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Investigation of *Bacillus zanthoxyli* UMK-PNF6 inoculation effect on paddy's growth, plant salinity stress tolerance, and its antimicrobial activities

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DECLARATION

I declare that this thesis entitled Investigation of *Bacillus zanthoxyli* UMK-PNF6 inoculation effect on paddy's growth, plant salinity stress tolerance, and its antimicrobial activities is the result of my research except as cited in the references.

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Investigation Of *Bacillus zanthoxyli* UMK-PNF6 Bacterial Inoculation Effect On Paddy's Growth, Plant Salinity Stress Tolerance, And Its Antimicrobial Activities

ABSTRACT

Bacillus zanthoxyli UMK-PNF6 a Plant Growth-Promoting Rhizobacteria (PGPR), is found in the rhizosphere, the area of soil that is directly impacted by plant roots. *Bacillus zanthoxyli* UMK-PNF6 is vital for boosting plant health and growth through a wide variety of methods. The key contributions of PGPR include nitrogen fixation, phosphate solubilization, enhanced stress tolerance, and biocontrol activity. Currently, salinity stress is a significant contributor, impacting roughly half of all irrigated land and 20% of all cultivated land, resulting in agricultural land degradation issues. Therefore, the current study is to investigate the potential of bacterial inoculation with *Bacillus zanthoxyli* UMK-PNF6 to promote paddy growth, improve plant tolerance to salinity stress, and display antimicrobial activity. In this experiment, paddy plants are treated with *Bacillus zanthoxyli* UMK-PNF6, and several growth parameters were observed such as chlorophyll content, leaf length grown (cm), and moisture content (%). The findings show that paddy growth parameters significantly improved after bacterial inoculation. Additionally, compared to untreated plants in saline circumstances, the paddy inoculated with *Bacillus zanthoxyli* UMK-PNF6 exhibits increased tolerance to salinity stress, as demonstrated by a higher chlorophyll content.

Keywords: PGPR, salinity stress, paddy growth

Penyiasatan *Bacillus zanthoxyli* UMK-PNF6 Kesan Inokulasi Bakteria Terhadap Pertumbuhan Padi, Toleransi Tekanan Saliniti Tumbuhan, Dan Aktiviti Antimikrobnya

ABSTRAK

Bacillus zanthoxyli UMK-PNF6 merupakan Rhizobakteria Penggalak Pertumbuhan Tumbuhan (PGPR), terdapat di rizosfera, kawasan tanah yang terjejas secara langsung oleh akar tumbuhan. *Bacillus zanthoxyli* UMK-PNF6 adalah penting untuk meningkatkan kesihatan dan pertumbuhan tumbuhan melalui pelbagai kaedah. Sumbangan utama PGPR termasuk penetapan nitrogen, pelarutan fosfat, toleransi tekanan yang dipertingkatkan dan aktiviti kawalan bio. Pada masa ini, tekanan kemasinan merupakan penyumbang penting, memberi kesan kepada kira-kira separuh daripada semua tanah pengairan dan 20% daripada semua tanah yang ditanam, mengakibatkan isu kemerosotan tanah pertanian. Oleh itu, kajian semasa adalah untuk menyiasat potensi inokulasi bakteria dengan *Bacillus zanthoxyli* UMK-PNF6 untuk menggalakkan pertumbuhan padi, meningkatkan toleransi tumbuhan terhadap tekanan kemasinan, dan memaparkan aktiviti antimikrob. Dalam eksperimen ini, pokok padi dirawat dengan *Bacillus zanthoxyli* UMK-PNF6, dan beberapa parameter pertumbuhan diperhatikan seperti kandungan klorofil, panjang daun tumbuh (cm), dan kandungan lembapan (%). Dapatan kajian menunjukkan bahawa parameter pertumbuhan padi meningkat dengan ketara selepas inokulasi bakteria. Di samping itu, berbanding dengan tumbuhan yang tidak dirawat dalam keadaan masin, padi yang disuntik dengan *Bacillus zanthoxyli* UMK-PNF6 mempamerkan peningkatan toleransi terhadap tekanan kemasinan, seperti yang ditunjukkan oleh kandungan klorofil yang lebih tinggi.

