CHARACTERISATION OF TOMATO ROOT ENDOPHYTIC BACTERIA AND THEIR EFFECTS WITH RESVERATROL TO ENHANCE THE GROWTH OF COWPEA (*Vigna unguiculata)*

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GOU/U22/BTG/163

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GODFREY OKOYE UNIVERSITY, ENUGU, NIGERIA

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A PROJECT RESEARCH SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMNTS FOR THE AWARD OF THE BACHELOR OF SCIENCE (B. Sc) DEGREE IN BIOTECHNOLOGY

SUPERVISOR: DR UBA, CHARLES U.

JULY, 2026

APPROVAL

This research titled "**Characterisation of Tomato Root Endophytic Bacteria and Their Effects with Resveratrol to Enhance the Growth of Cowpea (*Vigna unguiculata)***" has been assessed and approved by the Committees of Department of Biological sciences and Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University Enugu Nigeria.

Dr. Uba Charles U. Supervisor	____________________ Signature	____________________ Date
Prof Florence Akaneme External Examiner	____________________ Signature	____________________ Date
Dr. F. N. Olisaka Head of Department	____________________ Signature	____________________ Date
Prof. Chidi C. Uhuegbu Dean of FNASE	____________________ Signature	____________________ Date

CERTIFICATION

This is to certify that this research titled Characterisation of Tomato Root Endophytic Bacteria and Their Effects with Resveratrol to Enhance the Growth of Cowpea (*Vigna unguiculata)* was carried out by Nwambe Paschaline Chioma, GOU/U22/BTG/163 under supervision in the Department of Biological Sciences, Godfrey Okoye University.

Nwambe Paschaline Chioma	____________________	____________________
GOU/U22/BTG/163	Signature	Date

DEDICATION

This work is dedicated to Almighty God

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ABSTRACT

Cowpea (*Vigna unguiculata*) is one of the most important grain legumes cultivated in sub-Saharan Africa, serving as a major source of protein and income for millions of people. However, cowpea productivity is frequently limited by poor soil fertility, nutrient deficiencies, drought stress, and plant diseases. To address these challenges, tomato roots are recognized as a rich source of plant growth-promoting endophytic bacteria, which enhance growth in crop varieties. This study focused on the isolation and identification of endophytic bacteria from tomato roots using 16S rRNA sequencing and evaluating their effects with resveratrol as biofertilisers on the growth performance of cowpea. Bacterial samples were collected from the sterilised tomato root seedlings and cultured in Pikovskaya medium. The identified bacteria were then evaluated as biofertilisers alongside resveratrol extracted from red grape skin on four cowpea varieties under three treatments; control (T0), endophytic bacteria (T1), and endophytic bacteria with resveratrol (T2). The result of the 16S rRNA sequencing identified the three isolated bacteria as *Bacillus subtillis* with high sequence similarity with the reference seuence in the database. Also, mineral analysis carried out on the combined biofertilizer showed that silica is the predominant mineral (48.00%), followed by phosphorous (12.60%) while the lowest was copper (0.000036%). Additionally, the analysis of variance showed significant difference ($p<0.05$) for most parameters on the variety, treatment, and their interaction effect. The V4T0 had the highest mean germination time of 3.23 ± 0.40 followed by V3T0 (2.20 ± 0.19) while the lowest was V1T1 (1.18 ± 0.23). Regardless of the low germination time observed in the T1 and T2 treatments, further results showed they significantly improved seedling growth parameters such as seedling length, leaf length and width and higher germination index. These findings demonstrate that tomato endophytic *Bacillus subtillis* particularly when combined with resveratrol represents a promising biofertilizers strategy for or enhancing early cowpea establishment and reducing dependence on synthetic fertilizers.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Cowpea (*Vigna unguiculata*) is a common multipurpose legume crop farmed primarily in Africa, and it usually used in human and livestock diets in different areas of the world. It is also widely known to be one of the most resilient and versatile food crops among cultivated food crops (Singh, 2020). It is a member of the *Fabaceae* family, subtribe *Phaeseolinae*, *Vigna* genus, and *Catjang* section (Abebe and Alemayehu, 2022). It is a nutritious, underutilized vegetable legume crop that possesses the agricultural possibility to improve protein-calorie malnutrition, and it is a crop that can be cultivated under harsh conditions such as drought and sandy soils (Mekonnen *et al*., 2022). However, the growth productivity of cowpea is frequently limited by poor soil fertility, nutrient deficiencies, drought stress, and plant diseases regardless of its known adaptable nature to nutrient poor environments, which makes it an appropriate subject for the study of the growth effects of endophytic bacteria and resveratrol.

Tomato (hybrid cobra species) is an extensively cultivated horticultural plant valued for its edible fruits and economic importance. Tomatoes as a defence mechanism, possess a symbiotic relationship with the endophytic bacteria present in its phloem and these endophytes have shown to have beneficial effects on plants which include the promotion of plant growth and disease suppression. These benefits also involve the production of antimicrobial substances and bio stimulants (Tian *et al*, 2017). These attributes expressed in tomato plants make it appropriate hosts for the required microbes.

Endophytic bacteria are usually considered an intermediate group between saprophytic and pathogenic bacteria (Hallmann *et al*., 1997; Langner dos Santos *et al*., 2018). They belong to a larger group of microbes that undergo their life cycles entirely or partly inside the host plant (Langner dos Santos *et al*., 2018). They colonize the interior of plants, which usually occurs at a stage in the phenological development of the plant, and do not cause any visible damage to the host plant. In the host plant, they produce metabolites that help to increase the absorption of nutrients which improves biomass gain and plant growth.

Resveratrol is a naturally occurring bioactive polyphenol which has been universally detected in foods like; peanuts, grapes, blueberries, fern, red wine; since it was initially extracted from the root of white hellebore (*Veratrum grandiflorum*) in 1940 (Feng *et al*., 2022). It is a secondary metabolite that has gained a lot of attention in the fields of regenerative medicine and health care products due to its substantial biological traits including; anti-cancer, neuroprotection, anti-oxidation, anti-aging, anti-inflammation, and anti-glycation properties (Feng *et al*., 2022). It is also known to possess antibacterial activity and aid plant immune responses which are beneficial to plant growth (Kang *et al*., 2022).

Given these properties, endophytic bacteria and resveratrol could be incorporated into organic fertilizer production which presents multiple opportunities for the enhancement of nutrient production, growth quality and soil fertility in plants like cowpea. However, there is limited research and experimentation on the possibilities of these benefits from resveratrol and endophytic bacteria fertilizers in cowpea which justifies the need for further research and investigation.

1.2 Statement of the Problem

The constant improvement of the growth of cowpea is an agricultural necessity due to its strategic importance to food security in Nigeria and Africa as a whole. Fortunately, great efforts have already been made to adopt smart and sustainable biotechnological advances to favourably influence the production quality of cowpea (Olawale and Olubukola, 2021). As such, the potential for extensive research into the crop has skyrocketed in the scale of importance. Despite these advances, there are certain limitations that the crop faces which affect its strength as a food security crop. Cowpea possesses a low yield potential, quality assurance issues, biotic and abiotic stresses, cultural beliefs and policy regulations which in turn affects its productivity when compared to other legumes. (Mekonnen *et al*., 2022).

Most farmers have relied on chemical fertilizers as a solution to this problem, which led to the increasing trend of their usage. However, chemical fertilizers are unsustainable for long term use on crops as it causes air and water pollution, degrades the soil and reduces soil fertility and crop production (Gautam *et al*., 2024). This discovery has created a gap that organic fertilizers produced from bioactive materials like endophytic bacteria and resveratrol seek to close as they possess antioxidant, antimicrobial and growth enhancing properties to improve the growth quality of cowpea without causing permanent damage to the soil. Regardless of this discovery, there is limited research on the combined use of endophytic bacteria and resveratrol in the enhancement of growth quality in cowpea thereby creating a knowledge gap which this study seeks to address.

1.3 Aim and Objectives

Aim:

The aim of this study was to isolate and identify endophytic bacteria from tomato roots using 16S rRNA sequencing and evaluating their effects with resveratrol as biofertilizers on the growth performance of cowpea.

Objectives:

The objective of the study was to;

i. isolate and identify endophytic bacteria from tomato roots

ii. extract and isolate resveratrol from grape skin

iii. determine the nutrient composition of the formulated biofertilizer

iv. determine the effect of resveratrol combined with endophytic bacteria fertilizer on the growth of cowpea.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cowpea (*Vigna unguiculata*) Production and Agronomic Challenges

As a crop with its origins in southern Africa regions, cowpea has become widely established in the food systems and local cropping of more than 100 countries in both the tropics and subtropics (Singh, 2020). It is consumed as food in a variety of ways, containing up to 30% protein in its tender leaves, grains, and pods (Singh, 2020), as well as dietary fibre and vitamins. As warranted by its local popularity in various regions, it's been given indigenous names ranging from 'beans', 'ewa' and 'wake' in Nigeria to 'black eyed pea', 'southern pea' and 'crowder pea' in the United States and 'chowlee' and 'lobia' in Brazil, among others. Asides from being a food source, cowpea is also an income generating crop for farmers especially in the dry and harsh seasons due to its ability to adapt and grow in extreme weather conditions like drought as opposed to several other legumes (Kim *et al.*, 2025). An instance of this, is the cowpea farmers in northern Nigeria who experience significant increase in food security and household income because of the economic lucrative nature of the crop.

Irrespective its wide cultivation and acclaimed importance to agriculture, cowpea continues to experience an overall low productivity particularly in Africa. This is usually caused by several biotic and abiotic obstacles including; diseases, parasitic plants, nematodes and insect pests. Certain local varieties also experience low yield particularly due to their late maturity and prostrate growth habit which presents as a bigger constraint to the regions involved. In most farms in Africa, it is assumed that the adaptable nature of cowpea exempts it from the use of fertilizers to improve soil fertility and plant yield, however, this exemption has played a major role in the decline in cowpea production in Nigeria (Horn and Shimelis, 2020). This emphasises the need for suitable and sustainable bio stimulants (like resveratrol) in modern agriculture to improve the growth quality of cowpea.

2.2 Resveratrol: Biological Roles and Potential as a Plant Growth Elicitor

Resveratrol is a naturally occurring polyphenolic compound that is recognised for its antioxidant and regenerative properties. Phenolic contents play an important role in the achievement of high quality and quantity cowpea with availability of primary nutrients. It has been intensively studied for its possible use in the prevention and treatment of numerous chronic defects, with research spanning from in vitro and animal models to human clinical trials. In plants, it acts as either a phytoalexin or phytoanticipin depending on the species or plant organs considered (Courot, 2021). It also exhibits antifungal properties, and its biosynthesis is induced by biotic or abiotic stresses, however, its presence is limited to certain plant species that possess the STS (stilbene synthase) enzymes (Courot, 2021).

2.2.1 Sources of resveratrol

Resveratrol can be extracted from plants such as;

Grapes, Peanuts, Berries, Japanese knotweed (*Polygonum cuspidatum*), Cocoa, Sprouted legumes, Pistachio nuts, cacao, etc.

2.2.2 Functions of resveratrol in plants

Resveratrol is a known stress metabolite generated by grapevines in response to injury or fungal infection (Kang *et al*., 2022). Therefore, a concentration in plants can function as a useful indicator of disease resistance, since it contains both an inducible and a constitutive defence mechanism (Kang *et al*., 2022).

Furthermore, resveratrol plays a role in the response of plants to abiotic stress like; salinity, high temperatures, drought, etc. Certain studies have also shown that it improves the antioxidant defences of plants which enhances their ability to manage oxidative stress caused by these environmental factors. According to research by Hanzouli *et al*. (2024), resveratrol has been linked to the enhanced tolerance of grapevines to salt stress, which indicates its role in the maintenance of redox homeostasis and prevention of cell damage. Resveratrol is synthesized by an enzyme known as stilbene synthase (STS) in the phenylpropanoid pathway, which is found in various plant families. Its level in plants serves as an indicator of a plant health and its resistance to diseases (Courot, 2021, Hanzouli *et al*., 2024).

