defluvii*

Activity of keratinase produced by the bacterial isolates was determined by the modifed method of Cheng *et al.* (1995).

3.4 Bioconversion of Cow hoof and Chicken feather into keratin hydrolysate

Keratin substrate degradation was carried out in the fermentation medium using 5% of the inoculum size. The cultures were incubated at 37°C at 100rpm for up to 7 days. Thereafter, content of the whole flask was collected, centrifuged at 5000 rpm for 20 minutes and the

supernatant was heated for 30 minutes to kill unwanted microorganism that might be present. The non-heated and heated supernatant served as the crude keratinase and hydrolysate respectively, which were used without further purification.

3.5 Quantification of degradation (percentage keratin degradation)

The rate of degradation of father was calculated by intermittently removing the remaining feather substrate from the fermentation media at 24 h interval for 7 days and carefully cleaning in sterile water and dried at 70°C until constant weight is achieved.

Percentage degradation was calculated as follows;

$$\% \text{ of degraded feather} = \left(\frac{TF - RF}{TF}\right) * 100$$

TF represent the initial or total weight

RF represent the final degraded weight

keratinase and hydrolysate respectively, which were used without further purification.

3.6 Determination of degradation quantity (percentage of hoof degradation)

The degraded substrate percentage was determined by removing the remaining substrate sample from the fermentation media at 24 hours' interval for 7days, and washed with sterile water and dried at 70 °C until constant weight.

Percentage of degraded hoof was computed as follows:

% Of Degraded hoof = $\left(\frac{W1-W2}{W1}\right) * 100$

W1 represent initial weight of the hoof

W2 represent Final weight of the degraded hoof

3.7 Formulation of Calcium Nanocomposites from Feather and Hoof Hydrolysate

To synthesize Calcium nanoparticles from feather and hoof hydrolysate, 236.15M (0.236 g) of Calcium nitrate crystal pellets was dissolved in 1000 ml distilled water in a conical flask. The mixture can be gently heated to expedite dissolution. Once fully dissolved, produced hydrolysate was added to the calcium nitrate solution at a ratio 1:41 (200 ml of hydrolysate of choice (feather or hoof) to 800 ml of Calcium nitrate solution). The mixture was then heated in a water bath at 50°C for 2 h.

The resulting solution was designated as the stock nanocomposite and stored in bottle containers. It can be diluted to the desired concentrations for subsequent applications.

3.8 Evaluation of hydrolysate and nanoparticles as biofertilizers for Watermelon in a field experiment

The biofertilizer potential of feather hydrolysate and their nanoparticle formulations was assessed through a field experiment using *Citrullus lanatus* a succulent fruit, native to tropical Africa and cultivated around the world. The experimental plot was located at the

LAUTECH Botanical Garden Research plot (8° 9` 41 N, 4° 16` 35 E). The study spanned a period of four months.

Stock hydrolysates were prepared at concentrations of 75% and 100%, achieved by dilution with tap water. 30 beds were prepared within the experimental field such that it was group into 3 replicate and 10 treatment using randomized complete block design, the bed were prepared with the size of each bed being 2m x 2m, with a 0.5 m space gap between beds, the watermelon seeds were sown by burying them in the soil such that 9 stand were planted on each bed with spacing of 1m from each other, followed by wetting with water. The experimental plot area measured 24.5m x 8m. Germination was observed five days post-planting, and regular watering was maintained.

Two weeks after planting, the seedlings were thinned to two plants per stand. A total of 18 plants were then maintained per bed. The first bio fertilizer application was conducted using a foliar spray method, where 10ml of hydrolysate was diluted in 5ml distilled water. Both the hydrolysate and the nanoparticle formulations were applied at concentrations of 75%, 100% and 50%, 100% respectively. Treatments were designated as T1(water), T2(NPK), T3(100% HH), T4(75% HH), T5(100% FH), T6(75% FH), T7(100% HHCa), T8(50% HHCa), T9(100% FHCa), T10(50% FHCa). A negative control was also included, where plants were irrigated solely with water, without any fertilizer application.

Two weeks after the initial application, a second application was made using the soil drenching method, where 5ml of hydrolysate was diluted in 10ml of water. A positive control

treatment using NPK fertilizer (a commonly used synthetic fertilizer) was also applied at this stage, using the ring application method at a distance of 5 cm away from plant stem. A total of 18kg of NPK pellets were applied per plant, followed by soil coverage.

Throughout the experiment, observations were systematically recorded. These included the day of emergence, plant height measurements, leaf count, and the number of flowers and number of fruits produced by each plant were recorded weekly from the first application.