Kata kunci: PGPR, tekanan kemasinan, pertumbuhan padi

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LIST OF ABBREVIATIONS

B. zanthoxyli	*Bacillus zanthoxyli*
NaCI	Sodium Chloride
ATP	Adenosine Triphosphate
Sp.	Species
RNA	Ribonucleic Acid
E. coli	*Escherichia coli*
PGPR	Plant Growth-Promoting Rhizobacteria

LIST OF SYMBOLS

g	Gram
°C	Degree Celsius
µm	Micrometre
µl	Microlitre
mL	Millilitre
lbs	Pounds
g/mL	Gram per millilitre
g/L	Gram per litre
rpm	Revolution per minute
M	Molarity
mM	Millimolar
<	Less than
%	Percentage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In developing nations, agriculture serves as the foundation of the economic system and plays a significant part in the economy. Agriculture has long been associated with the production of essential food crops. Today's farming includes raising cattle, fruit, dairy, forestry, beekeeping, and others (Ismail, 2021). Recently, each country has faced the loss of agricultural land largely due to land degradation caused by erosion and climate change. Salt stress is considered an alarming issue to address when it can lower soil agricultural productivity and lowers crop yields. It is proven that 20% of all cultivated land and over half of all irrigated land are negatively impacted by salt stress, which reduces production below the genetic potential (Mahmood-ur-Rahman et al., 2019) as salt stress can influence plant productivity through modifications in respiration and photosynthesis.

Besides, plant pathogens comprising viruses, bacteria, fungi, and parasitic plants are one of the most significant factors to cause severe loss in terms of production in the agriculture sector (Al-Ani & Furtado, 2020). Plant pathogens have the potential to produce several mycotoxins which poses an issue of concern. These mycotoxins are often harmful to humans and livestock and are linked to a particular species (Schasteen, 2023). Hence, plant pathogens are becoming a major concern for economic and social stability.

Bacillus zanthoxyli is characterized as gram-stain positive, aerobic, asporogenous, rod-shaped, motile, and peritrichous flagella (M. Li et al., 2017). *Bacillus zanthoxyli* HS1 strain

is a promising option for regulating various stressors on vegetable plants. Tomato and paprika plants are shielded from infection by the soil drench of *B. zanthoxyli* HS1 against *Ralstonia solanacearum* and *Phytophthora capsici*, respectively. Following the application of a high saline solution, the root and shoot growths of tomato, cucumber, and cabbage plants treated with *B. zanthoxyli* HS1 are also greater than those of mock-treated plants. Furthermore, *B. zanthoxyli* HS1 pretreatment of cabbage plants prevents high salt stressors from degrading chloroplast pigments. These results imply that *B. zanthoxyli* HS1 stain prevents the development of diseases and provides vegetable plants resistance to salinity stress (Usmonov et al., 2021).

The characteristics of *Bacillus zanthoxyli are* known to be beneficial to the agricultural industry. With the challenges faced in the agricultural industry such as land limitation and climate change, the presence of *Bacillus zanthoxyli* able to make the plant grow efficiently especially to make the plant to be tougher and sturdier against harsh surroundings. For instance, plants can grow in high salt-content soil. This can be achieved through the inoculation of *Bacillus zanthoxyli* onto the seedlings.

1.2 Problem Statement

Erosion and climate change are the reasons behind the loss of agricultural land issues faced by each country nowadays. However, some cultivated land is facing issues with low production below the genetic potential which is caused by salt stress. Salinity in soil can be evoked by a few aspects notably poor drainage, inefficient irrigation, and an excessive build-up of soluble salt in the soil, which eventually inhibits crop growth and leads to crop death (Mahmood-ur-Rahman et al., 2019). The major responses of plants to this stress are their life cycle, including seed germination, seedling growth, vegetative growth, flowering, and

deterioration of photosynthetic function caused by ion toxicity, osmotic pressure, oxidative damage, and nutritional deficiencies (Mohamed et al., 2022).

The second problem to address is plant pathogen or soil-borne disease. Plant pathogens may result in diseases in the leaves, stems, roots, vascular system, and fruit. (Al-Ani & Furtado, 2020) which involves infection, colonization, and pathogen reproduction which is referred to as pathogenesis (Leach et al., 2014). Plant diseases establish close bonds with their hosts to obtain the nutrients necessary for their growth, survival, and reproduction. The plant pathogens disrupted the plant's vascular system, resulting in poor plant productivity, wilting, decreased energy production, and overall plant health. Consequently, plant diseases significantly lower the production of crops which unconsciously leads to threatening food security with the deterioration of wheat, corn, rice, barley, bran, flour, soybeans, peanuts, and other seeds. It is also reported as a harmful pathogen to humans and animals. In addition, food prices rise because of crop failures. Therefore, the predicament can be classified as a global challenge. Many initiatives have been taken to alleviate this crisis, somehow a more sustainable proposition is vital.