2.2.3 Biological effects of resveratrol in plants

Over the last 50 years, there has been extensive increase in the research on resveratrol due to its potential benefits to plants and human health such as the anti-bacterial, antioxidant and anti-inflammatory properties among others (Wagner *et al*., 2022). These benefits stem from the ability of resveratrol to initiate sirtuin-like protein deacetylases, redox sensing enzymes involved in regulating stress responses, aging processes, regulation of metabolism and longevity (Wagner *et al*., 2022). Although resveratrol has been extensively studied for its contribution to the improvement of human health, its biological function in plants is primarily associated with redox regulation, stress defence, fungal defence and developmental processes such as callus formation, wound healing and regeneration.

These benefits tend to differ according to how it is metabolised in the plant. These methods of metabolism include; while resveratrol undergoes the phenylpropanoid pathway, it functions as free resveratrol which improves antioxidant and anti-microbial activity in plants. It is usually highly concentrated in wound and stress sites, and it neutralises reactive oxygen species (ROS) directly. Also, when resveratrol is further metabolised through glycosylation (formation of piceid) it is released slowly into the plant tissues where it is more stable and it often stored in the vacuoles. Again, when metabolised through oligomerization (formation of dimers and trimers) it promotes the anti-microbial activity and plays an important role in the inhibition of pathogens. Finally, when metabolised though oxidation or polymerisation (creation of other stilbene derivatives), it creates structural reinforcement which contributes to the strengthening of the cell wall and improves long term defence against microorganisms. However, it is notable that these diverse methods of metabolism not only influence the defence responses of the plants but also affect cellular dedifferentiation and tissue regeneration processes thereby affecting the rate of effective wound healing and callus formation.

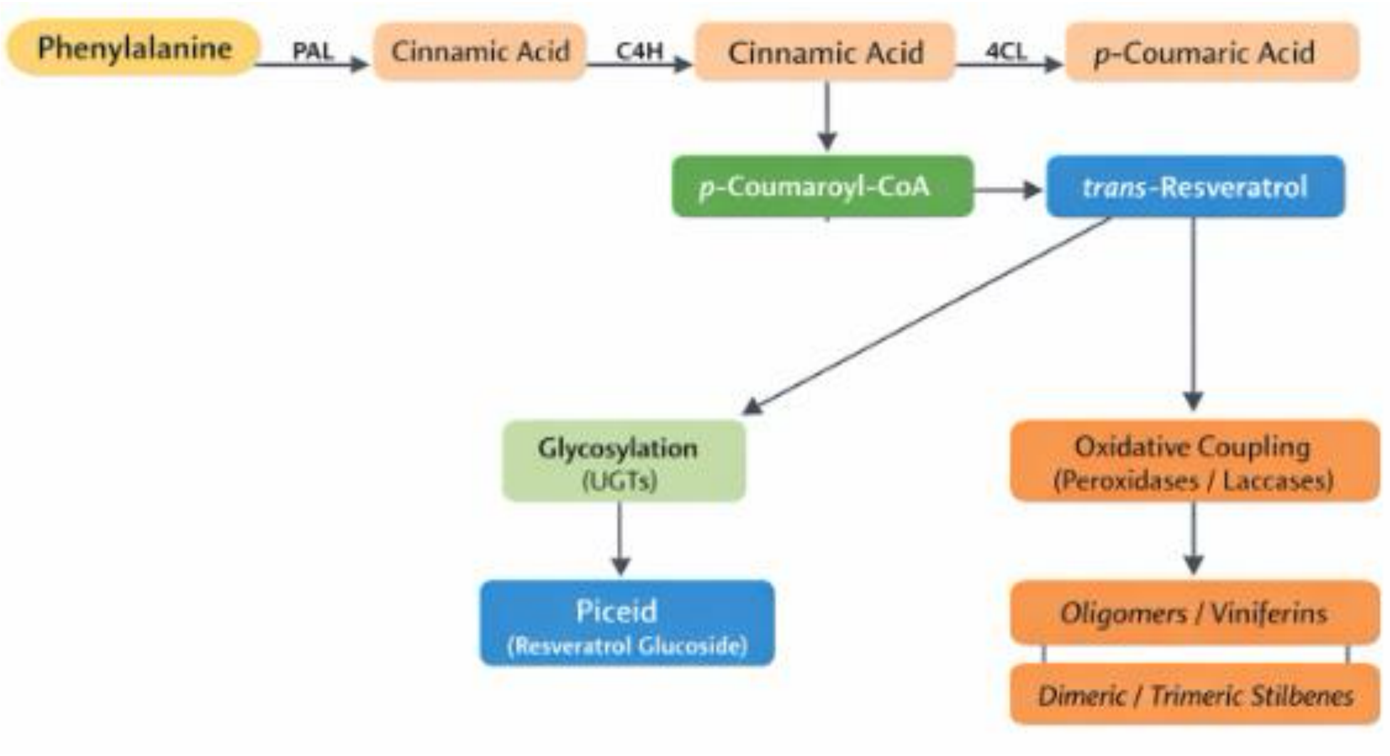


Figure 1: Metabolism pathway of resveratrol in plants
Source: Impact of Environmental Factors on Stilbene Biosynthesis, (Valletta *et al*., 2021).

Plants have devised various mechanisms of adaptation to ensure survival under unfavourable environmental and biological conditions with the involvement of the phenylpropanoid pathway and its bioactive derivatives particularly polyphenols like resveratrol which play crucial roles in plant stress tolerance (Rao and Zheng, 2025). Resveratrol plays an important role in the response of plants to biotic and abiotic stress (Liu *et al*., 2019; Wagner *et al*., 2022) such as; salinity, soil pH, pathogenic attacks, pollinators and more. The understanding of the regulation of the phenylpropanoid pathway under stress conditions is a very important factor in the development of stress resistant crops, ensuring food security and sustainable production (Paul *et al*., 2023; Saini *et al.*, 2024; Zagoskina *et al*., 2023; El-Saadony *et al*., 2024; Rao and Zheng, 2025). This pathway *metabolises* no less than 8000 types of bioactive compounds (Silva *et al*.,2023; Singla *et al.*, 2019; Rao and Zheng, 2025), which have been noted for their diverse roles in plant defence mechanisms and their antioxidant properties. Recent discoveries in molecular biology and metabolomics, reveals the in-depth regulation of this pathway and its mode of response to stimuli created by unfavourable environmental conditions (Salam *et al*., 2023; Rao and Zheng, 2025).

Abiotic stress on the other hand, which include salinity, drought, extreme temperatures, and heavy stress, significantly affects agricultural productivity and plant physiology (Yang *et al*., 2024; Nehra *et al*., 2024; Rao and Zheng, 2025). These stresses activate the production of reactive oxygen species (ROS), which leads to the impairment of physiological functions and oxidative damage (Rao and Zheng, 2025). In response to these stresses, plants actively pile up specific bioactive polyphenolic compounds such as resveratrol as part of the stress response mechanism (Paul *et al*., 2023; Salam *et al.*, 2023; Rao and Zheng, 2025). These piled compounds act as antioxidants which protect cellular components from oxidative damage induced by reactive oxygen species (ROS) (Zhang *et al*., 2019; Naikoo *et al*., 2019; Rao and Zheng, 2025). Studies have reported that the presence of resveratrol in plants helps to maintain the balance of mineral content, regulate proline and soluble sugar content to maintain osmotic balance and increases antioxidant enzyme activity to reduce the production of reactive oxygen species (Sun *et al*., 2024).

At the molecular level, there are natural signalling pathways and environmental factors that regulate the biosynthesis of resveratrol (Dubrovina and Kiselev, 2017). The phenylpropanoid pathway is responsible for synthesizing resveratrol and other primary and secondary metabolites, this process is regulated by key enzymes including phenylalanine ammonia-lyase (PAL) which is the entry step for carbon channelling, cinnamate 4-hydroxylase (C4H) and 4 - coumarate-CoA ligase (4CL) (Valletta *et al*., 2021). Under extreme stress conditions such as wounding, pathogenic invasion, oxidative stress and ultraviolet radiation, the expression of these biosynthetic genes are notably elevated through the signalling of stress responsive pathways which leads to an increase in accumulation of resveratrol in the affected sites (Chung *et al*., 2003; Dubrovina and Kiselev, 2017). The stilbene synthase (STS) functions as a key enzyme in the phenylpropanoid pathway, it prompts the formation of resveratrol from preceding molecules and studies have shown the expression of this enzyme significantly increases under stress conditions such as mechanical injury, oxidative challenges and pathogenic attack (Chung et al., 2003; Jiang *et al*., 2019).

Regardless of its known biological benefits, very limited research has been done to support the investigation of the effects of resveratrol as a phytochemical and its potential interaction with a plant associated microbiomes like endophytic bacteria.

2.3 Endophytic Bacteria: Diversity, Isolation, and Root Colonization.

Plants can develop associative relationships with other members of their ecosystem to improve growth in their environment. This often involves microorganisms (bacteria) as they are the most beneficial organisms that plants can develop associations with. Such bacteria are usually of a class of plant-beneficial bacteria that provide several advantages to their host plants (Afzal *et al*., 2019). Endophytic bacteria are bacteria that thrive inside plants, usually located in the vascular tissue or issue or intra and inter-cellular spaces and are found to colonize the roots or aerial parts of the plants. The relationship between endophytic bacteria and plants are characterised as symbiotic as they are mutually beneficial to each other (Langner dos Santos *et al.,* 2018). Their primary function in plants is to aid in the improvement of plant growth under normal and challenging conditions by affecting the growth quality and increasing biomass. They benefit the host plants directly by regulating stress related phytohormones and growth; and improving nutrient uptake. They also improve the health of plants indirectly by targeting pathogens and pests with antibiotics, nutrient limitations and priming plant defences (Afzal *et al*., 2019).

2.3.1 Mechanism of colonisation of tomato plant tissue by endophytic bacteria

Endophytic bacteria are associated with majority of crops for their promotion of growth and their ability to establish endophytic populations in various organs and tissues of the plants without any visible signs (Langner dos Santos *et al.,* 2018). The endophytic bacteria community is subdivided according to their different internal mechanisms of colonising plants. These different niches include passive, obligate and facultative endophytes. For this research, passive endophytes will be the niche of focus.

Passive endophytes are a type of endophytic bacteria that lives harmlessly within the tissues of plants. They internally inhabit plants and aid to mediate reactions related to its interaction to the host without evoking severe defence reactions or causing harm. They colonise the plants through the wounds that exist along the root hairs. They are also called associative bacteria due to being strongly attached to surface of plant roots. Plant roots have the highest frequency of endophytic bacteria and are considered the main pathway to microorganisms. However, other structures such as; stomata, nectaries, lenticels and tissue damage could be a path of entry for microorganisms (Langner dos Santos *et al.,* 2018).

Obligate endophytes are a type of endophytes which could be either fungi or bacteria. They depend entirely on their plant hosts for survival and live out most or all their lifecycle inside the plant without causing any harm to it. They rely solely on the metabolic resources and habitat provided by the plant to carry out important biological functions such as; reproduction and growth. They are usually transmitted by vertical colonisation through the seeds and other reproductive tissues of the host plant. They offer beneficial functions to the host plant, such as; enhancement of stress (salinity, drought, heat) tolerance, pest and pathogen resistance, and plant growth (Langner dos Santos *et al.,* 2018).

Facultative endophytes are type of endophytes that do not spend their entire life cycle within the host plant. They live freely in the soil and colonise the plants during some stage of their lifecycle through the rhizosphere (Langner dos Santos *et al.,* 2018) (Prinski *et al.,* 2019).

The interaction between host plants and endophytic bacteria occurs by bacteria mobility, growth of plants or passive colonisation mechanisms. Bacteria motility takes place via the process of chemotaxis which allows bacteria to move towards environmental cues, which in this case is the presence of exudates in the plant roots, based on the action of chemosensory pathways (Matilla and Krell, 2018).

*Solanum lycopersicum*b (tomato) is an extensively cultivated horticultural plant valued for its edible fruits and economic importance. Therefore, it is a suitable model for studying plant-microbe interactions (Dong *et al.*, 2019). It is one of the many crops that possess a symbiotic relationship with endophytes, particularly endophytic bacteria. A wide range of plant-growth-promoting rhizobacteria (endophytic bacteria) are known to be associated with the tomato rhizosphere (Cochard *et al.*, 2022). The rhizosphere is a hot spot for a wide range of microorganisms and is considered one of the most complex ecosystems on earth because of its high diversity and direct involvement in rhizobacteria especially plant-growth-promoting rhizobacteria, the growth of plants and the diseases that affect it (Dong *et al.*, 2019). The bacteria present in the rhizosphere of tomato, as well as other plants, are known to possess direct yield improvement traits such as phosphate and potassium solubilization, nitrogen fixation, siderophore production and phytohormone production (Cochard *et al*., 2022). These traits are known to play a role in disease prevention, the improvement of the growth quality and yield of tomato which makes them suitable components for the production of biofertilizers for sustainable modern agriculture.