Fig 3.1 An image showing the beds of Watermelon on the field

3.9 Determination of Proximate Compositions and Chlorophyll Contents of the Watermelon

The chlorophyll content of the leaves was determined according to the method of Wellburn (1994). Fresh leaves of each vegetable plant (20mg) was suspended in a tube containing 5 ml of 80% acetone and placed in a dark place for 72 hours. The absorbance reading of the extract was taken at 470, 646 and 663 nm on spectrophotometer with a resolution range of 1-4 nm. Chlorophyll a, chlorophyll b, carotenoids and total chlorophyll content will be determined using the following expression:

Chlorophyll a (mg/ml) = 12.21 *A*663 - 2.81 *A*646

Chlorophyll b (mg/ml) = 20.13 *A*646 – 5.03 *A*663

Total Carotenoid, C_{x+c} (mg/ml) = $\left(\frac{1000A470 - 3.27Cha - 104Chb}{198}\right)$

Total Chlorophyll (mg/ml) = 17.32 *A*646 + 7.18 *A*633

CHAPTER FOUR

4.1 Course of pH of *Aquamicrobium defluvii*

The course of pH during the hydrolysis of feathers and hooves by *Aquamicrobium defluvii* show as show in Figure 4.1. The change PH growth rose from the initial value of 4.9 to 8.8 reach maximum value of 8.8 during 120 h of fermentation while that of hoof rose from initial value of 4.7 to maximum value of 8.0 at 120h of fermentation. The shift in pH during keratinase production corresponds with the report of Gupta and Ramnani (2006), that environment with pH of 6 to 9 favors keratin degradation by most microorganisms. The rise in alkalinity of the fermentation medium could be attributed to the release of ammonium through deamination reaction that occurs during keratin hydrolysis (Riffel *et al.,* 2003).

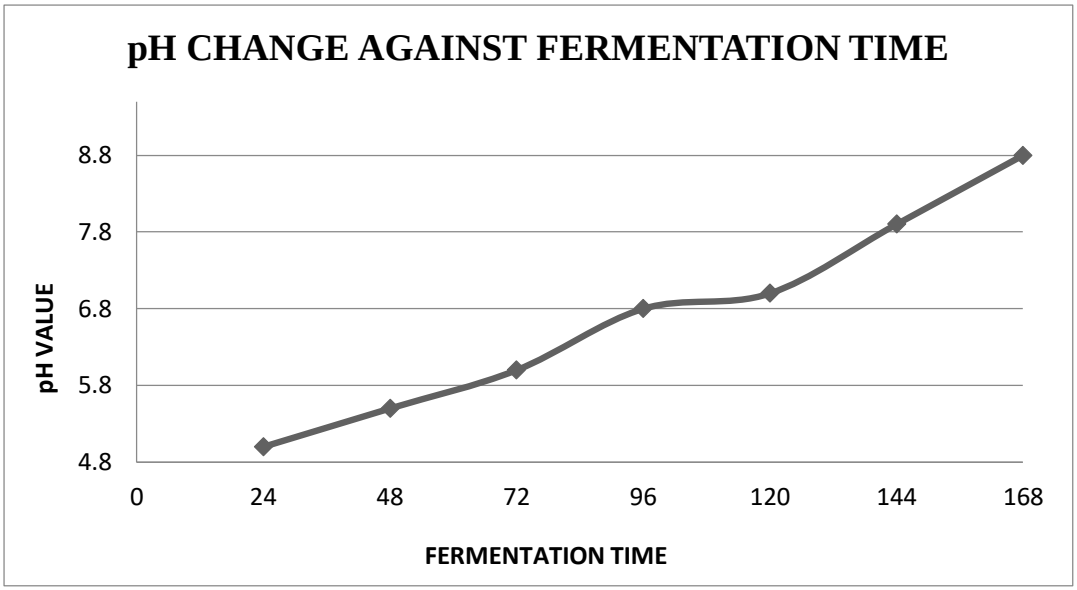

Fig. 4.1: The change in pH observed per fermentation time during the growth of isolate on feather substrate