In addition, *Bacillus zanthoxyli* UMK-PNF6 is a novel bacterial strain with potential applications in various fields, such as bioremediation, agriculture, and biopharmaceuticals. However, despite its promising characteristics, there is a lack of comprehensive understanding regarding its genetic makeup, metabolic pathways, and practical utility. Therefore, addressing these knowledge gaps is essential to fully exploit the capabilities of *Bacillus zanthoxyli* UMK-PNF6 at the same time facilitate its integration into biotechnological processes effectively.

1.3 Objectives

This research is carried out with the following objectives:

I. To evaluate the effect of the inoculation of locally isolated *Bacillus zanthoxyli* UMK-PNF6 in soil on paddy's growth.

II. To evaluate of paddy's growth inoculated with *Bacillus zanthoxyli* UMK-PNF6 in soil on its tolerance against salinity stress.

III. To determine the antimicrobial activity of *Bacillus zanthoxyli* UMK-PNF6 against Gram-positive and Gram-negative bacteria.

1.4 Scope of Study

This study was conducted using the bacterial strain *Bacillus zanthoxyli* UMK-PNF6, it is a rhizobacterium isolated from paddy fields by the previous study (Kamaruzaman et al., 2022). For the evaluation of the effect of the inoculation of *Bacillus zanthoxyli* UMK-PNF6 in soil on paddy's growth, it was observed through the performance of paddy's growth after 18 days. Data recorded included chlorophyll content, leaf length grown, and moisture content. Moreover, for the evaluation of paddy inoculated with *Bacillus zanthoxyli* UMK-PNF6 on plant's tolerance against salinity stress. The pot study was run with different concentrations of NaCl in the soil which were 50 mM, 100 mM, and 150 mM. 10 mL of salt solution was sprayed on the plant every 4 days. The evaluation was based on the plant's salinity stress tolerance which was observed from the performance of plant growth. For the evaluation of the antimicrobial activity, two microorganisms were utilized. It was done using the Gram-positive bacterium which is *Bacillus subtilis* meanwhile for the Gram-negative bacterium, *Escherichia coli* was used. The test was conducted using the disc diffusion method.

1.5 Significance of Study

As a result of this study, the effect of *Bacillus zanthoxyli* UMK-PNF6 on plant growth was determined. As it portrays positive results, *Bacillus zanthoxyli* UMK-PNF6 can be substituted as a biofertilizer replacing synthetic fertilizers that can reduce soil microorganisms and cause groundwater pollution (Fazelian & Yousefzadi, 2022). The utilization of biofertilizers can promote plant growth by improving nutrient acquisition (Maan & Garcha, 2021). Moreover, *Bacillus zanthoxyli* UMK-PNF6 on the plant's tolerance against salinity stress was determined. Given that it shows positive results, the implication of *Bacillus zanthoxyli* UMK-PNF6 in plants can be applied which leads to improved plant health and also increases plant resilience under salinity stress. Other than that, the antimicrobial activities of *Bacillus zanthoxyli* UMK-PNF6 were determined. If it shows positive results against microbes, it could potentially be a biological agent to fight against plant pathogens including viruses, bacteria, and fungi. Therefore, it could replace the usage of chemical pesticides. Even though pesticides have their benefits on agriculture, they still severely leave negative impacts on the environment. However using pesticides can contaminate soil, water, turf, and other vegetation (Aktar et al., 2009). Thus, a new sustainable initiative can be invented. Therefore, the usage of chemical pesticides can be cut down. In sync, the aim of sustainable crop production can be achieved. Overall, this research can provide views on possibilities for the use of *Bacillus zanthoxyli* as an antimicrobial agent under salinity stress conditions, at the same time leaving an immense contribution to the agricultural industry. Particularly, it can help improve sustainable crop production, increase yield, and level up the toughness of plants towards the surroundings.

