

SEED BACTERIZATION WITH PLANT GROWTH-PROMOTING
ENDOPHYTES: IMPACT ON OKRA PRODUCTIVITY, NUTRACEUTICALS
AND ANTIMICROBIAL PROPERTIES

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ABSTRACT

The growing challenges of population expansion, climate change, rising food costs, and unpredictable rainfall patterns have intensified the need for sustainable agricultural practices that boost crop productivity while minimising environmental impacts. One promising strategy involves the combined use of plant growth-promoting endophytic bacteria (PGPEB) with the environmentally friendly hydroponic technique to enhance plant growth and functional quality. This study investigated the influence of seed bacterization with three PGPEB strains, *Kosakonia cowanii* (KC), *Bacillus licheniformis* (BL), and *Cupriavidus metallidurans* (CM), previously isolated from *Myrothamnus flabellifolius*, on the productivity, nutraceutical accumulation, and antimicrobial activity of okra (*Abelmoschus esculentus* L. Moench) under hydroponic (Kratky method) conditions. Okra seeds were soaked in single and combined bacterial inocula, germinated under dark conditions, and cultivated in a randomised complete block design with three plants per pot, four replications, and eight treatments. Results revealed significant improvements ($P < 0.001$) in seed germination percentage and seedling vigour index among inoculated treatments compared to controls, with KC and CM demonstrating strong individual and synergistic effects. BL was notably more effective when used in combination with other bacteria. Furthermore, methanolic extracts of okra pods from bacterized plants exhibited enhanced nutraceutical properties, including elevated total phenolic content (TPC), antioxidant activity (up to 91% DPPH inhibition), and increased inhibition of α -amylase (92%) and α -glucosidase (88%), key enzymes linked to diabetes management. Thin-layer chromatography confirmed the presence of carotenes, while polysaccharides were detected across all treatments. Antimicrobial assays revealed substantial activity against pathogens such as *Mycobacterium avium*, *Salmonella typhi*, *Candida albicans*, and *Staphylococcus aureus*, with the strongest effects observed in methanolic extracts of BL-CM consortia. These findings underscore the potential of PGPEB consortia as eco-friendly bio-stimulants to enhance okra seedling establishment, yield functional bioactive compounds, and contribute to sustainable agriculture and novel nutraceutical and antimicrobial development. Further exploration of PGPEB-based biofertilizers may offer solutions for addressing chronic diseases and rising antibiotic resistance.

Keywords: Plant Growth-Promoting Endophytic Bacteria (PGPEB); Hydroponics; Okra (*Abelmoschus esculentus*); Seed Germination; Seedling Vigour Index; Nutraceuticals; Antioxidant; Antidiabetic; Antimicrobial Activity.

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LIST OF ABBREVIATIONS AND/OR ACRONYMS

ABTS	-2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ACCD	-1-Amino-cyclopropane-1-carboxylate deaminase
ANOVA	-Analysis of Variance
ASA	-Abscisic acid
BL	- <i>Bacillus licheniformis</i>
Ca	-Calcium
CK	-Cytokines
CM	- <i>Cupriavidus metallidurans</i>
CO	-Cyclooxygenase
DNA	-Deoxyribonucleic acid
DPHH	-1,1-diphenyl-2-picrylhydrazyl
DNS	-Dinitrosalicylic acid
DWC	-Deep Water Culture
Fe	-Iron
GAE	-Gallic Acid Equivalent
GP	-Germination Percentage
H₂O	-Water
H₂O₂	-Hydrogen peroxide
HCN	-Hydrogen Cyanide

IC₅₀	-Half-maximal Inhibitory Concentration
IAA	-Indole-3-acetic acid
IL-6	-Interleukin-6
ISR	-Induced systemic resistance
K	-Potassium
KC	- <i>Kosakonia cowanii</i>
LOX	-Lipoxygenase
MDA	-Malondialdehyde
MBC	-Minimum Bactericidal Concentration
MFC	-Minimum Fungicidal Concentration
Mg	-Magnesium
MIC	-Minimum Inhibitory Concentration
Mn	-Manganese
MHB	-Muller-Hinton Broth
Na	-Sodium
Na₂CO₃	-Sodium Carbonate
NFT	-Nutrient Film Technique
NH₄⁺	-Ammonium
NO	-Nitrogen Oxide
O₂	-Oxygen

O₃	-Ozone
OS	-Oxidative stress
P	-Phosphorus
pH	-Potential of Hydrogen
PGPB	-Plant growth-promoting bacteria
PGPEB	-Plant growth-promoting endophytic bacteria
PGPEs	-Plant growth-promoting endophytes
PGPM	-Plant growth-promoting microbe
PNP-G	-p-Nitrophenyl glucopyranoside
RCBD	-Randomised Complete Block Design
Rf	-Retention Factor
RNA	-Ribonucleic acid
ROS	-Reactive oxygen species
SOD	-Superoxide dismutase
SPSS	-Statistical Product and Services Solutions
SVI	-Seedling Vigour Index
TFC	-Total Flavonoid Content
TINFα	-Tumour necrosis factor-alpha
TLC	-Thin Layer Chromatography
TPC	-Total Phenolic Content

UV -Ultraviolet

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DEDICATION

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DECLARATION

I, Thembakazi Kamelu Ginindza, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof, has not been submitted for a degree at any other institution. No part of this thesis/dissertation may be reproduced, stored in any retrieval system, or transmitted in any form, or by means (e.g. electronic, mechanical, photocopying, recording or otherwise) without the prior permission of the author, or The University of Namibia on that behalf.

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October 2025

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CHAPTER 1: INTRODUCTION

1.1 Background of the study

Extensively distributed in tropical and subtropical regions, with a growing popularity in Southern Africa, okra (*Abelmoschus esculentus* L. Moench, Malvaceae) is a multipurpose vegetable with noteworthy nutritional and medicinal significance (Elkhalifa *et al.*, 2021). Recent studies have shown that okra's functional food components make it a source of antioxidant, antidiabetic, anti-inflammatory, anti-fatigue, antimicrobial, and other functions (Chen *et al.*, 2015; Elkhalifa *et al.*, 2021; Khan *et al.*, 2022). In the management of various chronic diseases, there is a growing shift from conventional therapies toward the relatively safer approach of phytotherapy (Elkhalifa *et al.*, 2021). Okra (*A. esculentus*) also gained popularity due to its potential antimicrobial capabilities, which have been demonstrated in numerous studies (Luthfi *et al.*, 2020; Korani *et al.*, 2021; Lin *et al.*, 2022). Extracts from okra plant components, including leaves, seeds, and pods, possess inhibitory effects on a variety of pathogenic microbes, indicating a possible natural alternative for combating infections (Nicault *et al.*, 2021). Research has shown that okra extracts are excellent at targeting bacterial and fungal strains, supporting their usage in traditional medicine and prospective pharmaceutical uses (Nicault *et al.*, 2021; Guebebia *et al.*, 2023). Thus, the commercial cultivation of okra has increased in recent years. As a result, there is a need to boost yield per unit area to improve production (Khan *et al.*, 2022).

Overall, okra is considered an easily available, low-cost vegetable crop, but its conventional production in arid and semi-arid countries such as Namibia is hampered by a hot semi-arid climate with an unimodal rainfall pattern and poor sandy soils leading to poor crop productivity (Elkhalifa *et al.*, 2021). For higher plant productivity

and resource use efficiency per unit area, soilless agriculture such as hydroponics is an effective substitute. Hydroponics is one of the efficient agricultural practices that are environmentally friendly and allow for precise control of nutrient availability to plants and up to 90% water use efficiency (Ahmed *et al.*, 2021; Khan *et al.*, 2022). An illustrative example is the Kratky hydroponic system, which represents a non-circulating, passive method of soilless cultivation. In this system, plants are grown in containers with their roots suspended in a nutrient-rich solution. As the nutrient solution is gradually depleted through plant uptake and evaporation, an air gap naturally forms, facilitating oxygenation of the root zone without the need for pumps or active aeration (Kratky 2009). Additionally, seed bacterization can be effectively used to enhance agricultural productivity (Lin *et al.*, 2022). Seed bacterization is the treatment of seeds with cultures of bacteria previously tested for plant growth-promoting traits and is considered a precise and cost-effective method to deliver plant growth-promoting bacteria (PGPB) inoculants (Lin *et al.*, 2022; Salehin *et al.*, 2023). PGPB mediate the cycling of nutrients, their enhanced mobilisation and facilitate their uptake, leading to increased root growth, biomass and yield of the plant (Salehin *et al.*, 2023). However, the effect of seed bacterization on seed emergence, plant productivity, nutritional quality and antimicrobial activity of plants cultivated under hydroponic conditions remains scarce (Stegelmeier *et al.*, 2022). Considering the above, the present study will unravel the prospective effects of selected desert-adapted *Myrothamnus flabellifolius* PGPEB on seed emergence, plant productivity, nutraceutical components accumulation and antimicrobial activity of okra in a Kratky hydroponic system.

1.2 Statement of the problem

Hydroponic cultivation, while efficient in water use and space, often lacks the complex rhizosphere interactions found in soil-based systems, making plants more dependent on synthetic nutrient solutions and prone to stress-related yield reduction. Moreover, the absence of native beneficial microbes may limit nutrient cycling, plant resilience, and secondary metabolite production. Recent evidence has highlighted the promise of plant growth-promoting bacteria (PGPB), including endophytes, in enhancing nutrient uptake, stress tolerance, and crop yield in hydroponic systems. However, there is a gap in research exploring how such bacteria affect nutrient dynamics, yield quality, and bioactive compound accumulation in okra, a nutritionally valuable crop. Addressing this gap could lead to sustainable innovations in hydroponic cultivation, improving crop productivity and harnessing the nutraceutical and antimicrobial potential of okra for human health.

1.3 Objectives

1. To assess the effects of seed bacterization with single and different consortia of PGPEB isolated from a desert plant, *M. flabellifolius*, on the germination of okra seeds.
2. To investigate the efficacy of single and different consortia of PGPEB in increasing the accumulation of nutraceutical components and antimicrobial activity in okra under the Kratky hydroponics system.

1.4 Significance of the study

Assessing the effects of seed bacterization with various consortia of plant growth-promoting endophytes (PGPEB) isolated from *M. flabellifolius* will contribute to understanding endophyte-okra interactions and their practical applications in the Kratky hydroponics system. Additionally, investigating the potential increase in nutraceutical components within okra through these endophyte consortia can offer promising prospects for boosting the crop's health benefits in a hydroponic context. Furthermore, since okra pod extracts have demonstrated significant antimicrobial activity against various pathogenic microorganisms, integrating these extracts with endophyte consortia may enhance the overall antimicrobial profile of the okra crop. This combined approach could not only improve the plant's disease resistance and growth but also potentially amplify its health benefits by leveraging both its inherent antimicrobial properties and those conferred by beneficial endophytes.

1.5 Limitation

The study's results are specific to the chosen hydroponic system, nutrient solution composition, growth conditions and specific endophytes from the resurrection plant *M. flabellifolius*.

1.6 Delimitation

The study mainly focused on a limited number of plant growth-promoting endophytes and a soilless experiment in a greenhouse.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Around two-thirds of the world's population relies on plant-based materials for medicinal and healing capabilities (Elkhalifa *et al.*, 2021). The current sharp increase in demand for plant nutraceutical products is largely attributable to their therapeutic impact on a variety of ailments, including cardiovascular and digestive diseases, as well as their anti-cancer, anti-diabetic, antioxidant, anti-fatigue, anti-inflammatory and antimicrobial effects (Khan *et al.*, 2022). The cultivation of okra (*Abelmoschus esculentus* L. Moench), a plant known for its high nutritional value and medicinal properties, has drawn the interest of agricultural and botany researchers over the years (Premalatha and Vijayaraghavan, 2017; Salehin *et al.*, 2023).

The purpose of this literature review is to investigate the existing body of knowledge on the effect of seed bacterization with plant growth-promoting endophytes isolated from *Myrothamnus flabellifolius* on the productivity, nutraceutical accumulation and antimicrobial activity of okra grown under hydroponic conditions. The review will look into studies that have investigated similar interactions in other plants, the mechanisms through which endophytes enhance plant growth, and the potential benefits and challenges of applying these findings to okra cultivation.

2.2 Plant Growth-Promoting Endophytic Bacteria

Plant growth-promoting endophytic bacteria (PGPEB) are a subset of rhizospheric bacteria that reside in plant internal tissues and are known to promote plant growth (Cueva-Yesquén *et al.*, 2021; Bohra *et al.*, 2022). They are considered an essential component of the host tissue system and can be found in practically every plant species (Rana *et al.*, 2020; Bohra *et al.*, 2022). PGPEB have advantageous properties such as

improving nutrient bioavailability and regulating hormone levels in plants (Cueva-Yesquén *et al.*, 2021; Huang *et al.*, 2023). They are capable of producing hydrolytic enzymes such as pectinase, xylanases, cellulose, and proteinase, which aid in their penetration into plant tissues (Rana *et al.*, 2020).

PGPEB may enhance plant growth using both direct and indirect mechanisms as illustrated in **Figure 2.1** (Krishnan and Praveen, 2019). Direct mechanisms of plant growth promotion by PGPEB include macronutrient solubilisation (phosphorus, potassium, and zinc), atmospheric nitrogen fixation, phytohormone synthesis, and siderophore production (Bohra *et al.*, 2022; Pellegrini *et al.*, 2023). These mechanisms aid plants in acquiring nutrients from their surroundings and control plant growth by regulating plant growth hormones (Pellegrini *et al.*, 2023). PGPEB promotes plant development indirectly through antibiosis, hydrogen cyanide (HCN) generation, siderophore production, induced systemic resistance (ISR), and the production of a variety of cell wall-disintegrating enzymes (Patle *et al.*, 2018). These mechanisms aid in the suppression of plant pathogens through the production of antibiotics, ACC deaminase and lytic enzymes (Patle *et al.*, 2018; Lacava, Bogas and Cruz, 2022).

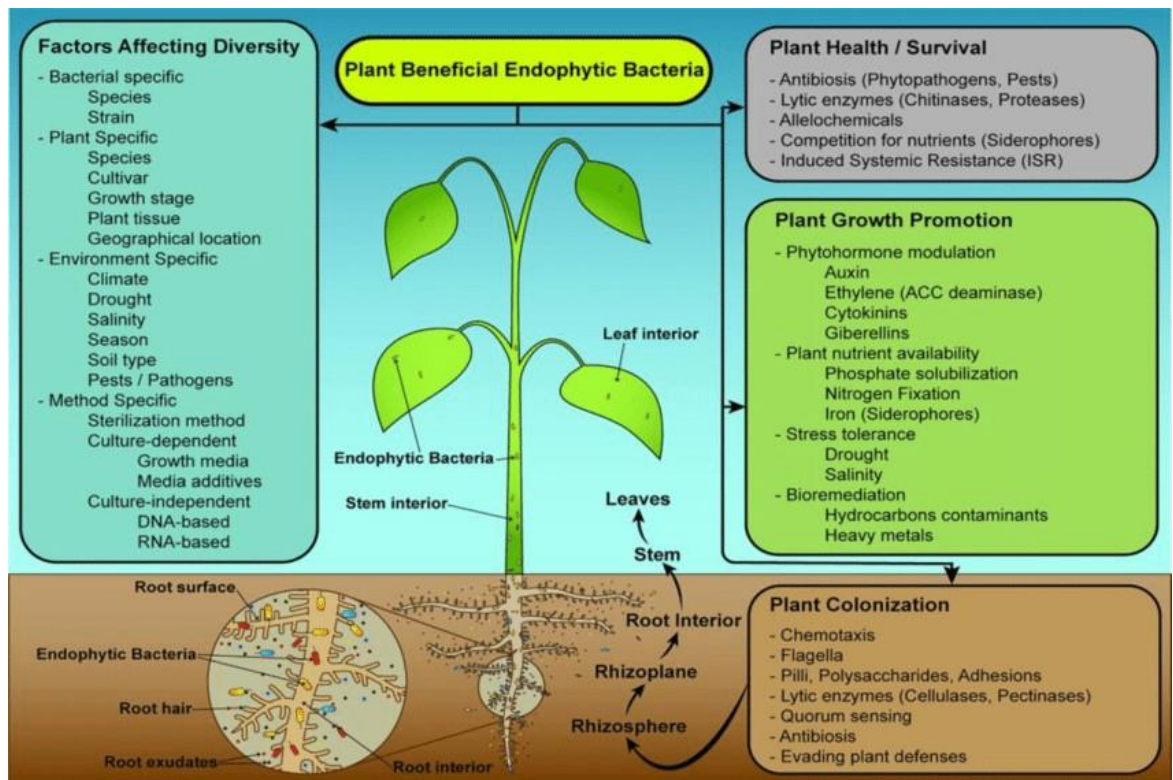


Figure 2.1: Endophytes and their role in plants (Krishnan and Praveen, 2019)

Plant growth-promoting endophytic bacteria can be applied in a variety of methods, depending on the needs of the plant and environment (Mahgoub *et al.*, 2021). Some examples of common methods used are:

Seed bacterization which is one of the most common ways of applying PGPEB. Before planting, the seeds are coated, soaked or sprayed with bacteria. This method keeps the bacteria close to the plant roots from the beginning, which improves the plant’s access to beneficial bacteria (Mahgoub *et al.*, 2021; Wang *et al.*, 2022).

Soil inoculation involves directly inoculating the soil by combining the bacteria with water. This method is especially useful for treating established plants or large areas (Mahgoub *et al.*, 2021; Wang *et al.*, 2022).

Foliar spray, the bacteria are combined with water and sprayed directly onto the plant’s leaves. This method is often used in combination with other methods to ensure that bacteria reach all parts of the plant (Rana *et al.*, 2020).

The drip irrigation technique involves introducing bacteria into the drip irrigation water. Bacteria are then dispersed as water is dripping into the soil, and this ensures that bacteria reach the root zone, in which they can be most efficiently utilised (Rana *et al.*, 2020; Mahgoub *et al.*, 2021).

Hydroponic system, in this method, bacteria are added directly to the nutrient solution. This keeps the bacteria in contact with the plant roots constantly (Mahgoub *et al.*, 2021).

2.3 Plant Growth-Promoting Endophytic Bacteria in Agriculture

The use of PGPEs in agriculture is gaining attention as a sustainable alternative to chemical fertilisers and pesticides, promoting plant health and productivity while reducing environmental impact (Lin, Zhang, *et al.*, 2022; Salehin *et al.*, 2023). Applying agriculturally important microbes to plant stressor mitigation has yielded promising results (Adeleke and Babalola, 2022). A better understanding of the biology of bacterial endophytic communities and their close interactions with the plant genetic network is paving the way for further investigations into the microbial impact on plant stress response, tolerance, and adaptation (Cueva-Yesquén *et al.*, 2021). Researchers have come to understand that an in-depth look at the interaction between endophytes and their host plants might be the key to developing multi-factor control strategies for the most common stress drivers affecting plants, particularly in adverse environments (Cueva-Yesquén *et al.*, 2021; Adeleke and Babalola, 2022).

Plants face a variety of abiotic stressors, including drought, salinity, heavy metals, and extreme temperatures. These stressors negatively affect plant physiology and morphology due to disruptions in the genetic control of cellular processes (Egamberdieva, Wirth, Alqarawi, *et al.*, 2017; Lacava, Bogas and Cruz, 2022). To counteract these stress effects, plants utilise numerous tolerance strategies and

pathways that are activated when metabolic changes occur (Lacava, Bogas and Cruz, 2022). Phytohormones, which are key growth regulators, significantly influence plant metabolism and play a crucial role in triggering plant defence mechanisms against stress (Majeed, Muhammad and Ahmad, 2018). The application of external phytohormones (**Figure 2.2**) has been used to enhance growth and metabolism under stressful conditions (Egamberdieva, Wirth, Alqarawi, *et al.*, 2017). Recent studies suggest that the microbial metabolic pathways responsible for the production of phytohormones associated with plant roots may serve as valuable targets for metabolic engineering aimed at enhancing host resistance to abiotic stress (Egamberdieva, Wirth, Shurigin, *et al.*, 2017; Lacava, Bogas and Cruz, 2022).

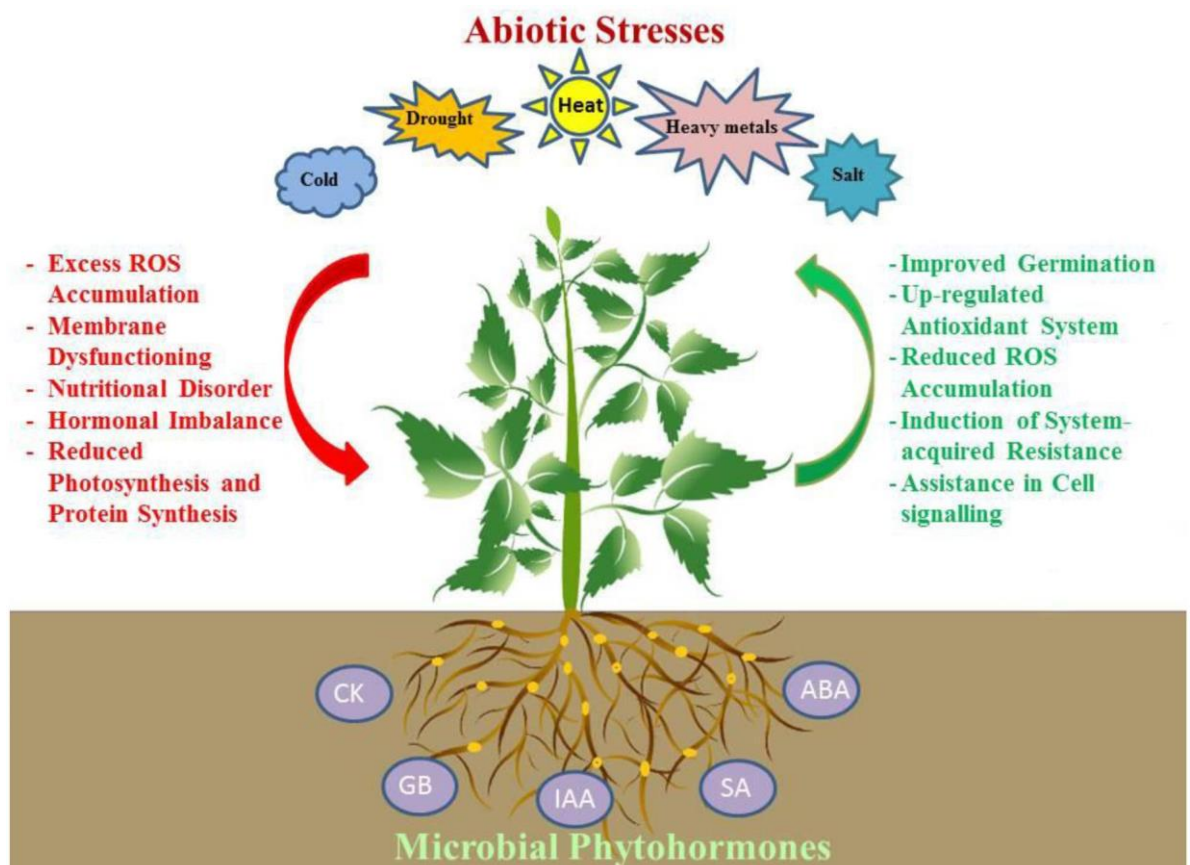


Figure 2.2: A summary of how microbial phytohormones contribute to plant stress tolerance

Numerous microbes associated with plant roots produce phytohormones such as cytokines (CK), gibberellin (GB), indole-3-acetic acid (IAA), salicylic acid (SA), and abscisic acid (ABA). These hormones aid plants in resisting stress by boosting their antioxidant capabilities, up-regulating the antioxidant system, and accumulating compatible osmolytes, thereby reducing damage caused by oxidative stress. They also enhance photosynthetic capacity and membrane stability, promote cell division and stomatal regulation, stimulate root system growth, and facilitate the acquisition of water and nutrients (Egamberdieva, Wirth, Alqarawi, *et al.*, 2017).

Plants can also be affected by biotic stress, which is induced by harmful microbes which hinder normal plant growth and have a wide range of adverse effects on crops worldwide (Chaudhary *et al.*, 2022; Lacava, Bogas and Cruz, 2022). Various biotic stressors like bacteria, viruses, fungi, insects, weeds, and nematodes are the primary sources of stress that lead to an increase in reactive oxygen species (ROS) (Lacava, Bogas and Cruz, 2022). These species impact the physiological and molecular functions of plants, resulting in reduced crop productivity (Chaudhary *et al.*, 2022; Lacava, Bogas and Cruz, 2022).

Bacterial endophytes offer a solution to these challenges faced in traditional farming. These environmentally friendly microbial entities colonise plant tissues without inflicting any harm (Majeed, Muhammad and Ahmad, 2018). They are known to assist plants in managing biotic stress (**Figure 2.3**) and enhancing crop and soil health (Pandey, Samanta and Yadav, 2019). Consequently, the use of endophytes as bio-fertilisers and biocontrol agents has emerged as an eco-friendly alternative to harmful chemicals for plant growth and biotic stress mitigation (Majeed, Muhammad and Ahmad, 2018; Chaudhary *et al.*, 2022).

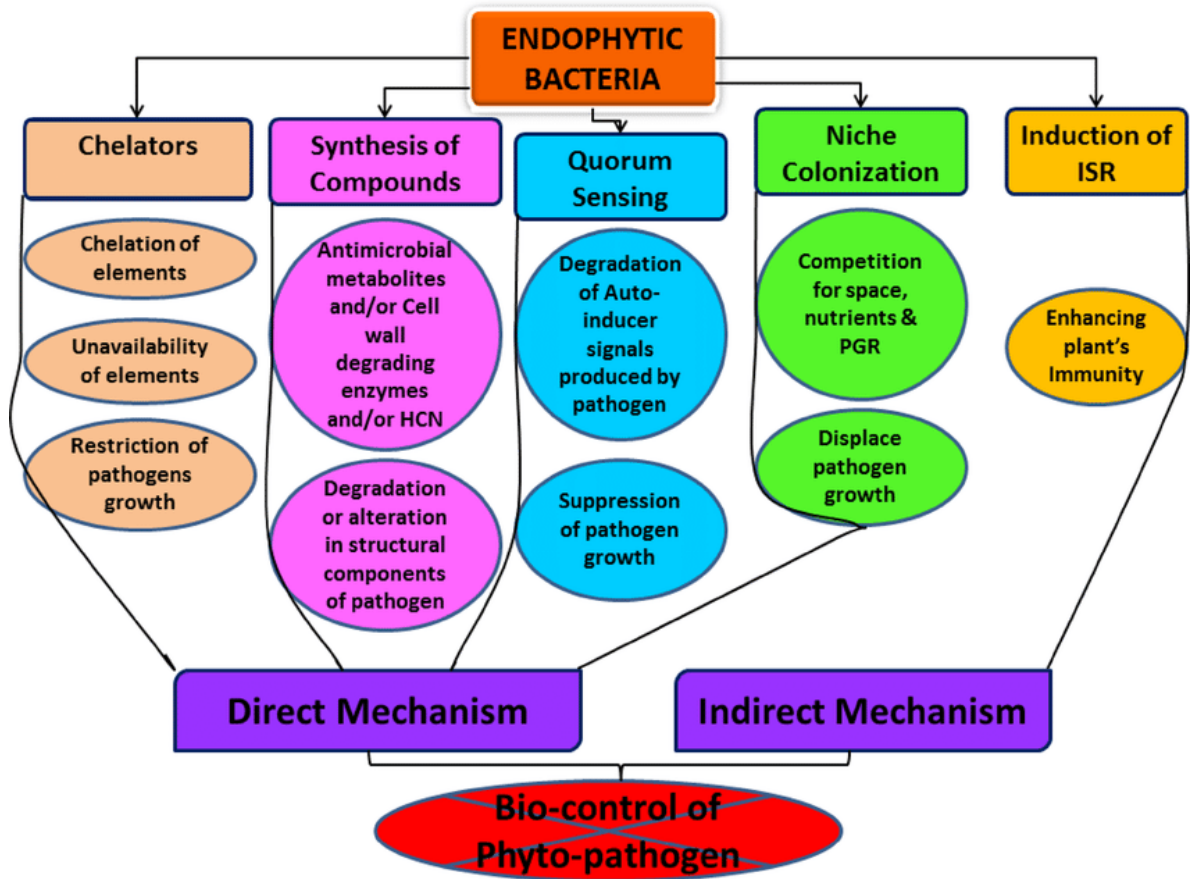


Figure 2.3: Mechanism of endophytic bacteria in biocontrol of phytopathogen (Pandey, Samanta and Yadav, 2019)

2.4 Resurrection Plant (*Myrothamnus flabellifolius*)

M. flabellifolius, commonly known as the resurrection plant in Namibia, is a fascinating species indigenous to the southern regions of Africa (Bentley, Moore and Farrant, 2019; Marks *et al.*, 2022; Tebele, Marks and Farrant, 2024). It is predominantly found in areas with severe environmental conditions, such as arid and semi-arid zones. It occurs in a wide area stretching from Namibia in the west, through Botswana, to Zimbabwe and the northern parts of South Africa (Bentley, Moore and Farrant, 2019). The plant is celebrated for its extraordinary ability to withstand desiccation and rejuvenate upon rehydration (Figure 2.4), demonstrating a unique adaptation to thrive in harsh climates (Marks *et al.*, 2022).