2.4 Mechanisms of Plant Growth Promotion by Endophytic Bacteria

Endophytic bacteria have been identified as one of the most promising plant growth-promoting microorganisms due to their ability to live inside plants without causing any diseases while developing symbiotic relationships with their hosts. In contrast to rhizospheric bacteria, endophytic bacteria inhabit the internal tissues of plants, allowing for more intimate contact between them and the physiological processes in plants. The capability of endophytic bacteria to boost the productivity of plants is due to direct and indirect mechanisms which include improving the processes of nutrient absorption and metabolism in plants and protecting them from biotic and abiotic stress factors. This feature makes endophytic bacteria attractive organisms for use in sustainable agriculture and biofertilizer production instead of the excessive use of chemical fertilizers (Ali *et al.*, 2024; Fanai *et al.*, 2024).

2.4.1 Direct plant growth promotion:

Biological Nitrogen Fixation

Nitrogen is the major limiting nutrient for crops since plants cannot absorb nitrogen from the atmosphere in its gaseous form (N_2). Some endophytic bacteria have the nitrogenase enzyme complex encoded by genes enabling them to fix nitrogen gas in the form of ammonia which the plant absorbs for protein production. Biological nitrogen fixation plays an especially significant role in legumes like *Vigna unguiculata* (cowpea) where it allows reducing dependence on chemical nitrogen fertilizers and improves soil fertility. Various endophytic bacteria such as *Bacillus, Paenibacillus, Pseudomonas, Azospirillum* and *Enterobacter* have shown their ability to fix nitrogen leading to increased plant biomass production (Mokrievich *et al.*, 2023; Semenzato and Fani, 2024).

Phosphate Solubilization

While phosphorus is plentiful in most soils used in agriculture, a significant part of it is found in forms that are insoluble and not available for uptake by plants. Phosphate solubilizing endophytic bacteria generate organic acids, namely gluconic, citric and oxalic acids that decrease the pH of the soil and transform insoluble phosphate compounds into soluble orthophosphates available for plants. This results in an enhanced development of the root system, photosynthesis, ATP generation and nucleic acid synthesis. Moreover, phosphate solubilizing bacteria improve the phosphorus efficiency of the plants and decrease their dependence on fertilizers (Pang *et al.*, 2024; Mokrievich *et al.*, 2023).

Phytohormone Generation

There are many endophytic bacteria capable of either generating phytohormones or controlling the levels of phytohormones within the plants. The most widespread phytohormone synthesized is indole-3-acetic acid (IAA) that encourages the development of roots and their branches and root hairs. This leads to an increased surface area of the root system available for water and nutrient absorption. Some endophytes can also synthesize cytokinin and gibberellin, the hormones responsible for cell division, stem growth, seed germination and leaf enlargement (Sun *et al.*, 2024; Fanai *et al.*, 2024).

Siderophore Production

Iron is a vital micronutrient that takes part in chlorophyll synthesis, respiration, and activation of enzymes; however, it is usually found in forms of poor solubility in soil used for agricultural purposes. Endophytic bacteria synthesize low-molecular-weight iron-chelating compounds called siderophores that can bind ferric iron (Fe^{3+}) and make it available for plants.

Siderophores not only enhance plant nutrition but also limit the iron availability to pathogenic bacteria, thus decreasing their population and maintaining good health status of plants. Thus, bacteria producing siderophores are considered useful elements of biofertilizers and biological control agents (Mokrievich *et al.*, 2023; Biswas *et al.*, 2024).

ACC Deaminase Activity

Drought, salinity, flooding and attack of pathogens induce synthesis of ethylene in plants. Even though ethylene acts as a signaling substance, high amounts of ethylene prevent root elongation and cause plant aging. Various endophytic bacteria synthesize 1-aminocyclopropane-1-carboxylate (ACC) deaminase that decomposes ACC a direct precursor of ethylene and decreases ethylene content in plants. This process ensures root development, nutrient uptake, and stress resistance of plants. Therefore, bacteria producing ACC deaminase have become significant organisms for increasing plant stress resistance under adverse climatic conditions (Fanai *et al*., 2024; Semenzato & Fani, 2024).

2.4.2 Indirect plant growth-promoting mechanisms

Besides providing nutrients and growth regulators, endophytic bacteria defend plants by various indirect means. The production of antifungal and antibacterial substances, antibiotics, and volatile organic compounds is a major strategy to counter fungi and other plant pathogens. The secretion of various hydrolytic enzymes such as chitinases, cellulases, proteases, and β-1,3-glucanases also results in the breakdown of pathogen cell walls, thus inhibiting disease establishment. The induction of induced systemic resistance (ISR) in the plants is another significant means of protection by which pathogens are rendered ineffective. Bacterial biofilm formation inside the plant tissues also helps in enhancing the ability of the bacteria to stay in the plant and engage in nutrient transfer and pathogen inhibition.

This combination of both direct and indirect means of action is responsible for making endophytic bacteria more widely used as eco-friendly biofertilizers nowadays. The ability to facilitate the uptake of nutrients by plants and regulate their phytohormone levels, stimulate antioxidant system development, and prevent pathogens is what makes endophytic bacteria a proper partner for new plant biostimulators, including resveratrol. It is therefore very important to know all about these mechanisms when studying the possible synergism between tomato root endophytic bacteria and resveratrol on the growth of *Vigna unguiculata*.

2.4.3 Characterisation of tomato root endophytic bacteria

The characterisation of endophytic bacteria is an important process in the identification and assessment of these organisms and their role as plant growth promoting bacteria. The isolated bacterial cultures undergo a number of analyses including morphological, microscopic, biochemical, and molecular examinations in order to determine their taxonomy and biological characteristics. In addition to identification of different bacterial species, the use of these techniques allows selection of promising isolates which could be used in agriculture, for example as biofertilizers and biological control agents. Even though the molecular approaches such as 16S rRNA sequencing have become the standard method of bacterial identification, traditional approaches of characterisation are still crucial since they give information about the physiology and metabolism of bacteria.

Morphological Characterisation

The morphological characterization of bacteria is the first step in the identification of bacteria, and it requires thorough observation of colonies growing on nutrient media. Observations made from these colonies often give preliminary data about the variety of bacteria before any further analysis. The common observations usually made include the size, shape, colour, elevation, edge, transparency, texture, consistence, and pigmentation of the colonies. However, none of these can be used alone for the exact identification of bacteria; rather, they help in discriminating bacteria and choosing representative colonies for further analysis.

Tomato roots have an extremely diverse population of endophytic bacteria. Research have found that colonies of bacteria isolated from tomato roots vary in color, such as creamy white to yellow or light brown in color and in shape, which include circular, irregular, smooth, wrinkled, convex, or flat in shape based on their genera.

Microscopic Characterisation

Bacterial microscopic analysis allows for further information on bacterial cell structures. Purified bacteria samples are analyzed using Gram staining to distinguish between Gram-positive and Gram-negative bacteria depending on the nature of their cell wall structure. The thick peptidoglycan layer of Gram-positive bacteria holds on to crystal violet dye, while the thin peptidoglycan layer of Gram-negative bacteria, which is surrounded by an outer membrane, takes up counterstain and appears pink.

Microscopy can be used to determine bacterial cell shapes, cellular arrangements, ability to form endospores, and whether they are motile or not. Typical endophytes found in tomato roots include Gram-positive rod-shaped bacteria that form heat-resistant endospores like *Bacillus*, while other bacteria such as *Pseudomonas* are Gram-negative motile rods with no spores.

Biochemical Characterisation

Biochemical characterization is used to assess the metabolic abilities of bacterial isolates through laboratory experiments. The assays help identify the presence of particular enzymes and metabolic pathways among closely related species of bacteria. Biochemical tests done for plant-associated bacteria include catalase test, oxidase test, citrate utilization test, urease test, indole test, methyl red, voges proskauer, gelatin liquefaction, starch hydrolysis, carbohydrate fermentation, and nitrate reduction tests.

Catalase test indicates whether the bacteria have the ability to break down hydrogen peroxide produced during aerobic respiration. Oxidase tests on the other hand identify those bacteria with cytochrome c oxidase. Citrate utilization test identifies whether the bacteria are able to use citrate as the only carbon source, while urease test helps identify the ability of the bacteria to break down urea to ammonia and carbon dioxide.

Apart from taxonomy-based classification, biochemical characterization helps determine certain characteristics that may prove beneficial in plant growth. Most of the endophytic isolates are checked for their ability to solubilize phosphate, produce siderophores, produce IAA, ACC deaminase activity, ammonia production, hydrogen cyanide production, and the secretion of extracellular enzymes. The possession of such traits is an important criterion for determining whether an isolate can be used as a biofertilizer or as a biological agent. Some of the recent studies that have been conducted on endophytes associated with tomato roots have revealed that several Bacillus and *Pseudomonas* isolates are capable of performing multiple activities related to plant growth promotion.

Molecular Characterisation

While traditional methods of characterization continue to be effective, molecular characterization is now considered the most accurate method of bacterial identification. Sequencing of 16S ribosomal RNA (16S rRNA) allows the identification of bacteria on the basis of conserved and hypervariable regions of nucleotides which exist in all prokaryotes. After isolation of DNA and its amplification and sequencing, nucleotide sequences are aligned against reference databases like GenBank or EzBioCloud to find out their nearest match. In several studies involving research of root endophytes of tomatoes, combination of morphological, biochemical, and molecular approaches to characterize bacteria has helped in identification of genera *Bacillus, Pseudomonas*, *Massilia, Streptomyces, Flavobacterium, Sphingomonas, Devosia,* and *Nocardioides.* The bacteria were linked with properties like phosphate solubilization, phytohormone production, nutrient recycling, stress tolerance, and plant disease inhibition.

Thus, complete characterization allows an integration of knowledge about the taxonomic status and functionality of tomato root endophytic bacteria. Using morphological, microscopic, biochemical, and molecular techniques not only helps in increasing the accuracy of identification but also allows us to choose the best bacterial strains for developing bio-fertilizers. In this current investigation, it is the complete characterization that provides the rationale for choosing tomato root endophytes that can interact with resveratrol for growth and production of *Vigna unguiculata*.

2.4.4 Isolation and purification of tomato root endophytic bacteria

The endophytic bacteria live inside the living plant tissues without causing any disease and play an important role in nutrient absorption, growth and development, abiotic stress tolerance, and plant disease resistance. As such, since these microorganisms live inside the plant tissues, the isolation process should be done cautiously and with great care to remove all epiphytic (living on the surface of) microorganisms while ensuring that the endophytes remain alive. Thus, the isolation step is among the most crucial processes in endophytes research since contamination by epiphytic microorganisms may cause mischaracterization and misunderstanding of the microbial diversity.

Collection of Plant Materials

Isolation of the endophytic bacteria is dependent to a large extent on the physiological status of the plant being employed. It is for this reason that healthy tomatoes (*Solanum lycopersicum*) are preferred since the damaged or infected tissues could be harboring opportunistic microorganisms which are non-endophytes. The roots are extracted with utmost care so as to prevent any damage to the tissues and are then transferred into the laboratory in a sterile and cool environment. Studies have demonstrated the effect of various environmental parameters like soil, irrigation, developmental phase of the plant, and fertilization on the endophytes of tomato roots.

2.5 Common Endophytic Bacteria Associated with Tomato Roots

Tomato roots (*Solanum lycopersicum*) are inhabited by a wide array of bacteria that perform a variety of essential functions, such as providing nutrients for plants, regulating plant growth, ensuring resistance to adverse environmental conditions and pathogens, and promoting disease suppression. While rhizospheric microorganisms reside on the surface of plant roots, endophytic bacteria colonize root tissues of plants without causing any disease symptoms. The diversity of these bacterial populations depends on several factors, such as the genotype of the host plant, its stage of development, soil properties, environmental conditions, and agricultural practices. Modern techniques of high-throughput sequencing revealed that the population structure of bacteria inhabiting tomato roots varies at different stages of plant development. The variations are related to the changing needs of the host plant and the interaction between plant immunity and beneficial microbes.