CHAPTER 2

LITERATURE REVIEW

2.1 Plant Growth Promoting Rhizobacteria

Plant growing in the field is a complex community with nuanced and stable partner interactions rather than an individual (Lundberg et al., 2012). Plants are constantly linked to a well-organized and controlled population of microbes (Smith et al., 2015). Every major plant structure, such as leaves, roots, flowers, fruits, and stems is linked to components of the phytomicrobiome, such as bacteria and fungi (Berg et al., 2014).

Plant growth-promoting rhizobacteria (PGPR) is the common term for beneficial bacteria that are free-living in the soil (Kloepper et al., 1989). A class of free-living bacteria known as plant growth-promoting rhizobacteria (PGPR) inhabits the rhizosphere and promotes root growth. Numerous genera of bacteria, with *Bacillus* and *Pseudomonas* species predominating, were identified as PGPR. PGPR has a direct impact on plant growth through the production of phytohormones, solubilizing inorganic phosphates, boosting iron nutrition by chelating siderophores and releasing volatile compounds that affect plant signaling pathways (Podile & Kishore, 2006).

Phosphorus (P) and nitrogen (N) are also essential to plant growth. Biofertilizers, or microbes that help plants acquire nutrients, work in several ways, such as increasing the surface area that plant roots can access, fixing nitrogen, phosphate-solubilization, producing siderophores, and producing hydrogen cyanide (HCN) (Pii et al., 2015). As a result, manipulating microbial activity offers a lot of possibilities for feeding crops the nutrients they

need. Siderophores are produced to dissolve iron from their surroundings and form a complex ferric-siderophore that can diffuse back to the cell surface (Andrews et al., 2003). Specific receptors on the membrane of both Gram-positive and Gram-negative microbes recognize the ferric-siderophore, stimulating the active transport process via the membrane (Boukhalfa & Crumbliss, 2002).

In addition, plant growth and development are largely regulated by phytohormones. In reaction to environmental stimuli that might otherwise restrict plant growth or turn deadly if untreated, they function as biological signals as well (Fahad et al., 2015). Numerous rhizosphere bacteria are known to release hormones that are absorbed by the roots or to alter the plant hormone balance to support growth and resilience to stress. Besides, auxins are produced by several PGPRs and have been shown to have particularly strong effects on the growth of roots (Gupta et al., 2015). It has been shown that the auxin-producing PGPR causes longer roots, increases root biomass, reduces the size and density of stomata (Llorente et al., 2016), and triggers genes that promote plant development through the auxin response. Additionally, it has been demonstrated that they cause transcriptional alterations in genes linked to hormones, defense, and cell walls (Ruzzi & Aroca, 2015).

The hydrolysis of 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC deaminase, which reduces the quantity of ethylene, is also one of the primary methods utilized by PGPR bacteria to relieve stress. ACC deaminase can therefore lessen the effects of salt stress. The benefits of inoculating pea plants with PGPR *Variovorax paradoxus* 5C-2, results in the production of ACC deaminase, as well as improved photosynthetic rate, electron transport, reduced stomatal resistance, and xylem balancing pressure, balanced ion homeostasis through increased K^+ flow to shoots and Na^+ deposition on roots, and increased biomass under salt stress at 70 and 130 mM NaCl. (Wang et al., 2016).

2.2 Bacillus zanthoxyli

Soil is vital for the evolution of life on Earth as it contains both biotic and abiotic components. Since soil is made up of various elements, every type of soil has unique qualities and structures. Because of the interdependence of soil's physical, chemical, and biological characteristics, soil serves as a useful substrate for plant growth in addition to other purposes. It influences the structure, growth, and productivity of the soil in terms of biological qualities (Kalev & Toor, 2018).

The most significant microorganisms are bacteria. The primary functions of microorganisms in paddy soils are methanogenesis, methane oxidation, and elemental biogeochemical cycling (Khoshru et al., 2023). According to research conducted in China, the most prevalent bacterial species are linked to the core microbiota of rice rhizospheres under typical crop management practices. In addition to 15 other genera, *Anaeromyxobacter, Arenimona, Arthrobacter, Bacillus, and Bellilinea* are all recognized to support the development and well-being of rice plants (Zhou et al., 2020).