Figure 2.4: *M. flabellifolius* “Resurrection” elapsed time in hours

M. flabellifolius is known by various local names such as mufandichimuka (in Shona), umfavuke (in Ndebele), uvukakwabafile (in Zulu), or moritela tshwene (in Setswana). These names highlight the plant’s unique and extraordinary traits (Nantapo and Marume, 2022). The plant is known for its strong production of secondary compounds linked to defence and tolerance, many of which have significant medicinal uses both historically and in the present day (Bentley, Moore and Farrant, 2019; Tebele, Marks and Farrant, 2024).

The plant is a remedy for numerous health issues, including asthma, backaches, kidney problems and microbial infections (Bentley, Moore and Farrant, 2019). In Botswana, locals grind parts of the shoot into powder and brew it as tea (Setshogo and Mbereki, 2011). In Southern Zimbabwe, the leaves and twigs of the plant are boiled and consumed as a treatment for colds and other chest discomforts (Maroyi, 2013). The Nama people in Namibia reportedly use leaf extracts of *M. flabellifolius* to aid in wound healing and to treat asthma and other chest ailments (Van Wyk, 2009). Beyond its traditional and medicinal uses, *M. flabellifolius* is gaining global interest for

potential uses in cosmetics, pharmaceuticals, and agriculture. Several international companies are currently investigating the potential of *M. flabellifolius* extracts for product development (Erhabor *et al.*, 2020).

Recent studies have investigated the potential of *M. flabellifolius* as a reservoir of endophytic bacteria (Marks *et al.*, 2022; Tebele, Marks and Farrant, 2024). The distinctive ecological niche of *M. flabellifolius*, characterised by its capacity to endure extreme drought conditions, implies that endophytic bacteria associated with this plant may possess attributes that aid in stress and drought tolerance, nutrient uptake, disease resistance, soil health and plant growth promotion (Marks *et al.*, 2022). Endophytic isolates obtained from this robust plant, such as *K. cowanii*, *B. licheniformis*, and *C. metallidurans*, exhibit promising features, making them potential candidates for bacterial inoculants in agricultural and environmental applications (Tebele, Marks and Farrant, 2024).

2.5 Okra as a Nutraceutical and Medicinal Plant

Okra is a herbaceous annual plant with an upright growth pattern. It can usually reach up to 1.8 metres in height. The plant has palmately lobed leaves and pale yellow flowers with a scarlet or maroon centre that resembles hibiscus flowers (Elkhalifa *et al.*, 2021). Okra's long, tapering green pods, which constitute the plant's edible portion, are what give it its unique appearance (**Figure 2.5**). These pods are known for their mucilaginous texture (Khan *et al.*, 2022).



Figure 2.5: Okra plants with flowers and pods growing in a Kratky hydroponics system

Okra is a nutritious powerhouse and a proficient source of vitamins A, E, and C. It also contains essential minerals, such as sodium, potassium, magnesium, and calcium, and trace elements such as zinc, iron, and nickel (Elkhalifa *et al.*, 2021; Guebebia *et al.*, 2023). Okra is regarded as a useful supplement (**Figure 2.6**) to enhance human nutrition and health because of its noteworthy nutritional value (Khan *et al.*, 2022; Guebebia *et al.*, 2023). Along with its significant nutritional value, the okra plant possesses certain therapeutic values, which make it a potential candidate for the use of a variety of nutraceuticals (Guebebia *et al.*, 2023).



Figure 2.6: Okra dietary supplement is said to help maintain a healthy blood sugar level, maintain a healthy cholesterol range, provide vital organ support and may provide immune system support (Retrieved from <https://www.natureherballife.com> 2024)

Okra seeds and pods contain phenolic compounds, such as flavonoids and catechins, which are used as anti-fatigue, antioxidants and antidiabetics (Sarkar, Christopher and Shetty, 2022). The pods also contain tocopherols (vitamin E), which are known as powerful reducing agents and have been extensively used as antioxidants (Khan *et al.*, 2022). Bioactive compounds such as flavonoids, alkaloids, saponins, and tannins found in okra pods are said to play a vital role in the antimicrobial activity of okra (Korani *et al.*, 2021). Okra pods have also been shown to have anti-diabetic properties. Zhang *et al.* (2024) demonstrated the *in vitro* inhibitory impact of polysaccharides found in okra pods' aqueous extract on α -glucosidase and α -amylase, indicating okra pods' potential to regulate blood sugar levels. Naturally occurring plant pigments such

as chlorophyll and carotenoids, endowed with the healing of wounds and anti-inflammatory properties, have been found in okra pods (Khan *et al.*, 2022).

2.5.1 Antioxidant and Anti-fatigue properties of okra pods

Okra pods, consumed globally as a vegetable, are recognised for their antioxidant potential due to their high polyphenol content, particularly in the seeds (Woumbo *et al.*, 2022). These polyphenols, constituting 29.5% of the seeds, contribute to antioxidant activity by reducing malondialdehyde (MDA) levels and increasing superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels (Wu *et al.*, 2020; Zainuddin *et al.*, 2022). Key phenolic compounds in okra include quercetin-3-O-gentiobioside, isoquercitrin, rutin, protocatechuic acid, and a catechin derivative, with quercetin-3-O-gentiobioside being the most abundant and a major contributor to antioxidant capacity. This compound also inhibits digestive enzymes like lipase, α -glucosidase, and α -amylase (Alblihd *et al.*, 2023; Kwok *et al.*, 2025). Studies have documented the free radical-scavenging and ferric-reducing capabilities of okra pods (Wu *et al.*, 2020; Woumbo *et al.*, 2022; Zainuddin *et al.*, 2022). Okra extracts, obtained through cold extraction and boiling, exhibit notable antioxidant activity (Wu *et al.*, 2020). Okra seeds are rich in phenols, such as procyanidin B1 and B2, which are involved in DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging activity (Woumbo *et al.*, 2022). Specific glucopyranoside compounds identified in okra also contribute to its antioxidant and ferric-reducing activities (Wu *et al.*, 2020).

Polyphenols in okra function through three main antioxidant mechanisms: serving as electron or hydrogen donors, combining with transition metal ions to produce antioxidants, and producing electrophilic or oxidative metabolites that trigger the expression of genes encoding antioxidant enzymes (Wu *et al.*, 2020; Guebebia *et al.*,

2023). The antioxidant capacity of okra is directly proportional to its total phenolic (TP) and total flavonoid (TF) contents, with studies confirming that higher TP and TF levels enhance reducing power and free radical scavenging ability (Wu *et al.*, 2020; Zainuddin *et al.*, 2022; Guebebia *et al.*, 2023).

Fatigue is a prevalent condition characterised by ongoing tiredness and a diminished ability to perform both physical and mental tasks. It can stem from various sources, such as lifestyle choices, health conditions, and psychological stress (Kwok *et al.*, 2025). Recent research has investigated natural solutions to alleviate fatigue, highlighting okra (*Abelmoschus esculentus*) pod extracts as a promising option due to their abundance of bioactive compounds (Al-Shawi *et al.*, 2021; Liu *et al.*, 2021). Okra pods are rich in polysaccharides, phenolic compounds, and flavonoids, which are thought to play a role in their anti-fatigue effects. These compounds have potent antioxidant properties, helping to mitigate oxidative stress, a common contributor to fatigue (Liao *et al.*, 2019; Liu *et al.*, 2021). Research indicates that okra pod extracts can significantly lower blood lactic acid and urea nitrogen levels, both of which are indicators of fatigue (Liu *et al.*, 2021). Furthermore, these extracts have been shown to increase liver glycogen storage, providing an easily accessible energy source during extended physical activity (Kwok *et al.*, 2025).

2.5.2 The anti-diabetic potential of okra pods

Diabetes is a progressive metabolic disorder affecting a significant portion of the global population. Epidemiological data from 2000 indicated that 2.8% of the world's population was diabetic, with projections suggesting this could rise to 4.4% by 2030 (Ahmad *et al.*, 2025). This disease impacts individuals across all age groups and ethnicities, leading to complications such as blindness, heart attacks, strokes, kidney

failure, gangrene, neuropathy, and even death, thereby imposing a heavy burden on families and society (Ahmad *et al.*, 2025; Kwok *et al.*, 2025).

After ingestion, starchy foods are broken down into free sugars by two key enzymes, α -amylase and α -glucosidase, which are then absorbed by the intestines (Mokgalaboni *et al.*, 2023; Kwok *et al.*, 2025). Inhibiting these enzymes can prevent the rise in blood glucose levels following a carbohydrate-rich meal, offering a strategy to manage hyperglycaemia in type-2 diabetes patients (Mokgalaboni *et al.*, 2023). However, current inhibitors can cause side effects like diarrhoea, flatulence, abdominal distention, and meteorism due to strong inhibition of pancreatic α -amylase, leading to abnormal bacterial fermentation of undigested carbohydrates in the colon (Mokgalaboni *et al.*, 2023; Ahmad *et al.*, 2025). Plant-derived amylolytic inhibitors have shown milder effects on α -amylase and stronger inhibition of α -glucosidase, making them effective therapeutic agents for type-2 diabetes with fewer side effects (Mokgalaboni *et al.*, 2023).

Recent studies have highlighted okra's potential in diabetes management, attributed to its rich content of bioactive compounds such as phenolic compounds, flavonoids, mucilage, and pectin (**Figure 2.7**). These compounds help reduce oxidative stress and inflammation, which are crucial in diabetes pathogenesis (Elkhalifa *et al.*, 2021; Sarkar, Christopher and Shetty, 2022). For example, quercetin and kaempferol, two prominent flavonoids in okra, have been found to enhance insulin sensitivity and lower blood glucose levels by modulating key signalling pathways involved in glucose metabolism (Khan *et al.*, 2022). Mucilage, a polysaccharide-rich component of okra, plays a vital role in its anti-diabetic effects by regulating blood sugar levels through the delayed absorption of glucose from the intestines. This delayed absorption prevents rapid spikes in blood sugar levels, benefiting diabetic patients (Liu *et al.*, 2021). Additionally, mucilage has been shown to improve gut health, an increasingly recognised factor in diabetes management. Pectin, another polysaccharide in okra, contributes to its anti-diabetic potential by enhancing insulin sensitivity and glucose

metabolism (Elkhalifa *et al.*, 2021; Woumbo *et al.*, 2022). Pectin forms a gel-like substance in the gut, slowing the digestion and absorption of carbohydrates, leading to a more gradual release of glucose into the bloodstream. This mechanism helps maintain stable blood sugar levels and reduces the risk of hyperglycaemia (Elkhalifa *et al.*, 2021; Liu *et al.*, 2021).

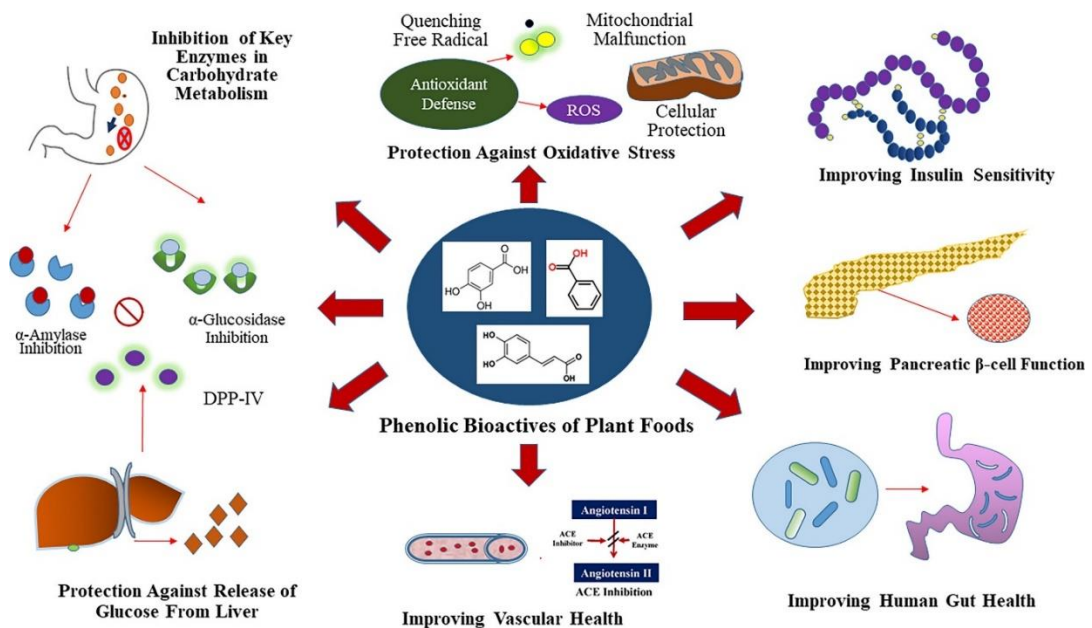


Figure 2.7: Glycemic Control Benefit Relevant Functional Properties of Plant Phenolic Compounds (Sarkar, Christopher and Shetty, 2022)

Okra pod extracts have been found to inhibit alpha-amylase activity, slowing carbohydrate digestion and glucose absorption. This inhibition is mainly due to polyphenols and flavonoids, which bind to the enzyme's active site, preventing the breakdown of carbohydrates (Elkhalifa *et al.*, 2021; Khan *et al.*, 2022). Alpha-glucosidase, located in the small intestine, further breaks down disaccharides into monosaccharides like glucose for absorption (Khan *et al.*, 2022). Okra pod extracts inhibit alpha-glucosidase in a similar way to alpha-amylase, with bioactive compounds like quercetin binding to the enzyme's active site, reducing its ability to hydrolyse disaccharides (Elkhalifa *et al.*, 2021). This competitive inhibition slows the release of

glucose into the bloodstream, aiding in the management of postprandial hyperglycaemia (Elkhalifa *et al.*, 2021; Khan *et al.*, 2022).

2.5.3 Anti-inflammatory potential of okra extracts

Inflammation is the body's response to potentially damaging stimuli such as pathogens, damaged cells, or irritants (Rudrapal *et al.*, 2022). It involves immune cell activation, cytokine release, and the production of other inflammatory mediators. During inflammation, immune cells such as macrophages and neutrophils produce reactive oxygen species (ROS) to eliminate pathogens (Luthfi *et al.*, 2020; Rudrapal *et al.*, 2022). However, high ROS can harm host tissues, causing further inflammation. In essence, oxidative stress and inflammation are two interconnected processes. Oxidative stress can cause and intensify inflammation, and inflammation can enhance ROS production, resulting in a vicious cycle that contributes to the progression of many chronic diseases (Rudrapal *et al.*, 2022). Okra is believed to have anti-inflammatory properties, which are attributed to its various bioactive compounds like polyphenols (**Figure 2.8**), flavonoids, saponins, and especially carotenoids (Luthfi *et al.*, 2020; Rudrapal *et al.*, 2022). These compounds can modulate inflammatory responses by inhibiting the production of pro-inflammatory cytokines and reducing oxidative stress, a key factor in inflammation (Luthfi *et al.*, 2020; Liu *et al.*, 2021). Research has shown that carotenoids in okra can lower the expression of inflammatory markers like tumour necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) (Luthfi *et al.*, 2020; Elkhalifa *et al.*, 2021). Additionally, carotenoids can boost the activity of antioxidant enzymes, protecting cells from oxidative damage and reducing inflammation (Luthfi *et al.*, 2020).

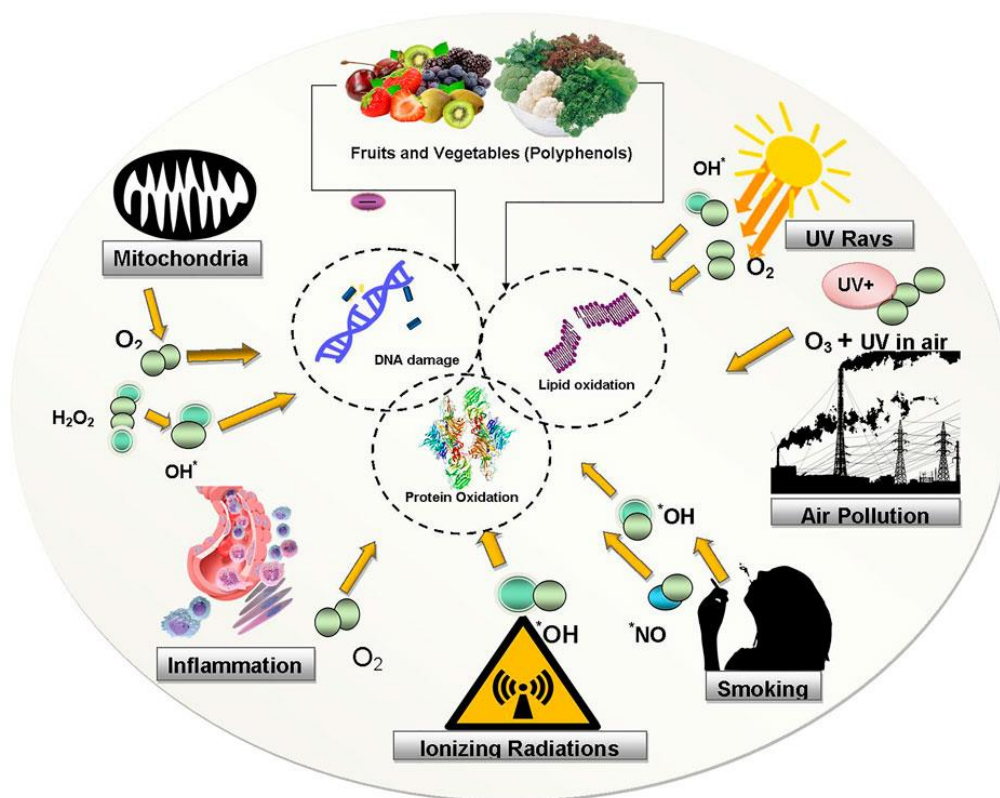


Figure 2.8: The role of dietary polyphenols in oxidative stress (O/S) and reactive oxygen species (ROS). OS/ROS generated from various sources (environmental/biological) cause oxidations of lipid, protein and DNA molecules. Abbreviations: UV: ultraviolet rays, OH: hydroxyl, NO: nitrogen oxide, O₂: oxygen, O₃: Ozone, H₂O₂: hydrogen peroxide (Rudrapal *et al.*, 2022)

Okra's anti-inflammatory properties are further supported by its ability to inhibit key enzymes involved in the inflammatory process (Luthfi *et al.*, 2020; Liu *et al.*, 2021). For example, okra extracts have been found to inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, which are crucial in the synthesis of pro-inflammatory mediators. This inhibition helps reduce inflammation and its associated symptoms (Luthfi *et al.*, 2020). Moreover, the antioxidant properties of carotenoids in okra contribute to its anti-inflammatory effects by scavenging reactive oxygen species (ROS) and reducing oxidative stress. This dual role of carotenoids as both antioxidants

and anti-inflammatory agents makes okra a valuable dietary component for managing inflammation (Luthfi *et al.*, 2020; Elkhalfa *et al.*, 2021).

2.5.4 Antimicrobial potential of okra extracts

The antimicrobial potential of okra pod extracts is attributed to a variety of bioactive compounds, including flavonoids, alkaloids, saponins, tannins, carotenoids, and phenolic compounds (Korani *et al.*, 2021; Sipahi *et al.*, 2022; Guebebia *et al.*, 2023). These compounds have demonstrated the ability to inhibit the growth of several pathogenic microorganisms. Flavonoids in okra extracts, such as quercetin, are known to interfere with bacterial cell wall synthesis and function, effectively inhibiting the growth of both gram-positive and gram-negative bacteria (Luthfi *et al.*, 2020; Guebebia *et al.*, 2023). Alkaloids contribute to antimicrobial activity by disrupting the integrity of bacterial cell membranes, leading to cell lysis and death (Guebebia *et al.*, 2023). Saponins in okra extracts exhibit antimicrobial properties by forming complexes with sterols in fungal cell membranes, causing increased permeability, cell death and inhibition of spore germination. This mechanism is particularly effective against fungi like *Candida albicans* (Korani *et al.*, 2021). Tannins act by precipitating microbial proteins, which inhibit the growth and proliferation of bacteria and fungi (Korani *et al.*, 2021; Sipahi *et al.*, 2022). Carotenoids, such as beta-carotene, have been shown to disrupt microbial cell membranes, while phenolic compounds, including various polyphenols, enhance the overall antimicrobial efficacy by interfering with microbial enzymes and cellular functions (Luthfi *et al.*, 2020; Guebebia *et al.*, 2023). Research has indicated that okra pod extracts possess significant antibacterial activity against bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* and *Bacillus cereus* (Luthfi *et al.*, 2020; Romdhane *et al.*, 2020). The lipid fraction of okra, particularly palmitic and stearic acids, plays a major role in its antibacterial

properties by disrupting bacterial cell membranes, leading to cell lysis and death (Guebebia *et al.*, 2023). Furthermore, okra mucilage, a polysaccharide-rich component, has demonstrated antimicrobial effects against various bacterial strains by forming a protective barrier that inhibits bacterial adhesion and colonisation on surfaces, thus preventing infection (Lin *et al.*, 2022; Guebebia *et al.*, 2023). This property makes okra mucilage a potential candidate for use in antimicrobial coatings and wound dressings (Luthfi *et al.*, 2020). Overall, the combined action of these bioactive compounds in okra extracts results in significant antimicrobial activity against a range of bacterial and fungal pathogens.

2.6 Challenges of okra production

Okra is a traditional crop that requires relatively low agronomic inputs and can contribute substantially to sustainable agricultural production (Elkhalifa *et al.*, 2021; Khan *et al.*, 2022). Since okra is an easily available and low-cost vegetable, it could become an ideal alternative source of nutrition in less-developed countries. However, its cultivation in countries such as Namibia faces several challenges, particularly in the context of biotic stress (pests and diseases), and abiotic stress (drought, temperature, salinity, etc.) (Ahmad *et al.*, 2022). Drought is a significant challenge for okra production. Okra's growth, biomass, yield and nutrient content can all be significantly affected by drought stress (Elkhalifa *et al.*, 2021; Ahmad *et al.*, 2022). Between 30 and 100 % of yield can be lost, particularly if stress strikes between the blooming and pod-filling stages of the plant (Ahmad *et al.*, 2022). Dietary fibre development may also be decreased by drought stress (Ahmad *et al.*, 2022; Khan *et al.*, 2022).

On the other hand, ash and mucilage content can rise in mild drought circumstances (Liu *et al.*, 2021). These stressors can also impact okra's nutraceutical content. For example, okra might have a higher protein content when it is stressed by drought (Liu

et al., 2021; Ahmad *et al.*, 2022). To fully understand the effect of these stressors on the nutraceutical content of okra, further investigation is required. Abiotic stressors are already one of the primary issues limiting global food production (Ahmad *et al.*, 2022). Abiotic stress factors regularly threaten the livelihood of a huge proportion of the population in underdeveloped nations where agriculture is still done on a subsistence basis (Liu *et al.*, 2021; Ahmad *et al.*, 2022). Okra is particularly susceptible to hardpan soils, and soil compaction can severely limit plant development (Ahmad *et al.*, 2022). According to a participatory rural appraisal, some of the most significant limits to okra yield apart from drought are pests, diseases and lack of improved varieties (Ibitoye and Kolawole, 2022). Diseases were identified as a production obstacle by 75% of respondents, while insect pests were identified as a significant constraint by 74%, followed by drought by 71%, and a lack of improved varieties by 67%. Yellow Vein Mosaic Disease (YVMD), okra enation leaf curl virus, *Cercospora* leaf spot/blight, and powdery mildew are the most common okra diseases (Pandey, Samanta and Yadav, 2019; Ibitoye and Kolawole, 2022). Jassids, mites, and spotted bollworms are among the most disastrous okra pests (Kumari, Naresh and Hs, 2019; Ibitoye and Kolawole, 2022). These challenges may have a substantial negative impact on the okra plant's productivity and nutraceutical accumulation, which may affect the crop's quality and quantity. Thus, strategies to mitigate these stresses, which include developing drought and disease-resistant cultivars, using bio-fertilisers, and adopting efficient, eco-friendly farming techniques such as hydroponics, are essential for ensuring the sustainability of okra production.

2.7 Hydroponic systems and plant growth-promoting bacteria

Hydroponics is a technique for growing plants without soil, using nutrient-rich water solutions. This innovative method has become popular due to its environmental

benefits and resource efficiency (Ahmed *et al.*, 2021). Hydroponic systems can be used in various settings, they can be set up in small spaces and stacked vertically, maximising land use and making them a versatile and sustainable option for modern agriculture (Ahmed *et al.*, 2021; Nkcukankcuka *et al.*, 2022). One of the main advantages of hydroponics over traditional soil-based farming is water conservation. Hydroponic systems use up to 90% less water than conventional methods because the water is recirculated and reused (Ahmed *et al.*, 2021). This is especially important in areas with water scarcity, such as Namibia. Additionally, hydroponics eliminates the need for soil, reducing the risk of soil-borne diseases and pests, and minimising the use of pesticides and herbicides (Nkcukankcuka *et al.*, 2022). Hydroponic farming also allows for precise control over nutrient delivery, ensuring plants receive the exact nutrients they need for optimal growth. This precision reduces nutrient runoff and environmental pollution (Ahmed *et al.*, 2021; Nkcukankcuka *et al.*, 2022).

A wide range of vegetables can be cultivated using hydroponics, including leafy greens, herbs, tomatoes, and peppers (Nkcukankcuka *et al.*, 2022). Okra is a particularly adaptable crop that thrives in hydroponic systems (Sousa *et al.*, 2023). The controlled environment provided by hydroponics ensures optimal growth conditions, leading to higher yields and superior-quality produce (Olutola, Elijah and Dare Femi, 2020). Okra plants flourish in various hydroponic setups such as deep water culture (DWC), nutrient film technique (NFT), drip systems, and the Kratky method (Kratky, 2009; Olutola, Elijah and Dare Femi, 2020; Sousa *et al.*, 2023). The Kratky hydroponic system, developed by Dr. Bernard Kratky, is a non-circulating passive method where plants are grown in a container with their roots suspended in a nutrient-rich solution. As the solution evaporates, it creates a natural vacuum that draws oxygen-rich air into the roots (Kratky, 2009).

Despite the advantages of hydroponics such as reduced water use and controlled nutrient delivery the system has notable limitations. Hydroponic crops often lack access to native soil microbiota, which play crucial roles in nutrient mineralization, pathogen resistance, and plant vigour (Stegelmeier *et al.*, 2022; Szekely and Jijakli, 2022). This can lead to reduced efficiency in nutrient utilization and an overreliance on chemical inputs, which compromises long-term sustainability and increases production costs. Furthermore, environmental stressors such as salinity or nutrient imbalances are more difficult to buffer in hydroponic setups, potentially resulting in stunted growth or lower yields (Stegelmeier *et al.*, 2022; Szekely and Jijakli, 2022). These challenges underscore the need for microbial-based interventions to improve the biological functionality of hydroponic systems. Bacterial inoculants, particularly plant growth-promoting bacteria (PGPB), may play a crucial role in enhancing the efficiency and sustainability of hydroponic systems (**Table 2.1**).

Table 2.1: Summarizes key studies that explored the use of plant growth-promoting bacteria (PGPB) consortia in hydroponic systems. These studies demonstrate how various bacterial strains can enhance crop productivity, nutrient uptake, and stress resilience across different hydroponic media.

Study	Crop	Bacterial Strains (Consortium)	Growth Medium	Observed Effects
Stegelmeier <i>et al.</i> , 2022	Lettuce	<i>Bacillus subtilis</i> , <i>Azospirillum brasilense</i>	NFT hydroponic	↑ biomass, ↑ chlorophyll, ↓ nitrate accumulation
Szekely & Jijakli, 2022	Tomato	<i>Pseudomonas fluorescens</i> , <i>Bacillus megaterium</i>	DWC system	↑ yield, ↑ root growth, ↑ resistance to <i>Pythium</i>
Singh <i>et al.</i> , 2021	Spinach	<i>Azotobacter sp.</i> , <i>P. putida</i>	Soilless cocopeat	↑ nutrient uptake, ↑ antioxidant enzyme activity

These beneficial bacteria can significantly increase crop yields by improving nutrient uptake, reducing plant stress, and promoting overall plant health (Stegelmeier *et al.*, 2022). PGPB can be introduced into hydroponic systems through various methods, including seed inoculation, root dipping, and adding directly to the nutrient solution (Paradiso *et al.*, 2017; Stegelmeier *et al.*, 2022). One of the key benefits of using bacterial inoculants in hydroponics is their ability to enhance nutrient availability. For example, certain bacteria can solubilise phosphorus, making it more accessible to plants. Others can fix atmospheric nitrogen, providing an essential nutrient for plant growth (Szekely and Jijakli, 2022). Additionally, PGPB can produce plant hormones such as auxins and gibberellins, which promote root growth and overall plant development (Stegelmeier *et al.*, 2022). Moreover, bacterial inoculants help maintain root health (**Figure 2.9**) and keep the nutrient solution disease-free (Szekely and Jijakli, 2022). Beneficial bacteria can outcompete harmful pathogens, reducing the incidence of root diseases and promoting a healthier root system. This not only enhances plant growth but also contributes to the overall sustainability of hydroponic systems (Stegelmeier *et al.*, 2022; Szekely and Jijakli, 2022).