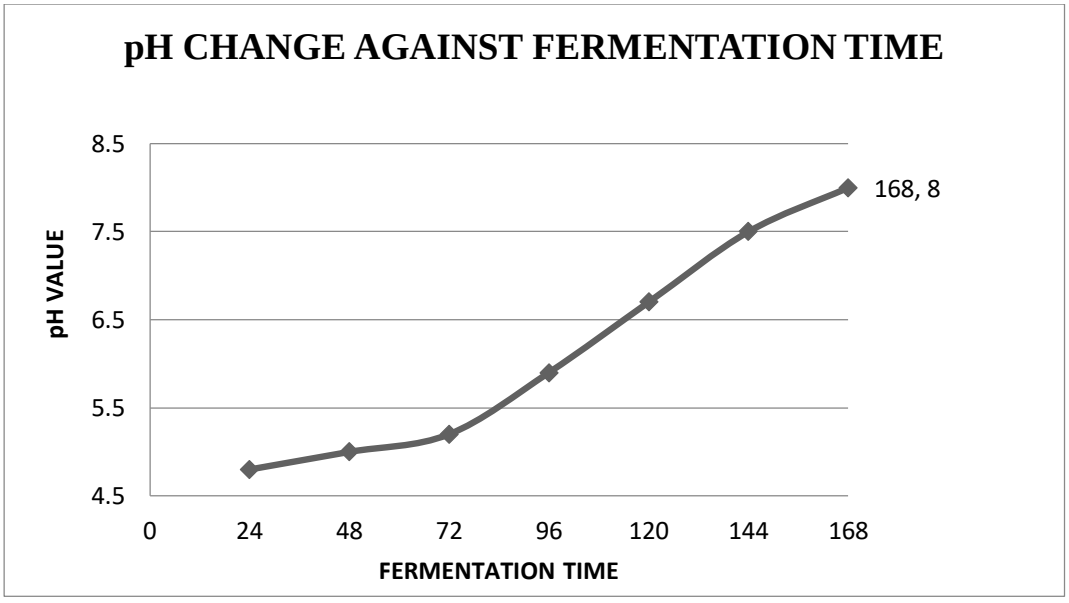

Fig. 4.2: The change in pH observed per fermentation time during the growth of isolate on hoof substrate

4.2 Conversion of keratin substrates into hydrolysate and Percentage keratin degradation

At 168 h of fermentation, ***A. defluvii*** effectively degraded feather and hoof substrates as shown in (plate 4.1 and plate 4.2). The efficiency of keratin degradation by *A. defluvii* depends on various environmental factors, such as temperature, pH, and substrate concentration. The degradation of feather and hoof keratin substrates was observed over 168 hours using submerged fermentation. The microbial strain *Aquamicrobium defluvii* effectively reduced the keratin content in both substrates, with a degradation efficiency of approximately 80% for feathers and 70% for hooves by the end of the period **(**see **Plates 4.1 and 4.2**). This aligns with reports by Adelere and Lateef (2019), who documented similar degradation efficiencies in *Bacillus spp*. when applied to feather waste also according to Peng *et al*, (2019), *Bacillus licheniformis* and *Stenotrophomonas maltophilia* degrade 50 g/L chicken feather waste in batches, and the degradation rates were 35.4% and 22.8% in 96 h, respectively.

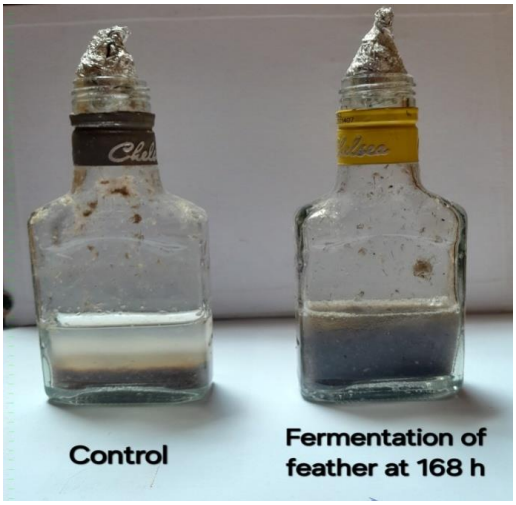

Plate 4.1 Biodegradation of poultry feather by isolate at 168 h.

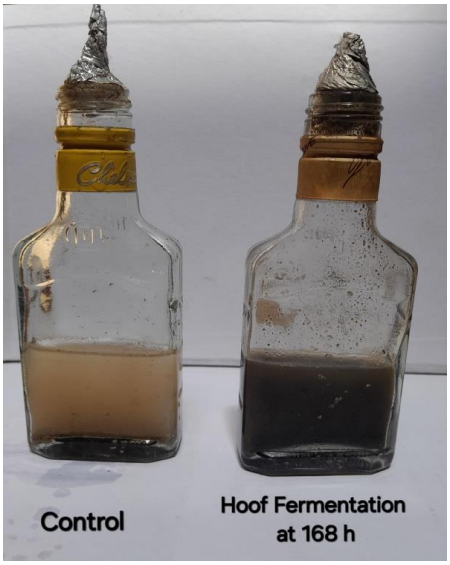

Plate 4.2 Biodegradation of cow hoof by isolate at 168 h.

4.3 Evaluation of feather and hoof hydrolysate and their nanocomposite on Watermelon shoot length:

The shoot of watermelon plants can positively impact fruit yield and quality. Taller plants, with their increased leaf surface area, can enhance photosynthesis, leading to higher sugar content in the fruits. In table 4.1, a month after planting, the length of watermelon treated with water supersedes that of every other treatment including NPK, 50% HHCa and 100% FH with 1.23,2.79 and 1.17-fold enhancement respectively. In the same week, water was slightly close to that of 100% HH with 3.3% increment.