Among many bacterial genera reported for tomato roots, *Bacillus* and *Pseudomonas* stand out due to their numerous plant growth-promoting traits. Other commonly isolated bacterial genera include *Enterobacter*, *Paenibacillus*, *Serratia*, Microbacterium, *Streptomyces*, *Sphingomonas*, *Massilia*, *Devosia*, and *Nocardioides*. These bacteria perform various roles, such as nutrient cycling, phosphate solubilisation, phytohormone production, biological nitrogen fixation, siderophore production, pathogen suppression, and increased plant resistance to environmental stresses. Therefore, roots of tomato have gained importance as rich sources of microorganisms that can be used for developing microbial biofertilizers and biocontrol agents.

2.5.1 *Bacillus* species

The bacteria belonging to the genus *Bacillus* are some of the most well-studied plant growth-promoting endophytes due to their ability to survive under various environmental conditions by forming heat resistant spores. Their viability in harsh environmental conditions and during storage make them ideal candidates for producing commercial biofertilizers. Many distinct types of *Bacillus spp*. isolated from roots of tomato have been found capable of synthesizing IAA, solubilisation of inorganic phosphates, formation of siderophores, ACC deaminase, and secretion of extracellular enzymes including cellulases, proteases, and chitinases. Apart from assisting in nutrient uptake, these enzymes also break down cell walls of fungal pathogens, reducing the incidence of diseases in tomato. In addition to their nutritional benefits, *Bacillus species* stimulate induced systemic resistance (ISR), enabling plants to activate defense responses more rapidly following pathogen invasion. Studies have reported that tomato plants inoculated with *Bacillus* isolates exhibit improved root architecture, increased chlorophyll content, enhanced biomass accumulation, and greater tolerance to drought and salinity stress. Because of these characteristics, *Bacillus specie*s have become one of the most commercially successful bacterial groups used in agricultural bioinoculants.

2.5.2 *Pseudomonas* species

The other dominant genera of tomato root endophytes are those belonging to the genus *Pseudomonas*, noted for their remarkable capacity for metabolism and efficient colonization of the plant tissues. The Gram-negative bacteria of *Pseudomonas* family produce a variety of secondary metabolites such as antibiotics, hydrogen cyanide, siderophores, biosurfactants, and VOCs, which are inhibitory against a broad spectrum of fungi and bacteria pathogenic to plants. *Pseudomonas species* also produce IAA, solubilize phosphate, and enhance nutrient assimilation. Several research have shown that inoculation of tomatoes with *Pseudomonas species* greatly reduces the occurrence of diseases such as Fusarium wilt, Rhizoctonia root rot, and early blight at the same time encouraging the growth of the plant. They readily colonize the interiors of the plant and exhibit antagonism against pathogens making them ideal candidates for disease management programs.

2.5.3 *Enterobacter* species

Enterobacter species are often recovered from the roots of tomatoes and have received great attention due to their ability to stimulate plant growth through various means. The majority of isolates have the ability to fix nitrogen, solubilize phosphorus, synthesize siderophores, and produce phytohormones. They facilitate nutrient uptake, while at the same time increasing the elongation and branching of roots; hence, increased water and mineral uptake. Additionally, some isolates of *Enterobacter* show antagonistic properties towards soil-borne pathogens by producing antimicrobial compounds and competition for ecological niches within the plant tissues. Their diverse capabilities have motivated their exploration as eco-friendly substitutes for chemical fertilizers and pesticides.

2.5.4 *Paenibacillus* and Other Beneficial Endophytes

In addition to *Bacillus*, *Pseudomonas*, and *Enterobacter*, there are other bacterial genera that make substantial contributions to the root microbiome of tomatoes. Species belonging to the genus *Paenibacillus* have been found to synthesize cellulases, nitrogen fixating enzymes, phosphate solubilizing substances, and antibiotics. Similarly, the bacteria belonging to the genus *Streptomyces* synthesize various antibiotics that inhibit fungi, while the bacteria belonging to the genus *Sphingomonas* help in stress tolerance and detoxification of environmental pollutants. Some of the genera recently identified by metagenomic studies that are part of tomato root endophytic microbiome include *Massilia*, *Devosia*, *Flavobacterium*, *Nocardioides*, and *Microbacterium*. Although these genera have not been studied as much as the bacteria belonging to *Bacillus* and *Pseudomonas* genera, recent research has shown that they contribute to nutrient recycling and plant hormone regulation.

Considering the positive traits displayed by the endophytes of tomato roots, it can be concluded that the microorganisms do not only inhabit tomato but can also grow and benefit the development of other agricultural plants. Several plant growth-promoting bacteria exhibit wide host ranges and have managed to facilitate growth in leguminous, cereal, and horticultural crops after artificial inoculation. The cross-host capacity is what gives scientists the reason to explore the possibility of using tomato endophytic bacteria as microbial inoculants for *Vigna unguiculata*. In addition to the capability to enhance nutrient uptake, hormone regulation, pathogen inhibition, and stress resistance, their activity could be supplemented by antioxidants from resveratrol, which could bring synergistic benefits for the development of the plant. However, there is very minimal on this area of study are the time of this research.

2.6 16S rRNA-Based Molecular Identification of Bacterial Isolates

The threshold standards for bacterial identification and classification have been updated and replaced with the adoption of 16S rRNA sequencing techniques since the 1970s (Ragan *et al*., 2014; Fitzgerald, 2019; Faniyan *et al*., 2023). These techniques have revolutionized bacteriology by allowing for the phytogenic classification of prokaryotes based on their similarities; allows for the fast identification of prokaryotic organisms down to its species level using bacterial genome sequencing methods (Faniyan *et al.*, 2023). It has been used for several decades as a supplement to culture-based methods in clinical laboratory and has been of particular use in instances where bacteria may appear to be unidentifiable use to being uncommon, biochemically inactive or fastidious (Watts *et al.*, 2017).

The method of 16S rRNA gene sequencing, commonly utilized in bacterial identification, classification and quantization, involves the hybridization of small ribosomal subunit genetic marker sequences universally across all bacteria (Faniyan *et al*., 2023). These sequences also contain hyper variable regions with varying lengths and conservation (Watts *et al.*, 2017), that have sites in specific regions in the variable domains or conserved regions that allow for bacterial species differentiation (Faniyan *et al*., 2023). The 16S rRNA sequencing for identification of bacteria on next-generation sequencers involved PCR amplification of the desired region of the 16S rRNA gene followed by library preparation, sequencing and analysis of the sequence data (Watts *et al.*, 2017). Sequence diversity, which provides the power to discriminate against bacteria species, varies across the 16S rRNA gene (Neefs *et al*., 1993; Ashelford *et al*., 2005; Watts *et al.*, 2017). These differences between 16S rRNA sequences have enhanced our understanding of the evolution of bacteria and highlighted 16S rRNA as a versatile tool for bacterial phylogeny, ecology and taxonomy studies.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site and Plant Material

This research experiment was carried out in Godfrey Okoye University biotechnology laboratory, Thinkers Corner, Enugu state during the period from March 2026 – June 2026 to study of the effect of bacteria endophytes and resveratrol on the growth of cowpea (*Vigna unguiculata*). The location of the experiment was 235° SW, 6°28'11''N latitude and 7°31'35''E longitude with a 190m elevation. The plant materials used for the experiment included; red grapes to produce resveratrol, the root of three-week-old tomato seedlings, and cowpea seeds.

3.2 Preparation of Resveratrol

Resveratrol was extracted from grape skins with the use of a simple solvent extraction method for the isolation of phenolic compounds from grapes. Fresh grape skins were first separated from the pulp and washed with distilled water to eradicate surface contaminants. The skins were then air-dried to remove moisture. After drying, they were ground into a fine powder using a mortar and pestle to increase the surface area for extraction. Approximately 5 g of the powdered grape skin was weighed and transferred into a clean conical flask. About 50 mL of 80% ethanol was added as the extraction solvent because resveratrol is soluble in alcohol-based solvents. The mixture was then stirred continuously for 1–2 hours at room temperature to allow the solvent to dissolve the phenolic compounds present in the grape skins.

After extraction, the mixture was filtered using Whatman filter paper to separate the liquid extract from the plant residue. The filtrate containing the dissolved resveratrol and other phenolic compounds was collected in a clean flask. The solvent was then concentrated by gentle evaporation using a water bath at approximately 40°C until a smaller volume of extract remained. The concentrated extract contained resveratrol and other polyphenolic compounds from the grape skin. Finally, the extract was transferred into a labelled container and stored in a refrigerator at about 4°C until further analysis or use.

3.3 Experimental Design and Planting Procedure

The experiment was laid out in a complete randomized design (CRD) for both the screenhouse and laboratory experiment. The soil for the experiment for the screenhouse was collected from the root of a mango tree located inside the Sacred Heart Hostel, Godfrey Okoye University on 21st May 2026 to prepare it for planting. 500ml of soil was measured into 36 planting bags and set for planting. The experiment consisted of 4 variieties of cowpea seeds; each one was represented with alphanumerical symbols; V1: 'Akidi' cowpea; V2: Honey cowpea; V3: Kampala cowpea; V4: White iron beans. Each variety was planted in 9 bags containing 5 seeds, with three bags in each group representing a treatment.

However, for the determination of germination parameters, the sterilized Petri dishes were placed in the laboratory and lined with paper towels for planting. 20 seeds per plate for each variety was planted into the Petri dishes in 3 replication labelled control (T0) and 10ml of distilled water was added. Another 120 seeds of each variant were soaked in endophytic bacteria organic fertilizer for 45 minutes before being left to dry for 20 minutes. After drying, the seeds were placed in the Petri dishes labelled T1 and T2 containing 20 seeds per plate for each variant. Then, 5ml of organic fertilizer and 5ml of distilled water was added to each plate and covered with another piece of paper towel. The planting process was replicated three times for each variant and factor.

3.4 Isolation of Endophytic Bacteria

The tomato plants used for the isolation of bacteria endophytes was collected from Odegba in Enugu State - 6°27'6" N 7°28'52" E with a 200m elevation. The bacteria were isolated from the roots of a three-week-old hybrid cobra tomato seedling. Collected tomato roots were individually disinfected by soaking in 70% ethanol for 1min, immersion in 1% sodium hypochlorite for 10 min and then in 70% ethanol for 30sec followed by rinsing with sterile distilled water and air-dried. Processed samples were chopped up and placed on nutrient agar and incubated at 28°C for 48hours (Abdallah R.A.B. *et al*., 2018). A total of 4 colonies were isolated, subcultured into Pikovskaya agar (PVK) and incubated at 28°C for 96 hours. A portion of the organisms were then inoculated into slate cultures for storage.

3.5 Morphological Characterization of Bacteria Isolates

The pure cultures were observed for morphological characteristics on the basis of color, shape, margin, elevation and appearance. Gram staining was carried out using the crystal violet gram staining method. The slides were viewed with the light microscope under oil immersion.

3.6 Organic Fertilizer Production from Bacteria Isolates

1500ml of Pikovskaya (PVK) broth was produced and sterilized in 3 conical flasks.

The PVK agar was composed of; Glucose 0.60g, Tri-calcium phosphate 0.30g, Agar 0.90g, Ammonium sulphate 0.03g, Sodium chloride 0.012g, Magnesium sulphate 0.03g, Potassium chloride 0.012g, Yeast extract 0.03g, Manganese sulphate 0.00012g, Iron sulphate 0.00012g

3 pure colonies were inoculated into each conical flasks containing 500ml of PVK broth and allowed to grow for 7 days.

3.7 Experimental Treatments

The experiment consisted of three different treatments:

T0: Control (No fertilizer)

T1: Organic fertilizer (endophytic bacteria)

T2: Resveratrol extract + Organic fertilizer (endophytic bacteria)

3.8 DNA Extraction

- 50–100 mg (wet weight) of bacterial cells that had been resuspended in up to 200 µl of water or isotonic buffer were added to a ZR BashingBead™ Lysis Tube (0.1 mm & 0.5 mm).
- 750 µl BashingBead™ Buffer was added to the tube.
- It was secured in a bead beater fitted with a 2 ml tube holder assembly and processed at maximum speed for ≥ 5 minutes.