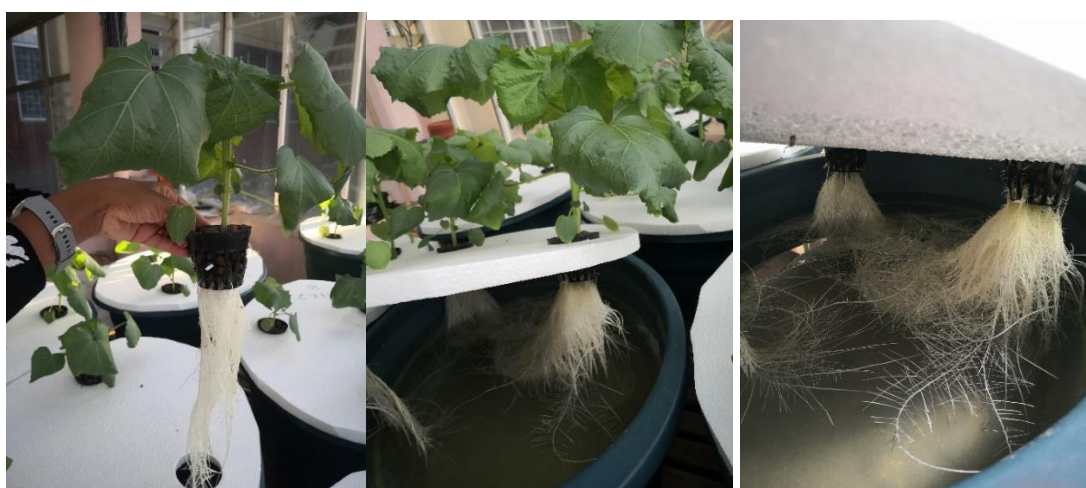


Figure 2.9: Okra plants inoculated with PGPEB grown in a Kratky hydroponics system showing a healthy root system.

2.7.1 Challenges of using bacterized seeds in hydroponics

Recent research has demonstrated the effectiveness of plant growth-promoting bacteria (PGPB) in enhancing crop growth, nutrient uptake, and stress resilience in soil-based conditions. For instance, PGPB applications have led to significant increases in biomass and grain yield across major crops such as maize, wheat, and soybean, while also reducing heavy metal uptake in contaminated soils (Xiao *et al.*, 2024). Studies involving consortia of strains like *Azospirillum brasilense*, *Bacillus subtilis*, and cyanobacterial bioactivators have shown notable improvements in nitrogen cycling, grain protein content, and the overall physiological performance of crops under abiotic stress, including drought and salinity (Belkacem-Hanfi *et al.*, 2022; Chaves *et al.*, 2021). These findings highlight the wide-ranging agronomic benefits of PGPB in conventional soil systems and provide a basis for exploring similar microbial interventions in controlled, soilless environments. Conversely, the application of PGPB in hydroponic systems remains in development, with emerging evidence indicating both promising outcomes and ongoing challenges. For example, inoculation with *Pseudomonas fluorescens* and *Bacillus megaterium* has been shown to improve biomass production, nutrient-use efficiency, and disease resistance in tomato and lettuce cultivated in deep-water and nutrient film technique (NFT) systems (Szekely and Jijakli, 2022; Stegelmeier *et al.*, 2022). However, the absence of native soil microbial communities, instability of microbial colonisation in inert substrates, and lack of standardised protocols for microbial delivery hinder the consistency of these benefits (Olutola, Elijah and Dare Femi, 2020). Consequently, maintaining a stable and effective population of PGPB around the plant roots can be challenging. The survival and activity of these beneficial bacteria can be affected by factors such as nutrient solution composition, pH levels, and the physical environment of the

hydroponic system (Olutola, Elijah and Dare Femi, 2020; Ikiz, Dasgan and Gruda, 2024). To ensure that PGPB can flourish and reliably benefit the plants, careful management is essential. This might involve developing specialised formulations to enhance the stability and survival of PGPB in hydroponic systems. These could include protective carriers or encapsulation techniques that shield the bacteria from harsh conditions (Olutola, Elijah and Dare Femi, 2020). Additionally, modifying nutrient solution composition to create a more favourable environment for PGPB, adjusting pH levels, nutrient concentrations, and adding specific compounds to support bacterial growth may be beneficial. Promoting biofilm formation, where bacterial communities adhere to surfaces and provide a stable environment for PGPB, can also improve their resilience and effectiveness in hydroponic systems (Stegelmeier *et al.*, 2022; Ikiz, Dasgan and Gruda, 2024). Implementing a regular schedule for PGPB inoculation can help sustain their population and activity consistently around the plant roots (Stegelmeier *et al.*, 2022).

It is essential to customise PGPB strains to suit the specific plant species, but this can be quite challenging. Microbial-plant interactions vary greatly, so some PGPB strains may not work well for certain plants. Additionally, the effectiveness of PGPB depends on the needs and characteristics of the particular plant species as well as the hydroponic environmental conditions (Olutola, Elijah and Dare Femi, 2020; Stegelmeier *et al.*, 2022). Such PGPB strains which are not compatible with the plants selected may result in suboptimal effects or worse, undesirable effects. For this reason, proper investigations, as well as experiments and trials, should be performed to determine the effective strains of PGPB that will have the highest possible compatibility for plants grown by hydroponic techniques. This includes trials whereby many strains will be tested to determine which ones are compatible with which plants when using

hydroponics (Olutola, Elijah and Dare Femi, 2020; Stegelmeier *et al.*, 2022). Constructing specific consortia of PGPB that are suitable for a particular crop species being grown. These consortia consist of different strains which can have complementary actions on various processes beneficial to the plant (Stegelmeier *et al.*, 2022). This may mean changing some bacterial strains to improve their performance during colonisation of the roots of the plants and supplying favourable activity to the plant host (Stegelmeier *et al.*, 2022; Ikiz, Dasgan and Gruda, 2024). Hydroponic systems should incorporate ongoing monitoring frameworks to evaluate the performance and activity of the applied plant growth-promoting bacteria (PGPB). Such data-driven assessments are essential for making informed adjustments that optimise the interactions between PGPB and host plants (Stegelmeier *et al.*, 2022).

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CHAPTER 3: *MYROTHAMNUS* ENDOPHYTES: UNLOCKING OKRA'S GERMINATION POTENTIAL

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ABSTRACT

Harnessing beneficial microorganisms that mobilise nutrients offers a promising alternative to chemical fertilisers, supporting more sustainable agricultural systems. This study investigated the potential of endophytic plant growth-promoting bacteria (PGPB) (specifically *Kosakonia cowanii*, *Bacillus licheniformis*, and *Cupriavidus metallidurans*) isolated from the desert plant *Myrothamnus flabellifolius*. These bacteria exhibit several key plant growth-promoting traits, including nitrogen fixation (*nifH*), the ability to utilise ACC (1-aminocyclopropane-1-carboxylate) deaminase, phosphate solubilisation, ammonium (NH₄⁺) production, siderophore production, and indole-3-acetic acid (IAA) synthesis. Their effects on germination and seedling vigour of okra (*Abelmoschus esculentus*) were assessed by inoculating seeds (individually and in consortia) with these bacteria and growing them under hydroponic conditions. The experiment was conducted in a greenhouse using a randomised complete block design (RCBD), with three plants per pot, four replicates, seven treatments and a control. Results revealed statistically significant improvements in seed germination percentage ($P < 0.001$) and seedling vigour index ($P < 0.001$) compared to uninoculated controls. *K. cowanii* and *C. metallidurans* demonstrated strong potential as both individual and combined inoculants, while *B. licheniformis* was most effective when used in combination with other strains. The study's findings highlight the capacity of endophytic bacteria to enhance seed germination and seedling establishment, supporting their potential application as biofertilisers for sustainable crop production.

Key Words: Seed Germination, Seedling Vigour Index, Plant Growth-Promoting Endophytic Bacteria, Hydroponics and Okra (*Abelmoschus esculentus*).

3.1 INTRODUCTION

The use of plant growth-promoting bacteria (PGPB) has emerged as a promising strategy for enhancing agricultural crop productivity and sustainability in modern agriculture (Guha and Biswas, 2024). Among the various types of PGPB, endophytic bacteria have garnered noticeable attention. This is due to their unique ability to colonise plant tissues without causing harm while promoting plant growth and enhancing resilience under diverse environmental conditions (Guha and Biswas, 2024). *Myrothamnus flabellifolius*, a resurrection plant native to arid regions, is hypothesised to harbour a wide variety of such endophytes, potentially offering significant applications for agriculture. This hypothesis is grounded in a recent metatranscriptomic study by Tebele, Marks and Farrant (2025), which profiled root-associated microbiomes during dehydration and rehydration cycles and found that bacterial transcripts dominated the root endosphere and rhizoplane. Under desiccation, structural groups such as Actinobacteria significantly increased their transcript abundance, and key stress-related pathways including antioxidant enzymes, trehalose biosynthesis, and hormonal regulation were strongly upregulated, indicating active microbial adaptation to extreme water loss (Tebele, Marks and Farrant, 2025). These findings provide the first functional evidence that the internal microbiome of *M. flabellifolius* is not only present but transcriptionally engaged in stress response, supporting the hypothesis that it harbours beneficial endophytes. Despite growing interest, the agricultural potential of endophytic bacteria from this unique plant remains largely unexplored (Truyens *et al.*, 2015; Guha and Biswas, 2024).

Seed bacterization, a process of inoculation of seeds with beneficial bacteria, has been shown to enhance seed germination, seedling vigour, and overall plant health (Wang *et al.*, 2022). This technique facilitates early symbiotic relationships between plants

and endophytes, enhancing nutrient uptake, increased production of growth-promoting hormones, and enhanced protection against pathogens (Mahgoub *et al.*, 2021; Wang *et al.*, 2022). Endophytes facilitate these benefits through several mechanisms. They produce phytohormones such as auxins, gibberellins, and cytokines, which stimulate germination and promote root and shoot growth (Egamberdieva *et al.*, 2017). Additionally, these bacteria solubilise and mobilise essential nutrients like phosphorus and nitrogen, thereby increasing their bioavailability to the germinating seed (Bohra *et al.*, 2022). This nutrient mobilisation is mediated by the secretion of organic acids, phosphatases, and siderophores, all of which enhance plant nutrient uptake (Majeed, Muhammad and Ahmad, 2018). Beyond nutrient dynamics, endophytic bacteria exhibit significant potential in mitigating abiotic stresses, including drought and salinity, through the production of stress-related enzymes and metabolites (Majeed, Muhammad and Ahmad, 2018). Moreover, they produce antimicrobial compounds that protect seeds from pathogenic microbes, thereby enhancing seedling resilience and vigour (Egamberdieva *et al.*, 2017). Additionally, certain endophytes regulate reactive oxygen species (ROS) levels, maintaining a balance that supports seed dormancy, seed dormancy release and cell expansion necessary for germination (Majeed, Muhammad and Ahmad, 2018). Furthermore, endophytes can trigger induced systemic resistance (ISR), priming of the plant's immune system and enhancing its defence mechanisms against a broad spectrum of pathogens, which ultimately contribute to improved plant health and productivity (Majeed, Muhammad and Ahmad, 2018). The application of PGPB, both as single strains and consortia, isolated from *M. flabellifolius*, presents a unique opportunity to investigate their effects on the germination and early growth of economically important crops such as okra.

Okra is valued for its nutritional profile and economic benefits, but often suffers from poor seed germination and seedling establishment, especially under adverse environmental conditions (Ahmad *et al.*, 2022). Introducing PGPB through seed bacterization with PGPB offers a potential solution to address these challenges by improving germination rates and promoting vigorous seedling growth (Egamberdieva *et al.*, 2017).. While the efficacy of PGPB in enhancing the germination and growth of various crops has been investigated, the specific effects of endophytic bacteria from *M. flabellifolius* on okra have yet to be investigated. This study aimed to investigate the effects of seed bacterization using both single and consortia of PGPB isolated from *M. flabellifolius* on okra seed germination and seedling vigour index. This research seeks to quantify the potential benefits of these endophytic bacteria in enhancing okra cultivation. The findings are expected to contribute to the development of sustainable agricultural practices, particularly in regions facing environmental challenges.

3.2 MATERIALS AND METHODS

3.2.1 Seed Bacterization and Germination

Bacterial inoculants were prepared from glycerol stocks of three strains—*Kosakonia cowanii* (KC), *Bacillus licheniformis* (BL), and *Cupriavidus metallidurans* (CM)—originally isolated from the desert plant *Myrothamnus flabellifolius*. Each strain was cultured in sterile broth media according to the protocol described by Zafar *et al.* (2012). Seven treatment groups were established, comprising individual strains and their various consortia (**Table 3.1**) and the uninoculated control (T₀), consisted of sterile media broth. The bacterial suspension's turbidity was standardised to 1×10^6 cells mL⁻¹ using McFarland standards (McFarland, 1907) to ensure consistent inoculum density. Okra seeds were surface sterilised using the protocol described by

Somasegaran and Hoben (1994) to eliminate external microbial contaminants. Seed viability was confirmed via the tetrazolium chloride test (Zafar *et al.*, 2012). Viable Okra seeds were soaked in respective bacterial suspensions for 48 hours, while control seeds were soaked in sterile broth.

Table 3.1: Bacterial endophytes treatments isolated from the Desert Plant *M. flabellifolius*

Treatment	Treatment Label
Control (uninoculated)	T ₀
<i>Kosakonia cowanii</i>	KC
<i>Bacillus licheniformis</i>	BL
<i>Cupriavidus metallidurans</i>	CM
<i>K. cowanii</i> and <i>B. licheniformis</i>	KC-BL
<i>K. cowanii</i> and <i>C. metallidurans</i>	KC-CM
<i>B. licheniformis</i> and <i>C. metallidurans</i>	BL-CM
<i>K. cowanii</i> , <i>B. licheniformis</i> and <i>C. metallidurans</i>	KC-BL-CM

Post-treatment seeds were placed in sterile petri dishes lined with cotton wool moistened with either sterile broth (negative control) or respective PGPB endophyte inoculants (treatments). Each treatment was replicated four times with three seeds per replicate. The petri dishes were incubated in the dark at room temperature ($25 \pm 2^\circ\text{C}$) for about 8 to 14 days. Seed germination rate was monitored daily by calculating the seed germination percentage (GP) using the equation:

$$\text{Germination percentage (\%)} = (\text{Number of germinated seeds} / \text{Total number of seeds}) \times 100.$$

3.2.2 Greenhouse experiment

Following germination in petri dishes, okra seedlings were transferred to the greenhouse environment (**Figure 3.1**) and grown in a Kratky hydroponics system. The system comprised 40 L basins filled with nutritive hydroponic nutrient solution (Starke Ayres (PTY) LTD, South Africa), prepared according to the manufacturer's

guidelines. The nutrient composition per 500 g included: 65 g/kg N; 27 g/kg P; 130 g/kg K; 70 g/kg Ca; 20 mg/kg Cu; 1500 mg/kg Fe; 10 mg/kg Mo; 22 g/kg Mg; 240 mg/kg Mn; 75 mg/kg S and 240 mg/kg B. The nutrient solution's pH was maintained between 6.0 and 7.0 using sodium bicarbonate or lemon juice as needed, with regular monitoring to ensure optimal conditions. To assess the impact of seed bacterization on okra seedling vigour under hydroponics conditions, plant height (cm) was measured three weeks after transplanting. This interval allowed all plant seedlings to acclimate to the hydroponic environment, ensuring reliable and unbiased measurements. The seedling vigour index (SVI) was calculated using the following equation:

Seedling Vigour Index (SVI) = Seed germination percentage (%) × Seedling height (cm)



Figure 3.1: Okra seedlings transferred from Petri dishes into the Kratky hydroponics system in the greenhouse

3.3 DATA ANALYSIS

The experiment was arranged in a randomised complete block design (RCBD) with four replications per treatment. Each hydroponic basin contained three plants, with seven bacterial treatments and an uninoculated control. All results in this study are

presented as the mean \pm the standard deviation of three independent replicates. Statistical significance was assessed at a 5% probability level ($P < 0.005$). Normality testing via the Shapiro-Wilk test revealed non-normal distribution for seed germination percentage data ($P \leq 0.05$), prompting the use of the Kruskal-Wallis test to compare treatment effects (mean statistical differences), followed by Dunn's post-hoc test for pairwise comparisons. For normally distributed seedling vigour index data, One-way ANOVA with Turkey's HSD post-hoc test was used. All statistical analyses were performed using IBM SPSS Statistics (Version 29).

3.4 RESULTS

3.4.1 Germination percentage (%) and seedling vigour Index (SVI) of okra seeds soaked in single and different consortia of plant growth-promoting endophytic bacteria

Seed germination percentages and seedling vigour indices were significantly influenced by bacterial endophyte inoculation. The uninoculated control (T_0) exhibited a baseline germination percentage of 51.4% while inoculated treatments showed marked improvements, with the consortium KC-BL (*K. cowanii* + *B. licheniformis*) achieving the highest germination (70.1%), followed by CM (*C. metallidurans*, 69.5%) and KC (*K. cowanii*, 66.7%) (**Figure 3.2**).

In contrast, BL (*B. licheniformis*) had the lowest germination rate among inoculated treatments (52.1%), though still marginally higher than T_0 . Seedling vigour index (SVI) mirrored these trends: T_0 recorded the lowest SVI (513.01), while KC-BL demonstrated the highest vigour (846.46), followed by KC (767.05) and KC-CM (736.28) (**Figure 3.3**). Notably, the triple consortium KC-BL-CM showed the lowest SVI (525.36) among inoculated treatments, only slightly exceeding T_0 . Further

analysis revealed non-normal distribution for germination data (Shapiro-Wilk, $P = 0.015$), prompting the use of the Kruskal-Wallis test, which confirmed significant differences between treatments ($P = 0.001$). Key pairwise comparisons included BL vs. T_0 ($P \leq 0.001$) and BL vs. KC-BL ($P = 0.003$). For normally distributed SVI data (Shapiro-Wilk, $P = 0.333$), one-way ANOVA indicated significant treatment effects ($P \leq 0.001$), with post-hoc Tukey's HSD tests highlighting contrasts such as T_0 vs. KC-BL ($P \leq 0.001$) and KC-BL vs. KC-BL-CM ($P \leq 0.001$). Full statistical details are provided in **Appendix Tables 3.3** and **3.7**.

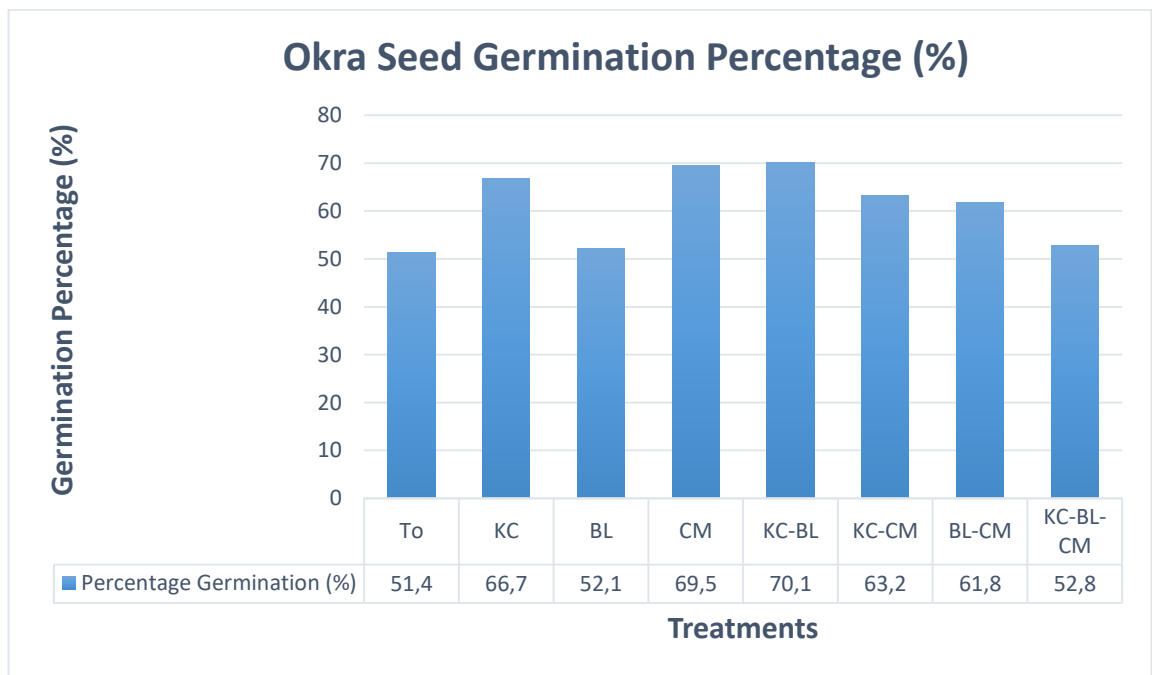


Figure 3.2: Effects of plant growth-promoting endophytic bacteria on the seed germination percentage of okra.

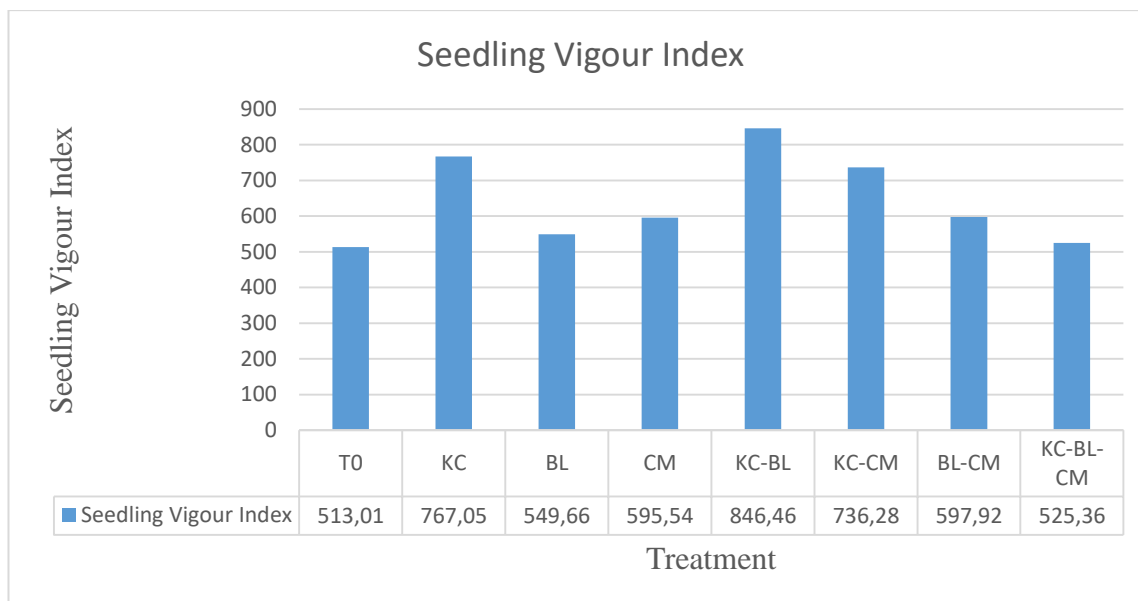


Figure 3.3: Effects of plant growth-promoting endophytic bacteria on okra seedling vigour.

3.5 DISCUSSION

The effects of bacterial endophyte inoculation on okra seed germination and seedling vigour were evaluated using single strains and consortia of plant growth-promoting bacteria (PGPB) isolated from *M. flabellifolius*. The present study demonstrated the significant impact of bacterial endophyte inoculation on okra seed germination and seedling vigour, with results revealing substantial variations in these parameters across different treatments. The uninoculated sample T_0 (control) displayed a baseline germination rate of 51.4%, consistent with challenges reported in okra cultivation under suboptimal conditions by Ahmad *et al.* (2022). Inoculated treatments, however, showed statistically significant improved germination rates (Kruskal-Wallis test, $P = 0.001$) with the KC-BL consortium (*K. cowanii* + *B. licheniformis*) achieving the highest germination (70.1%) and seedling vigour index (SVI: 846.46). The observed enhancement in okra seed germination and seedling vigour through bacterial inoculation aligns with a growing body of evidence supporting the efficacy of

microbial treatments. For instance, *Pseudomonas fluorescens* increased tomato seed germination from 81% (control) to 90% (Niknam and Dbawan, 2003), while *Meyerozyma guilliermondii* boosted durum wheat germination from 47% to 93% via indole-3-acetic acid (IAA)-mediated cell elongation and root development (Kthiri *et al.*, 2021). Similarly, consortia of *P. fluorescens*, *P. putida*, *K. aerogenes*, and *B. cereus* elevated carrot germination by 115–125% compared to controls (Fiodor *et al.*, 2023), and *Mixta theicola* SAR improved maize germination by 27% (Hagaggi and Mohamed, 2020). These improvements are attributed to microbial production of phytohormones (e.g., IAA, gibberellins) and hydrolytic enzymes that mobilise nutrients and stimulate growth (Sammauria *et al.*, 2020; Mei *et al.*, 2021).

In this study, the strain-specific efficacy of endophytes was evident, with pairwise comparisons (Dunn's test) revealing stark contrasts: BL (*B. licheniformis*) vs. T₀ ($P \leq 0.001$) and BL vs. KC-BL ($P = 0.003$). The superior performance of the KC-BL consortium (*K. cowanii* + *B. licheniformis*) suggests synergistic interactions, likely driven by complementary traits. *K. cowanii*'s nitrogen fixation (*nifH*) and IAA production synergised with *B. licheniformis*' ACC deaminase activity, which mitigates ethylene-induced stress (Egamberdieva *et al.*, 2017; Fiodor *et al.*, 2023). Such synergies are critical in hydroponic systems, where nutrient availability and stress tolerance directly influence seedling establishment. These findings mirror successes in *Brachiaria decumbens*, where bacterial inoculation increased germination vigour by 61% and speed by 40% (Oliveira *et al.*, 2022), and highlight the importance of tailored microbial combinations to avoid antagonism in complex consortia. Microbial consortia and single-strain inoculants have demonstrated significant potential in enhancing germination rates and early seedling development across diverse crops. For instance, co-inoculation of *Bradyrhizobium yuanmingense* PR3 and *Paenibacillus* sp.

SPR11 increased black gram (*Vigna mungo*) seed germination by 40–45% under 300 mM saline stress, alongside improved root (84.45%) and shoot growth (90.15%) compared to uninoculated controls (Tiwari *et al.*, 2025). Similarly, *C. metallidurans* enhanced photosynthetic performance and biomass in tomato, maize, and wheat by modulating stomatal density and rhizosphere acidification, independent of auxin or ethylene pathways (Ali *et al.*, 2021; Tang *et al.*, 2024). These findings underscore the multifunctional roles of PGPM in overcoming abiotic stressors while promoting germination.

Mechanistically, bacterial inoculation often enhances germination through phytohormone synthesis, nutrient solubilisation, and stress resilience. For example, *K. cowanii* and *B. licheniformis*, key components of the KC-BL consortium in this study, produce auxins (IAA) and ACC deaminase, which mitigate ethylene-induced stress and stimulate root elongation (Gupta and Pandey, 2019; Gamalero and Glick, 2022). Such traits align with observations in pearl millet (*Pennisetum glaucum*), where *K. cowanii* colonisation restored seedling growth compromised by seed endophyte removal (Kumar *et al.*, 2021). Similarly, *P. fluorescens* and *B. cereus* increased carrot seed germination by 115–125% via IAA-mediated cell enlargement and phosphate solubilisation (Fiodor *et al.*, 2023). These mechanisms are critical in hydroponic systems, where nutrient availability and root architecture directly influence seedling vigour.

In this study, the KC-BL consortium achieved the highest germination rate (70.1%, 18.7% increase over control) and seedling vigour index (SVI: 846.46 vs. control: 513.01). This mirrors findings in wheat (*Triticum aestivum*), where microbial consortia improved germination indices by 16.83% and peak values by 23.16% through synergistic siderophore production and nutrient mobilisation (Roshani *et al.*, 2020).

The superior performance of KC-BL over single-strain treatments (e.g., *C. metallidurans* [69.5%] and *K. cowanii* [66.7%]) highlights the importance of microbial synergy, as seen in tomato systems where *Trichoderma harzianum* + *P. fluorescens* increased germination by 48% compared to individual strains (Anbazhagan *et al.*, 2020). However, the underperformance of *B. licheniformis* alone (52.1%) suggests that certain *Bacillus* spp. require co-inoculation to fully express growth-promoting traits, a phenomenon noted in saline-stressed black gram (Tiwari *et al.*, 2025).

The enhanced seedling vigour observed here is critical for early resource capture and stress tolerance, consistent with global trends. For example, bio-priming with *M. guilliermondii* increased durum wheat germination from 47% to 93% via IAA-driven root initiation (Kthiri *et al.*, 2021), while *Mixta theicola* improved maize germination by 27% (Hagaggi and Mohamed, 2020). These outcomes emphasise the role of microbial inoculants in promoting rapid seedling establishment, which is vital for crop resilience in variable climates (Gamalero and Glick, 2022).

3.6 CONCLUSION AND RECOMMENDATION

The KC-BL consortium's efficacy in okra cultivation underscores the potential of tailored microbial partnerships to enhance sustainable agriculture. By integrating nutrient solubilisation, phytohormone production, and stress mitigation, such consortia address both germination challenges and seedling vigour deficits. Future research should prioritise metabolic profiling of microbial interactions and field trials under abiotic stressors to optimise consortium design, building on frameworks established in recent seed microbiome studies (Kimotho and Maina, 2024).

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APPENDICES

Appendix Table 3.1: Seed germination percentage and seedling vigour index of okra.