After 6 weeks, table 4.1 shows that the plants treated with NPK outperformed 100% FH, 100% HH and even 50% HHCa with significant differences of,1.09, 0.88 and 1.08- fold enhancement respectively. NPK have a subtle difference with 100% HH with 4.2% increment. The type and timing of nutrient availability had a marked influence on watermelon shoot growth across treatments.

Initial observations at week four showed water treatment encouraging the greatest early shoot growth, potentially due to residual soil nutrients or moisture availability and also soil type, which could have facilitated early root establishment and shoot elongation. This early advantage in plant height with water may also point to the initial stage being less nutrient-dependent, with the plants relying more on soil conditions and water availability.

By the sixth week, however, NPK treatment surpassed all others, showing that synthetic nutrients provided a more readily available source of essential elements like nitrogen, phosphorus, and potassium, which are quickly absorbed and utilized by the plants. Tejada and Gonzalez (2018) conducted a research on organic versus inorganic fertilizers and found that while organic fertilizers contribute to steady, sustainable growth, inorganic fertilizers such as NPK often provide an initial growth boost due to the rapid availability of nutrients. This aligns with this research that NPK surpassed hydrolysates in shoot growth by the sixth week in the watermelon study. A study by Canellas *et al*. (2015) also shows the effects of organic hydrolysates on plant growth, concluding that these biostimulants enhance growth by providing amino acids and organic matter that promote microbial activity in soil. However, 100% HH, 100% FH and 50% HHCa, showed a moderate impact, which indicates that although they release nutrients more slowly, they still provide growth support, albeit at a rate that may not match the immediate availability of synthetic fertilizers.

Table 4.1: Evaluation of shoot height after four weeks of planting

TREATMENT	WEEK 4	WEEK 6
WATER	26.92 ±1.92^{a}	131.22 ±5.25^{ab}
NPK	21.87 ±0.54^{bc}	155.22 ±15.09^{a}
100% HH	26.02 ±1.89^{ab}	148.72 ±9.62^{a}
75% HH	21.28 ±0.92^{bc}	125.89 ±9.12^{ab}
100% FH	23.03 ±2.19^{abc}	142.89 ±5.72^{ab}
75% FH	21.34 ±1.58^{bc}	127.17 ±14.40^{ab}
100% HHCa	21.49 ±1.37^{bc}	131.83 ± 17.57^{ab}
50% HHCa	24.13 ±1.58^{abc}	143.67 ±3.98^{ab}
100% FHCa	22.11 ±2.06^{abc}	110.84 ±6.58^{b}
50% FHCa	20.11 ±0.81^{c}	125.56 ±5.41^{ab}

Values with the same superscript within a column are not significantly different (p<0.05)

SH Shoot height

4.4 The effect of feather and hoof hydrolysate and their nanocomposite on Watermelon leaf production:

A larger leaf surface area enhances a plant's ability to capture solar energy, leading to increased photosynthesis and higher sugar production, which contributes to the sweetness and flavor of the fruit. In Table 4.2, the data indicates that plants treated with 100% HH exhibited the highest mean number of leaves, with a 1.08-fold increase over water and a 1.14-fold increase over NPK treatments after four weeks. Similarly, the 100% FHCa treatment also showed notable improvements, surpassing water by 1.03 times and NPK by 1.0 times.

At week 6, NPK treatment demonstrated a significant advantage with a 1.21-fold increase compared to water and other treatments, including 100% HH and 100% FH, which recorded 1.06 and 1.08-fold enhancements, respectively. NPK remains the most effective treatment for promoting leaf development at later stages but, both 100% HH and 100% FH treatments also contributed positively to leaf number, surpassing water controls.

The overall trends in leaf number indicate that hydrolysate applications, particularly 100% HH and FH formulations, are effective in promoting vegetative growth. The consistent performance of these treatments can be attributed to the enhanced nutrient availability and microbial activity in the soil, resulting in improved plant health and resilience. According to Ertani *et al*. (2009) the effect of protein hydrolysates on leaf area and number in various crops, concluding that organic treatments enhanced vegetative growth by providing amino acids and

peptides that support microbial activity in the soil. Their results align with the observation that HH and FH treatments increased leaf count early on, supporting sustainable growth by enhancing nutrient cycling and soil health.

While NPK is optimal for leaf growth, the use of organic amendments such as 100% HH and 100% FH can significantly improve leaf development and, consequently, overall plant yield potential. The integration of biofertilizers like feather hydrolysate into agricultural practices not only supports sustainable farming but also enhances crop productivity by leveraging their growth-promoting properties.