- The ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) was centrifuged in a microcentrifuge at 10,000 × g for 1 minute.
- 400 µl supernatant was transferred to a Zymo-Spin™ III-F Filter in a collection tube and centrifuged at 8,000 × g for 1 minute.
- 1,200 µl of Genomic Lysis Buffer was added to the filtrate in the collection tube.
- A total of 800 µl of the mixture from the previous step was transferred to a Zymo-Spin™ IICR Column® in a collection tube and centrifuged at 10,000 × g for 1 minute.
- The flow through from the collection tube was discarded and Step 6 repeated.
- Subsequently, 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin™ ICR column in a new collection tube and centrifuged at 10,000 × g for 1 minute.
- Thereafter, 500 µl of g-DNA Wash Buffer was added to the Zymo-Spin™ IICR Column and centrifuged at 10,000 × g for 1 minute.
- The Zymo-Spin™ IICR Column was then transferred to a clean 1.5 ml microcentrifuge tube, and 100 µl (35 µl minimum) DNA Elution Buffer was added directly to the column matrix and centrifuged at 10,000 × g for 30 seconds to elute the DNA.

3.9 Sample Preparation for Mineral Analysis

The bio fertilizer was coated with platinum coating of electrically conducting material, deposited on the sample either by low-vacuum sputter coating or by high-vacuum evaporation. Scanning Electron Microscope (SEM) instruments place the specimen in a relative high-pressure chamber where the working distance is short, and the electron optical column was differentially pumped to keep vacuum adequately low at the electron gun. The high-pressure region around the sample in the ESEM neutralised charge and provided an amplification of the secondary electron signal. Low-voltage SEM was conducted in an FEG-SEM because the field emission guns (FEG) are capable of producing high primary electron brightness and small spot size even at low accelerating potentials.

Embedding in a resin with further polishing to a mirror-like finish was used for both biological and materials specimens when imaging in backscattered electrons or when doing quantitative X-rays microanalysis.

CHAPTER FOUR

RESULT

4.1 Isolation and Identification of Endophytic Bacteria

The results of morphological characterization of the isolates showed three bacterial organisms (Table 4.1). Isolate 1 had a cream color, irregular shape, raised elevation, wavy margin, and a rough surface. Isolate 2 had a cream colour, irregular shape, flat elevation, wavy margin and a dry surface. Isolate 3 also had a cream colour, irregular shape, flat elevation, wavy margin, and a slightly wrinkled surface. Additionally, phosphate solubilizing activity was observed as a clear zone (halo) along the streak lines quantitatively. However, there were no visible distinct, measurable halos around isolated individual colonies, preventing quantitative solubilization of index (SI) measurements.

Table 4.1: Morphological characteristics of the isolates

Isolate	Colour	Shape	Margin	Elevation	Surface	Organism
Isolate 1	Cream	Irregular	Wavy	Raised	Rough	Bacteria
Isolate 2	Cream	Irregular	Wavy	Flat	Dry	Bacteria
Isolate 3	Cream	Irregular	Wavy	Flat	Slightly wrinkled	Bacteria

4.2 Analysis of Variance (ANOVA) for Germination and Early Seedling Growth Traits for the Cowpea Study.

The analysis of variance highlighted significant difference ($p < 0.5$) for most parameters on variety, treatment level and their interactions (Table 4.2). Variety had a notable effect on days to first germination (11.17***), days to 50% germination time (5.73**), germination percentage (10.49***), mean germination time (5.73**), germination index (10.64***), seedling length (4.82**), leaf width (6.63**), and the number of leaves (3.50*). Also, the treatment level had significant effects on days to first germination (84.50***), days to 50% germination (72.85***), germination percentage (35.71***), mean germination time (60.69***), germination index (121.67***), seedling length (18.60***), leaf width (4.26*), and the number of leaves (13.65***). However, significant interaction was shown on Variety × Treatment interaction on germination time (11.17***), 50% germination time (7.46***), germination percentage (5.96***), mean germination time (2.68*) and germination index (5.94***) which indicates that the varieties response to the endophytic bacteria and resveratrol biofertilizers.

Table 4.2: Tabular representation of Analysis of variance (ANOVA) for germination and early seedlings growth trait for cowpea study

Parameters	Df	T1	T50	GP	MGT	GI	SL	LL	LW	NOL
Variety	3	11.17***	5.73**	10.49***	5.73**	10.64***	4.82**	1.15ns	6.63**	3.50*
Trea. level	2	84.50***	72.85***	35.71***	60.69***	121.67***	18.60***	0.07ns	4.26*	13.65***
Variety × Treatment level	6	11.17***	7.46***	5.96***	2.68*	5.94***	0.69ns	0.12ns	0.98ns	1.00ns
Error	24	0.06	0.17	57.60	0.07	2.80	5.74	1.08	0.31	4.50

Legend: Df = Degree of freedom; GP = germination percentage; MTG = mean germination time; GI =germination index; SL =Seedling length; LL = leaf length; LW = leaf width; NOL = number of leaves; ns =not significant

4.3 Mean ± Standard Deviation of Germination and Early Seedling Growth for Studied Cowpea

According to the results of the mean analysis shown in Table 4.3, it is indicated that all the plants were germinated by day 2 of planting. However, it is notable that all treated plants germinated on day 1 after planting as opposed to the controls (V1T0, V2T0, V4T0) which germinated on day 2 except V3T0 which germinated on day 1. V4TO had the longest time to germination (T50) followed closely by V2T0. V1T0, V3T0 and V3T2 had the intermediate time to germination while T1 groups, V1T2, V2T2, and V4T2 had the shortest time to germination.

V1T0, V1T1, V2T1, V2T2 are shown below to have the highest germination percentage (GP), V1T2, V3T1, V4T1, V4T2 and V3T2 had an intermediate germination time while most of the controls (V2T0, V3T0, V4T0) show the lowest germination percentage.

The V4T0 had the highest mean germination time (MGT) followed closely by V3T2 and the rest of the T0 group while the T1 group and the rest of the T2 group had the lowest mean germination time.

Table 4.3 Tabular representation of mean ± standard deviation of germination and early seedling growth for studied cowpea

Treatment	T1 (days)	T50 (days)	GP (%)	MGT (days)	GI	SL (cm)	LL (cm)	LW (cm)	NOL
V1T0	2.00 ± 0.00	2.22 ± 0.47	91.67 ± 7.64	2.61 ± 0.11	7.52 ± 0.94	11.50 ± 0.50	6.17 ± 0.76	3.27 ± 0.46	6.67 ± 1.15
V1T1	1.00 ± 0.00	1.00 ± 0.00	90.00 ± 0.00	1.18 ± 0.23	15.78 ± 1.57	15.50 ± 0.87	6.03 ± 0.45	3.73 ± 0.68	9.00 ± 1.00
V1T2	1.00 ± 0.00	1.28 ± 0.25	85.00 ± 10.00	1.76 ± 0.09	12.25 ± 1.63	18.33 ± 1.53	5.47 ± 0.57	3.40 ± 0.36	11.67 ± 4.04
V2T0	2.67 ± 0.58	3.90 ± 0.10	56.67 ± 7.64	2.61 ± 0.35	0.89 ± 0.51	12.07 ± 1.37	6.53 ± 0.87	3.50 ± 0.44	4.00 ± 0.00
V2T1	1.00 ± 0.00	1.33 ± 0.58	95.00 ± 8.66	1.72 ± 0.26	14.72 ± 2.80	16.00 ± 1.00	6.40 ± 0.53	3.60 ± 0.36	9.00 ± 4.58
V2T2	1.00 ± 0.00	1.17 ± 0.29	93.33 ± 2.89	1.69 ± 0.28	14.17 ± 2.02	16.83 ± 2.84	6.50 ± 1.32	3.43 ± 0.75	7.33 ± 2.52
V3T0	1.00 ± 0.00	2.43 ± 0.21	51.67 ± 2.89	2.63 ± 0.06	4.50 ± 0.50	10.83 ± 1.61	5.67 ± 0.58	3.47 ± 0.25	4.00 ± 0.00
V3T1	1.00 ± 0.00	1.53 ± 0.71	81.67 ± 2.89	1.93 ± 0.38	11.11 ± 1.64	18.07 ± 1.01	5.67 ± 0.76	4.60 ± 0.40	10.00 ± 1.00
V3T2	1.00 ± 0.00	2.18 ± 0.50	75.00 ± 0.00	2.20 ± 0.19	8.42 ± 2.53	15.83 ± 3.33	5.80 ± 1.68	3.73 ± 0.64	7.67 ± 2.52
V4T0	2.67 ± 0.58	4.08 ± 0.29	56.67 ± 7.64	3.23 ± 0.40	1.91 ± 1.04	15.33 ± 5.86	6.33 ± 2.08	3.83 ± 1.15	4.00 ± 0.00
V4T1	1.00 ± 0.00	1.27 ± 0.46	86.67 ± 7.64	1.82 ± 0.22	12.36 ± 1.96	20.17 ± 1.26	6.47 ± 0.50	4.80 ± 0.20	6.67 ± 0.58
V4T2	1.00 ± 0.00	1.40 ± 0.53	86.67 ± 12.58	1.82 ± 0.31	12.14 ± 1.00	20.00 ± 2.00	6.33 ± 0.85	4.87 ± 0.23	7.33 ± 0.58

Key: GP = germination percentage, MGT = mean germination time, GI= germination index, SL=Seedling length, LL=leaf length, LW indicates the leaf width, and NOL =number of leaves.

4.4 Boxplot Analysis of Treatment Variables

Below is a boxplot showing the treatment mean for the cowpea study according to different morphological parameters; with multiple letters indicating significant variation and similar letters indicating insignificant variables. T1, days to first germination; T50, days to 50% germination; GP, germination percentage; MGT, the mean germination time; GI, the germination index; SL, the Seedling length, LL, the leaf length; LW, the leaf width; and NOL, the number of leaves.

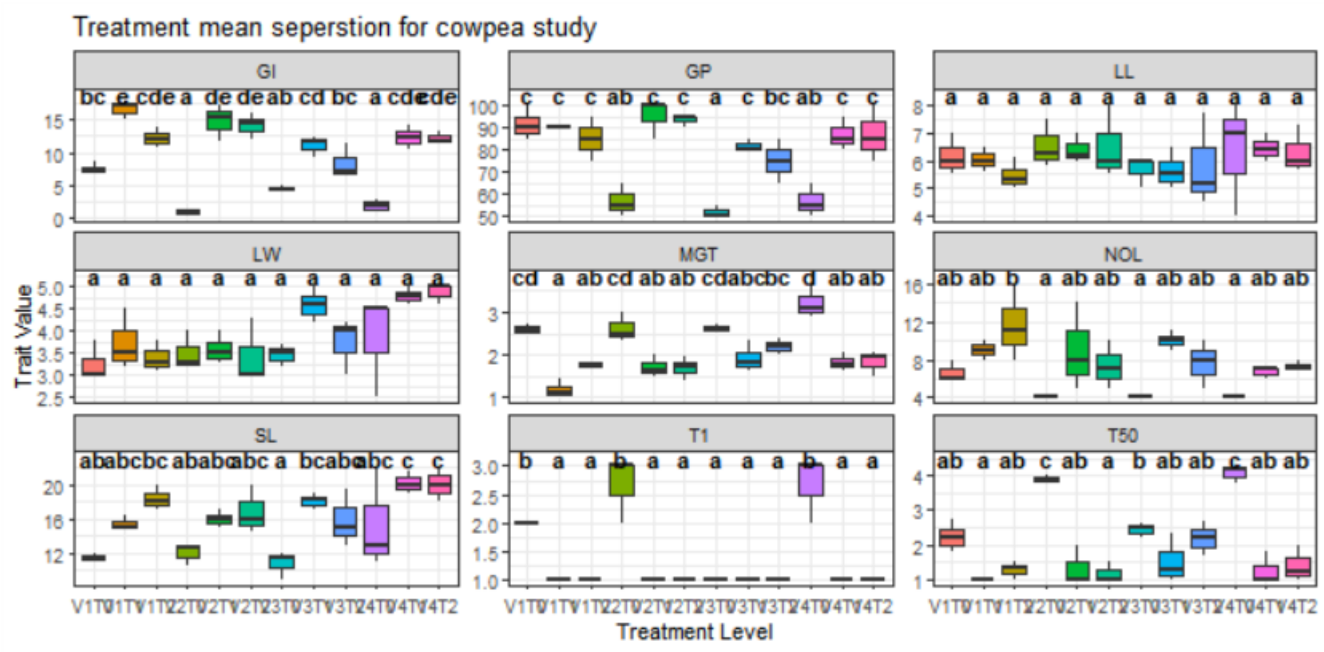


Figure 4.1 Boxplot representation of treatment variables

4.5 Hierarchical Clustering of Cowpea Varieties Treatments

These clusters are grouped according to plants with similar responses to treatments or the lack of them. The plants in cluster one expressed high germination percentage (GP), low mean germination time (MGT), average germination index (GI), high seedling length (SL), high leaf length (LL), average leaf width (LW) and the one of the highest number of leaves (NOL) recorded.