Treatment	Percentage Germination (%)	Seedling Vigour Index
1	51,4	513.01
2	66,7	767.05
3	52,1	549.66
4	69,5	595.54
5	70,1	846.46
6	63,2	736.28
7	61,8	597.92
8	52,8	525.36

Appendix Table 3.2: Shapiro-Wilk test of normality of the percentage germination of okra seeds.

Tests of Normality					
Kolmogorov-Smirnov ^a			Shapiro-Wilk		
Statistic	Df	Sig.	Statistic	df	Sig.
,166	48	,002	,939	48	,015

a. Lilliefors Significance Correction

Appendix Table 3.3: Significant statistical difference between the treatments in the percentage germination of bacterial-inoculated Okra seeds via Kruskal-Wallis tests.

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig. ^{a,b}	Decision
1	The distribution of Germination% is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	,001	Reject the null hypothesis.

a. The significance level is .050.

b. Asymptotic significance is displayed.

Appendix Table 3.4: Pair-wise alignment test for seed germination percentage.

		Pairwise Comparisons of Treatment				
Sample 1-Sample 2		Test		Std. Test		Adj. Sig.
		Statistic	Std. Error	Statistic	Sig.	^a
BL-	KC-BL-CM	-2,000	8,023	-,249	,803	1,000
BL-	BL-CM	-9,333	8,023	-1,163	,245	1,000
BL-	KC-CM	-13,500	8,023	-1,683	,092	1,000
BL-	KC	16,583	8,023	2,067	,039	1,000
BL-	CM	-21,417	8,023	-2,669	,008	,213
BL-	KC-BL	-23,583	8,023	-2,939	,003	,092
BL-	To	29,583	8,023	3,687	<,001	,006
KC-BL-CM-	BL-CM	7,333	8,023	,914	,361	1,000
KC-BL-CM-	KC-CM	11,500	8,023	1,433	,152	1,000
KC-BL-CM-	KC	14,583	8,023	1,818	,069	1,000
KC-BL-CM-	CM	19,417	8,023	2,420	,016	,435
KC-BL-CM-	KC-BL	21,583	8,023	2,690	,007	,200
KC-BL-CM-	To	27,583	8,023	3,438	<,001	,016
BL-CM-	KC-CM	4,167	8,023	,519	,604	1,000
BL-CM-	KC	7,250	8,023	,904	,366	1,000
BL-CM-	CM	12,083	8,023	1,506	,132	1,000
BL-CM-	KC-BL	14,250	8,023	1,776	,076	1,000
BL-CM-	To	20,250	8,023	2,524	,012	,325
KC-CM-	KC	3,083	8,023	,384	,701	1,000
KC-CM-	CM	7,917	8,023	,987	,324	1,000
KC-CM-	KC-BL	10,083	8,023	1,257	,209	1,000
KC-CM-	To	16,083	8,023	2,005	,045	1,000
KC-	CM	-4,833	8,023	-,602	,547	1,000
KC-	KC-BL	-7,000	8,023	-,872	,383	1,000
KC-	To	13,000	8,023	1,620	,105	1,000
CM-	KC-BL	-2,167	8,023	-,270	,787	1,000
CM-	To	8,167	8,023	1,018	,309	1,000
KC-BL-	To	6,000	8,023	,748	,455	1,000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix Table 3.5: Shapiro-Wilk test for normality of the seedling vigour Index of Okra bacterial inoculated seeds.

Tests of Normality						
	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
Seedling Vigour Index	,160	32	,036	,963	32	,333

a. Lilliefors Significance Correction

Appendix Table 3.6: One-way ANOVA test results for Seedling Vigour Index.

ANOVA					
Seedling Vigour Index					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	447301,219	7	63900,174	7,995	<,001
Within Groups	191827,250	24	7992,802		
Total	639128,469	31			

Appendix Table 3.7: Post-Hoc Tukey test for significant statistical difference

between the treatments of the seedling vigour of Okra bacterial inoculated seeds.

Multiple Comparisons						
Dependent Variable: Seedling Vigour Index						
Tukey HSD						
(I)	(J)	Mean	Std.	Sig.	95% Confidence Interval	
Treatment	Treatment	Difference (I-J)			Error	Lower Bound

To	KC	-253,750*	63,217	,010	-463,12	-44,38
	BL	-36,750	63,217	,999	-246,12	172,62
	CM	-82,750	63,217	,887	-292,12	126,62
	KC-BL	-333,000*	63,217	<,001	-542,37	-123,63
	KC-CM	-237,250*	63,217	,019	-446,62	-27,88
	BL-CM	-84,750	63,217	,874	-294,12	124,62
	KC-BL-CM	-12,000	63,217	1,000	-221,37	197,37
KC	To	253,750*	63,217	,010	44,38	463,12
	BL	217,000*	63,217	,038	7,63	426,37
	CM	171,000	63,217	,169	-38,37	380,37
	KC-BL	-79,250	63,217	,907	-288,62	130,12
	KC-CM	16,500	63,217	1,000	-192,87	225,87
	BL-CM	169,000	63,217	,179	-40,37	378,37
	KC-BL-CM	241,750*	63,217	,016	32,38	451,12
BL	To	36,750	63,217	,999	-172,62	246,12
	KC	-217,000*	63,217	,038	-426,37	-7,63
	CM	-46,000	63,217	,995	-255,37	163,37
	KC-BL	-296,250*	63,217	,002	-505,62	-86,88
	KC-CM	-200,500	63,217	,067	-409,87	8,87
	BL-CM	-48,000	63,217	,994	-257,37	161,37
	KC-BL-CM	24,750	63,217	1,000	-184,62	234,12
CM	To	82,750	63,217	,887	-126,62	292,12
	KC	-171,000	63,217	,169	-380,37	38,37
	BL	46,000	63,217	,995	-163,37	255,37
	KC-BL	-250,250*	63,217	,012	-459,62	-40,88
	KC-CM	-154,500	63,217	,266	-363,87	54,87
	BL-CM	-2,000	63,217	1,000	-211,37	207,37
	KC-BL-CM	70,750	63,217	,946	-138,62	280,12
KC-BL	To	333,000*	63,217	<,001	123,63	542,37
	KC	79,250	63,217	,907	-130,12	288,62
	BL	296,250*	63,217	,002	86,88	505,62
	CM	250,250*	63,217	,012	40,88	459,62
	KC-CM	95,750	63,217	,792	-113,62	305,12
	BL-CM	248,250*	63,217	,012	38,88	457,62
	KC-BL-CM	321,000*	63,217	<,001	111,63	530,37
KC-CM	To	237,250*	63,217	,019	27,88	446,62
	KC	-16,500	63,217	1,000	-225,87	192,87
	BL	200,500	63,217	,067	-8,87	409,87
	CM	154,500	63,217	,266	-54,87	363,87
	KC-BL	-95,750	63,217	,792	-305,12	113,62
	BL-CM	152,500	63,217	,280	-56,87	361,87

	KC-BL-CM	225,250*	63,217	,029	15,88	434,62
BL-CM	To	84,750	63,217	,874	-124,62	294,12
	KC	-169,000	63,217	,179	-378,37	40,37
	BL	48,000	63,217	,994	-161,37	257,37
	CM	2,000	63,217	1,000	-207,37	211,37
	KC-BL	-248,250*	63,217	,012	-457,62	-38,88
	KC-CM	-152,500	63,217	,280	-361,87	56,87
	KC-BL-CM	72,750	63,217	,938	-136,62	282,12
KC-BL-CM	To	12,000	63,217	1,000	-197,37	221,37
	KC	-241,750*	63,217	,016	-451,12	-32,38
	BL	-24,750	63,217	1,000	-234,12	184,62
	CM	-70,750	63,217	,946	-280,12	138,62
	KC-BL	-321,000*	63,217	<,001	-530,37	-111,63
	KC-CM	-225,250*	63,217	,029	-434,62	-15,88
	BL-CM	-72,750	63,217	,938	-282,12	136,62

*. The mean difference is significant at the 0.05 level.

**CHAPTER 4: EFFECTS OF SINGLE AND MIXED ENDOPHYTE
INOCULATIONS ON NUTRACEUTICAL AND ANTIMICROBIAL TRAITS
IN OKRA (*Abelmoschus esculentus* L. MOENCH)**

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ABSTRACT

Plant-based therapies are increasingly valued for their safety and minimal side effects. Okra (*Abelmoschus esculentus* L. Moench), a nutrient-rich crop with documented antioxidant, anti-inflammatory, and antimicrobial properties, holds promise for the management of chronic diseases such as diabetes and cardiovascular disorders. This study explored a novel approach to enhance the nutraceutical profile of okra by integrating plant growth-promoting endophytic bacteria (PGPEB) with hydroponic cultivation, specifically the Kratky method. Three PGPEB strains—*Kosakonia cowanii* (KC), *Bacillus licheniformis* (BL), and *Cupriavidus metallidurans* (CM), isolated from *Myrothamnus flabellifolius* were applied as single or combined inoculants to hydroponically grown okra in a randomized block design (three plants per pot, four replicates). Methanolic, acetic, and aqueous extracts of okra pods were analysed for antioxidant (DPPH radical scavenging, total phenolic content [TPC]), antidiabetic (α -amylase/ α -glucosidase inhibition), anti-inflammatory (carotenoids, polysaccharides), and antimicrobial (agar disc diffusion, MIC/MBC assays) activities. Bacterial consortia showed significantly elevated TPC and antioxidant capacity compared to controls, with KC-CM methanolic extracts showing the highest TPC (230.71 mg GAE/100 g). The antioxidant activity peaked at 91% (BL alone) and 88% (KC-CM/BL-CM consortia). The antidiabetic effects were pronounced in KC-BL (92% α -amylase inhibition) and KC-CM/BL-CM (88% α -glucosidase inhibition). Methanolic extracts exhibited robust antimicrobial activity, notably against *Mycobacterium avium* (13.00 mm inhibition zone). *Salmonella typhi*, *Candida albicans*, and *Staphylococcus aureus* showed high susceptibility (6.00–11.33 mm zones), with BL-CM consortium methanolic extracts demonstrating the lowest MIC (>1.25 mg/mL). These results

suggest that PGPEB consortia is a potent bio-stimulants for enhancing the functional quality of okra in hydroponic systems, offering a sustainable strategy to boost phytochemical yields for nutraceutical and antimicrobial applications. Future research should optimize consortium formulations and explore pharmacological scalability to address chronic disease and antibiotic resistance challenges.

Keywords: Plant growth-promoting endophytic bacteria (PGPEB); Hydroponics; *Abelmoschus esculentus*; Nutraceuticals; Antidiabetic; Antioxidant; Anti-fatigue; Anti-inflammatory and Antimicrobial.

4.1 INTRODUCTION

Okra (*Abelmoschus esculentus*), the most well-known and utilised species of the Malvaceae, is a crop of significant nutritional, nutraceutical and therapeutic value, with its pods and seeds serving as a rich source of essential vitamins (A, E, and C), minerals, trace elements and bioactive compounds (Elkhalifa *et al.*, 2021; Khan *et al.*, 2022). These bioactive components not only contribute to okra's nutritional profile but also position it as a potential natural alternative to synthetic antimicrobial agents (Luthfi *et al.*, 2020). The nutraceutical properties of okra are multifaceted. The anti-diabetic properties of okra are primarily ascribed to polyphenols and mucilage content, which have been shown to regulate blood sugar levels by inhibiting digestive enzymes and slowing glucose absorption (Alblihd *et al.*, 2023; Ahmad *et al.*, 2025; Kwok *et al.*, 2025).

Okra's anti-inflammatory benefits are attributed to carotenoids, which reduce inflammation and promote overall health (Elkhalifa *et al.*, 2021; Lee and Joo, 2021; Khan *et al.*, 2022). Lee and Joo (2021) reported that Okra polysaccharides exhibit potent anti-inflammatory effects by modulating the expression of pro-inflammatory cytokines and enhancing antioxidant enzyme activities. Furthermore, Okra's high polyphenol content contributes to its antioxidant and anti-fatigue capabilities. These compounds aid in neutralising free radicals, reducing oxidative stress and promoting cellular health (Xiong *et al.*, 2021; Kwok *et al.*, 2025). The study Lee and Joo (2021) further provided compelling evidence for okra's antioxidant capabilities, demonstrating that extracts from this plant exhibit potent free radical scavenging activity and effectively inhibit lipid peroxidation.

The global challenge of antimicrobial resistance has intensified the search for novel therapeutic alternatives, with plant-derived compounds emerging as promising

candidates (Luthfi *et al.*, 2020; Nicault *et al.*, 2021). Okra, a plant rich in bioactive components such as flavonoids and polyphenols, has demonstrated significant activity against a broad spectrum of pathogens, including bacteria and fungi (Luthfi *et al.*, 2020; Nicault *et al.*, 2021; Guebebia *et al.*, 2023). These natural compounds exhibit diverse mechanisms of action, including disruption of microbial cell walls, enzyme inhibition and interference with microbial DNA replication (Guebebia *et al.*, 2023). For instance, quercetin, a flavonoid found in okra, has been shown to inhibit DNA gyrase and disrupt bacterial cell membrane integrity (Nguyen and Bhattacharya, 2022). Similarly, catechin, another polyphenol in okra, demonstrates antibacterial activity by interfering with bacterial cell wall synthesis (Woumbo *et al.*, 2022). The multifaceted antimicrobial properties of okra's bioactive compounds position it as a potential alternative or adjunct to conventional antibiotics, particularly in combating antibiotic-resistant strains (Hafeez *et al.*, 2020; Korani *et al.*, 2021). Recent studies have explored synergistic effects between plant-derived compounds and conventional antibiotics, with Bonincontro *et al.* (2023) demonstrating that compounds enhanced ciprofloxacin's activity against multidrug-resistant *Pseudomonas aeruginosa*, suggesting promising avenues for combination therapies.

Recent research has increasingly focused on the symbiotic relationship between plants and plant growth-promoting endophytic bacteria (PGPB) to enhance plant development and the accumulation of bioactive compounds in crops like okra (Salehin *et al.*, 2023). These beneficial microorganisms colonise plant tissues, improving nutrient uptake, stress tolerance, and disease resistance (Lin *et al.*, 2022; Salehin *et al.*, 2023). Applying single strains or consortia of PGPB inoculants has shown the potential to stimulate the production of secondary metabolites in plants, potentially leading to increased nutraceutical components and enhanced antimicrobial activity (Lin *et al.*,

2022). Consentino *et al.* (2022) reported that PGPB inoculation significantly increased the phenolic content and antioxidant activity in lettuce, while Nguyen and Bhattacharya (2022) observed enhanced production of flavonoids in medicinal plants following PGPB treatment. The integration of PGPB inoculants with hydroponic systems, particularly the Kratky method, presents a promising approach to optimising okra's nutraceutical profile and antimicrobial properties (Stegelmeier *et al.*, 2022; Szekely and Jijakli, 2022). Hydroponics, a soil-less cultivation technique, provides precise control over nutrient supply and environmental conditions, resulting in optimal plant development and productivity (Ahmed *et al.*, 2021). The Kratky hydroponics system, a passive method requiring minimal maintenance, is particularly ideal for resource-limited environments (Ahmed *et al.*, 2021). Ikiz, Dasgan and Gruda (2024) investigated hydroponically grown lettuce inoculated with PGPB, observing enhanced growth and improved nutrient uptake in treated plants. Masmoudi *et al.* (2024) extended this research to hydroponic tomatoes, treating them with endophytic bacteria. They observed a significant increase in antioxidant content in treated tomatoes, demonstrating that PGPB benefits extend beyond growth promotion to include improvements in crop nutritional quality. The integration of PGPB inoculants into hydroponic systems presents a promising approach to enhance the nutraceutical and antimicrobial properties of crops such as okra (Stegelmeier *et al.*, 2022). This strategy could potentially lead to the development of more effective natural therapies for chronic illnesses and microbial infections, addressing the growing concerns of antimicrobial resistance and the need for alternative therapeutic approaches (Korani *et al.*, 2021; Stegelmeier *et al.*, 2022).

Myrothamnus flabellifolius, commonly known as the resurrection plant, is indigenous to Southern Africa and predominantly found in areas with severe environmental

conditions, such as arid and semi-arid zones (Bentley, Moore and Farrant, 2019; Tebele, Marks and Farrant, 2024). It occurs in a wide area stretching from Namibia in the west, through Botswana, to Zimbabwe and the northern parts of South Africa (Bentley, Moore and Farrant, 2019). This plant is renowned for its extraordinary ability to withstand desiccation and rejuvenate upon rehydration, demonstrating a unique adaptation to thrive in harsh climates (Marks *et al.*, 2022). *M. flabellifolius* is characterised by its strong production of secondary compounds linked to defence and tolerance, many of which have significant medicinal applications (Bentley, Moore and Farrant, 2019). Recent studies have investigated *M. flabellifolius* as a potential reservoir of endophytic bacteria with attributes that may aid in stress tolerance, nutrient uptake, disease resistance and plant growth promotion (Marks *et al.*, 2022; Tebele, Marks and Farrant, 2024). By exploring interactions between *M. flabellifolius*-derived endophytic bacteria and okra in a hydroponic system, this research seeks to contribute to sustainable agriculture and the development of natural antimicrobial agents.

Thus, this study aimed to evaluate the comparative effects of single and consortia of plant growth-promoting endophytic bacteria (PGPEB) inoculants isolated from *M. flabellifolius* on the accumulation of nutraceutical components and the antimicrobial activity of okra grown in the Kratky hydroponic system.

4.2 MATERIALS AND METHODS

4.2.1 Okra Seed Inoculation

Bacterial inoculants preparation and Seed Bacterization

Endophytic bacterial inoculants were prepared from glycerol stocks of three previously isolated and purified bacterial strains, namely *Kosakonia cowanii* (KC), *Bacillus licheniformis* (BL), and *Cupriavidus metallidurans* (CM). These strains were originally

isolated from the desert plant *Myrothamnus flabellifolius* and obtained from the Department of Biochemistry, Microbiology and Biotechnology at the University of Namibia. Before cryopreservation, each strain was sub-cultured and confirmed to be pure based on uniform colony morphology and repeated plating. In the present study, Gram staining was performed to confirm the expected cell morphology and Gram reaction of each strain. Strains were selected based on plant growth-promoting (PGP) traits: nitrogen fixation (*nifH*), ACC deaminase activity, phosphate solubilisation, ammonium (NH₄⁺) production (excluding KC), siderophore production (excluding BL), and indole-3-acetic acid (IAA) production. Okra seeds (*A. esculentus* L. Moench) were surface-sterilised via sequential rinsing in 95% ethanol (20 s), 2.5% NaCl (5 min), and six sterile-water washes (Somasegaran and Hoben, 1994). Viability was confirmed using the tetrazolium chloride assay (Zafar *et al.*, 2012). Seeds were inoculated by soaking for 48 h in individual or consortia treatments (**Table 4.1**), with CFU normalised to 0.5 McFarland standard (McFarland, 1907).

Table 4.1: Endophytic bacterial inoculants isolated from *M. flabellifolius* in singular and consortia form

Treatment	Treatment Label
Control (uninoculated)	T ₀
<i>Kosakonia cowanii</i>	KC
<i>Bacillus licheniformis</i>	BL
<i>Cupriavidus metallidurans</i>	CM
<i>K. cowanii</i> and <i>B. licheniformis</i>	KC-BL
<i>K. cowanii</i> and <i>C. metallidurans</i>	KC-CM
<i>B. licheniformis</i> and <i>C. metallidurans</i>	BL-CM
<i>K. cowanii</i> , <i>B. licheniformis</i> and <i>C. metallidurans</i>	KC-BL-CM

4.2.2 Growth and Harvesting

Seed germination and cultivation

Okra seeds were germinated in petri dishes lined with sterile cotton wool, moistened with either sterile broth (negative control) or plant growth-promoting bacterial (PGPB) endophyte inoculants (positive control). Petri dishes were incubated in the dark at room temperature and monitored daily for 8 to 14 days to assess germination. The germination rate was calculated as:

Germination percentage = (number of seeds germinated / total number of seeds) x 100.

Following germination, seedlings were transferred to a greenhouse and cultivated in a controlled hydroponic system. A completely randomised block design was employed with treatments including broth inoculated and uninoculated controls.

Harvesting and drying of okra pods

Fresh okra pods were harvested at the green to dark green stage with an average length of 8-10 cm (**Figure 4.1**). After collection, pods were thoroughly washed, air-dried at room temperature for 72 hrs and subsequently oven-dried at 40° C to a constant weight. Dried pods from each treatment group (T₀, KC, BL, CM, KC-BL, KC-CM, BL-CM, and KC-BL-CM) were ground to a fine powder using an electric grinder, sieved, and stored in airtight containers for further analysis (**Figure 4.1**).



Figure 4.1: Shows fresh okra pods harvested from the greenhouse, which were dried, ground into powder and stored in glass jars.

4.2.3 Extraction and Analysis of Okra Pod Bioactive Compounds

Preparation of okra pod extracts

Okra pod extracts were prepared using a modified protocol from Khan *et al.* (2022). Powdered samples from each of the seven treatments and control were combined at a concentration of 100 mg/mL with distilled water, methanol, or acetone and incubated for 24 hours at room temperature. The mixtures were subsequently centrifuged at 2000 rpm for 15 minutes, and the resulting supernatants were collected and concentrated via rotary evaporation (**Figure 4.2**). Evaporation was carried out under reduced pressure using solvent-specific parameters: 40 °C for methanol, 50 °C for acetone, and 60 °C for water, each at approximately 150–200 mbar with a rotation speed of 90 rpm. The concentrated extracts were then freeze-dried (lyophilized) at –50 °C under a vacuum of 0.1 mbar for 24 hours to obtain dry powdered extracts, which were stored in air-tight bottles at 4 °C for later use. The dried extracts were weighed, and the yields varied depending on the treatment and extraction solvent, ranging from 1.0 to 1.7 g for

distilled water extracts, 0.12 to 0.32 g for acetone extracts, and 0.5 to 1.2 g for methanol extracts. Resuspension of the dried extracts was carried out only prior to specific bioassays. For each assay, the extracts were reconstituted in their respective original extraction solvents (distilled water, methanol, or acetone) to the appropriate working concentrations as required by the test.



Figure 4.2: Extraction of okra pod extracts using different solvents (image 1) and the use of rotary evaporation (image 2) and freeze-drying (image 3) techniques to concentrate the extracts (image 4) for bioactive compounds analysis and antimicrobial testing.

Estimation of Total Phenolic Content (TPC)

The Okra extract TPC was quantified using the Folin-Ciocalteu method (Saeed, Khan and Shabbir, 2012). To prepare the working solution for total phenolic content determination, 1 mg of the lyophilized extract for each treatment was accurately weighed and dissolved in 1 mL of solvent (distilled water, acetone or methanol) to obtain a concentration of 1 mg/mL. From this solution, 1 mL was mixed with 1 mL of

Folin–Ciocalteu phenol reagent (Merck, Germany) and incubated for 5 minutes at room temperature and combined with 10 ml of 7% Na₂CO₃ solution and 13 ml of deionised distilled water. After 90 minutes at room temperature, the absorbance was measured at 750 nm using a 96-well plate reader. Gallic acid (Sigma-Aldrich, Germany) was used as a standard for a calibration curve with concentrations ranging from 0.5 to 0.0039 mg/mL. Total Phenolic Content was expressed as milligrams of Gallic acid equivalents (GAE) per 100 g of dried sample weight using the equation:

$$C = cV/m,$$

Where c is the Gallic acid concentration obtained from the calibration curve in mg/mL, V is the volume of the extract in mL, and m is the sample mass (g) of the extract.

DPPH free radical scavenging assay

Diphenyl-1-Picrylhydrazyl (DPPH) assay followed the method of Chanda and Dave (2009) with some modifications. The working reagent of 0.3 mM DPPH (Sigma-Aldrich, Germany) was prepared in methanol. 1 ml of extract (10 mg/ml) was mixed with 1 ml of prepared DPPH solution and incubated at room temperature for 10 minutes in the dark. After incubation, absorbance was measured at 517 nm. Ascorbic acid was used as the positive control. The antioxidant capacity for scavenging DPPH free radicals was determined by the equation below. The results were expressed as percentage inhibition.

$$\% \text{ Inhibition} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

Where A₀ = absorbance of control and
A₁ = absorbance of extract

Alpha-amylase inhibition assay

Alpha-amylase inhibitory activity of okra pod extracts was assessed using a modified standard method (Kazeem, Adamson and Ogunwande, 2013). In a 96-well plate, 100 μ l of extracts (1 mg/ml) was combined with 50 μ l of 0.02 M sodium phosphate buffer (pH 6.9) and 10 μ l of alpha-amylase solution (0.5 mg/ml). After pre-incubation at 37 °C for 20 minutes, 20 μ l of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added, followed by incubation at 37 °C for 30 minutes. The reaction was terminated with 100 μ l of dinitrosalysic acid (DNS) reagent, boiled for 5 min, diluted with 100 μ l distilled water, and the absorbance measured at 540 nm. Acarbose (Sigma-Aldrich, Germany) served as a positive control. The results were expressed as percentage inhibition using the formula below:

$$\% \text{ Inhibition} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

Where A_0 = absorbance of control and
 A_1 = absorbance of extract

Alpha-glucosidase inhibition assay

The α -glucosidase inhibitory activity was evaluated using the procedure described by Kazeem, Adamson and Ogunwande (2013). In a 96-well plate, 100 μ l of alpha-glucosidase (1.0 U/ml) was mixed with 100 μ l of different concentrations of okra pod extracts (0.078 – 10 mg/ml) and pre-incubated at room temperature for 10 minutes. Subsequently, 50 μ l of 3.0 mM p-nitrophenyl glucopyranoside (PNPG) (Sigma-Aldrich, Germany) dissolved in phosphate buffer (pH 6.9) was added, and the mixture was incubated at 37°C for 20 minutes. The reaction was terminated by adding 50 μ l of Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm. Acarbose at various concentrations (0.1-0.5 mg/ml) was used as the positive

control, and a solution without sample was used as the blank. The results were expressed as percentage inhibition, which was calculated using the formula below.

$$\% \text{ Inhibition} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

A_0 – absorbance of control
 A_1 – absorbance of extract

Screening for carotenoids

Carotenes were identified using the method described by Kagan and Flythe (2014). A mobile phase of petroleum ether, ethyl acetate and diethylamine (29:15:6, v/v/v) was prepared, and TLC chambers were filled to a depth of 0.5 cm. Samples extracted with distilled water, acetone and methanol were spotted onto TLC plates (two samples per solvent). Plates were visualised under UV light (254 nm), and the retention factors (Rf) were determined using the following equation:

$$Rf = \frac{\text{Distance travelled by sample}}{\text{Distance travelled by solvent}}$$

Polysaccharides screening

Polysaccharides from the okra pod extracts were screened using the phenol-sulfuric acid assay (Chen *et al.*, 2016). Briefly, 50 μ L of okra extract was mixed with 50 μ L of 5% phenol in a 96-well plate. After homogenising for 1 min, 150 μ L of sulphuric acid was added and the reaction proceeded for 10 min at room temperature. The mixture was incubated at 37°C for 20 minutes to develop the yellowish colour, indicating the presence of polysaccharides.

4.2.4 Antimicrobial Activity Assessment of Okra Pod Extracts

Test microorganisms and preparation of inoculum

The test microbes (*Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 13932, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 6539, *Shigella sonnei* ATCC 25931, *Mycobacterium avium* ATCC 25291, and *Candida albicans* ATCC 10231) were obtained from the Department of Biochemistry, Microbiology and Biotechnology of the University of Namibia. Stock cultures were maintained in nutrient broth at $-20\text{ }^{\circ}\text{C}$. Before use, each microorganism was revived by inoculating into fresh nutrient broth and incubating at $28\text{ }^{\circ}\text{C}$ for 24 hours. The activated cultures were subsequently streaked onto nutrient agar plates and incubated for another 24 hours to obtain fresh colonies for experimental use. Active cultures (inoculum) for the experiment were standardized by transferring a loopful of cells from the activated culture into distilled water and adjusting the turbidity to match the 0.5 McFarland standard, measured at 600 nm.

Determination of antimicrobial activity

The antimicrobial activity of the okra extracts was evaluated using the agar disc diffusion method, following the procedure described by Saravanakumar et al. (2021) with slight modifications. Sterile paper discs were impregnated with the respective okra extracts by soaking them for 24 hours at room temperature. Mueller-Hinton agar plates were prepared by evenly spreading $100\text{ }\mu\text{L}$ of each standardised bacterial suspension over the surface using a sterile glass spreader. After allowing the inoculum to absorb and dry for 10–15 minutes, the extract-soaked discs were aseptically placed onto the surface of the agar. Nitrofurantoin discs were used as a positive control, while discs treated with the extraction solvents (acetone, methanol, and distilled water) served as negative controls. The plates were incubated at $37\text{ }^{\circ}\text{C}$ for 24 hours, after

which the diameter of the zones of inhibition was measured in millimetres using a transparent ruler and recorded.

Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal/ fungicidal concentration (MBC/ MFC)

Minimum inhibitory concentration (MIC) screening was conducted in 96-well microplates using the broth microdilution assay, as described by Nicault *et al.* (2021), with minor modifications. Each sample was diluted to obtain four concentrations: 5, 2.5, 1.25, 0.625 mg/ml. Each sample was tested in triplicate. For bacterial assays, Mueller-Hinton broth (Merck, Germany) was used, while Sabouraud Dextrose broth (Merck, Germany) was used for the fungal assay involving *Candida albicans*. Approximately 100 µl of Muller-Hinton broth was added to each well, followed by 100 µl of each sample stock solution (10 mg/ml) in the first three wells, with serial dilutions to achieve the desired concentrations. Then, 5 µl of the specific standardised 0.5 McFarland solutions of the test microbe was added to each well. The plates were parafilmmed and incubated at 37°C for 24 hours. 5 µl of resazurin solution (0.015 mg/mL) was added to each well, and the plates were further incubated at 37 °C for 4 hours. Colour changes from colourless to pink were observed and recorded as an indication of bacterial or fungal growth at specific concentrations. For determination of minimum bactericidal or fungicidal concentrations (MBC/MFC), 5 µL from wells showing no visible growth (no colour change) were aseptically plated onto Mueller-Hinton agar (for bacteria) or Sabouraud Dextrose agar (for *C. albicans*) and incubated at 37 °C for 24 hours. Plates were then observed for any microbial growth to confirm bactericidal or fungicidal activity.

4.3 DATA ANALYSIS

Data is presented as mean \pm standard deviation of the three biological replicates. Statistical significance was set at $\alpha = 0.05$. Normality was assessed using the Kolmogorov-Smirnov test, which confirmed non-normal distribution of all the data in this study ($P \leq 0.05$). Non-parametric Kruskal-Wallis tests were employed to compare the treatment effects (single and consortium bacterial inoculants) on Total Phenolic Content (TPC), antimicrobial activity (zones of inhibitions), free radical scavenging activity (inhibition percentage and IC_{50}), Alpha-amylase and Alpha-glucosidase percentage inhibitions, as well as the half-maximal inhibition concentration (IC_{50}) of okra pod extracts. Post-hoc pairwise comparisons (Dunn's test) were conducted for statistically significant variables. All analyses were performed in SPSS v.29 (IBM Corp.), with IC_{50} values calculated via nonlinear regression in GraphPad Prism v.6.0.

4.4 RESULTS

4.4.1 Total Phenolic Content (TPC)

The total phenolic content of the different okra pod extracts, expressed in mgGAE/100 g, is seen in **Figure 4.3**. Among the solvents tested, methanol extracts consistently yielded significantly higher amounts of phenols than acetone and distilled water extracts ($P \leq 0.001$). Specifically, the methanol control (T_0) recorded the highest TPC at 318.58 mgGAE/100g. Within the single inoculant treatments, the aqueous BL extract had the highest TPC (160.59 mgGAE/100g), closely followed by methanolic BL (153.43 mgGAE/100g), and acetonetic BL had a lower value of 84.32 mgGAE/100g. For the consortia treatments, the methanolic KC-CM showed the greatest TPC (230.71 mgGAE/100g), followed by KC-BL (214.99 mgGAE/100g) and acetonetic KC-BL-CM

extract (114.12 mgGAE/100g). Overall, the consortia treatments exhibited the highest TPC over the single inoculants.

Across all treatments, methanol extracts had TPC values ranging from 133.66 to 318.58 mgGAE/100g, while distilled water extracts ranged from 124.45 to 161.43 mgGAE/100g, and acetone extracts ranged from 84.24 to 167.29 mgGAE/100g (**Figure 4.3**). The lowest TPC was observed in the acetone control (To) extracts at 84.24 mgGAE/100g, indicating the positive influence of bacterial inoculants on the accumulation of phenols in okra pods.

Statistical analysis confirmed significant differences among the solvents at $P \leq 0.001$ (**Appendix Table 4.2**), with a notable pairwise difference observed only between acetone and methanol ($P = 0.02$) (**Appendix Table 4.3**). However, there were no significant differences in TPC among the various treatments ($P=0.488$) (**Appendix Table 4.4**). Overall, these results highlight the superior efficiency of methanol as a solvent for phenolic extraction and demonstrate the enhanced effectiveness of microbial consortia over single inoculants in promoting phenolic compounds accumulation.

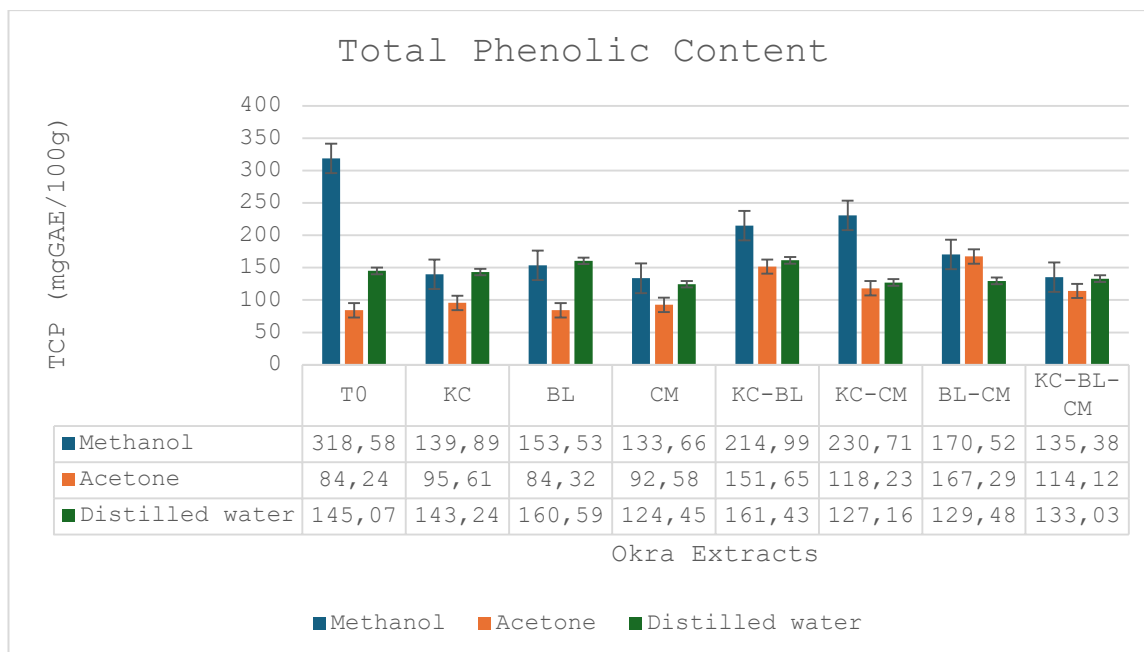


Figure 4.3: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on the accumulation of Total Phenolic content in okra pod extracts grown under hydroponic conditions.

4.4.2 Free radical scavenging activity (DPPH assay)

The free radical scavenging activity of okra pod extracts treated with seven different bacterial inoculants, both as single strains (KC, BL and CM) and as consortia (KC-BL, KC-CM, BL-CM and KC-BL-CM) and the uninoculated positive control (T₀) was assessed at different concentrations as illustrated in **Figure 4.4, 4.5 and 4.6**. Analysis revealed that the control (T₀) exhibited the highest percentage inhibition of free radicals, achieving 92% at the concentration of 330 µg/ml. Within the single inoculant treatments, methanolic BL extracts exhibited the highest inhibition at 91%, followed by methanolic KC and CM, each with 89% inhibition at the same concentration. The lowest inhibition among single inoculant treatments was observed in the aqueous BL extract, with only 14% inhibition at 2.6 µg/ml. In the case of consortia treatments, both methanolic KC-CM and BL-CM extracts showed the highest inhibition rates at 88%, while methanolic KC-BL achieved 87% at 330µg/ml. The lowest inhibition among the

consortia was recorded for KC-BL-CM at 2% at 5.2 $\mu\text{g/ml}$. No free radical scavenging activity (0%) was observed from acetonic extracts at the lowest concentration (2.6 $\mu\text{g/ml}$), nor in BL-CM at 5.2 $\mu\text{g/ml}$.

Statistical analysis indicated that the differences among treatments were statistically significant ($P \leq 0.001$, **Appendix Table 4.9**). The methanolic extracts displayed percentage inhibition ranging from 57% (BL at 2.6 $\mu\text{g/ml}$) to 92% (T_0 at 330 $\mu\text{g/ml}$) as shown in **Appendix Table 4.5**. In comparison, acetonic extracts exhibited inhibition values from 0% (all the samples at 2.6 $\mu\text{g/ml}$) to 69% (T_0 at 330 $\mu\text{g/ml}$) (**Appendix Table 4.6**), while distilled water extracts ranged from 8% (BL-CM at 2.6 $\mu\text{g/ml}$) to 69% (KC at 330 $\mu\text{g/ml}$) (**Appendix Table 4.7**). Among the solvents tested, methanol consistently exhibited the highest free radical inhibition. The percentage radical significant differences in inhibition percentage were observed between distilled water and Methanol at ($P \leq 0.001$), as well as between Acetone and Methanol ($P \leq 0.001$) (**Appendix Table 4.8**).

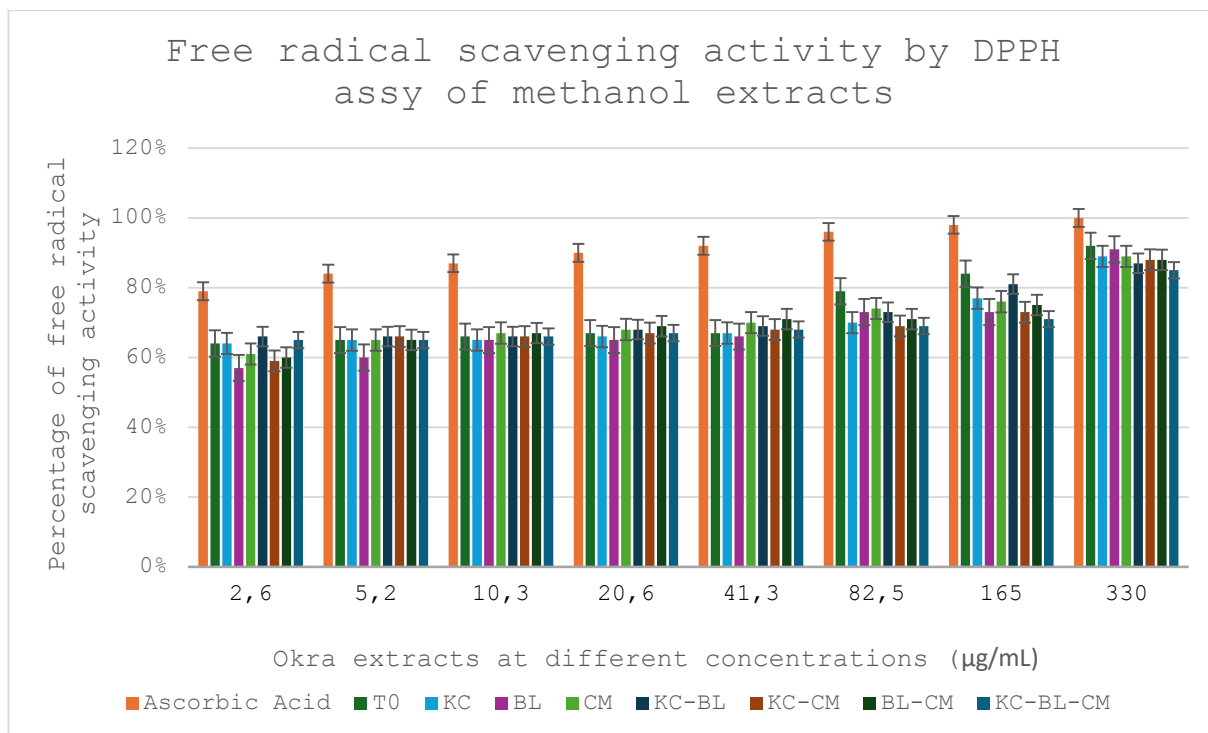


Figure 4.4: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on the Free radical scavenging activity (percentage inhibition) by DPPH assay of okra pod extracts at different concentrations.

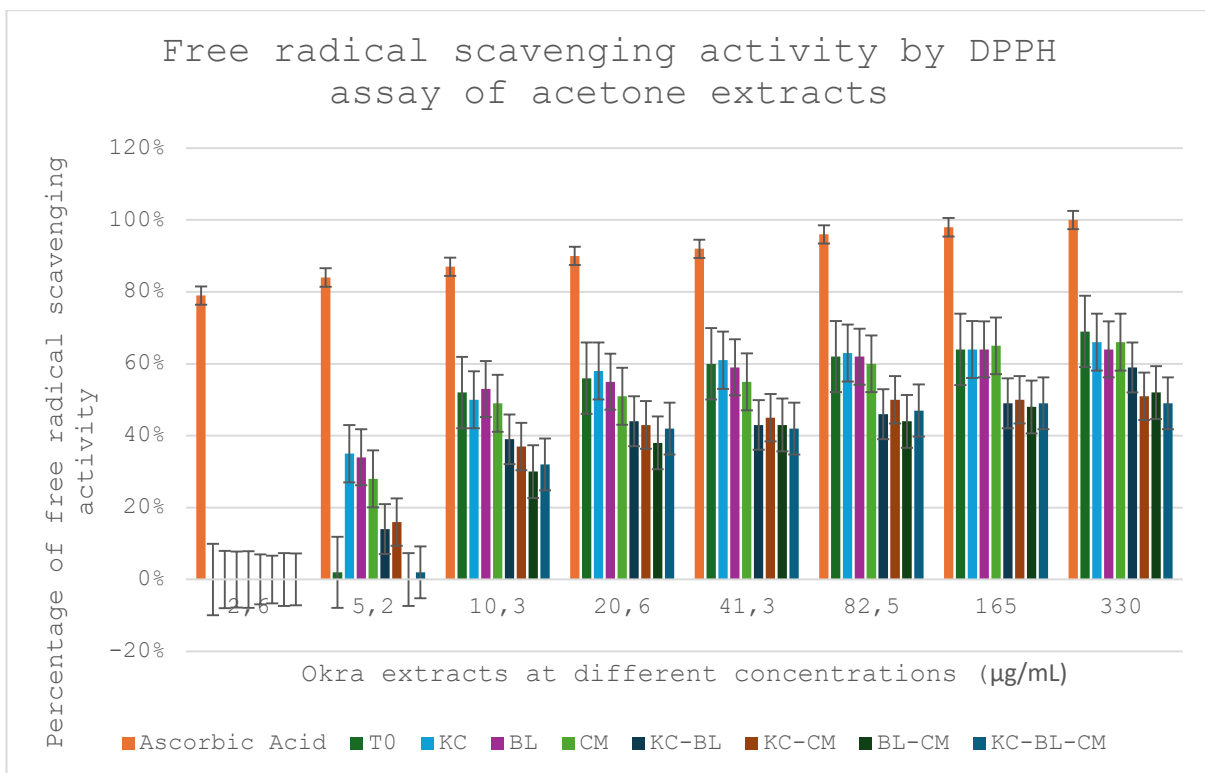


Figure 4.5: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on the Free radical scavenging activity (percentage inhibition) by DPPH assay of okra pod extracts at different concentrations.

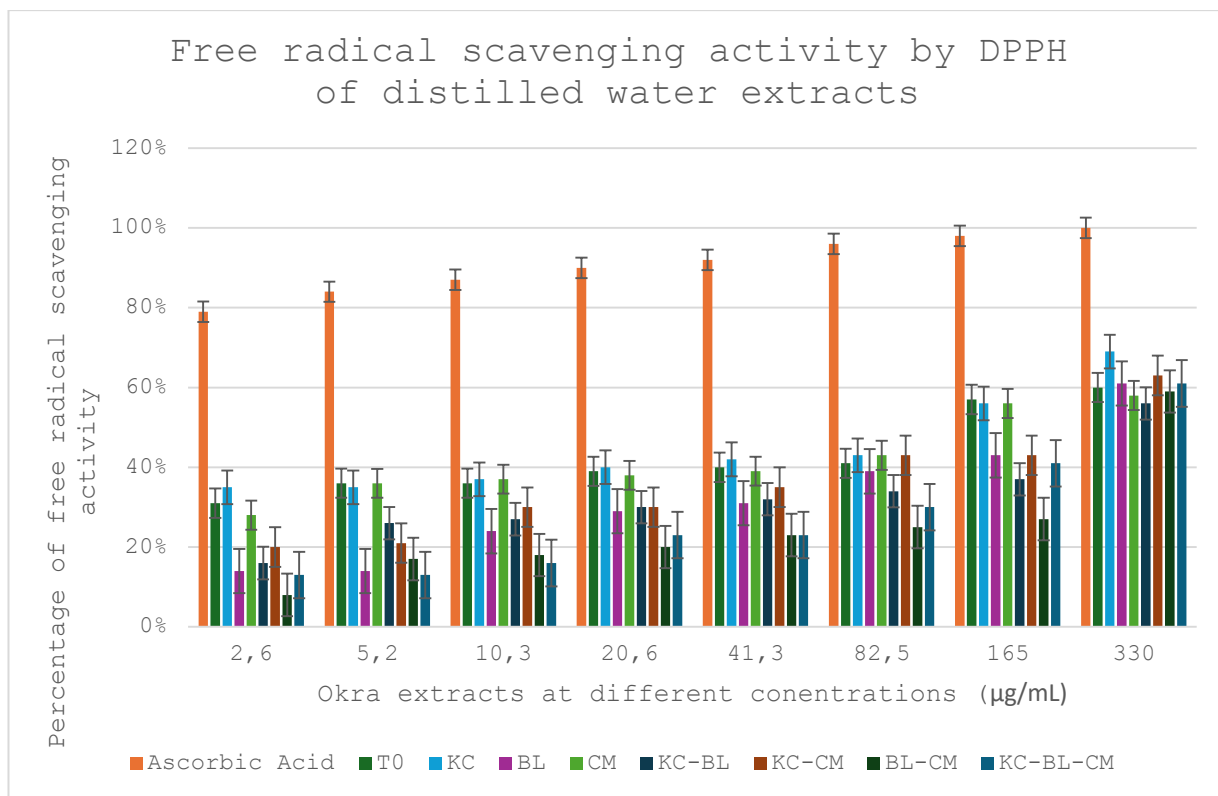


Figure 4.6: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on the Free radical scavenging activity (percentage inhibition) by DPPH assay of okra pod extracts at different concentrations.

The half-maximal inhibition concentration (IC_{50}) values for the different extracts are summarised in **Table 4.2**. Methanolic extracts exhibited the lowest overall IC_{50} value (0.4418 $\mu\text{g/mL}$), indicating the strongest antioxidant activity, followed by acetone (28.21 $\mu\text{g/mL}$) and distilled water (75 $\mu\text{g/mL}$). The reference antioxidant, ascorbic acid, showed an IC_{50} below the lowest concentration tested (<2.6 $\mu\text{g/mL}$). Several methanolic extracts, especially KC, KC-BL, and KC-CM, also exhibited IC_{50} values below 2.6 $\mu\text{g/mL}$, comparable to ascorbic acid. Across all solvents, KC-CM consistently showed the lowest IC_{50} (<2.6 $\mu\text{g/mL}$) in both methanol and acetone and 180.8 $\mu\text{g/L}$ in distilled water. Conversely, the aqueous KC-BL-CM extract exhibited the highest IC_{50} (229.8 $\mu\text{g/mL}$). Overall, significant differences in IC_{50} values were

observed between solvents ($P=0.043$), with a specific difference between methanol and distilled water extracts ($P=0.014$). However, differences among the various inoculant treatments were not statistically significant. These findings indicate that methanol is the most effective solvent for extracting antioxidant compounds from okra pods, and certain bacterial inoculant combinations, particularly KC-CM, may enhance antioxidant activity in specific extraction conditions.

Table 4.2: IC₅₀ standard deviation for free radical scavenging activity by DPPH assay for okra extracts, extracted with three different solvents

Sample	IC ₅₀ (µg/mL)		
	Methanol	Acetone	Distilled water
T ₀	0.6667 ± 0.5749	36.53 ± 7.685	120.1 ± 15.585
KC	<2.6	28.21 ± 6.005	75.09 ± 10.125
BL	1.304 ± 0.6635	30.72 ± 7.21	205.6 ± 7.7
CM	0.4418 ± 0.2996	36.62 ± 5.685	130 ± 11.725
KC-BL	<2.6	112.2 ± 41	<2.6
KC-CM	<2.6	<2.6	180.8 ± 13.55
BL-CM	0.8924 ± 0.2316	217.8 ± 11.65	<2.6
KC-BL-CM	2.231 ± 0.8551	<2.6	229.8 ± 11.55

4.4.3 Alpha-amylase inhibition assay

The α -amylase inhibition assay revealed a clear concentration-dependent response across all okra pod extracts, with the highest percentage inhibition constantly observed at the maximum tested concentration of 2500 µg/mL (**Figure 4.7, 4.8 and 4.9**). Among the various treatments, the methanolic KC-BL extract exhibited the highest overall percentage inhibition, reaching 92% at 2500 µg/mL. In general, methanolic extracts outperformed acetonic and aqueous extracts in terms of α -amylase inhibitory activity. For single inoculant treatments, the highest percentage inhibition was recorded for acetonic KC and BL extracts, each achieving 87% at 2500 µg/mL, followed by CM and BL with 86% inhibition at 2500 µg/mL and 1250 µg/mL, respectively. In contrast,

the lowest inhibition among single inoculant treatments was observed in the aqueous BL extract with only 58% at 19.53 $\mu\text{g/mL}$. The consortia treatments indicated the highest inhibition percentage of 92% from methanolic KC-BL at 2500 $\mu\text{g/mL}$, followed by BL-CM and KC-BL with 91% at 1250 $\mu\text{g/mL}$, and the lowest was aqueous KC-CM with 12% at 19.53 $\mu\text{g/mL}$. Hence, the results indicate a heightened effect of bacterial inoculants on alpha-amylase inhibition compared to the control, which had an overall percentage inhibition of 88%.

A detailed analysis of the inhibition potential across solvents revealed that methanolic extracts ranged from 58% (T_0 at 19.53 $\mu\text{g/mL}$) to 92% (KC-BL at 2500 $\mu\text{g/mL}$) (**Appendix table 4.10**). Acetonic extracts displayed a broader range with no inhibition detected at the lowest concentration (0 at 19.53 $\mu\text{g/mL}$) for all samples, but reaching up to 90% inhibition (KC-BL at 2500 $\mu\text{g/mL}$) (**Appendix table 4.11**). Distilled water extracts exhibited intermediate efficacy, ranging from 12% (KC-CM at 19.53 $\mu\text{g/mL}$) to 88% (KC-BL at 2500 $\mu\text{g/mL}$). For comparison, the reference inhibitor acarbose (positive control) demonstrated 89% inhibition at 19.53 $\mu\text{g/mL}$ and 100% at 2500 $\mu\text{g/mL}$ (**Appendix Table 4.12**), indicating the potential effects of okra pod extracts as natural α -amylase inhibitors.

Statistical analysis revealed significant differences in percentage inhibition between distilled water and methanol ($P=0.003$) and distilled water and Acetone ($P=0.009$) (**Appendix Table 4.13**). In addition, significant differences were observed between the control (T_0) and the treatments, including KC-BL ($P \leq 0.001$), KC-CM ($P = 0.013$), KC-BL-CM ($P = 0.012$), and BL-CM ($P = 0.005$). Additional significant differences were also observed between both treatments in singular and consortia treatments including CM and KC-BL ($P \leq 0.001$), CM and KC-CM ($P = 0.029$), CM and KC-BL-CM ($P = 0.022$), CM and BL-CM ($P = 0.012$), BL and KC-CM ($P = 0.049$), BL and

KC-BL-CM ($P = 0.045$), BL and BL-CM ($P = 0.021$), BL and KC-BL ($P = 0.002$), KC and BL-CM ($P = 0.026$) and KC and KC-BL ($P = 0.002$) (**Appendix Table 4.14**).

These findings suggest that both the choice of solvent and the specific treatment significantly influence the α -amylase inhibitory potential of the extracts.

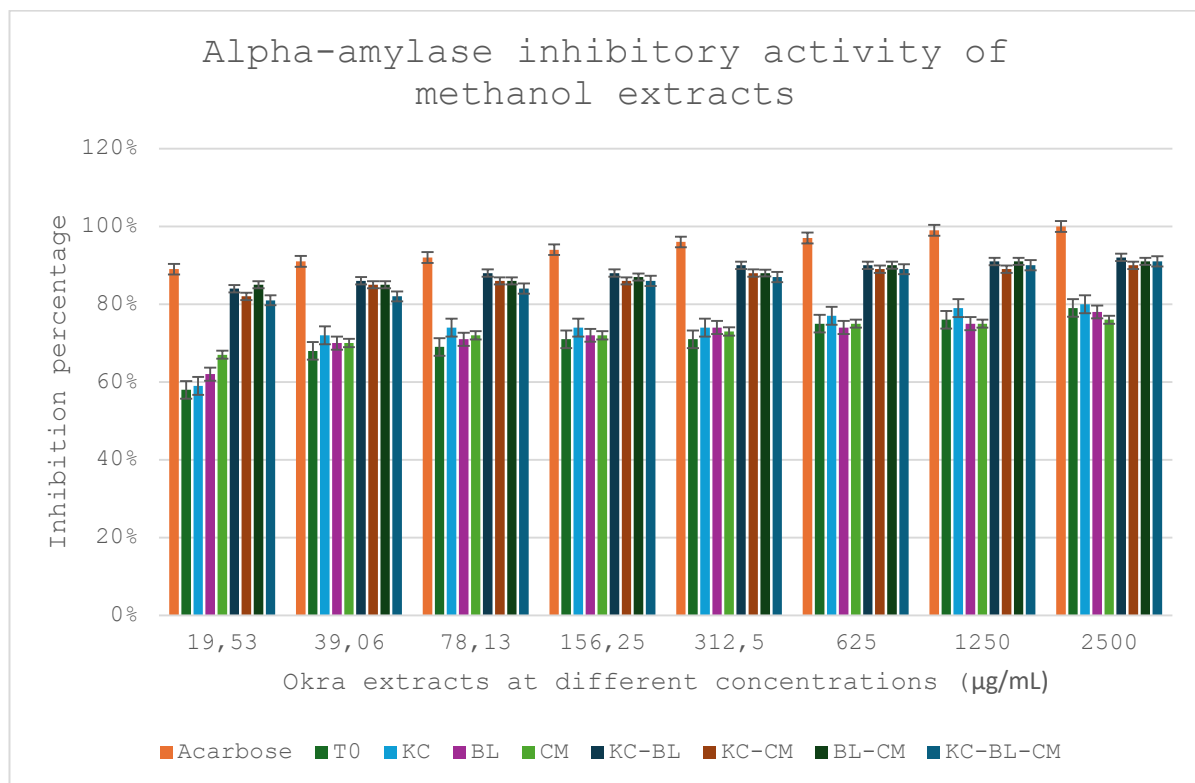


Figure 4.7: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on Alpha-amylase inhibitory activity of okra pod extracts at different concentrations.

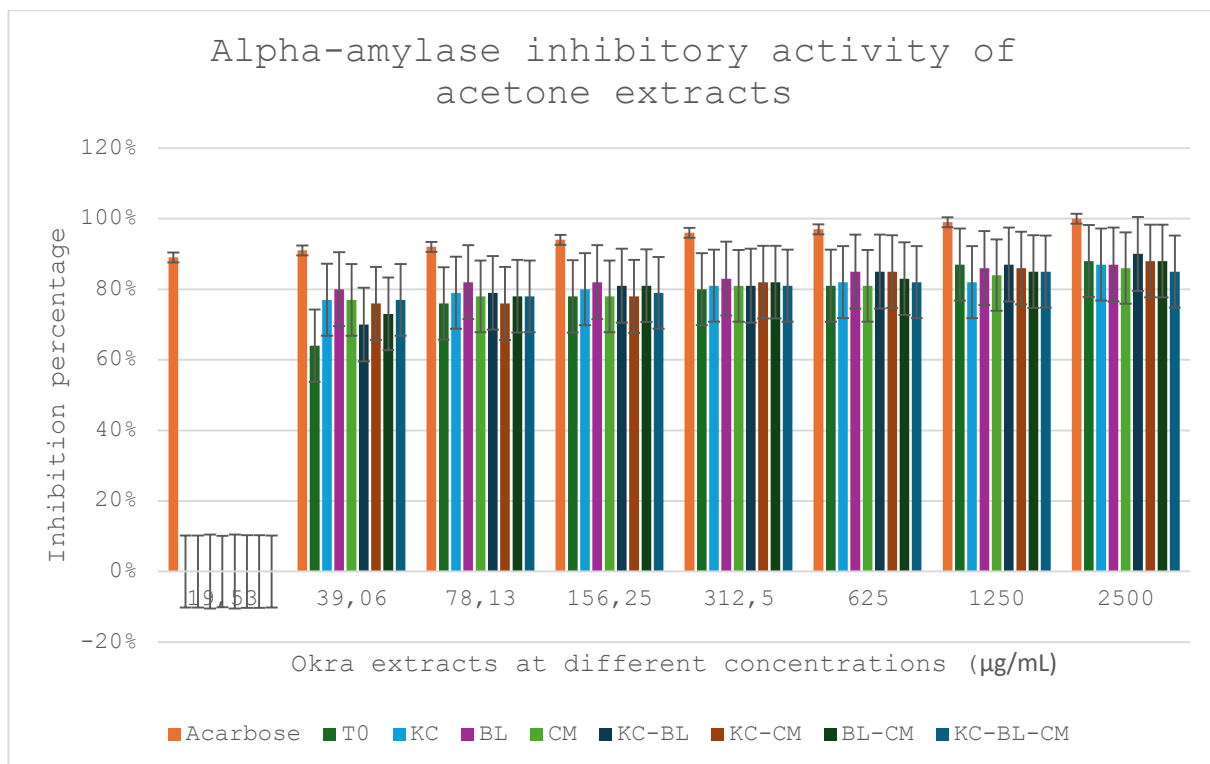


Figure 4.8: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on Alpha-amylase inhibitory activity of okra pod extracts at different concentrations.