Table 4.2: Number of leaves counted four weeks after germination

TREATMENT	WEEK4	WEEK6
WATER	12.07 ±0.96[a]	65.00 ±2.78[abc]
NPK	11.44 ±0.82[a]	78.44 ±7.10[a]
100% HH	13.00 ±0.87[a]	74.00 ±5.01[ab]
75% HH	10.78 ±0.99[a]	61.67 ±4.50[abc]
100% FH	10.78 ±1.19[a]	72.78 ±5.16[abc]
75% FH	11.33 ±0.71[a]	59.44 ±7.16[bc]
100% HHCa	10.78 ±0.60[a]	65.78 ±8.80[abc]
50% HHCa	12.11 ±0.86[a]	68.33 ±2.70[abc]
100% FHCa	12.44 ±1.63[a]	55.90 ±3.40[c]
50% FHCa	11.54 ±0.30[a]	66.38 ±1.76[abc]

Values with the same superscript within a column are not significantly different ($p<0.05$)

4.5 The role of feather and hoof hydrolysate and their nanocomposite on flowering of Watermelon:

The number of flowers produced by watermelon plants is crucial for determining fruit yield, as each female flower has the potential to develop into fruit. In Table 4.3, the data shows that the 100% FHCa treatment yielded the highest mean flower count, significantly surpassing water and 100% HHCa with enhancements of 1.31 and 4.22-fold, respectively. This remarkable performance suggests that the application of feather hydrolysate may provide essential nutrients or growth-promoting compounds that enhance flower development.

While 100% HH also demonstrated a solid mean flower count of 4.00, it did not reach the same level as 100% FHCa or NPK, indicating that while hydrolysate applications are beneficial, their efficacy can vary based on concentration and formulation. The fact that both 100% HH and NPK treatments resulted in similar flower counts (4.00 and 4.22, respectively) underscores the importance of optimized formulations in promoting floral development.

The small standard deviations observed in the 100% HH and 100% FHCa treatments imply a high consistency in flower production within these groups, indicating that these treatments reliably enhance flower development compared to the more variable responses seen in other treatments. The stark contrast between 100% FHCa and water, along with the close performance of 100% HHCa to 100% HH, highlights the need to understand the underlying mechanisms driving these differences.

It is worth noting that the low flower counts in the 75% HH, 100% FH, and 75% FH treatments suggest that reduced application rates of hydrolysates may not provide sufficient nutrients for optimal flower production. This emphasizes the importance of using appropriate concentrations to achieve desired outcomes. Pascual *et al*. (2010) compared organic and synthetic fertilizers, observing that while synthetic fertilizers like NPK promote rapid flowering due to readily available nutrients, organic treatments contribute to more sustainable and consistent flower production. This result corresponds with the observed similarity in flower counts between NPK and 100% HH, suggesting that optimized organic formulations can effectively support flower development, although they may act differently than NPK.

Overall, the results indicate that while synthetic fertilizers like NPK can promote flower development, hydrolysate treatments such as 100% FHCa and 100% HH present a viable alternative, with the potential for greater sustainability in agricultural practices. Future research should further explore the specific components of feather hydrolysates that contribute to improved flower production, as well as the effects of varying application rates to maximize crop yield.

Table 4.3: Evaluation of flower count recorded from the time of emergence

TREATMENT	No. of Flower
WATER	3.22 ± 0.40^{ab}
NPK	4.22 ± 0.55^{a}
100% HH	4.00 ± 0.62^{a}
75% HH	1.44 ± 0.29^{abc}
100% FH	1.78 ± 0.49^{bc}
75% FH	1.78 ± 0.49^{bc}
100% HHCa	1.00 ± 0.29^{c}
50% HHCa	3.00 ± 0.82^{ab}
100% FHCa	4.22 ± 0.88^{a}
50% FHCa	2.72 ± 0.21^{abc}

Values with the same superscript within a column are not significantly different ($p < 0.05$)

4.6 The effect of feather and hoof hydrolysate and their nanocomposite on fruiting of *Citrullus Lanatus*:

The number of fruits produced by watermelon plants is a key indicator of overall yield, and the data presented in Table 4.4 illustrates the significant disparities among the treatments. Notably, NPK emerged as the most effective treatment, yielding a mean of 2.11 fruits per plant, which represents a substantial advantage over all other treatments, including water and the feather hydrolysate applications (100% FHCa and 50% HHCa), which produced significantly lower counts with enhancements of only 1.26-fold compared to NPK. Similarly, Ezekiel *et al*. (2017) found that while protein hydrolysates improve plant health and early growth, they may not always enhance fruit yield to the same degree as synthetic fertilizers like NPK tend to provide more consistent and higher yields, this finding reflects the lower fruit yields observed with hydrolysate treatments like 100% FHCa and 50% HHCa.