The plants in cluster two also expressed high germination percentage (GP), low mean germination time (MGT), high germination index (GI), high seedling length (SL), high leaf length (LL), highest leaf width (LW) recorded and the one of the highest number of leaves (NOL).

The plants in cluster three expressed the highest germination percentage (GP), average mean germination time (MGT), average germination index (GI), high seedling length (SL), high leaf length (LL), high leaf width (LW), and high number of leaves (NOL).

The plants in cluster four (which consisted of two of the four controls) expressed low germination percentage (GP), high mean germination time (MGT), low germination index (GI), average seedling length (SL), high leaf length (LL), average leaf width (LW) and low number of leaves (NOL).

The plants in cluster five (which consisted of the other two controls) expressed an average germination percentage (GP), high mean germination time (MGT), low germination index (GI), low seedling length (SL), high leaf length (LL), average leaf width (LW) and low number of leaves (NOL).

These results further emphasize the advantage expressed by fertilized plants over unfertilized controls in significant parameters.

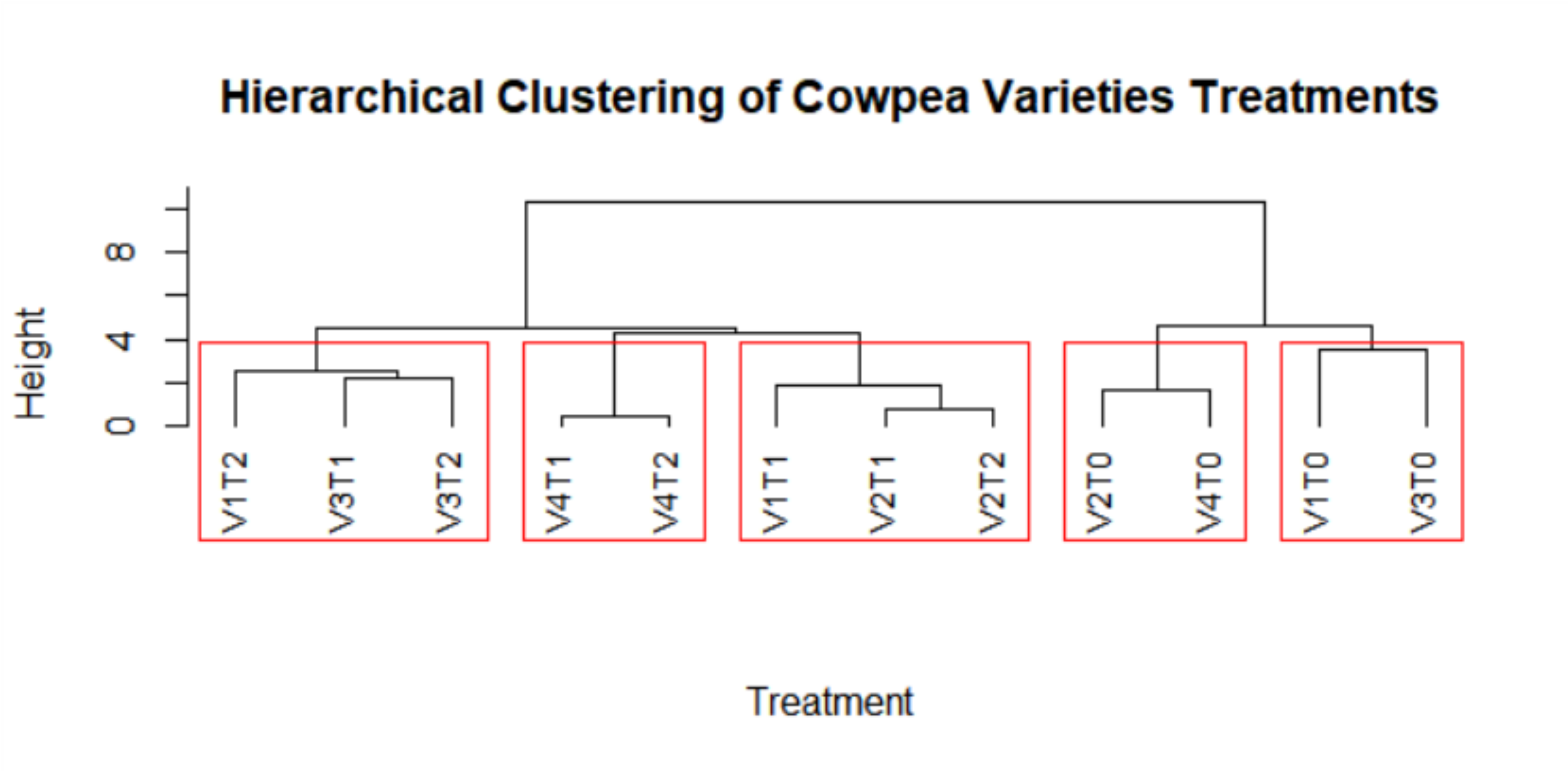


Figure 4.2 Hierarchical clustering of cowpea varieties treatments

4.7 Mineral Analysis of Resveratrol Extract and Endophytic Bacteria Bio Fertilizer

The table below represents the minerals present in a combined mineral analysis of resveratrol and endophytic bacteria. It shows the high amount of zinc in the mixture and the lack of nitrogen. These minerals also played a significant role in the growth of the plants.

Table 4.4 Tabular representation of minerals present in resveratrol extract and endophytic bacteria fertilizer.

Sample	Phosphorus (%)	Potassium (%)	Nitrogen (%)	Iron (%)	Zinc (%)	Manganese (%)	Copper (%)	Sodium (%)	Silica (%)	Calcium (%)
Fertilizer	12.60	0.44	0.00	0.00046	0.0122	0.000115	0.000036	0.0001	48.00	0.000295

4.8 16S rRNA identification of Bacterial Isolate

Results from the 16S rRNA analysis shows the 100% match relationship between the three isolates and *Bacillus subtillis*. According to phylogeny table below, all the samples isolated from the root of the tomato seedling was identified as *Bacillus subtillis sp* which is a known plant growth-promoting rhizobacterium (PGPR) present in the root of tomato seedling plant, noted for improving the the growth conditions of plants such as; cowpea.

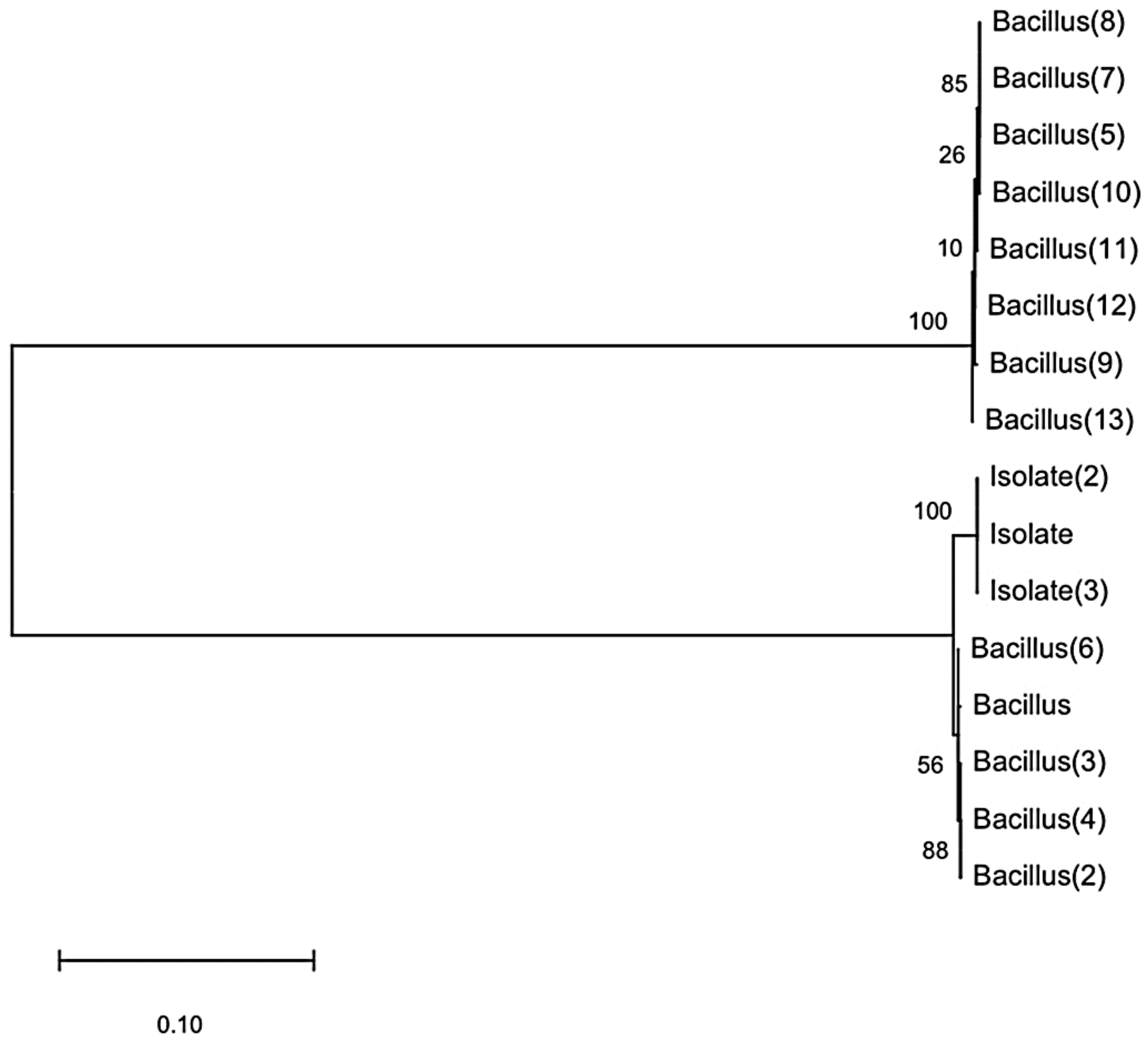


Figure 4.5 Phylogeny Tree Indicating the Relationship Between the Isolates and *Bacillus sp.*

CHAPTER FIVE

DISCUSSION AND CONCLUSION

The endophytic bacteria form part of the ecologically significant microorganisms that help enhance plant adaptation to various environments through processes including nutrient uptake, phytohormones production, biological control of plant pathogens, and enhanced biotic and abiotic stress tolerance, (Sun *et al.*, 2024). The bacteria live inside the plants without causing diseases, and they have been greatly studied for their environmentally friendly use as opposed to artificial fertilizers in improving plant growth while still maintaining soil fertility. The isolation of endophytic bacteria from the healthy tomato root is consistent with other literature sources suggesting that tomato roots act as significant sources of endophytes that can be used as biofertilizers. Consistent with the results obtained by Langner dos Santos *et al.* (2018), the successful extraction of endophytic bacteria from the roots of healthy tomatoes in the current study indicates that tomato plants act as potential sources of microorganisms that can be utilized as biofertilizers in sustainable agriculture practices.

Identification of the endophytic bacteria was important due to the similarities that exist among most of the bacterial species in terms of morphological and biochemical characteristics and hence conventional methods were not sufficient for accurate identification of bacterial species. In this case, identification of the bacterial species obtained from the tomato roots was possible by the use of molecular identification involving sequencing of the 16S rRNA gene. Sequencing of the 16S rRNA gene is the most acceptable molecular tool for identifying bacteria based on the presence of conservative and variable regions within the gene (Fanai *et al.*, 2024).