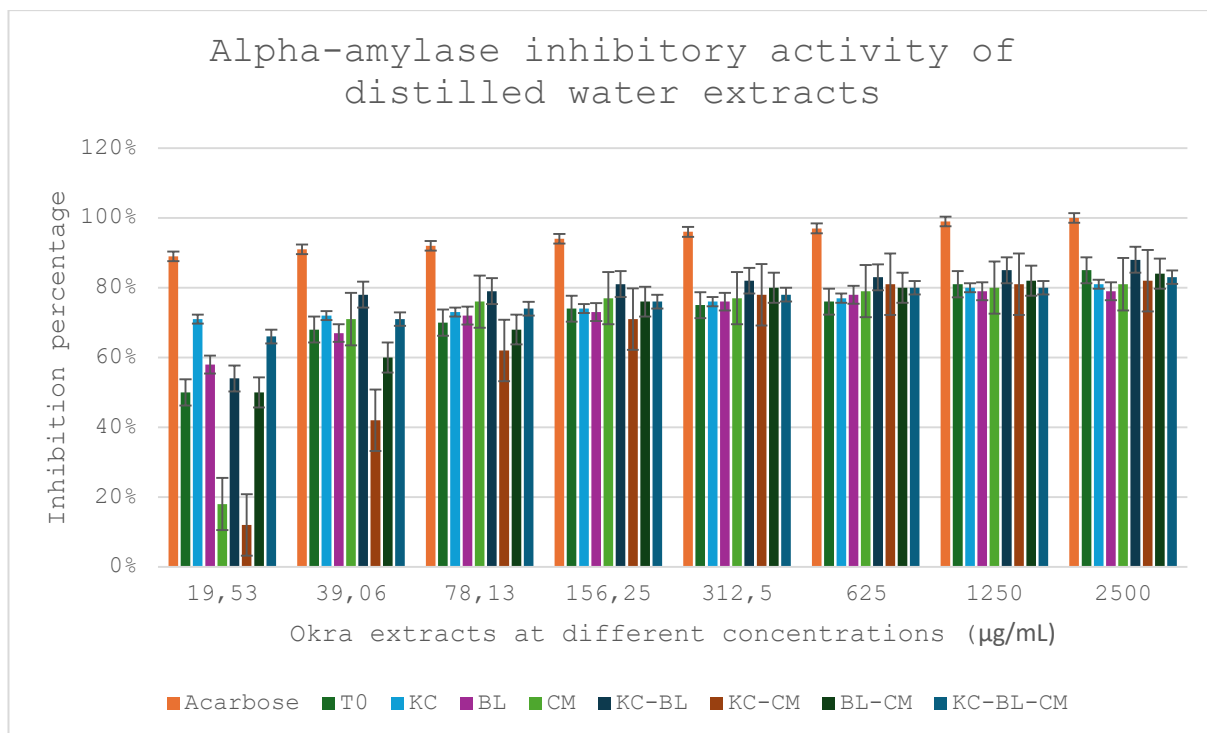


Figure 4.9: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on Alpha-amylase inhibitory activity of okra pod extracts at different concentrations.

The half-maximal inhibition concentration (IC_{50}) values for the okra extracts are presented in **Table 4.3**. Acarbose, used as a positive control, exhibited an IC_{50} below the lowest concentration used in this experiment ($< 19.53 \mu\text{g/mL}$). Similarly, methanolic BL and KC-BL extracts also showed IC_{50} values below the threshold ($< 19.53 \mu\text{g/mL}$), highlighting their strong inhibitory potential. The distilled water extract of CM exhibited the lowest IC_{50} ($0.0001492 \mu\text{g/mL}$), indicating the highest potency among all samples. Distilled water extracts had the overall lowest IC_{50} values, followed by methanolic and acetone extracts, respectively. On the contrary, sample KC-CM persistently exhibited the overall highest IC_{50} values across all solvents 1.1800 , 1.614 and $1.852 \mu\text{g/mL}$ for distilled water, methanol, and acetone extracts, respectively. Despite these variations, statistical analysis revealed no statistically significant difference in IC_{50} values between solvents ($P = 0.920$) or treatments ($P =$

0.138). These findings suggest that while all okra extracts demonstrate α -amylase inhibitory activity, the extraction solvent and specific bacterial inoculant influence the potency of this inhibition. Assuming that similar results observed with distilled water extracts remain the same in a saline environment. This can mean that the okra extract will have a higher potency in a physiological environment like in a human body. The exceptional performance of certain extracts, particularly those using distilled water, highlights their promises as natural α -amylase inhibitors and warrants further investigation into their potential applications in managing postprandial hyperglycaemia.

Table 4.3: IC₅₀ standard deviation for Alpha-amylase inhibitory activity for okra extracts, extracted with three different solvents

Sample	IC ₅₀ (μ g/mL)		
	Methanol	Acetone	Distilled water
T ₀	0.5647 \pm 0.14664	<19.53	<19.53
KC	0.1417 \pm 0.03490	0.0095 \pm 0.00316	0.09435 \pm 0.06277
BL	<19.53	<19.53	0.01977 \pm 0.01543
CM	0.001173 \pm 0.00019	0.1333 \pm 0.05201	0.0001493 \pm 0.00001
KC-BL	<19.53	<19.53	0.0727 \pm 0.013128
KC-CM	1.1800 \pm 0.001	1.614 \pm 0.01702	1.852 \pm 0.08437
BL-CM	0.000498 \pm 0.00016	0.2994 \pm 0.07474	<19.53
KC-BL-CM	0.008622 \pm 0.00322	0.03126 \pm 0.003823	<19.53

4.4.4 Alpha-glucosidase Inhibition Assay

The alpha-glucosidase inhibitory activity of okra pod extracts, derived from okra treated with seven bacterial inoculants applied either individually (KC, BL and CM) or in consortia (KC-BL, KC-CM, BL-CM and KC-BL-CM), was evaluated in comparison to acarbose, a standard inhibitor (**Figure 4.10, 4.11 and 4.12**). The uninoculated T₀ served as the control. The aqueous uninoculated control (T₀) exhibited the highest inhibition percentage of 89% when tested at 2500 μ g/ml. Among the single

inoculant treatments, the aqueous KC extract achieved the highest inhibition (88%) at 2500 µg/mL, closely followed by CM and BL, with 87% at the same concentration. For the consortia treatments, methanolic KC-CM and BL-CM extracts reached 88% inhibition at 2500 µg/mL, while KC-BL and KC-BL-CM reached 87% at 2500 µg/mL. In contrast, all acetonic samples extracts demonstrated no inhibitory activity (0 inhibition at the lowest concentration tested (19.53 µg/mL). Methanolic extracts exhibited inhibition ranging from 2% (BL-CM at 19.53 µg/mL) to 88% (KC-CM & BL-CM at 2500 µg/mL). Maximal inhibition was consistently observed at 2500 µg/mL (**Appendix Table 4.15**). Acetonic extracts overall demonstrated the lowest inhibition rates, ranging from 0 at the lowest concentration (all the samples at 19.53 µg/mL and others at 39.06 µg/mL and 78.13 µg/mL) to a maximum of 86% (KC-BL at 2500 µg/mL). Aqueous extracts showed inhibition from 0 (T₀ at 19.53 µg/mL) to 89% (T₀ at 2500 µg/mL) (**Appendix Tables 4.16 and 4.17**).

Methanolic and aqueous extracts exhibited comparable maximal inhibition. Methanolic extracts achieved above 50% inhibition across all treatments \geq 39.06 µg/mL. Similarly, aqueous extracts inhibited above 50% inhibition at 39.06 µg/mL with exceptions for T₀ (21%), BL (17%) and CM (45%). Kruskal-Wallis test indicated significant differences in inhibitory activity between acetone and distilled water ($P \leq 0.001$) as well as between acetone and methanol ($P = 0.007$) (**Appendix Table 4.18**). All okra treatments differed significantly from Acarbose ($P \leq 0.001$), but no significant differences were observed among the okra treatments themselves (**Appendix Table 4.19**).

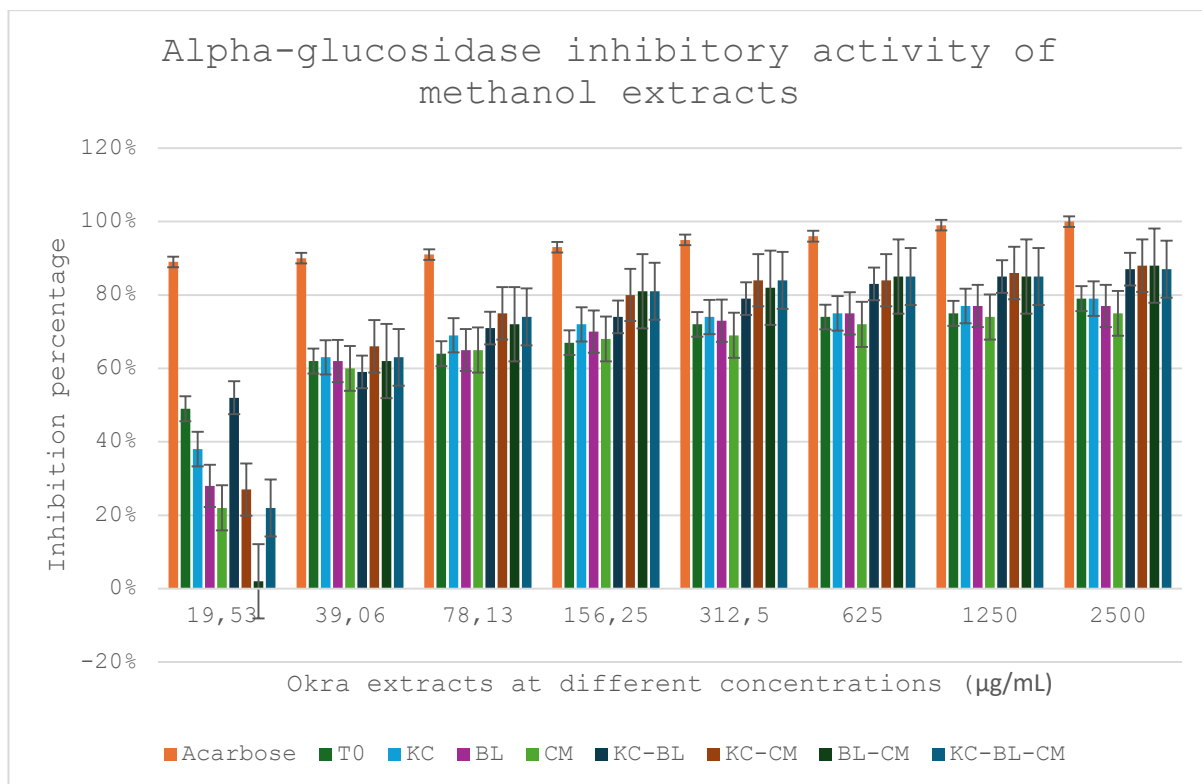


Figure 4.10: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on Alpha-glucosidase inhibitory activity of okra pod extracts at different concentrations.

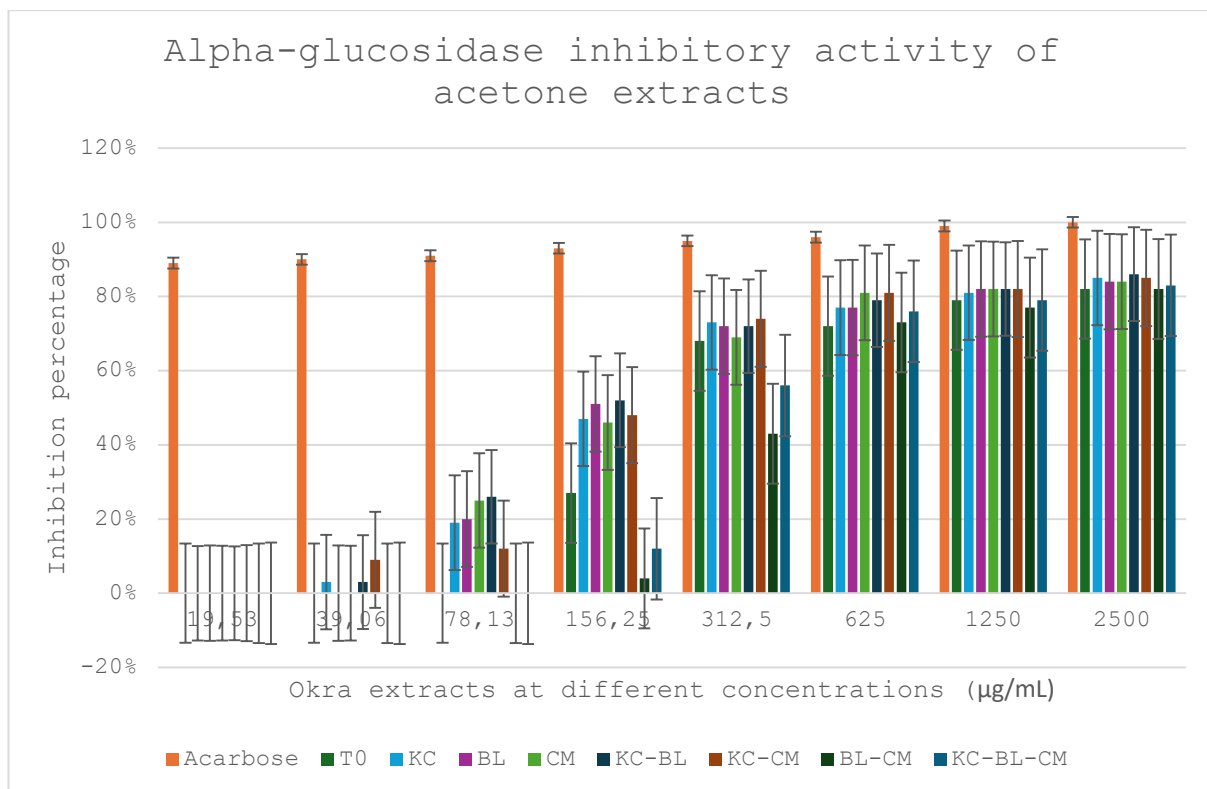


Figure 4.11: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on Alpha-glucosidase inhibitory activity of okra pod extracts at different concentrations.

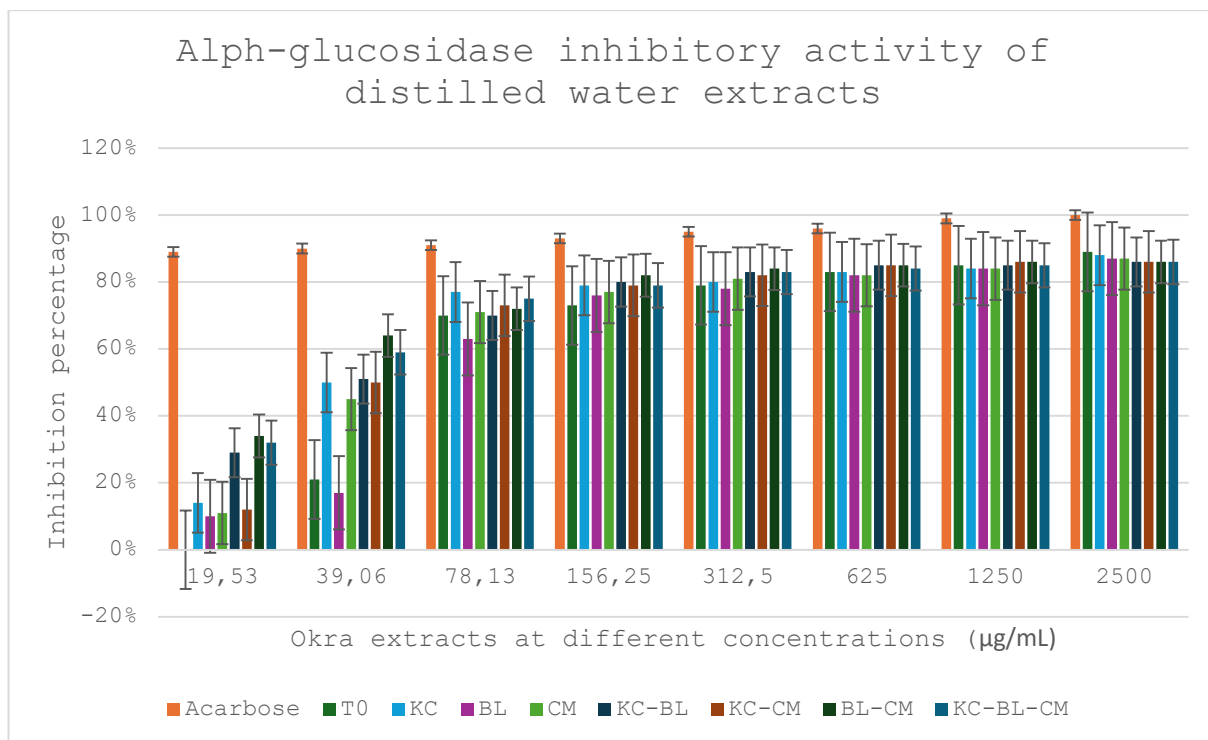


Figure 4.12: The effect of bacteria inoculants in singular form (KC, BL and CM) and consortia form (KC-BL, KC-CM, BL-CM and KC-BL-CM) on Alpha-glucosidase inhibitory activity of okra pod extracts at different concentrations.

The half-maximal inhibitory concentration (IC_{50}) results further supported these trends. Acarbose was used as a positive control, showing an IC_{50} below the lowest concentration tested ($< 19.53 \mu\text{g/mL}$). Comparably, the aqueous extract of KC and CM, and the methanolic extract of BL-CM showed an IC_{50} below this threshold, indicating a strong inhibitory potency. In contrast, the acetonetic extract BL-CM exhibited the highest IC_{50} value ($438.4 \mu\text{g/mL}$), reflecting lower activity. Overall, methanolic and aqueous extracts showed comparable inhibitory potency against α -glucosidase, while acetonetic extracts were less effective. Significant differences were observed between solvents ($P \leq 0.001$). Pairwise comparison showed significant differences between distilled water and acetone ($P \leq 0.001$) and methanol and acetone at $P = 0.002$. However, no statistically significant differences were found among treatments ($P = 0.918$). These findings suggest that both the choice of extraction

solvent and the specific bacterial treatment play important roles in determining the inhibitory potential of Okra pod extracts, with methanol and water proving to be the most effective solvents for extracting bioactive compounds with antidiabetic potential.

Table 4.4: IC₅₀ standard deviation for Alpha-glucosidase inhibitory activity for okra extracts, extracted with three different solvents

Sample	IC ₅₀ (µg/mL)		
	Methanol	Acetone	Distilled water
T ₀	3.279 ± 0.10147	261.3 ± 26.05	9.005 ± 0.16729
KC	0.2186 ± 0.08944	194.5 ± 9.1	<19.53
BL	1.501 ± 0.03126	186 ± 13.95	13.98 ± 6.741
CM	0.9499 ± 0.09669	146.7 ± 33.315	<19.53
KC-BL	4.61 ± 0.04648	109.3 ± 18.285	0.03557 ± 0.00382
KC-CM	0.09768 ± 0.00354	124.7 ± 19.05	0.1115 ± 0.00356
BL-CM	<19.53	438.4 ± 38.9	0.0006 ± 0.00016
KC-BL-CM	0.00303 ± 0.00033	349.7 ± 18.05	0.03351 ± 0.00520

4.4.5 Carotenes and polysaccharides screening

Thin layer Chromatography (TLC) was used to screen for carotenes in the okra extracts. The presence of carotenes was only detected in the acetone extracts. As illustrated in **Figure 4.13**, distinct yellow-orange spots, with an average R_f value of 0.90 (**Table 4.5**), were observed across all acetone-extracted samples (T₀, KC, BL, CM, KC-BL, KC-CM, BL-CM and KC-BL-CM), confirming the presence of carotenoids. The intensity of these spots correlated with carotenoid concentration, with uniform prominence notes in all treatments. No carotenoids were detected in methanolic or aqueous extracts, highlighting acetone's efficacy as a solvent for non-polar phytochemical recovery.



Figure 4.13: TLC results for okra pod extracts that were extracted with acetone for the eight different treatments (T₀, KC, BL, CM, KC-BL, KC-CM, BL-CM and KC-BL-CM).

Table 4.5: Rf value standard deviation of carotenes detected in acetone extracts of okra inoculated with plant growth-promoting endophytic bacteria

Sample	Rf values of the carotenes detected in the TLC screens
T ₀ (Control (uninoculated))	0.93±0.022
KC (<i>Kosakonia cowanii</i>)	0.94±0.022
BL (<i>Bacillus licheniformis</i>)	0.91±0.022
CM (<i>Cupriavidus metallidurans</i>)	0.93±0.022
KC-BL (<i>K. cowanii</i> and <i>B. licheniformis</i>)	0.94±0.022
KC-CM (<i>K. cowanii</i> and <i>C. metallidurans</i>)	0.94±0.022
BL-CM (<i>B. licheniformis</i> and <i>C. metallidurans</i>)	0.89±0.022
KC-BL-CM (<i>K. cowanii</i> , <i>B. licheniformis</i> and <i>C. metallidurans</i>)	0.89±0.022

Phenol-Sulfuric Acid Assay for Polysaccharides Detection

The phenol sulphuric acid assay was used to qualitatively determine the presence of polysaccharides in okra pods. A visible colour transition to amber brown in the reaction solution (**Figure 4.14**) confirmed the presence of polysaccharides across all tested extracts.

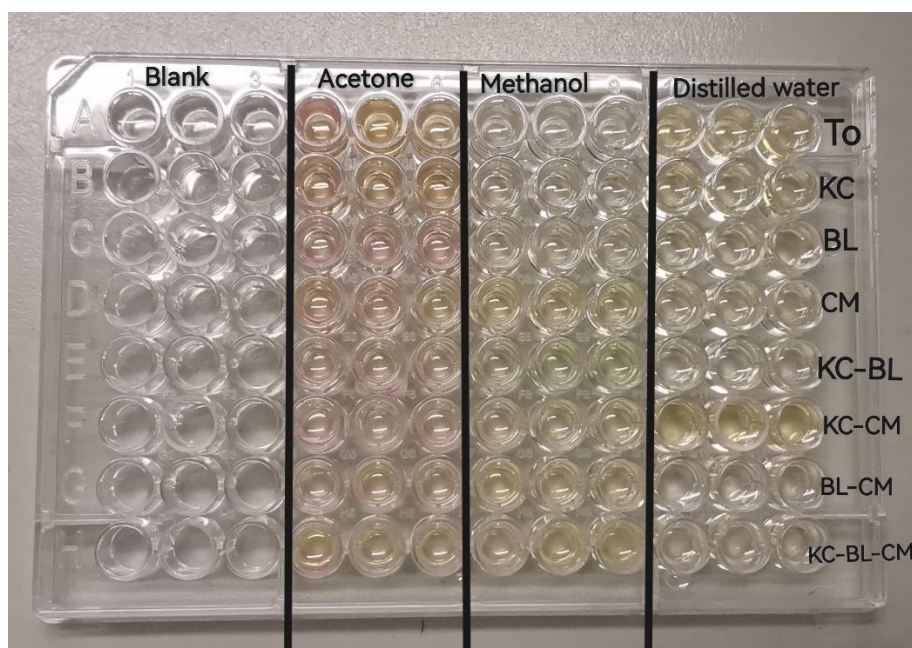


Figure 4.14: A 96-well plate indicating the presence of polysaccharides in all solvents and all treatments with an amber brown colour.

4.4.6 Antimicrobial Activity Assessment of Okra Pod Extracts

The antimicrobial activity of okra extracts was evaluated using three different solvents (acetone, methanol and distilled water) (**Appendix Table 4.20**) against a panel of seven human pathogens, including three gram-negative bacteria (*Escherichia coli*, *Shigella sonnei* and *Salmonella typhi*), three gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes* and *Mycobacterium avium*) and one fungus (*Candida albicans*). As presented in **Table 4.6**, the okra extracts demonstrated varying inhibitory

activity depending on the solvent used and the microorganisms tested. Methanolic extracts consistently exhibited the largest zones of inhibition, followed by acetone and then distilled water extracts. BL-CM methanolic extract showed the highest inhibition (13.00 ± 0.00 mm) against *M. avium* (**Figure 4.15** and **Table 4.6**). Acetonic KC, CM and KC-BL extracts displayed substantial activity with inhibition zones of approximately 10mm (**Appendix Table 4.20**) against *S. typhi* and *M. avium* bacteria. The uninoculated sample (T_0) also indicated potential inhibition, especially against *S. aureus*. The aqueous extracts, while generally less potent, demonstrated an overall broad-spectrum activity across the tested microorganisms (**Appendix Table 4.20**). Among all pathogens, *S. typhi*, *C. albicans*, and *S. aureus* were the most susceptible pathogens, with inhibition zones ranging from 6.00 ± 0.00 mm to 11.33 ± 0.58 mm for *S. typhi*; 6.00 ± 0.00 mm to 9.67 ± 0.58 mm for *C. albicans* and 6.00 ± 0.00 mm to 11.33 ± 0.58 mm for *S. aureus*. *Escherichia coli* demonstrated the highest zone with acetone and distilled water extracts, whereas *M. avium* was the most sensitive to methanolic extracts. No inhibition was observed against *S. sonnei* with the acetone extracts. Okra extracts treated with *B. licheniformis* (BL), particularly for the consortia, exhibited the overall highest average inhibition zones across all solvents, although methanolic single inocula were also effective. The positive control nitrofurantoin produced significantly greater inhibition zones (from 13 ± 0.00 mm against *S. aureus* to 23 ± 0.00 mm against *C. albicans*) than any other okra extract ($P \leq 0.001$), with zones of inhibition ranging from 0.00 ± 0.00 mm against *S. sonnei* in acetonic extracts to 13.00 ± 0.00 mm by BL-CM against *M. avium* in methanolic extract. Significant differences were observed in inhibition zones amongst the test microbes ($P \leq 0.005$), specifically between *S. sonnei* and *M. avium*. Additionally, the choice of extraction solvent significantly influenced antimicrobial activity ($P \leq 0.001$),

with methanol exhibiting significantly higher inhibition zones against distilled water extracts.

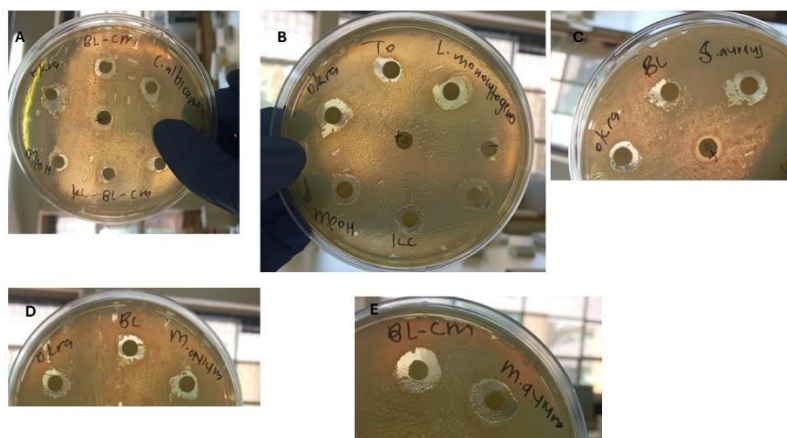


Figure 4.15: Zones of inhibition of different methanolic extracts against the indicated test microbes

Table 4.6: Inhibition zones of Okra pod extracts observed after 24 hours of incubation via the disc diffusion method.

Samples	Test microorganisms						
	<i>E.coli</i>	<i>S. sonnei</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>M. avium</i>
Nitrofurantoin	16.00±0.00	15±0.00	14±0.00	23±0.00	13±0.00	18±0.00	14±0.00
Methanol							
TO	9.00±0.58	10.00±0.00	8.33±0.58	9.33±0.58	8.00±0.00	10±0.00	7.00±0.00
KC	7.00±0.58	8.00±0.00	8.00±0.58	7.00±0.00	7.33±0.58	10.00±0.00	7.00±0.00
BL	9.00±0.00	6.67±0.58	11.33±0.58	8.00±0.00	11.33±0.58	10.33±0.58	10.00±0.00
CM	7.00±0.00	8.00±0.00	7.00±0.00	6.33±0.58	7.00±0.00	6.00±0.00	7.00±0.00
KC-BL	0.00±0.00	6.00±0.00	8.67±0.58	7.67±0.58	8.00±0.00	8.00±0.00	8.00±0.00
KC-CM	7.00±0.00	6.00±0.00	8.00±0.00	6.33±0.58	8.00±0.00	8.00±0.00	8.00±0.00
BL-CM	0.00±0.00	7.67±0.58	8.00±0.00	9.67±0.58	10.00±0.00	0.00±0.00	13.00±0.00
KC-BL-CM	10.67±1.00	9.33±0.58	6.67±0.58	7.67±0.58	7.33±0.58	0.00±0.00	7.00±0.00

Appendix Table 4.21 shows the Minimum Inhibitory Concentration (MIC) analysis, which supported these findings, with methanolic extracts demonstrating the greatest

potency and antimicrobial activity (MIC values ranging from > 1.25 mg/ml to > 5 mg/ml). Whereas aqueous extracts generally exhibited the highest MIC concentration up to 10 mg/ml for most pathogens, except for KC and BL against *E.coli*, which had an MIC of 5mg/mL. *M. avium* displayed the highest susceptibility across all solvent extracts as evidenced by low MIC values. The presence of bacterial growth indicated by a pink/red colour (**Figure 4.16**) was used to determine the lowest concentration for microbial inhibition. Extracts from okra samples inoculated with *K. cowanii* and *B. licheniformis* as single inoculants, particularly in methanol, exhibited low MIC values, suggesting that these bacterial endophytes enhance the antimicrobial properties of okra, although consortia treatments also showed promising results.