Despite the promising results from NPK, it is essential to critically assess the overall fruit yield across all treatments. The fruit counts for water, 100% HH, 75% HH, 100% FH, and 75% FH treatments all hovered around 1.22 or lower, with many of them showing no improvement over the water treatment. This minimal fruit production reflects a concerning trend, particularly when considering that these treatments were expected to enhance plant performance through the provision of nutrients and growth-promoting properties.

Furthermore, the occurrence of fruit rot, which limited the final fruit count, raises significant questions about the overall viability of the treatments employed and. It is possible

that inadequate nutrient balance or unfavorable environmental conditions contributed to this degradation, leading to lower fruit viability and ultimately impacting yield.

The 20% increment in mean average fruit count between NPK and the next best treatment (50% HHCa) highlights a significant gap that indicates the need for further optimization of the treatments.

Table 4.4: Evaluation of Initial fruit count

TREATMENT	No. of Fruits
WATER	1.22 ± 0.15^{bc}
NPK	2.11 ± 0.26^{a}
100% HH	1.22 ± 0.32^{bc}
75% HH	0.89 ± 0.26^{bc}
100% FH	0.89 ± 0.26^{bc}
75% FH	1.11 ± 0.20^{bc}
100% HHCa	0.67 ± 0.24^{c}
50% HHCa	1.67 ± 0.44^{ab}
100% FHCa	1.67 ± 0.44^{ab}
50% FHCa	1.27 ± 0.09^{bc}

Values with the same superscript within a column are not significantly different ($p<0.05$)

In general, the primary objective of this research was to evaluate the effectiveness of biofertilizers derived from feather and hoof hydrolysates on watermelon yield compared to conventional NPK fertilizer and water. Unfortunately, the findings of our study indicate that the application of these biofertilizers did not achieve the anticipated improvements in plant growth and fruit yield. But, in many cases, the gap of performance of the biofertilizers was narrow compared to that of the controls (water and NPK).

Moreover, the limited improvement seen in plant performance relative to water emphasizes the need for a critical reevaluation of the formulations used in this study. It is possible that the concentrations of the hydrolysates were either too low or not optimized for the specific needs of watermelon plants. Additionally, environmental factors such as soil health, moisture levels, and pest pressures may have further compounded the challenges faced during the growing season. Environmental factors such as rainfall could also have played a role, especially in aiding the breakdown and release of nutrients from biofertilizers. Additionally, higher temperatures would favor microbial activity, speeding up the fermentation of organic matter in hoof and feather treatments and, in turn, nutrient release.

While the aim of this research was to promote the use of sustainable, organic alternatives to synthetic fertilizers, the results have shown that, in this instance, the biofertilizers did not meet expectations. This outcome underscores the complexity of plant nutrient management and the importance of comprehensive testing of organic amendments

under varying environmental conditions and also the environmental condition that efficiently promote the growth of Watermelon.

4.7 Evaluation of chlorophyll and carotenoid content obtained from leaves of watermelon

Chlorophyll is an important photosynthetic pigment to the plant, largely determining photosynthetic capacity and hence plant growth. According to Simkin, *et al* in 2022 chlorophyll is essential for photosynthesis, greater chlorophyll levels can translate into higher biomass, leading to enhanced energy production and improved yield during harvest.

In table 4.5, T10 appears to be the most effective in promoting chlorophyll accumulation, with the highest values for both chlorophyll a, chlorophyll b, and total chlorophyll, total chlorophyll values range from 13.90 mg/ml (T9) to 23.97 mg/ml (T10) with T5, T6, and T10 showing relatively high total chlorophyll content, with T10 showing a notably higher value than all other treatments. These results are in accordance with the results reported by Tahir *et al*. (2018), Azarmi *et al*. (2009) in cucumber.

Carotenoids, key for photoprotection, appear in lower concentrations across most treatments as seen in Table 4.5, with several below detection levels (T4, T5, T6, and T10). This finding may indicate that under high chlorophyll conditions, photoprotection is either inherently sufficient or that excess light dissipation is minimized, particularly since watermelon plants are highly sunlight-adaptive. In T9, where carotenoid levels are highest (1.21 mg/ml),

photoprotection seems more robust. Such a peak might signal a stronger response to oxidative stress or high-light conditions, aligning with research showing that carotenoids manage excess light, helping to prevent chlorophyll degradation.

These findings align with recent studies, like those by Scheer (2022) and Demmig-Adams *et al*. (2020), which explain how sun-loving plants like watermelon regulate pigment composition to optimize photosynthesis while protecting from photodamage. High chlorophyll with limited carotenoids indicates that the treatments support robust photosynthetic efficiency without overstressing photoprotective pathways—ideal for watermelon's growth under bright conditions.