The bacteria obtained in the current study were identified as members of the strain *Bacillus subtilis*. This identification is supported by many research findings in the past that have suggested *Bacillus subtilis* as one of the major bacterial endophytes in the roots of tomatoes. The bacteria belonging to the genus *Bacillus* are known to be beneficial to plants due to their capability of producing indole-3-acetic acid (IAA), phosphate solubilization, siderophore synthesis, production of antimicrobial compounds, and induction of systemic resistance in plants.

Resveratrol is a polyphenolic substance found in plants that is generated in reaction to external stressors including attack by pathogens, exposure to UV radiation, drought, high salt concentrations, and physical injury. In addition to being an antioxidant, resveratrol is a signalling substance that takes part in regulation of defensive reactions in plants and reduces oxidative stress through scavenging reactive oxygen species (ROS). Maintaining redox homeostasis of cells under stress conditions ensures enhanced physiological performance and may promote effective symbiosis between plants and microorganisms (Kang *et al*., 2022).

A combination of *Bacillus subtilis* and resveratrol led to a beneficial impact on the growth of cowpeas, as evidenced from the higher germination percentage, germination index, and length of seedlings. This implies that there is a likelihood of these two biological factors having worked through complementary ways. Whereas *Bacillus subtilis* could have increased plant growth through nutrient release, phytohormone release, and better root growth, resveratrol could have provided better antioxidant defenses during seed germination and seedling formation stages. Even though there is little information about this combination, there is a strong biological rationale based on the physiological role played by PGPRs and antioxidants. The current results are also supported by earlier studies showing that the endophytic *Bacillus strains* enhance growth and reduce diseases in various economically valuable crops. For instance, *Bacillus subtilis* was found to enhance the growth of tomato and reduce soil-borne diseases in chickpea and cowpea by a number of plant growth promoting mechanisms. This is a possible indication that tomato-isolated *Bacillus subtilis* could easily establish itself as an endophyte in other crop plants like *Vigna unguiculata.*

While the findings from this research are promising, there are some shortcomings which should be noted. For instance, functional traits like nitrogen fixing ability, indole-3-acetic acid production, siderophores production, ACC deaminase activity, and phosphate solubilizing ability have not been quantitatively determined (Biswas *et al*., 2024). Moreover, the current study has concentrated mostly on the early growth characteristics without determining the effects on subsequent growth stages such as blooming, podding, grain productivity and nutrition content. These attributes need to be studied in order to have a better idea about the growth promoting mechanisms. Also, the effects of resveratrol at different concentrations and comparisons between various endophytic bacteria and *B. subtilis* will make it clearer whether there is a synergic effect between phytochemical biostimulants and bacteria.

In general, this research indicates that the bacteria isolated from tomato roots belonging to the genus *Bacillus subtilis* have substantial prospects in being used as biofertilizers for sustainable cultivation of crops. The use of the isolated bacteria along with resveratrol resulted in enhanced growth characteristics of cowpeas, implying the presence of a possible synergy between the beneficial endophytic bacteria and antioxidants derived from plants. While more greenhouse and field studies recommended in order to confirm these results in different environments, the current study offers important preliminary information on this issue.

REFERENCES

Abebe, B. K., Alemayehu, M. T. (2022). A Review of the Nutritional Use of Cowpea (*Vigna unguiculata L.* Walp) for Human and Animal Diets. *Journal of Agriculture and Food Research*, 10 (100383).

Abdallah, R. A., Jabnoun-Khiareddine, H., Nefzi, A., and Daami-Remadi, M. (2018). Evaluation of the Growth-Promoting Potential of Endophytic Bacteria Recovered from Healthy Tomato Plants. *Journal of Horticulture*; 5(2).

Afzal, I., Shinwari, Z. K., Sikandar, S., and Shahzad, S. (2019). Plant Beneficial Endophytic Bacteria: Mechanisms, Diversity, Host Range, and Genetic Determinants. *Elsevier (Microbiological Research)*, 221: 36-49.

Ashelford, K.E., Chuzhanova, N.A., Fry, J.C., Jones, A.J. and Weightman, A.J. (2005). At Least 1 In 20 16S RNA Sequence Records Currently Held in Public Repositories Is Estimated to Contain Substantial Anomalies. *Applied Environmental Microbiology,* 71:7724-7736.

Balkrishna, A., Gautam, A. K., Sharma, N., Arya, V., Khelwade, V., and Arya, D. (2024). Effects Of Organic Fertilizers on Growth and Yield of Field Crop Cowpea. *Asian Journal of Agriculture;* 6(1): 32-40.

Cochard, B., Giroud, B., Crovadore, J., Chablais, R., Arminjon, L., and Lefort, F. (2022). Endophytic PGPR from Tomato Roots: Isolation, In Vitro Characterization and In Vivo Evaluation of Treated Tomatoes (*Solanum lycopersicum* L.). *Multidisciplinary Digital Publishing Institute (Microorganisms),* 10(4): 765.

Courot, E. (2021) Resveratrol in Plants. *Multidisciplinary Digital Publishing Institute Plants): Special Issue.*

Dong, C., Wang, L., Li, Q., and Shang, Q. (2019). Bacterial Communities in the Rhizosphere, Phyllosphere and Endosphere of Tomato Plants. *Public Library of Science* (PLOS One).

Faniyan, O., Akpe, V., and Cock, I. (2023). Analysing Bacterial Species from Different Environments Using Direct 16S rRNA Gene Seqencing Methods. *Pharmacognosy Communications*, 13(1): 24-33.

Fitzgerald, D. M., and Rosenberg, S. M. (2019). What is Mutation? Chapter in the Series: How Microbes 'Jeopardize' the Modern Synthesis. *Public Library of Science Genet,* 15(4).

Gutam, N., Ghimire, S., Kafle, S., and Dawadi, B. (2024). Efficacy of Bio Fertilizers and Chemical Fertilizers on Growth and Yield of Cowpea Varieties. *Technology in Agronomy,* 4 (e007).

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W. F., and Kloepper, J. W. (1997). Bacterial Endophytes in Agricultural Corps. *J Microbiol*, 43(10): 895-914.

Horn, L. N., and Shimelis, H. (2020). Production Constraints and Breeding Approaches for Cowpea Improvement for Drought Prone Agro-Ecologies in Sub Saharan Africa. *Elsevier (Annals of Agricultural Sciences)*, 65(1): 83-91.

Kang, J. E., Yoo N., Jeon, B. J., Kim, B. S., and Chung, E. H. (2022). Resveratrol Oligomers, Plant-Produced Natural Products with Anti-virulence and Plant Immune-Priming Roles. *Frontiers in Plant Science;* 13.

Kim, D., Ochar, K., Iwar, K., Ha, B., and Kim, S. (2025). Cowpea (*Vigna unguiculata L.*) Production, Genetic Resources and Strategic Breeding Priorities for Sustainable Food Security: A Review. *Frontiers in Plant Science and Plant Breeding,* 16.

Langner dos Santos, M., Berlitz, D. L., Wiest, S. L. F., Schunemann, R., Knaak, N., and Fiuza, L. M. (2018). Benefits Associated with the Interaction of Endophytic Bacteria and Plants. *Brazilian Archives of Biology and Technology,* 61.

Matilla, M., and Krell, T. (2018). The Effects of Bacterial Chemotaxis on Host Infection and Pathogenicity. *FEMS Microbiology Reviews*, 42(1).

Mekonnen, T. W., Gerraro, A. S., Mbuma, N. W., and Labuschagne, M. T. (2022). Breeding of Vegetable Cowpea for Nutrition and Climate Resilience in Sub-Saharan Africa: Progress, Opportunities, and Challenges. *Multidisciplinary Digital Publishing Institute (Plants)*, 11 (12): 1583.

Neefs, J.-M., Van de Peer, Y., De Rijk, P., Chapelle, S. and De Wachter, R. (1993) Compilation of Small Ribosomal Subunirna Structures. *Public Medical Journal,* 21: 3025-3049.

Omomowo, O. I., and Babalola, O. O. (2021). Constraints and Prospects of Improving Cowpea Productivity to Ensure Food, Nutritional Security and Environmental Sustainability. *Frontier Plant Science*, 12.

Pinski, A., Betekhtin, A., Hupert-Kocurek, K., Mur, L., and Hasterok, R. (2019). Defining The Genetic Basis of Plant Endophytic Bacteria Interactions. *International Journal of Molecular Sciences*, 20(8): 1947.

Regan, M. A., Bernard, G., and Chan, C. X. (2014). Molecular Phytogenetics Before Sequences: Oligonucleotide Cataloges as K-mer Spectra. *RNA Biology*, 11(3): 76-85.

Singh, B. (2020). *Cowpea: The food legume of the 21st century*. John Wiley and Sons': Technology and Engineering.

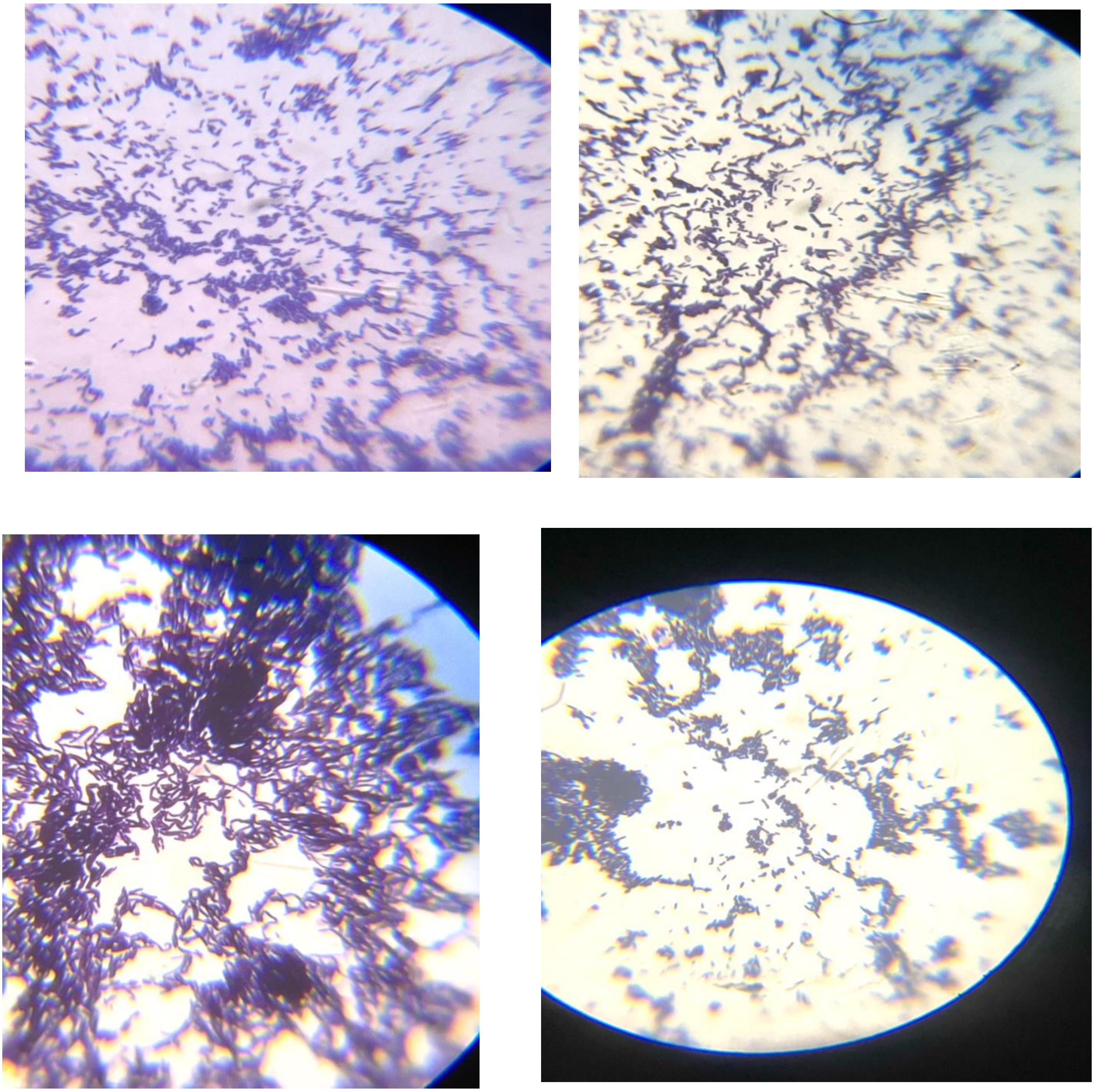
Tian, B., Zhang, C., Ye, Y., Wen, J., Wu, Y., Wang, H., Li, H., Cai, S., Cai, W., Cheng, Z., Lei, S., Ma, R., Lu, C., Cao, Y., Xu, X., and Zhang, K. (2017). Beneficial Traits of Bacterial Endophytes Belonging to the Core Communities of the Tomato Root Microbiome. *Elsevier (Agriculture, Ecosystems and Environment),* 247: 149-156.