Table 4.7: Minimal Inhibitory Concentration (MIC) in mg/ml of Okra pod extracts against selected human pathogens.

Samples	Test microorganisms						
	<i>E.coli</i>	<i>S. sonnei</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>M. avium</i>
Methanol							
T0	> 2.5	> 2.5	> 5	> 2.5	> 5	> 2.5	> 5
KC	> 1.25	> 1.25	> 5	> 2.5	> 2.5	> 2.5	> 2.5
BL	> 1.25	> 1.25	> 2.5	> 5	> 5	> 2.5	> 1.25
CM	> 1.25	> 1.25	> 2.5	> 5	> 5	> 2.5	> 5
KC-BL	-	> 2.5	> 5	> 2.5	> 2.5	> 2.5	> 2.5
KC-CM	> 5	> 2.5	> 5	> 5	> 1.25	> 2.5	> 2.5
BL-CM	-	> 1.25	> 5	> 5	> 5	-	> 1.25
KC-BL-CM	> 2.5	> 5	> 5	> 2.5	> 2.5	-	> 1.25

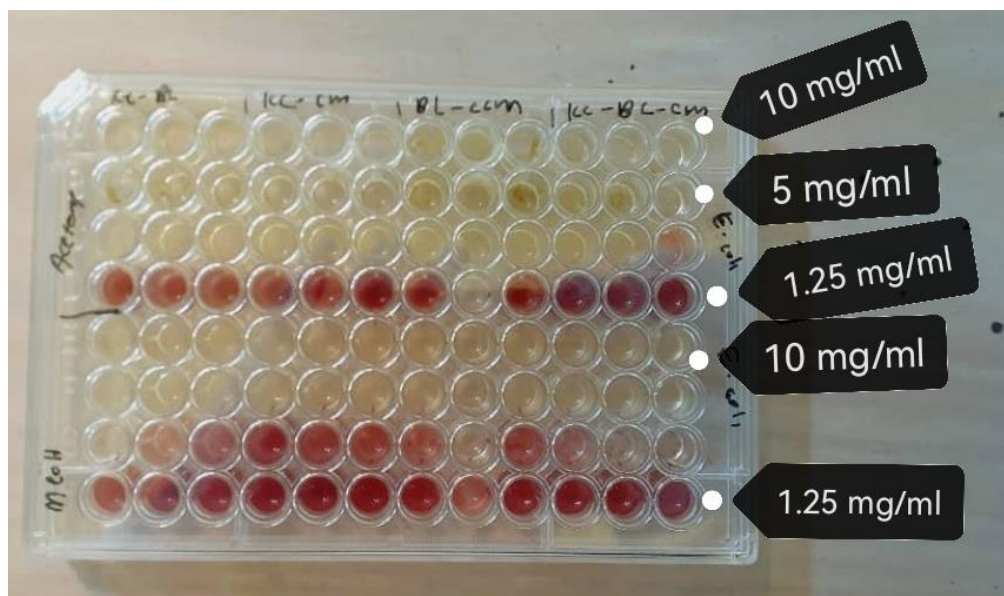


Figure 4.16 Minimum inhibitory concentration (MIC) of acetonic and methanolic extracts from KC-BL until KC-BL-CM against *E.coli*.

Assessment of the minimal Bactericidal / Fungicidal concentration (MBC/ MFC) (**Appendix Table 4.22**) revealed that distilled water extracts were primarily bacteriostatic and fungistatic, while acetone and methanol extracts were generally bactericidal and fungicidal. *C. albicans* exhibited the overall lowest MFC and was observed to be fungicidal across all the acetone and methanol extracts. Overall, all the test microbes were susceptible to cidal effects when treated with acetone and/ or methanol extracts. These findings collectively indicate that Okra extracts, particularly those prepared with methanol and enhanced by specific bacterial inoculants, possess significant antimicrobial potential and warrant further investigation for their application as natural antimicrobial agents.

Table 4.8: Minimal Bactericidal / Fungicidal concentration (MBC/ MFC) in mg/ml of Okra pod extracts against selected human pathogens.

Samples	Test microorganisms						
	<i>E. coli</i>	<i>S. sonnei</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>M. avium</i>
Methanol							
T0	10; Cidal	10; Cidal	5; Cidal	2.5; Cidal	5; Cidal	10;Cidal	10; Cidal
KC	10; Cidal	10; Cidal	5; Cidal	5; Cidal	10; Cidal	10; Cidal	2.5; Cidal
BL	5; Cidal	10; Cidal	10; Cidal	5; Cidal	10; Static	10; Static	5; Cidal
CM	10; Cidal	10; Static	10; Cidal	5; Cidal	10; Cidal	10; Static	5; Cidal
KC-BL	-	10; Cidal	5; Cidal	2.5; Cidal	5; Cidal	10; Cidal	10; Cidal
KC-CM	10; Cidal	5; Cidal	5; Cidal	5; Cidal	10; Cidal	10; Cidal	5; Cidal
BL-CM	-	5; Cidal	10; Cidal	5; Cidal	10;Cidal	-	10; Cidal
KC-BL-CM	5; Cidal	10; Cidal	5; Cidal	2.5; Cidal	10; Cidal	-	2.5; Cidal

4.5 DISCUSSION

The observed enhancement of total phenolic content (TPC) in okra pod extracts through bacterial inoculation aligns with contemporary studies on plant-microbe interactions and their role in phytochemical biosynthesis. Methanolic extracts of the KC-CM consortium exhibited the highest TPC (230.71 mg GAE/100 g), a finding consistent with research demonstrating that microbial consortia synergistically stimulate phenolic compound accumulation in plants through stress-induced metabolic pathways (Lin *et al.*, 2022). For instance, microbial consortia have been shown to upregulate phenylpropanoid pathway enzymes, such as phenylalanine ammonia-lyase (PAL), which catalyses phenolic biosynthesis (Kumar *et al.*, 2023). This mechanism may explain the superior TPC in mixed inoculations compared to single-strain treatments.

The efficacy of *B. licheniformis* (BL) in elevating TPC (evident in aqueous BL (160.59 mg GAE/100 g) and methanolic BL (153.53 mg GAE/100 g) extracts) is supported by

its documented role in enhancing antioxidant activity and secondary metabolite production. Recent studies attribute this to BL's ability to produce lipopeptides and siderophores, which induce systemic resistance and phenolic synthesis in host plants (Lin *et al.*, 2022; Saiyam *et al.*, 2024). Similarly, the performance of *K. cowanii* (KC) in consortia aligns with its reported ability to modulate plant metabolism, potentially through auxin-like compounds that enhance nutrient uptake and stress tolerance (Flores *et al.*, 2023). The solvent-dependent variation in TPC (methanol > aqueous > acetone) reflects methanol's polarity, which optimises the extraction of both hydrophilic and moderately hydrophobic phenolics, such as hydroxycinnamic acids and flavonoids (da Silva *et al.*, 2021). This aligns with global trends in phytochemical extraction, where methanol is prioritised for its efficiency in recovering bioactive compounds (Wahyuningsih *et al.*, 2020; El Mannoubi, 2023). The low TPC in acetone extracts, despite their efficacy for non-polar carotenoids, underscores the solvent-specific selectivity for phytochemical classes (El Mannoubi, 2023).

The uninoculated control (T_0) in methanol exhibited the highest TPC (318.58 mg GAE/100 g), suggesting that intrinsic okra phenolic biosynthesis may dominate under non-inoculated conditions. However, microbial inoculants (particularly consortia) appear to fine-tune phenolic profiles, as seen in potato studies where microbial treatments increased shikimic acid and rutin levels by 3.47- and 1.72-fold, respectively (Kumar *et al.*, 2023). This highlights the potential of tailored microbial partnerships to enhance specific bioactive compounds without suppressing intrinsic plant metabolism.

This study assessed the free radical scavenging activity using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay and ascorbic acid as the standard. The results revealed that the percentage inhibition for methanol extracts ranged from 57% to 92%,

for acetone extracts from 0% to 69%, and distilled water extracts from 8% to 69%, with methanol showing the overall highest radical inhibition percentage among the solvents. The free radical scavenging activity of okra pod extracts, evaluated using the DPPH assay, demonstrated significant solvent-dependent and inoculant-mediated variations. Methanol emerged as the most effective solvent, with inhibition percentages ranging from 57% (BL at 2.6 $\mu\text{g/mL}$) to 92% (T0 at 330 $\mu\text{g/mL}$), outperforming acetone and distilled water extracts ($P \leq 0.001$) (**Appendix Tables 4.5–4.7**). These findings align with recent studies highlighting methanol's superior ability to solubilise phenolic acids and flavonoids, which are critical for radical scavenging (Wahyuningsih *et al.*, 2020; da Silva *et al.*, 2021). For instance, Wahyuningsih *et al.* (2020) reported methanol extracts of okra pods with IC_{50} values of 35.21 $\mu\text{g/mL}$, corroborating its efficiency in extracting bioactive antioxidants. Methanol's intermediate polarity optimally solubilises hydrophilic (e.g., hydroxycinnamic acids) and moderately hydrophobic (e.g., quercetin derivatives) antioxidants, explaining its superior performance. Acetone, while effective for non-polar carotenoids, underperformed in phenolic extraction, reflecting its limited ability to disrupt hydrogen bonds in plant cell walls (da Silva *et al.*, 2021; El Mannoubi, 2023). Aqueous extracts exhibited broad-spectrum but weaker activity due to reduced solubility of non-polar antioxidants. Bacterial inoculants, particularly consortia like KC-CM and BL-CM, enhanced antioxidant activity, achieving inhibition percentages of 88% in methanolic extracts. Single inoculants such as methanolic BL also showed high efficacy (91%), consistent with *B. licheniformis*' documented role in producing lipopeptides that enhance antioxidant pathways (Lin *et al.*, 2022; Saiyam *et al.*, 2024). The synergy in consortia likely stems from stress-induced phenolic biosynthesis, as

microbial interactions upregulate enzymes like phenylalanine ammonia-lyase (PAL) (Kumar *et al.*, 2023).

Methanolic extracts exhibited the lowest IC₅₀ values (<2.6 µg/mL for KC, KC-BL, and KC-CM), indicating potency comparable to ascorbic acid (<2.6 µg/mL). In contrast, aqueous KC-BL-CM showed the highest IC₅₀ (229.8 µg/mL), highlighting solvent polarity as a critical determinant of bioactivity (Wahyuningsih *et al.*, 2020). The IC₅₀ trends align with studies demonstrating methanol's efficiency in extracting quercetin and catechin, which donate hydrogen atoms to stabilise DPPH radicals (Khan *et al.*, 2022; Zhang *et al.*, 2024). The antioxidant activity of okra extracts is attributed to synergistic interactions between phenolics, polysaccharides, and vitamin E (tocopherols). Polysaccharides, identified via phenol-sulfuric acid assay (**Figure 4.16**), contribute to radical scavenging through hydrogen donation, while vitamin E inhibits lipid peroxidation (Zhang *et al.*, 2024). The absence of significant differences among treatments ($P = 0.138$) suggests microbial effects are secondary to solvent efficacy, though consortia like KC-CM show promise for targeted bioactive compound enhancement.

The α -amylase inhibitory activity of okra pod extracts was systematically evaluated across a concentration gradient, revealing a clear dose-dependent inhibition, with the highest activity consistently observed at the maximum tested concentration of 2500 µg/mL. Among all extracts, those prepared with methanol exhibited superior inhibitory effects compared to acetic and aqueous extracts, with the methanolic KC-BL consortium achieving the highest inhibition at 92% at 2500 µg/mL. This finding underscores the effectiveness of methanol as a solvent for extracting bioactive α -amylase inhibitors from okra pods, a result that is consistent with recent studies demonstrating the strong antidiabetic potential of polyphenol-rich okra extracts and

their ability to inhibit digestive enzymes such as α -amylase and α -glucosidase in a concentration-dependent manner (Wahyuningsih *et al.*, 2020; Liu *et al.*, 2021; Guebebia *et al.*, 2023).

A closer examination of solvent-dependent inhibition patterns revealed that methanolic extracts displayed inhibition ranging from 58% (T_0 at 19.53 $\mu\text{g/mL}$) to 92% (KC-BL at 2500 $\mu\text{g/mL}$), reflecting methanol's strong ability to solubilise polar inhibitors such as flavonoids and phenolic acids. Acetonic extracts showed a broader and more variable range, with no inhibition detected at the lowest concentration (0% at 19.53 $\mu\text{g/mL}$) but reaching up to 90% inhibition (KC-BL at 2500 $\mu\text{g/mL}$). Aqueous extracts demonstrated intermediate efficacy, with inhibition values ranging from 12% (KC-CM at 19.53 $\mu\text{g/mL}$) to 88% (KC-BL at 2500 $\mu\text{g/mL}$), likely due to the extraction of hydrophilic polysaccharides and other water-soluble bioactive compounds. These results are in agreement with recent comparative studies showing that the whole okra pod, which is rich in phenolics and flavonoids, exhibits significantly higher radical scavenging and enzyme inhibitory activity than seeds or bark alone (Woumbo *et al.*, 2022; Molehin *et al.*, 2024).

Regarding the effects of bacterial inoculants, single inoculant treatments such as acetonic KC and BL extracts achieved 87% inhibition at 2500 $\mu\text{g/mL}$, while aqueous BL extracts showed minimal activity at lower concentrations (58% at 19.53 $\mu\text{g/mL}$). In consortia treatments, methanolic KC-BL extracts again exhibited the highest inhibition (92%), outperforming the uninoculated control (88%), which suggests a synergistic effect of microbial consortia in enhancing α -amylase inhibitory activity. Such findings support the growing evidence that both the phytochemical profile and the microbial environment can modulate the functional properties of okra extracts (Guebebia *et al.*, 2023). Statistical analysis revealed significant differences in

inhibition percentages between solvents, specifically between methanol and distilled water ($P = 0.003$) and between distilled water and acetone ($P = 0.009$). Treatments involving KC-BL, KC-CM, and BL-CM also differed significantly from the control ($P \leq 0.001$ – 0.005), while differences among the various inoculant treatments themselves were not statistically significant ($P = 0.138$), indicating that solvent choice has a more substantial impact than the specific bacterial treatment.

The half-maximal inhibitory concentration (IC_{50}) analysis further supported these findings. Methanolic extracts exhibited the lowest IC_{50} values ($<2.6 \mu\text{g/mL}$ for BL and KC-BL), which are comparable to the positive control acarbose ($<19.53 \mu\text{g/mL}$), indicating potent α -amylase inhibition. The distilled water CM extract showed exceptional potency with an IC_{50} of $0.0001492 \mu\text{g/mL}$, while KC-CM extracts exhibited the highest IC_{50} values across all solvents (1.1800 – $1.852 \mu\text{g/mL}$). However, statistical analysis indicated no significant differences in IC_{50} values between solvents ($P = 0.920$) or treatments ($P = 0.138$), emphasising the intrinsic bioactivity of okra over microbial modulation (Panighel *et al.*, 2022).

The α -glucosidase inhibitory activity of okra pod extracts, derived from plants treated with bacterial inoculants, demonstrated significant solvent-dependent and inoculant-mediated variations. Methanol and aqueous extracts consistently outperformed acetonetic extracts, with the uninoculated control (T_0) in aqueous extracts exhibiting the highest inhibition (89% at $2500 \mu\text{g/mL}$). These findings align with studies highlighting methanol's efficacy in extracting polar bioactive compounds like flavonoids and phenolic acids, which are critical for enzyme inhibition (Wu *et al.*, 2020; El Mannoubi, 2023). Methanol's intermediate polarity enables efficient extraction of hydrophilic antioxidants, such as polysaccharides and polyphenols, which contribute to α -glucosidase inhibition (Zhang *et al.*, 2024). Aqueous extracts also showed strong

activity, likely due to water-soluble polysaccharides like rhamnogalacturonans, which delay glucose absorption by inhibiting carbohydrate-hydrolysing enzymes (Abdel-Razek *et al.*, 2023; Khan *et al.*, 2023). Acetonic extracts underperformed, reflecting the limited solubility of polar bioactive compounds in non-polar solvents (El Mannoubi, 2023). These results corroborate findings by da Silva *et al.* (2021), who reported methanol's superiority in recovering phenolic compounds linked to antidiabetic activity. Consortia treatments, particularly KC-CM and BL-CM in methanol, achieved 88% inhibition, suggesting microbial synergy enhances phenolic biosynthesis. *B. licheniformis* (BL) is known to produce lipopeptides that upregulate phenylpropanoid pathways, increasing flavonoid and phenolic yields (Lin *et al.*, 2022; Saiyam *et al.*, 2024). While *K. cowanii* (KC) and *C. metallidurans* (CM) lack direct reports on antidiabetic activity, their role in nutrient mobilisation may indirectly boost phytochemical accumulation (Flores *et al.*, 2023). Methanolic and aqueous extracts exhibited the lowest IC₅₀ values (e.g., 0.0001492 µg/mL for aqueous CM), comparable to acarbose (<19.53 µg/mL), indicating potent inhibition. This aligns with Wahyuningsih *et al.* (2020), who attributed low IC₅₀ values to synergistic interactions between polysaccharides and polyphenols. Acetonic extracts showed reduced efficacy (IC₅₀ up to 438.4 µg/mL), consistent with their poor extraction of polar inhibitors (Zhang *et al.*, 2024). Significant differences between solvents ($P \leq 0.001$) underscore methanol's dominance, while the lack of treatment-specific significance ($P = 0.918$) suggests microbial effects are secondary to solvent choice. These findings emphasise the importance of solvent selection in optimising okra's antidiabetic potential. The results support okra's role in managing postprandial hyperglycaemia, particularly through bacterial consortia-enhanced extracts, which may reduce reliance on synthetic drugs like acarbose (Mokgalaboni *et al.*, 2023; Hsiao, Lee and Wu, 2024).

Okra is also rich in carotene, a precursor of vitamin A synthesis known to modulate inflammatory responses by inhibiting the production of pro-inflammatory cytokines and reducing oxidative stress, a key factor in inflammation (Luthfi *et al.*, 2020; Liu *et al.*, 2021). Carotenoids are biologically active, fat-soluble compounds found in plants, including beta-carotene, lycopene, lutein, and xanthophyll. These compounds are typically yellow, orange, or red, and nearly 600 known carotenoid species are found in vegetables and fruits (Luthfi *et al.*, 2020). In addition to their role as common organic food components that impart specific colouration to plants, carotenoids and chlorophylls are also believed to possess anti-inflammatory properties (Romdhane *et al.*, 2020). Thin-layer chromatography (TLC) revealed the presence of carotenes exclusively in acetone extracts of okra pods, as evidenced by distinct yellow-orange spots with an average Rf value of 0.90. The intensity of these spots correlated with carotene concentration, demonstrating acetone's efficacy as a solvent for non-polar phytochemical recovery.

Methanolic and aqueous extracts showed no detectable carotenes, consistent with the principle of "like dissolves like," where non-polar carotenes preferentially dissolve in non-polar solvents like acetone (Romdhane *et al.*, 2020; Liu *et al.*, 2021). These findings align with studies highlighting acetone's superiority in extracting lipophilic compounds such as β -carotene and lycopene from plant matrices (Liu *et al.*, 2021; Zhang *et al.*, 2024). The uniform Rf values across all treatments (T₀, KC, BL, CM, KC-BL, KC-CM, BL-CM, KC-BL-CM) suggest that bacterial inoculants did not alter carotene profiles, though *Bacillus licheniformis* (BL) has been linked to enhanced carotene accumulation in other plant systems (Lin *et al.*, 2022). The absence of literature on *Kosakonia cowanii* (KC) and *Cupriavidus metallidurans* (CM) in

carotene modulation underscores the need for further research into microbial effects on okra's phytochemical composition.

The phenol-sulfuric acid assay confirmed the presence of polysaccharides in all extracts, with a visible amber-brown colour transition in reaction solutions. This colourimetric shift, proportional to carbohydrate concentration, is characteristic of polysaccharides such as rhamnogalacturonans and arabinogalactans, which are abundant in okra mucilage (Guebebia *et al.*, 2023; Zhang *et al.*, 2024). The assay's reliability was validated by recent optimisations of the phenol-sulfuric acid method, which account for monosaccharide-specific correction factors to improve accuracy (Yue *et al.*, 2022). Methanol and aqueous extracts exhibited comparable polysaccharide content, reflecting the solubility of hydrophilic polymers in polar solvents. Acetone extracts, while effective for carotene recovery, showed lower polysaccharide yields due to the limited solubility of polar compounds. These results align with studies demonstrating okra polysaccharides' role in antioxidant and antidiabetic activities, mediated through hydrogen-donating mechanisms (Wu *et al.*, 2020; Zhang *et al.*, 2024).

The solvent-dependent recovery of carotenes and polysaccharides underscores the importance of extraction protocols in phytochemical studies. Acetone's non-polar nature optimises carotene extraction, while methanol and water excel in recovering polar antioxidants and polysaccharides. Bacterial inoculants, particularly consortia like KC-CM and BL-CM, showed no significant impact on carotene profiles but may subtly enhance polysaccharide yields through stress-induced metabolic pathways (Lin *et al.*, 2022; Guebebia *et al.*, 2023).

The antimicrobial activity of okra (*A. esculentus*) extracts, evaluated against seven human pathogens using acetone, methanol, and distilled water as solvents,

demonstrated significant solvent-dependent and inoculant-mediated variations. Methanolic extracts exhibited the highest zones of inhibition (13.00 ± 0.00 mm against *M. avium*), followed by acetone and distilled water extracts. This aligns with studies highlighting methanol's superior ability to extract polar bioactive compounds, such as phenolic acids and flavonoids, which disrupt microbial cell membranes and inhibit growth (Hafeez *et al.*, 2020; da Silva *et al.*, 2021). For instance, Hafeez *et al.* (2020) reported methanolic okra leaf extracts as the most potent against *Pasteurella multocida*, attributing this to methanol's polarity, which enhances the solubility of antimicrobial phytochemicals. Acetonic extracts showed moderate activity (e.g., ≈ 10 mm zones against *S. typhi* and *M. avium*), likely due to the limited extraction of polar compounds (da Silva *et al.*, 2021). Aqueous extracts, while broad-spectrum, were less potent, consistent with reduced solubility of lipophilic antimicrobial agents in water (Guebebia *et al.*, 2023).

S. typhi, *C. albicans*, and *S. aureus* were the most susceptible pathogens across all solvents, with inhibition zones ranging from 6.00 ± 0.00 mm to 11.33 ± 0.58 mm. This mirrors findings by Sipahi *et al.* (2022), who noted okra's strong antifungal activity against *C. albicans*. Notably, *B. licheniformis* (BL) inoculated samples, particularly consortia like BL-CM, exhibited enhanced antimicrobial potency (MIC as low as >1.25 mg/mL in methanol extracts). This aligns with studies showing microbial inoculants like *Bacillus* spp. induce stress-mediated synthesis of secondary metabolites, including antimicrobial lipopeptides (Lin *et al.*, 2022). Methanolic extracts demonstrated the lowest MIC values (>1.25 – 5 mg/mL), indicating potent antimicrobial activity, while aqueous extracts required higher concentrations (up to 10 mg/mL). *M. avium* was the most susceptible pathogen across solvents, with consistently low MIC values, likely due to its lipid-rich cell wall, which is vulnerable

to phenolic compounds (Lin *et al.*, 2022). Distilled water extracts were bacteriostatic/fungistatic, whereas acetone and methanol extracts were bactericidal/fungicidal, particularly against *C. albicans* (MFC = 1 mg/mL). These results corroborate findings by Astutiningsih, Kristanti and Ariani (2023), who reported okra's fungicidal activity against *C. albicans* via membrane disruption.

The superior efficacy of methanol aligns with its role in extracting flavonoids and polysaccharides, which synergistically inhibit microbial growth (Wahyuningsih *et al.*, 2020). The lack of significant differences among treatments ($P = 0.138$) suggests microbial inoculants primarily enhance bioactive compound yield rather than altering antimicrobial mechanisms. However, consortia like KC-CM and BL-CM showed promise, likely due to stress-induced upregulation of phenylpropanoid pathways (Lin *et al.*, 2022). Okra extracts, particularly methanolic ones, exhibit significant antimicrobial potential against clinically relevant pathogens. The synergy between solvent polarity and microbial inoculants highlights opportunities for optimising extraction protocols. Future research should focus on isolating specific bioactive compounds and validating their efficacy *in vivo*.

4.6 CONCLUSION AND RECOMMENDATION

This study demonstrated that bacterial consortia treatments, particularly those involving *K. cowanii* and *C. metallidurans* (KC-CM), as well as *K. cowanii* and *B. licheniformis* (KC-BL), significantly enhanced the accumulation of total phenolic content (TPC), antioxidant activity, and α -amylase and α -glucosidase inhibitory activities in *A. esculentus*. The use of consortia confirmed the synergistic potential of microbial interactions to stimulate the biosynthesis of secondary metabolites, likely through the upregulation of phenylpropanoid pathways and activation of stress-response mechanisms. Among single-strain treatments, *B. licheniformis* showed

notable efficacy in enhancing phenolic accumulation. Previous research (Jeong *et al.*, 2021) supports these findings, demonstrating improved growth and productivity in *Arabidopsis thaliana* inoculated with *K. cowanii*. Both *K. cowanii* and *B. licheniformis* exhibit key plant growth-promoting traits such as nitrogen fixation (*nifH*), ACC deaminase activity, phosphate solubilisation, siderophore production, and IAA biosynthesis, although *B. licheniformis* uniquely produces ammonium (NH_4^+). The presence of ACC deaminase, as highlighted by Lin *et al.* (2022), is particularly important for enhancing plant stress tolerance and productivity.

Given the rising global concern over fatigue, inflammatory diseases, diabetes, microbial infections, and the escalation of antimicrobial resistance, the search for alternative therapeutics is increasingly urgent. In this context, okra (*A. esculentus*) emerges as a promising source of pharmacologically active compounds with documented antioxidant, anti-fatigue, anti-inflammatory, antidiabetic, and antimicrobial properties. The choice of extraction solvent was critical to maximising bioactivity. Methanol consistently outperformed distilled water and acetone, yielding higher levels of phenolics, antioxidant activities (as measured by DPPH assay), and enzyme inhibitory activities. Methanolic extracts also exhibited the strongest antimicrobial activity across all treatment groups, emphasising the role of solvent polarity in enhancing the recovery of bioactive compounds.

Furthermore, the study confirms that okra extracts derived from plants inoculated with *K. cowanii*, *B. licheniformis*, or their consortia possess substantial antimicrobial activity against *S. typhi*, *E. coli*, *S. aureus*, and *C. albicans*. These findings align with previous reports on okra's broad-spectrum antimicrobial potential and substantiate its value as a functional food. Okra is rich in vitamins, minerals, fibre, flavonoids, and polyphenols, which contribute to its overall health benefits. It supports digestive

health, helps regulate blood sugar levels, and possesses anti-inflammatory and immunomodulatory properties, reinforcing its potential in the prevention and management of infections

This study advocates for the strategic use of bacterial inoculants to enhance okra's nutraceutical and antimicrobial properties, offering a sustainable approach to developing new bioactive therapies. By harnessing the synergistic potential of plant-microbe interactions, okra can be positioned as a potent dietary component and a natural source of antimicrobial agents to address the growing challenge of infectious diseases.

Based on these findings, the following recommendations are proposed: long-term field studies are necessary to validate the efficacy and consistency of PGPEB consortia under hydroponic conditions, and advocacy efforts should be intensified to promote microbial bio-stimulants as sustainable agricultural inputs, contributing to food security and environmental health. However, a key limitation of this study is the lack of quantification of enzyme inhibition using appropriate biochemical assays, which would have provided deeper insight into the specific mechanisms underpinning plant-microbe interactions. Future research should incorporate such analyses to strengthen the understanding of functional microbial contributions.

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APPENDICES

Appendix I: Ethical Clearance Certificate



UNAM
UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SOS-0234 Date: 23AUGUST 2024

This Ethical Clearance Certificate is issued by the University of Namibia Ethics Committee (REC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the ethics committee.

Title of Project: INVESTIGATING THE INFLUENCE OF SEED BACTERIZATION WITH PLANT GROWTH-PROMOTING ENDOPHYTES ON OKRA (*Abelmoschus esculentus* L. MOENCH) PRODUCTIVITY AND NUTRACEUTICALS ACCUMULATION UNDER CONTROLLED CONDITIONS

Student: MRS. THEMBAKAZI GININDZA

Student Number: 202040658


Supervisor(s): DR. JEAN DAMASCENE UZABAKIRIHO
DR. CELINE MUKAKALISA

Centre for Research Services

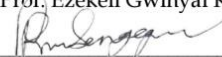
Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the ethics committee. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the ethics committee.
3. The Principal Researcher must report issues of ethical compliance to the ethics committee (through the Chairperson) at the end of the Project or as may be requested by the ethics committee.
4. The ethics committee retains the right to:
 - i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
 - ii) Request for an ethical compliance report at any point during the course of the research.