The genus *Bacillus* is created based on their 16S rRNA gene sequences (Ash et al., 1993). *Bacillus* species are rod-shaped, endospore-forming, and aerobic or facultatively anaerobic. They are widely distributed in nature (Makino, 1986). *Zanthoxyli* of *Zanthoxylum*, referring to the plant *Zanthoxylum* simulants, five endospore-forming, nitrogen-fixing strains were recovered from the rhizosphere soils of *Zanthoxylum* simulants grown in Beijing, China. (Ma et al., 2007). It is reported that there is nitrogenase activity in five newly developed strains in which the enzyme is responsible for catalyzing nitrogen fixation, which is the reduction of nitrogen (N_2) to ammonia (NH_3). Meanwhile the morphology of *Bacillus zanthoxyli*, it is divided into a few aspects such as gram stain, cell length and width, cell shape, motility, and pigment production. It's a rod-shaped gram-positive bacteria with a length of 4-4.8 µm and a width of 0.35-0.4 µm. *Bacillus zanthoxyli* is motile and does not produce pigment (Barberán et

al., 2017). Other than that, *Bacillus zanthoxyli* is classified as halophile where it can tolerate saline conditions such as under sodium chloride NaCl which makes it a promising capability to lessen the negative consequences of salt stress on plants. It is also a mesophilic bacteria that can grow at 4-37 ℃ and optimum at 30 ℃. However, for pH, it can grow at 4.2-10 and optimum at 7.1. In addition, it has oxygen tolerance. It is a very important aspect since paddy is planted in waterlogged soil which the transfer of oxygen is limited. It is known that *Bacillus zanthoxyli* is a facultative anaerobe, where it can grow either with or without free oxygen. When *Bacillus zanthoxyli* is present in an oxygen-rich environment, it produces ATP through aerobic respiration but, when the oxygen content is low, it can obtain the energy it needs to survive through anaerobic respiration or fermentation (Simon, 2013).

Furthermore, *Bacillus zanthoxyli* exhibits the ability to solubilize potassium and phosphate by breaking down insoluble potassium and phosphate compounds, making them more accessible to plants. It also helps to expel the excess Na^+ from aboveground parts and absorb more K+ in roots to maintain ion homeostasis in tall fescue (Y. Li et al., 2022). *Bacillus zanthoxyli* also displays biocontrol abilities against plant infections. It generates antimicrobial agents that stop the spread of harmful infections and defend plants against disease (Usmonov et al., 2021). Additionally, pathogen colonization and dissemination are restricted by *Bacillus zanthoxyli*'s competition with them for resources and available space. By lowering dependency on artificial materials and fostering sustainable pest management techniques, this biocontrol activity provides a sustainable replacement for chemical-based pesticides.

2.3 Plant's Salinity Stress Tolerance and Its Importance

One significant environmental stressor that has an impact on plant growth and development is salt stress. Salt stress raises intracellular osmotic pressure and can lead to a hazardous buildup of sodium (Zhao et al., 2021). Salinity stress has an impact on many

parts of plant growth and ultimately lowers crop yields overall. It influences, among other things, spike development, plant growth and development, seed germination, and reproductive growth (Korenblum et al., 2020). Salt stress negatively impacts plant development and metabolism due to the build-up of ions (Na^+ and K^+). Plant physiology and metabolism are significantly impacted by ionic toxicity, osmotic stress, ion imbalance, and decreased water potential, all of which are brought on by salt stress. It primarily impacts plant development and growth's physiological, morphological, and biochemical processes (Bistgani et al., 2019).

It has been demonstrated that applying Plant Growth Promoting Rhizobacteria (PGPR) reduces abiotic stressors in plants, which would improve sustainable agriculture (Ayaz et al., 2021). According to earlier research, plants can grow more quickly in salty environments when exposed to PGPRs such as *Pesudomonas* and *Bacillus* species that have been isolated from saline soil (Ali et al., 2022). Because of their strong metabolic or genetic foundation and ability to form spores, *Bacillus* species have demonstrated exceptional resistance to this abiotic stress, improving plants' ability to withstand salt soils (Ali et al., 2022). The ability of Bacillus strains to withstand salt stress is mostly related to genes that belong to many families, including membrane transport, osmotic control, signal transduction, oxidative stress, and antioxidant enzymes (Hoffmann et al., 2008)

Cui et al. (2016) reported that the expression of genes under salt stress leads to specific transcriptional modifications that enhance membrane function, antioxidant enzyme activity, and intracellular water maintenance through the regulation of many signaling pathways (Cui et al., 2016). Apart from these fundamental genetic characteristics, *Bacillus*'s ability to form biofilms is a crucial characteristic for colonization and the production of secondary metabolites in unfavorable environments (Yan et al., 2017).