Table 4.5: Evaluation of chlorophyll and carotenoid content obtained from leaves of watermelon using spectrophotometer at different wavelength three weeks after planting

TREATMENT	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Total Chlorophyll	Total Carotenoid
T1	6.29	9.27	15.90 ± 0.19a	0.53
T2	6.68	9.09	16.46 ± 0.66a	0.62
T3	6.66	9.97	16.93 ± 1.50a	0.11
T4	6.95	10.42	17.67 ± 0.17a	Below detection
T5	8.48	12.31	21.29 ± 7.11a	Below detection
T6	8.26	12.02	20.50 ± 6.62a	Below detection
T7	6.20	9.44	15.85 ± 0.10a	0.53
T8	6.59	9.79	16.70 ± 0.59	0.024
T9	5.36	7.62	13.90 ± 0.87a	1.21
T10	9.43	14.14	23.97 ± 3.46a	Below detection

Values with the same superscript within a column are not significantly different ($p<0.05$)

CHAPTER FIVE

5.1 CONCLUSION

This research aimed to evaluate the potential of feather and hoof hydrolysate-derived biofertilizers as sustainable alternatives to conventional NPK fertilizers in watermelon cultivation. While the study did not yield the expected positive results in terms of plant growth and fruit yield, it offers valuable insights for future research and practical applications. The narrow performance gap with conventional fertilizers suggests that with further optimization, biofertilizers could offer a sustainable and effective alternative. To maximize the efficacy of these biofertilizers, future research should focus on optimizing nutrient concentrations, application timing, climate and soil conditions. By addressing these factors, we can harness the power of biofertilizers to promote sustainable agriculture and reduce reliance on synthetic inputs.

5.2 RECOMMENDATION

1. Explore the impact of soil type, pH, and environmental conditions on biofertilizer efficacy
2. Conduct further research to determine the optimal nutrient concentrations in biofertilizers for maximizing plant growth and yield.
3. Conducting long-term studies to assess the cumulative effects of biofertilizer application on soil health, crop productivity, and environmental sustainability.

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APPENDIX

Raw chlorophyll data and their respective wavelength

TREATMENT	ABS @470	ABS @646nm	ABS @663nm
T1 a	1.096	0.650	0.620
T1 b	1.081	0.668	0.630
T2 a	0.970	0.619	0.562
T2 b	1.209	0.703	0.686
T3 a	0.952	0.645	0.594
T3 b	1.090	0.755	0.745
T4 a	1.050	0.739	0.700
T4 b	1.009	0.720	0.700
T5 a	0.958	0.599	0.530
T5 b	0.350	1.172	1.135
T6 a	1.052	0.605	0.557
T6 b	0.527	1.122	1.069
T7 a	1.010	0.618	0.584
T7 b	1.201	0.688	0.679
T8 a	1.030	0.673	0.627

T8 b	1.040	0.709	0.691
T9 a	0.919	0.583	0.511
T9 b	1.180	0.533	0.525
T10 a	0.605	1.133	1.088
T10 b	0.911	0.848	0.811

Vegetative properties (plant shoot length and leaf number)

First week first data (3 weeks post application)

TREATMENTS	PL (PER REPLICATE)			LN (PER REPLICATE)		
T1	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	21.5	**19.5**	**22.5**	**11**	**10**	**13**
	32.5	**27**	**35**	**16**	**14**	**10**
	34.5	**25.2**	**24.6**	**18**	**10**	**12**
T2	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	22.5	**19.9**	**22**	**12**	**12**	**10**
	20.8	**22.5**	**25.5**	**11**	**12**	**17**
	21	**21.9**	**20.7**	**11**	**8**	**10**
T3	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	25.4	**35**	**18.5**	**14**	**18**	**10**
	28	**24**	**23**	**11**	**12**	**11**
	22.5	**22.8**	**35**	**13**	**12**	**16**
T4	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**

	17.9	**24.5**	**19.5**	**9**	**13**	**10**
	18	**22.1**	**24**	**9**	**11**	**11**
	19.5	**21**	**25**	**10**	**11**	**13**
T5	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	23.5	**24.5**	**14.9**	**6**	**13**	**8**
	22	**33**	**16.5**	**15**	**17**	**9**
	22.7	**33**	**17.2**	**11**	**9**	**9**
T6	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	17.1	**28.5**	**26.4**	**9**	**12**	**14**
	17.5	**25.9**	**14.4**	**8**	**14**	**11**
	21.9	**21**	**21.2**	**11**	**13**	**10**
T7	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	24.2	**27**	**18**	**13**	**10**	**9**
	26.5	**20.2**	**15.5**	**12**	**14**	**9**
	23.5	**21.3**	**17.2**	**14**	**10**	**10**
T8	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	24.5	**18.6**	**28.5**	**11**	**10**	**15**
	28.5	**21.6**	**24.5**	**15**	**11**	**12**
	32	**19.5**	**19.5**	**16**	**10**	**9**
T9	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	28	**19.5**	**18.7**	**14**	**8**	**12**
	32.2	**17.5**	**19.5**	**24**	**10**	**10**
	29.9	**18.5**	**15.2**	**15**	**9**	**10**
T10	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	19.5	**19.5**	**24**	**8**	**8**	**12**
	15.5	**22.7**	**21**	**6**	**12**	**11**