The ethics committee wishes you the best in your research.



Prof. Ezekeil Gwinyai Kwembeya (Chairperson Ethics Committee)



Prof. Davis Mumbengegwi (Head, Multidisciplinary Research)

Appendix Table 4.1: Normality Test for Total Phenolic Content

Tests of Normality

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
TPC (mgGAE/100g)	,198	24	,016	,841	24	,001

a. Lilliefors Significance Correction

Appendix Table 4.2: Statistical differences for TPC (mgGAE/100g) between the solvents for the okra extracts.

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig. ^{a,b}	Decision
1	The distribution of TPC (mgGAE/100g) is the same across categories of Solvent.	Independent-Samples Kruskal-Wallis Test	,010	Reject the null hypothesis.

a. The significance level is ,050.

b. Asymptotic significance is displayed.

Appendix Table 4.3: Pairwise comparison between the solvents for TPC

(mgGAE/100g) of okra extracts.

Pairwise Comparisons of Solvent

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
Acetone-Distilled water	-5,375	3,536	-1,520	,128	,385
Acetone-Methanol	10,750	3,536	3,041	,002	,007
Distilled water-Methanol	5,375	3,536	1,520	,128	,385

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix Table 4.4: Statistical difference for TPC (mgGAE/100g) between the different treatments of okra extracts.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig. ^{a,b}	Decision
1	The distribution of TPC (mgGAE/100g) is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	,488	Retain the null hypothesis.

a. The significance level is ,050.

b. Asymptotic significance is displayed.

Appendix Table 4.5: DPPH free radical scavenging activity (percentage inhibition)

of different samples of okra extracts at different concentrations, extracted with methanol.

Concentration µg/mL	2.6	5.2	10.3	20.6	41.3	82.5	165	330
Ascorbic Acid	79%	84%	87%	90%	92%	96%	98%	100%
T ₀	64%	65%	66%	67%	67%	79%	84%	92%
KC	64%	65%	65%	66%	67%	70%	77%	89%
BL	57%	60%	65%	65%	66%	73%	73%	91%
CM	61%	65%	67%	68%	70%	74%	76%	89%
KC-BL	66%	66%	66%	68%	69%	73%	81%	87%
KC-CM	59%	66%	66%	67%	68%	69%	73%	88%
BL-CM	60%	65%	67%	69%	71%	71%	75%	88%
KC-BL-CM	65%	65%	66%	67%	68%	69%	71%	85%

Appendix Table 4.6: DPPH free radical scavenging activity (percentage inhibition)

of different samples of okra extracts at different concentrations, extracted with acetone

Concentration µg/mL	2.6	5.2	10.3	20.6	41.3	82.5	165	330
Ascorbic Acid	79%	84%	87%	90%	92%	96%	98%	100%
T ₀	0%	2%	52%	56%	60%	62%	64%	69%
KC	0%	35%	50%	58%	61%	63%	64%	66%
BL	0%	34%	53%	55%	59%	62%	64%	64%
CM	0%	28%	49%	51%	55%	60%	65%	66%
KC-BL	0%	14%	39%	44%	43%	46%	49%	59%
KC-CM	0%	16%	37%	43%	45%	50%	50%	51%
BL-CM	0%	0%	30%	38%	43%	44%	48%	52%
KC-BL-CM	0%	2%	32%	42%	42%	47%	49%	49%

Appendix Table 4.7: DPPH free radical scavenging activity (percentage inhibition)

of different samples of okra extracts at different concentrations, extracted with distilled water.

Concentration µg/mL	2.6	5.2	10.3	20.6	41.3	82.5	165	330
Ascorbic Acid	79%	84%	87%	90%	92%	96%	98%	100%
T ₀	31%	36%	36%	39%	40%	41%	57%	60%
KC	35%	35%	37%	40%	42%	43%	56%	69%
BL	14%	14%	24%	29%	31%	39%	43%	61%
CM	28%	36%	37%	38%	39%	43%	56%	58%
KC-BL	16%	26%	27%	30%	32%	34%	37%	56%
KC-CM	20%	21%	30%	30%	35%	43%	43%	63%
BL-CM	8%	17%	18%	20%	23%	25%	27%	59%
KC-BL-CM	13%	13%	16%	23%	23%	30%	41%	61%

Appendix Table 4.8: Pairwise Comparisons of DPPH Percentage inhibition between solvents

Pairwise Comparisons of Solvent					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
Distilled Water-Acetone	18,514	10,413	1,778	,075	,226
Distilled Water-Methanol	91,549	10,413	8,791	<,001	,000
Acetone-Methanol	73,035	10,413	7,013	<,001	,000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix Table 4.9: Pairwise Comparisons of DPPH% % inhibition between the treatments

Pairwise Comparisons of Treatment					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
BL-CM-KC-BL-CM	-1,375	18,037	-,076	,939	1,000
BL-CM-KC-BL	8,208	18,037	,455	,649	1,000
BL-CM-KC-CM	8,646	18,037	,479	,632	1,000
BL-CM-BL	12,771	18,037	,708	,479	1,000
BL-CM-CM	22,813	18,037	1,265	,206	1,000
BL-CM-To	24,500	18,037	1,358	,174	1,000
BL-CM-KC	26,354	18,037	1,461	,144	1,000
BL-CM-Ascorbic acid	-117,146	18,037	-6,495	<,001	,000
KC-BL-CM-KC-BL	6,833	18,037	,379	,705	1,000
KC-BL-CM-KC-CM	7,271	18,037	,403	,687	1,000
KC-BL-CM-BL	11,396	18,037	,632	,528	1,000
KC-BL-CM-CM	21,438	18,037	1,189	,235	1,000
KC-BL-CM-To	23,125	18,037	1,282	,200	1,000
KC-BL-CM-KC	24,979	18,037	1,385	,166	1,000
KC-BL-CM-Ascorbic acid	-115,771	18,037	-6,419	<,001	,000

KC-BL-KC-CM	-,438	18,037	-,024	,981	1,000
KC-BL-BL	4,563	18,037	,253	,800	1,000
KC-BL-CM	14,604	18,037	,810	,418	1,000
KC-BL-To	16,292	18,037	,903	,366	1,000
KC-BL-KC	18,146	18,037	1,006	,314	1,000
KC-BL-Ascorbic acid	-108,938	18,037	-6,040	<,001	,000
KC-CM-BL	4,125	18,037	,229	,819	1,000
KC-CM-CM	14,167	18,037	,785	,432	1,000
KC-CM-To	15,854	18,037	,879	,379	1,000
KC-CM-KC	17,708	18,037	,982	,326	1,000
KC-CM-Ascorbic acid	-108,500	18,037	-6,016	<,001	,000
BL-CM	-10,042	18,037	-,557	,578	1,000
BL-To	11,729	18,037	,650	,516	1,000
BL-KC	13,583	18,037	,753	,451	1,000
BL-Ascorbic acid	-104,375	18,037	-5,787	<,001	,000
CM-To	1,688	18,037	,094	,925	1,000
CM-KC	3,542	18,037	,196	,844	1,000
CM-Ascorbic acid	-94,333	18,037	-5,230	<,001	,000
To-KC	-1,854	18,037	-,103	,918	1,000
To-Ascorbic acid	-92,646	18,037	-5,137	<,001	,000
KC-Ascorbic acid	-90,792	18,037	-5,034	<,001	,000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix Table 4.10: Alpha-amylase inhibitory activity (percentage inhibition) of different samples of okra extracts at different concentrations, extracted with methanol

Concentration µg/mL	19.53	39.06	78.13	156.25	312.5	625	1250	2500
Acarbose	89%	91%	92%	94%	96%	97%	99%	100%
To	58%	68%	69%	71%	71%	75%	76%	79%
KC	59%	72%	74%	74%	74%	77%	79%	80%
BL	62%	70%	71%	72%	74%	74%	75%	78%
CM	67%	70%	72%	72%	73%	75%	75%	76%
KC-BL	84%	86%	88%	88%	90%	90%	91%	92%
KC-CM	82%	85%	86%	86%	88%	89%	89%	90%
BL-CM	85%	85%	86%	87%	88%	90%	91%	91%

KC-BL-CM	81%	82%	84%	86%	87%	89%	90%	91%
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Appendix Table 4.11: Alpha-amylase inhibitory activity (percentage inhibition) of different samples of okra extracts at different concentrations, extracted with acetone

Concentration µg/mL	19.53	39.06	78.13	156.25	312.5	625	1250	2500
Acarbose	89%	91%	92%	94%	96%	97%	99%	100%
T ₀	0%	64%	76%	78%	80%	81%	87%	88%
KC	0%	77%	79%	80%	81%	82%	82%	87%
BL	0%	80%	82%	82%	83%	85%	86%	87%
CM	0%	77%	78%	78%	81%	81%	84%	86%
KC-BL	0%	70%	79%	81%	81%	85%	87%	90%
KC-CM	0%	76%	76%	78%	82%	85%	86%	88%
BL-CM	0%	73%	78%	81%	82%	83%	85%	88%
KC-BL-CM	0%	77%	78%	79%	81%	82%	85%	85%

Appendix Table 4.12: Alpha-amylase inhibitory activity (percentage inhibition) of different samples of okra extracts at different concentrations, extracted with distilled water.

Concentration µg/mL	19.53	39.06	78.13	156.25	312.5	625	1250	2500
Acarbose	89%	91%	92%	94%	96%	97%	99%	100%
T ₀	50%	68%	70%	74%	75%	76%	81%	85%
KC	71%	72%	73%	74%	76%	77%	80%	81%
BL	58%	67%	72%	73%	76%	78%	79%	79%
CM	18%	71%	76%	77%	77%	79%	80%	81%
KC-BL	54%	78%	79%	81%	82%	83%	85%	88%
KC-CM	12%	42%	62%	71%	78%	81%	81%	82%
BL-CM	50%	60%	68%	76%	80%	80%	82%	84%
KC-BL-CM	66%	71%	74%	76%	78%	80%	80%	83%

Appendix Table 4.13: Pairwise Comparisons of Alpha-amylase % inhibition

between solvents.

Pairwise Comparisons of Solvent					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
Distilled water-Acetone	27,083	10,408	2,602	,009	,028
Distilled water-Methanol	31,083	10,408	2,986	,003	,008
Acetone-Methanol	4,000	10,408	,384	,701	1,000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix Table 4.14: Pairwise Comparisons of Alpha-amylase % inhibition

between treatments.

Pairwise Comparisons of Treatment					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
To-CM	-5,229	18,028	-,290	,772	1,000
To-BL	-9,083	18,028	-,504	,614	1,000
To-KC	-10,333	18,028	-,573	,567	1,000
To-KC-CM	-44,646	18,028	-2,476	,013	,478
To-KC-BL-CM	-45,208	18,028	-2,508	,012	,438
To-BL-CM	-50,542	18,028	-2,804	,005	,182
To-KC-BL	-65,625	18,028	-3,640	<,001	,010
To-Acarbose	-134,583	18,028	-7,465	<,001	,000
CM-BL	3,854	18,028	,214	,831	1,000
CM-KC	5,104	18,028	,283	,777	1,000
CM-KC-CM	-39,417	18,028	-2,186	,029	1,000
CM-KC-BL-CM	-39,979	18,028	-2,218	,027	,957
CM-BL-CM	-45,313	18,028	-2,513	,012	,430
CM-KC-BL	-60,396	18,028	-3,350	<,001	,029
CM-Acarbose	-129,354	18,028	-7,175	<,001	,000
BL-KC	1,250	18,028	,069	,945	1,000

BL-KC-CM	-35,563	18,028	-1,973	,049	1,000
BL-KC-BL-CM	-36,125	18,028	-2,004	,045	1,000
BL-BL-CM	-41,458	18,028	-2,300	,021	,773
BL-KC-BL	-56,542	18,028	-3,136	,002	,062
BL-Acarbose	-125,500	18,028	-6,961	<,001	,000
KC-KC-CM	-34,313	18,028	-1,903	,057	1,000
KC-KC-BL-CM	-34,875	18,028	-1,934	,053	1,000
KC-BL-CM	-40,208	18,028	-2,230	,026	,926
KC-KC-BL	-55,292	18,028	-3,067	,002	,078
KC-Acarbose	-124,250	18,028	-6,892	<,001	,000
KC-CM-KC-BL-CM	-,563	18,028	-,031	,975	1,000
KC-CM-BL-CM	-5,896	18,028	-,327	,744	1,000
KC-CM-KC-BL	20,979	18,028	1,164	,245	1,000
KC-CM-Acarbose	-89,938	18,028	-4,989	<,001	,000
KC-BL-CM-BL-CM	5,333	18,028	,296	,767	1,000
KC-BL-CM-KC-BL	20,417	18,028	1,132	,257	1,000
KC-BL-CM-Acarbose	-89,375	18,028	-4,958	<,001	,000
BL-CM-KC-BL	15,083	18,028	,837	,403	1,000
BL-CM-Acarbose	-84,042	18,028	-4,662	<,001	,000
KC-BL-Acarbose	-68,958	18,028	-3,825	<,001	,005

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix Table 4.15: Alpha-glucosidase inhibitory activity (percentage inhibition)

of different samples of okra extracts at different concentrations, extracted with methanol.

Concentration µg/mL	19.53	39.06	78.13	156.25	312.5	625	1250	2500
Acarbose	89%	90%	91%	93%	95%	96%	99%	100%
T ₀	49%	62%	64%	67%	72%	74%	75%	79%
KC	38%	63%	69%	72%	74%	75%	77%	79%
BL	28%	62%	65%	70%	73%	75%	77%	77%
CM	22%	60%	65%	68%	69%	72%	74%	75%
KC-BL	52%	59%	71%	74%	79%	83%	85%	87%
KC-CM	27%	66%	75%	80%	84%	84%	86%	88%
BL-CM	2%	62%	72%	81%	82%	85%	85%	88%

KC-BL-CM	22%	63%	74%	81%	84%	85%	85%	87%
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Appendix Table 4.16: Alpha-glucosidase inhibitory activity (percentage inhibition)

of different samples of okra extracts at different concentrations, extracted with acetone.

Concentration µg/mL	19.53	39.06	78.13	156.25	312.5	625	1250	2500
Acarbose	89%	90%	91%	93%	95%	96%	99%	100%
T ₀	0%	0%	0%	27%	68%	72%	79%	82%
KC	0%	3%	19%	47%	73%	77%	81%	85%
BL	0%	0%	20%	51%	72%	77%	82%	84%
CM	0%	0%	25%	46%	69%	81%	82%	84%
KC-BL	0%	3%	26%	52%	72%	79%	82%	86%
KC-CM	0%	9%	12%	48%	74%	81%	82%	85%
BL-CM	0%	0%	0%	4%	43%	73%	77%	82%
KC-BL-CM	0%	0%	0%	12%	56%	76%	79%	83%

Appendix Table 4.17: Alpha-glucosidase inhibitory activity (percentage inhibition)

of different samples of okra extracts at different concentrations, extracted with distilled water.

Concentration µg/mL	19.53	39.06	78.13	156.25	312.5	625	1250	2500
Acarbose	89%	90%	91%	93%	95%	96%	99%	100%
T ₀	0%	21%	70%	73%	79%	83%	85%	89%
KC	14%	50%	77%	79%	80%	83%	84%	88%
BL	10%	17%	63%	76%	78%	82%	84%	87%
CM	11%	45%	71%	77%	81%	82%	84%	87%
KC-BL	29%	51%	70%	80%	83%	85%	85%	86%
KC-CM	12%	50%	73%	79%	82%	85%	86%	86%
BL-CM	34%	64%	72%	82%	84%	85%	86%	86%
KC-BL-CM	32%	59%	75%	79%	83%	84%	85%	86%

Appendix Table 4.18: Pairwise Comparisons of Alpha-glucosidase % inhibition

between solvents.

Pairwise Comparisons of Solvent					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
Acetone-Methanol	28,035	10,409	2,693	,007	,021
Acetone-Distilled water	-40,403	10,409	-3,881	<,001	,000
Methanol-Distilled water	-12,368	10,409	-1,188	,235	,704

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix Table 4.19: Pairwise Comparisons of Alpha-glucosidase % Inhibition

between treatments.

Pairwise Comparisons of Treatment					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
To-CM	-3,938	18,030	-,218	,827	1,000
To-BL	-4,313	18,030	-,239	,811	1,000
To-KC	-11,146	18,030	-,618	,536	1,000
To-BL-CM	-18,458	18,030	-1,024	,306	1,000
To-KC-BL-CM	-20,667	18,030	-1,146	,252	1,000
To-KC-BL	-22,854	18,030	-1,268	,205	1,000
To-KC-CM	-27,021	18,030	-1,499	,134	1,000
To-Acarbose	-121,479	18,030	-6,738	<,001	,000
CM-BL	,375	18,030	,021	,983	1,000
CM-KC	7,208	18,030	,400	,689	1,000
CM-BL-CM	-14,521	18,030	-,805	,421	1,000
CM-KC-BL-CM	-16,729	18,030	-,928	,353	1,000
CM-KC-BL	-18,917	18,030	-1,049	,294	1,000
CM-KC-CM	-23,083	18,030	-1,280	,200	1,000
CM-Acarbose	-117,542	18,030	-6,519	<,001	,000
BL-KC	6,833	18,030	,379	,705	1,000

BL-BL-CM	-14,146	18,030	-,785	,433	1,000
BL-KC-BL-CM	-16,354	18,030	-,907	,364	1,000
BL-KC-BL	-18,542	18,030	-1,028	,304	1,000
BL-KC-CM	-22,708	18,030	-1,260	,208	1,000
BL-Acarbose	-117,167	18,030	-6,499	<,001	,000
KC-BL-CM	-7,313	18,030	-,406	,685	1,000
KC-KC-BL-CM	-9,521	18,030	-,528	,597	1,000
KC-KC-BL	-11,708	18,030	-,649	,516	1,000
KC-KC-CM	-15,875	18,030	-,880	,379	1,000
KC-Acarbose	-110,333	18,030	-6,120	<,001	,000
BL-CM-KC-BL-CM	-2,208	18,030	-,122	,903	1,000
BL-CM-KC-BL	4,396	18,030	,244	,807	1,000
BL-CM-KC-CM	8,563	18,030	,475	,635	1,000
BL-CM-Acarbose	-103,021	18,030	-5,714	<,001	,000
KC-BL-CM-KC-BL	2,188	18,030	,121	,903	1,000
KC-BL-CM-KC-CM	6,354	18,030	,352	,725	1,000
KC-BL-CM-Acarbose	-100,813	18,030	-5,591	<,001	,000
KC-BL-KC-CM	-4,167	18,030	-,231	,817	1,000
KC-BL-Acarbose	-98,625	18,030	-5,470	<,001	,000
KC-CM-Acarbose	-94,458	18,030	-5,239	<,001	,000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,050.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix Table 4.20: Inhibition zones of Okra extracts observed after 24 hours of incubation via the disc diffusion method

Samples	Test microorganisms						
	<i>E.coli</i>	<i>S. sonnei</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>M. avium</i>
Nitrofurantoin	16.00±0.00	15±0.00	14±0.00	23±0.00	13±0.00	18±0.00	14±0.00
Acetone							
TO	9.00±0.58	0.00±0.00	7.00±0.00	8.00±0.00	10.00±0.00	6.33±0.58	6.67±0.00
KC	8.00±0.00	0.00±0.00	10.33±0.00	8.33±0.00	7.67±0.58	7.00±0.00	8.67±0.58
BL	8.33±0.58	0.00±0.00	9.00±1.00	9.33±0.58	10.00±0.58	8.00±0.00	6.00±0.00
CM	8.67±0.58	0.00±0.00	7.33±0.58	8.33±0.58	9.00±0.00	8.00±0.58	10.00±0.58

KC-BL	9.33±0.00	0.00±0.00	7.33±0.58	8.00±0.00	6.67±0.58	8.33±0.58	10.00±0.00
KC-CM	7.00±0.58	0.00±0.00	8.33±0.58	6.00±0.00	10.00±0.00	9.00±0.00	8.00±0.58
BL-CM	6.33±0.58	0.00±0.00	7.67±1.00	8.00±0.00	10.00±0.58	9.00±0.58	7.00±0.58
KC-BL-CM	8.67±0.58	0.00±0.00	8.33±0.00	8.67±0.58	12±0.58	7.67±0.58	8.00±0.58
Methanol							
T0	9.00±0.58	10.00±0.00	8.33±0.58	9.33±0.58	8.00±0.00	10±0.00	7.00±0.00
KC	7.00±0.58	8.00±0.00	8.00±0.58	7.00±0.00	7.33±0.58	10.00±0.00	7.00±0.00
BL	9.00±0.00	6.67±0.58	11.33±0.58	8.00±0.00	11.33±0.58	10.33±0.58	10.00±0.00
CM	7.00±0.00	8.00±0.00	7.00±0.00	6.33±0.58	7.00±0.00	6.00±0.00	7.00±0.00
KC-BL	0.00±0.00	6.00±0.00	8.67±0.58	7.67±0.58	8.00±0.00	8.00±0.00	8.00±0.00
KC-CM	7.00±0.00	6.00±0.00	8.00±0.00	6.33±0.58	8.00±0.00	8.00±0.00	8.00±0.00
BL-CM	0.00±0.00	7.67±0.58	8.00±0.00	9.67±0.58	10.00±0.00	0.00±0.00	13.00±0.00
KC-BL-CM	10.67±1.00	9.33±0.58	6.67±0.58	7.67±0.58	7.33±0.58	0.00±0.00	7.00±0.00
Distilled Water							
T0	8.00±0.58	7.00±0.00	9.33±0.58	7.33±0.58	7.33±0.58	8.00±0.00	7.00±0.00
KC	7.00±0.58	7.00±0.58	8.00±0.00	7.00±0.00	7.33±0.58	7.33±0.58	6.00±0.00
BL	8.33±0.58	7.00±0.00	7.33±0.58	7.33±0.58	6.00±0.00	7.00±0.58	6.00±0.00
CM	8.33±0.58	7.33±0.58	7.33±0.58	7.00±0.00	6.00±0.00	8.00±0.00	6.00±0.00
KC-BL	8.67±0.58	8.33±0.58	8.67±0.58	6.67±0.58	7.33±0.58	8.33±0.58	7.00±0.00
KC-CM	8.00±0.00	6.33±0.58	8.33±0.58	6.00±0.00	6.33±0.58	6.67±0.58	6.67±0.58
BL-CM	6.33±0.58	6.00±0.00	6.00±0.00	7.67±0.58	7.00±0.00	6.67±0.58	7.00±0.00
KC-BL-CM	8.00±0.00	7.00±0.00	6.67±0.58	7.67±0.58	7.00±0.00	7.33±0.58	8.00±0.00

Appendix Table 4.21: Minimal Inhibitory Concentration (MIC) in mg/ml of Okra pod extracts against selected human pathogens.

Samples	Test microorganisms						
	<i>E.coli</i>	<i>S. sonnei</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>M. avium</i>
Acetone							
T0	>2.5	-	> 2.5	> 1.25	> 1.25	> 1.25	> 5
KC	> 5	-	> 5	> 5	> 1.25	> 1.25	> 2.5
BL	> 5	-	> 5	> 5	> 1.25	> 2.5	> 2.5
CM	> 5	-	> 2.5	> 2.5	> 5	> 5	> 2.5
KC-BL	> 2.5	-	> 5	> 5	> 1.25	> 1.25	> 2.5
KC-CM	> 2.5	-	> 5	> 5	> 2.5	> 1.25	> 2.5
BL-CM	> 2.5	-	> 5	> 5	> 5	> 1.25	> 1.25
KC-BL-CM	> 2.5	-	> 5	> 5	> 5	> 5	> 1.25
Methanol							

T0	> 2.5	> 2.5	> 5	> 2.5	> 5	> 2.5	> 5
KC	> 1.25	> 1.25	> 5	> 2.5	> 2.5	> 2.5	> 2.5
BL	> 1.25	> 1.25	> 2.5	> 5	> 5	> 2.5	> 1.25
CM	> 1.25	> 1.25	> 2.5	> 5	> 5	> 2.5	> 5
KC-BL	-	> 2.5	> 5	> 2.5	> 2.5	> 2.5	> 2.5
KC-CM	> 5	> 2.5	> 5	> 5	> 1.25	> 2.5	> 2.5
BL-CM	-	> 1.25	> 5	> 5	> 5	-	> 1.25
KC-BL-CM	> 2.5	> 5	> 5	> 2.5	> 2.5	-	> 1.25
Distilled Water							
T0	> 10	> 10	> 10	> 10	> 10	> 10	> 10
KC	> 5	> 10	> 10	> 10	> 10	> 10	> 10
BL	> 5	> 10	> 10	> 10	> 10	> 10	> 10
CM	> 10	> 10	> 10	> 10	> 10	> 10	> 10
KC-BL	> 10	> 10	> 10	> 10	> 10	> 10	> 10
KC-CM	> 10	> 10	> 10	> 10	> 10	> 10	> 10
BL-CM	> 10	> 10	> 10	> 10	> 10	> 10	> 10
KC-BL-CM	> 10	> 10	> 10	> 10	> 10	> 10	> 10

Appendix Table 4.22: Minimal Bactericidal / Fungicidal concentration (MBC/ MFC) in mg/ml of Okra pod extracts against selected human pathogens.

Samples	Test microorganisms						
	<i>E.coli</i>	<i>S. sonnei</i>	<i>S. typhi</i>	<i>C. albicans</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>M. avium</i>
Acetone							
T0	10; Static	-	10; Static	1.25; Cidal	10; Static	10; Cidal	10; Cidal
KC	10; Cidal	-	5; Cidal	5; Cidal	10; Static	10; Cidal	5; Cidal
BL	10; Static	-	10; Cidal	5; Cidal	10; Static	10; Static	5; Cidal
CM	10; Cidal	-	10; Cidal	5; Cidal	10; Cidal	10; Static	5; Cidal
KC-BL	5; Cidal	-	5; Cidal	5; Cidal	10; Static	10; Cidal	10; Static
KC-CM	10; Cidal	-	5; Cidal	5; Cidal	10; Static	10; Cidal	5; Cidal
BL-CM	10; Cidal	-	10; Cidal	5; Cidal	10; Static	10 Static	10; Cidal
KC-BL-CM	5; Cidal	-	5; Cidal	5; Cidal	10; Cidal	10; Static	2.5; Cidal
Methanol							
T0	10; Cidal	10; Cidal	5; Cidal	2.5; Cidal	5; Cidal	10; Cidal	10; Cidal
KC	10; Cidal	10; Cidal	5; Cidal	5; Cidal	10; Cidal	10; Cidal	2.5; Cidal
BL	5; Cidal	10; Cidal	10; Cidal	5; Cidal	10; Static	10; Static	5; Cidal
CM	10; Cidal	10; Static	10; Cidal	5; Cidal	10; Cidal	10; Static	5; Cidal
KC-BL	-	10; Cidal	5; Cidal	2.5; Cidal	5; Cidal	10; Cidal	10; Cidal
KC-CM	10; Cidal	5; Cidal	5; Cidal	5; Cidal	10; Cidal	10; Cidal	5; Cidal

BL-CM	-	5; Cidal	10; Cidal	5; Cidal	10;Cidal	-	10; Cidal
KC-BL-CM	5; Cidal	10; Cidal	5; Cidal	2.5; Cidal	10; Cidal	-	2.5; Cidal
Distilled Water							
T0	10; Static	10;Static	10; Static	10;Static	10;Static	10; Static	10;Static
KC	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static
BL	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static
CM	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static
KC-BL	10; Static	10;Static	10; Static	10;Static	10;Static	10; Static	10;Static
KC-CM	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static
BL-CM	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static
KC-BL-CM	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static	10; Static

CHAPTER 5: GENERAL CONCLUSION AND RECOMMENDATIONS

This study highlights the promising potential of plant growth-promoting endophytic bacterial (PGPEB) consortia, particularly combinations such as *K. cowanii* with *B. licheniformis* (KC-BL) and *C. metallidurans* (KC-CM), to significantly improve okra productivity, seedling vigour, and bioactive compound accumulation under controlled conditions. The observed enhancement in germination indices, total phenolic content, antioxidant capacity, and enzyme inhibitory activities underscores the synergistic capabilities of microbial partnerships in stimulating secondary metabolite biosynthesis, likely via the activation of phenylpropanoid pathways and stress-response mechanisms. Notably, methanolic extracts of inoculated plants demonstrated superior antimicrobial efficacy, further validating okra's therapeutic potential as a functional food with activity against pathogens such as *S. typhi*, *E. coli*, *S. aureus*, and *C. albicans*. These results reinforce the value of bio-inoculants as sustainable agricultural inputs that not only boost crop resilience and nutraceutical quality but also contribute to the development of alternative therapies amid growing global health challenges. The functional traits of the inoculated strains, including ACC deaminase activity, nitrogen fixation, and IAA production, play crucial roles in enhancing stress tolerance and metabolite accumulation.

Future research should expand on these findings by conducting metabolic profiling of plant-microbe interactions and validating the efficacy of these consortia through long-term field trials, particularly under abiotic stress conditions such as salinity and drought. Building on recent advances in seed microbiome research, such studies will be essential for optimising inoculant formulations and ensuring consistency across diverse agro-ecological settings. Furthermore, advocacy and policy support are needed

to mainstream microbial bio-stimulants in sustainable agriculture, thereby promoting food security, environmental stewardship, and public health.