Truong V., Jun M., and Jeong W. S. (2018). Role Of Resveratrol in Regulation of Cellular Defense Systems Against Oxidative Stress. National Library of Medicine; 44(1): 36-49.

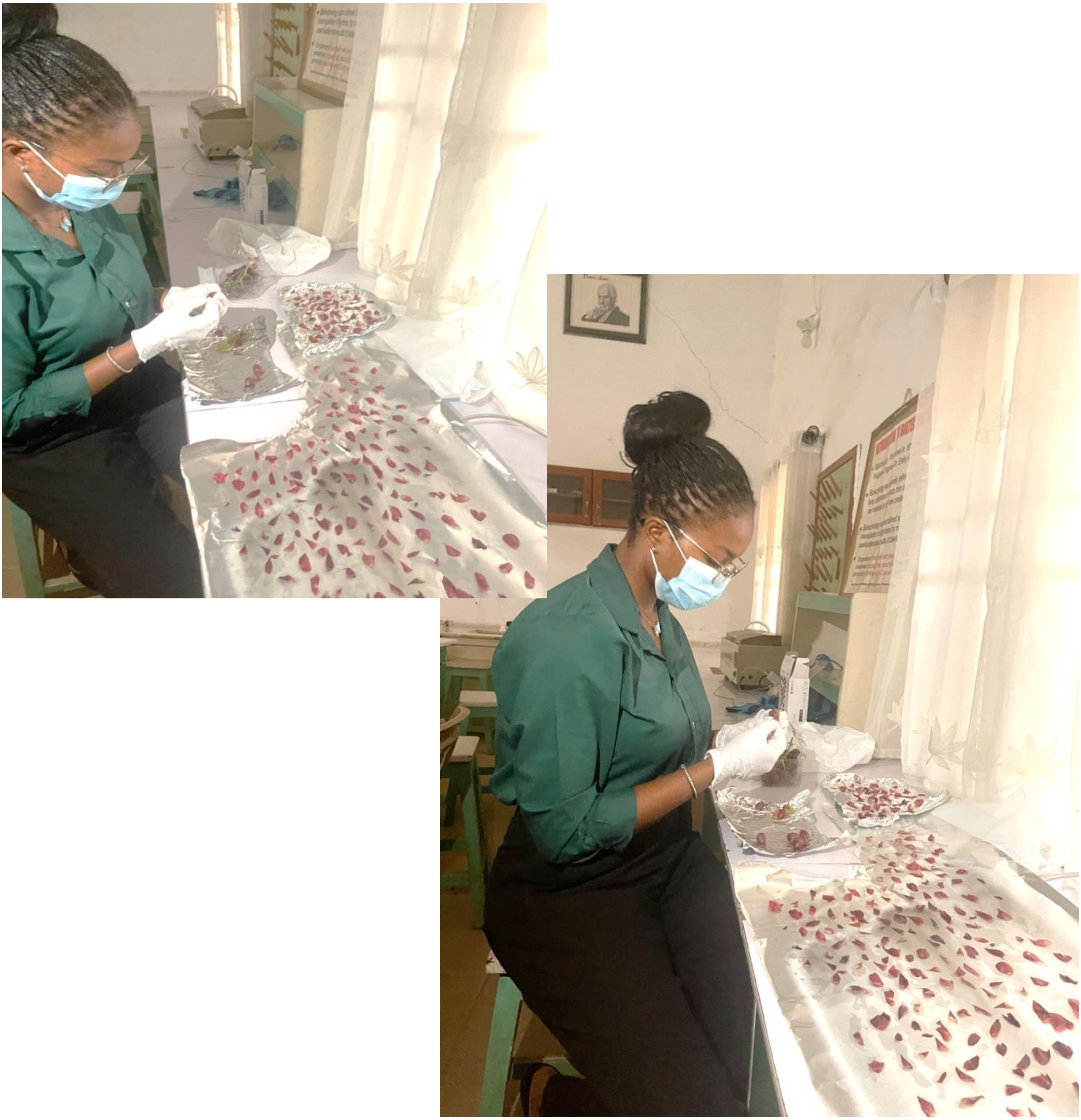
Wagner A. L., Fabrizio A. E. Ishii-Iwamoto E. L., and Abenavoli M. R. (2022). Resveratrol Exerts Beneficial Effects on the Growth and Metabolism of *Lactuca Sativa* L. *Science Direct: Plant Physiology and Biochemistry*; 171: 26-37.

Watts, G. S., Youens-Clark, K., Slepian, M. J., Wolk, D. M., Oshiro, M. M., Metzger, G.S., Dhingra, D., Cranmer, L. D., and Hurwitz, B. L. (2017). 16S rRNA Sequencing on a Benchtop Sequencer: Accuracy for Identification of Clinically Important Bacteria. *Journal of Applied Microbiology*, 123(6): 1584-1596.

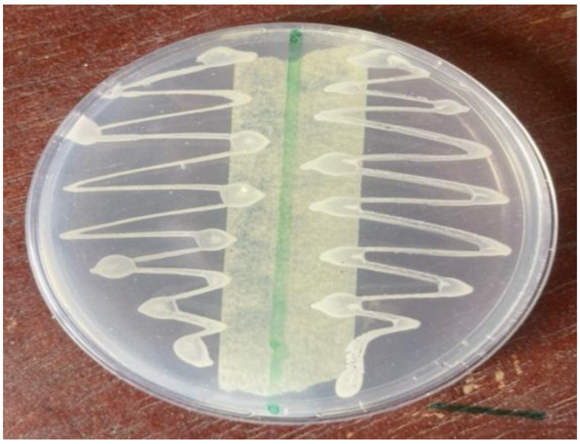
APPENDICES

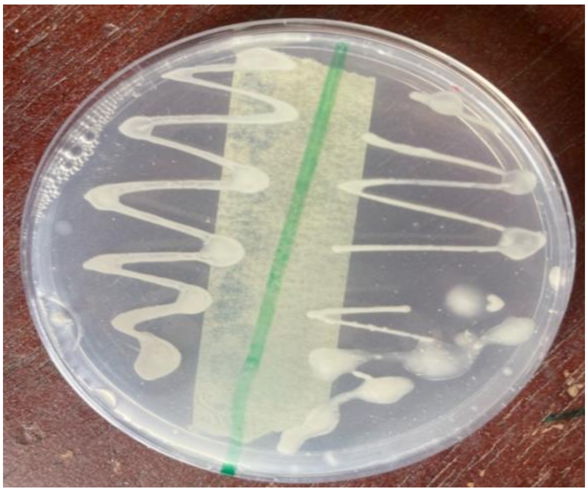


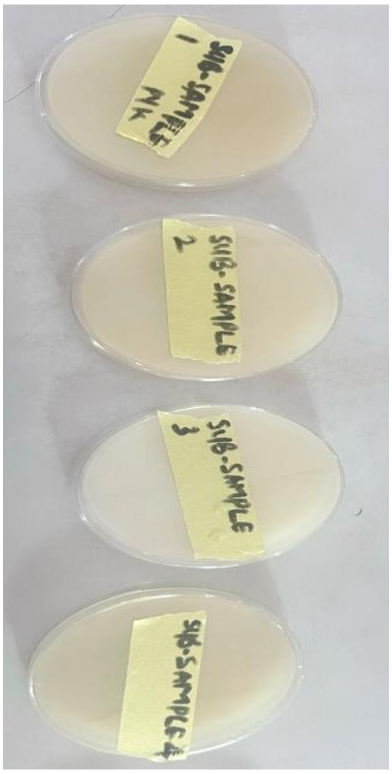
Appendix I Microscope view of isolated bacteria



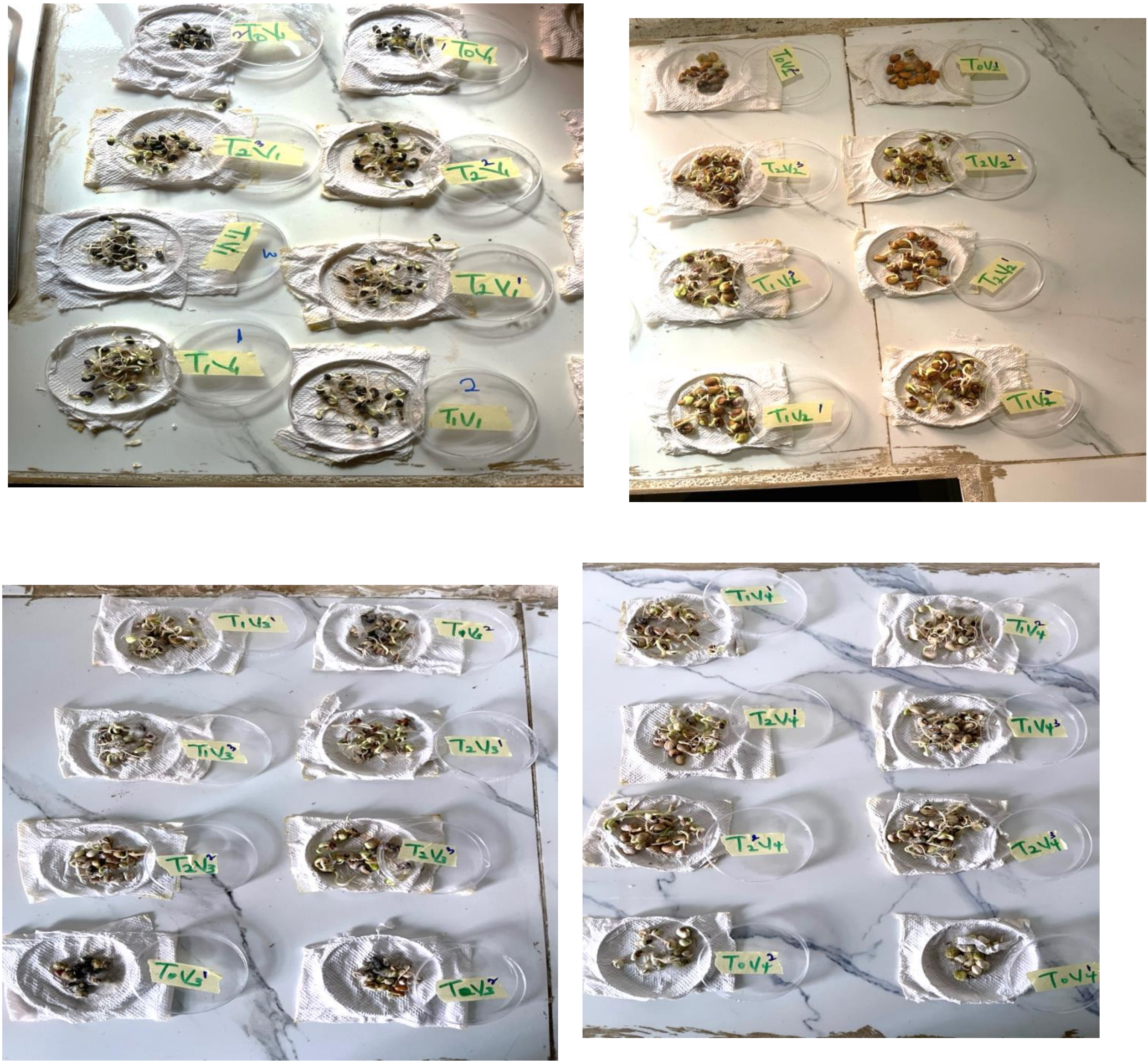
Appendix 2: The researcher peeling off the skin of red grapes







Appendix 3: Pictures showing the growth of isolated bacteria from tomato root



Appendix 4: The growth of cowpea seeds under different treatments in a Petri dish


Appendix 5: The growth of the control plants in 11 days and 4 days, respectively.

Appendix 6: Pictures showing the growth of the cowpea seeds under different treatments in 4 days.