	20.1	**18.7**	**20**	**12**	**11**	**11**

Third week second data (3 weeks post application)

TREATMENTS	PL (PER REPLICATE)			LN (PER REPLICATE)		
T1	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	138	**118**	**137**	**49**	**61**	**69**
	160	**104**	**132**	**69**	**60**	**66**
	140	**122**	**130**	**80**	**66**	**65**
T2	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	139	**145**	**153**	**69**	**62**	**77**
	126	**143**	**195**	**63**	**72**	**97**
	100	**140**	**256**	**67**	**71**	**128**
T3	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	137.5	**220**	**142**	**67**	**110**	**57**
	143	**124**	**124**	**71**	**76**	**71**
	139	**154**	**155**	**75**	**62**	**77**
T4	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	113	**145**	**106**	**56**	**72**	**53**
	100	**105**	**184**	**50**	**71**	**92**
	109	**143**	**128**	**55**	**42**	**64**
T5	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	138	**160**	**133**	**53**	**80**	**55**
	105	**153**	**149**	**81**	**78**	**66**

	161	**139**	**148**	**65**	**103**	**74**
T6	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	74	**130**	**155**	**30**	**65**	**53**
	74	**132**	**169**	**37**	**65**	**85**
	71.5	**174**	**165**	**36**	**82**	**82**
T7	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	138	**208**	**67**	**69**	**104**	**33**
	168	**150.5**	**73**	**84**	**75**	**37**
	160	**163**	**59**	**80**	**81**	**29**
T8	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	142	**156**	**160**	**67**	**72**	**80**
	142	**141**	**121**	**71**	**67**	**60**
	155	**137**	**139**	**76**	**53**	**69**
T9	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	131	**77**	**106**	**66**	**37**	**53**
	142	**99**	**106**	**71**	**49**	**58**
	128.6	**103**	**105**	**64**	**52**	**53**
T10	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	102	**128**	**140**	**51**	**64**	**70**
	129	**141**	**127**	**65**	**70**	**63**
	100	**118**	**145**	**50**	**56**	**73**

Raw data of number of flowers and fruits before rotten

TREATMENTS	NUMBER OF FLOWERS			NUMBER OF FRUITS		
T1	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	3	**3**	**3**	**1**	**1**	**1**
	6	**4**	**3**	**2**	**2**	**1**
	2	**2**	**3**	**1**	**1**	**1**
T2	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	6	**3**	**3**	**2**	**2**	**2**
	5	**4**	**2**	**2**	**2**	**1**
	5	**7**	**3**	**2**	**4**	**2**
T3	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	7	**3**	**4**	**2**	**2**	**2**
	2	**6**	**3**	**0**	**1**	**2**
	2	**6**	**3**	**0**	**0**	**2**
T4	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	2	**2**	**1**	**1**	**2**	**1**
	2	**0**	**2**	**2**	**0**	**1**
	2	**0**	**2**	**1**	**0**	**0**
T5	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	2	**5**	**0**	**2**	**0**	**0**
	1	**1**	**1**	**1**	**0**	**1**
	1	**3**	**2**	**1**	**1**	**2**
T6	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**

	0	**4**	**3**	**1**	**2**	**1**
	0	**2**	**2**	**1**	**2**	**1**
	0	**3**	**2**	**0**	**1**	**1**
T7	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	1	**2**	**0**	**1**	**2**	**0**
	2	**1**	**0**	**1**	**1**	**0**
	2	**1**	**0**	**0**	**1**	**0**
T8	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	7	**2**	**2**	**1**	**2**	**0**
	7	**2**	**1**	**1**	**1**	**0**
	4	**1**	**1**	**0**	**1**	**0**
T9	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	4	**0**	**4**	**14**	**8**	**12**
	7	**2**	**3**	**24**	**10**	**10**
	8	**7**	**3**	**15**	**9**	**10**
T10	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
	1	**2**	**5**	**1**	**1**	**1**
	1	**3**	**3**	**2**	**1**	**2**
	4	**1**	**3**	**1**	**1**	**1**