Where there is a lack of access to high-quality water, salt-tolerant plants can be helpful. The development of crops that produce well when planted with salty irrigation fluids, such as brackish subterranean well water, drain water, or even diluted sea water, should be taken into consideration. To raise the production of marginal areas, it may be able to increase the genetic resistance of crops to salt. The main purposes of salt-tolerant species may be to extend the varieties of crops that a farmer in a salt-affected region can grow, to improve the yields and consistency of crops now farmed in naturally salt-affected areas, or to supply crops suitable for marginal spots with few water supplies (Shannon Calvin, 1984).

2.4 Antimicrobial Activity of Genus Bacillus and Its Importance

Most *Bacillus* species produce an extensive array of antibiotic chemicals that put them at risk of competition with other microbes. Therefore, different strains of *Bacillus* or their separated antimicrobial components may help to suppress phytopathogenic microbes, resulting in a reduction in the use of chemical pesticides that may have detrimental effects on the environment. Because of their large host spectrum and soil-borne persistence, soilborne pathogens require substantial dosages of chemical fungicides for control (Naing et al., 2014). Human-pathogenic food-borne bacteria like *Salmonella* may be controlled after harvest by *Bacillus* due to its antibacterial properties (Allard et al., 2014). *Bacillus* produces volatile organic compounds (VOCs), peptides, and enzymes that function as antimicrobials. While antimicrobial peptides are extremely significant in agriculture, the purified or synthesized peptides also have realized potential uses in medicine and food processing. Purified or synthetic peptides have shown potential in medical treatment and food processing, even though antimicrobial peptides are highly important in agriculture. The breakdown of eukaryotic cell walls has been linked to the production of glucanases, chitinases, cellulases, and proteases by several species of soil-dwelling *Bacillus*. The fungal and other oomycete competitors' cell walls

can be attacked by these hydrolytic *Bacillus* enzymes. In the presence of widely used fungicides, for example, a chitinase from *Paenibacillus* sp. D1 also showed great stability, indicating the possibility of some hydrolytic enzymes as additions to chemical fungicides (Singh & Chhatpar, 2011). Volatile organic compounds work by improving the interactions between soil-dwelling microorganisms by diffusing through soil pores filled with air to reach physically distant organisms (Morath et al., 2012). The primary antifungal chemicals in the mixture are phenol, benzenes, aldehydes, keytones, and alcohol (Raza et al., 2015).

Antimicrobial resistance poses a serious concern to the world because it affects food security, safety, and the financial stability of millions of farming households. In addition, some fungal species can create hazardous compounds in the afflicted areas, which could pose a health risk to humans in addition to inflicting enormous economic losses (Tournas, 2005). The predicament faced in the agriculture industry is the loss of viable land. It is significant to optimize all the land available. Antimicrobials play a critical role in treating diseases of food-producing plants and help to ensure food security. It acts as a plant defense system against predation by microbes, insects, and herbivores (Cowan, 1999). Plant antimicrobials like salicylic acid and its derivatives can prevent the formation of bacterial capsules. Meanwhile, the decrease in the synthesis of microbial toxins is another way that plant metabolites have antimicrobial effects (Savoia, 2012). Moreover, the association's beneficial effects of antimicrobial agents combating resistant microorganisms result in novel, environmentally friendly options for the treatment of infectious diseases. (Nascimento et al., 2000).

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

3.1.1 Bacterial Strain

Bacillus zanthoxyli UMK-PNF6 is a plant-growth-promoting rhizobacteria. The bacterium was isolated from a paddy field in the previous study (Kamaruzaman et al., 2022). This strain demonstrated several plant growths promoting activities namely phosphate solubilization phytohormones production and nitrogen fixation. Throughout the study, the bacterium was maintained on Burk's and nutrient media.

3.1.2 Paddy Seed

Paddy seed MR297 obtained from Kemubu Agriculture Development Agency (KADA) was utilized in this study.

3.2 Methods

3.2.1 Evaluation of The Effect of Inoculation of *Bacillus zanthoxyli* UMK-PNF6 in Soil on Paddy's Growth

3.2.1.1 Preparation of Burk's Media and Nutrient Agar

For Burk's Media, all the compositions listed in Table 3.1 had been suspended in 1000 mL distilled water in the Schott bottle while for nutrient agar, it was prepared by suspending 35 g of the nutrient agar powder in 1000 mL distilled water. Next, sterilized by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes. The media was left for a few minutes to let the media cool before pouring it into the petri dish. The agar was left to harden before keeping it in the chiller in an upside-down condition. Nutrient Agar was prepared by suspending 35 g of the nutrient agar powder in 1000 mL of distilled water.

Table 3. 1 Composition of Burk's Media

Composition of Burk's Media	Concentration (g/L)
Magnesium Sulphate	0.2
Dipotassium Phosphate	0.8
Monopotassium Phosphate	0.2
Calcium Chloride	0.13
Ferric Chloride	0.0145
Sodium Molybdate	0.00025
Sucrose	20.0
Agar	15
Distilled Water	1000

3.2.1.2 Cultivation of *Bacillus zanthoxyli* UMK-PNF6 Bacteria on Burk's Medium Agar

The bacterial strain was inoculated on the Burk's media agar plate. Then, the inoculated plate was left in the incubator at 30 °C for 3 days.

3.2.1.3 Preparation of Nutrient Medium Broth

15.75 g of nutrient broth powder had been suspended in 500 mL of distilled water in the Schott bottle, and 50 mL of the mixture was poured into five different 150 mL Erlenmeyer flasks with a stopper made of gauze cloth, cotton, and aluminum foil, which then were sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.

3.2.1.4 Cultivation of *Bacillus zanthoxyli* UMK-PNF6 Bacteria into Nutrient Medium Broth

The cultured bacteria strain from agar medium was inoculated under aseptic conditions into the nutrient broth and incubated in an orbital shaking incubator at 180 rpm at 37 °C for 2 days until it reached OD_{600} = 0.5. The colony was used for the inoculation of *Bacillus zanthoxyli* UMK-PNF6 in soil.

3.2.1.5 Preparation of Seed

Seeds were added into the Schott bottle containing 200 mL of sterile distilled water and 4% of bleach from the total content which was 8 mL which later was shaken and soaked for 10 minutes. After 10 minutes, floating seeds indicated as bad seeds were thrown away. The good seeds were later rinsed with sterile distilled water for 3 times and soaked them overnight.

3.2.1.6 Germination Process

In a petri dish, a sterile filter paper was placed and later sprayed wet with sterile distilled water. The seeds were arranged on it and left for 5 to 10 Days. The paper was sprayed with sterile distilled water constantly to avoid drying out.

3.2.1.7 Soil Media Preparation

Soil media was prepared by mixing 80 g of topsoil and 40 g of cocopeat to achieve the 2:1 ratio. Soil was added into a plastic cup until it reached 3/4 of the cup. Tests on moisture content were conducted using a moisture analyzer to determine the amount of water added to soil media to reach 50% moisture content. However, for the treatment batch, 1% of cultured bacteria in broth media was added to the soil using a micropipette. Each cup was labeled accordingly. The media formulation was conducted a day prior to sowing.

3.2.1.8 Pot Study

The germinated seeds were transferred into the soil media. Then, at the bottom of each plastic cup poked with 10 holes. Initial height and initial weight were taken. The cups were arranged in a tray that had been filled with water level enough to cover the holes. The samples were left for 18 days with constant observation.

3.2.2 Evaluation of Paddy's Growth Inoculated with *Bacillus zanthoxyli* UMK-PNF6 in Soil on Its Tolerance Against Salinity Stress

3.2.2.1 Salt Stress Evaluation

The pot study for salinity stress is similar to the methodology mentioned in sections 3.2.1.4 to 3.2.1.8 however with the addition of NaCl solution into the soil. Different concentrations of NaCl solutions were prepared (50 mM, 100 mM, 150 mM) with 1 M of NaCl of stock solution through the dilution method. Every 4 days, the soil was sprayed with 10 mL of different concentrations of NaCl solutions.

3.2.3 Evaluation of Plant Growth

3.2.3.1 Chlorophyll Content

Each time a chlorophyll meter was used, the chlorophyll meter must be calibrated. Before introducing plant tissue, calibration was done by pressing on the finger rest to seal the head. A display appears after the meter beeps, indicating that the meter is ready for the first sample. The reading has been monitored by the 'N=' displayed at the top of the screen on the chlorophyll meter as shown in Figure 3.1.

Figure 3.1 Checking chlorophyll content using Chlorophyll SPAD meter

3.2.3.2 Leaf Length Grown (cm)

Using data from the plant's initial height, the leaf length grown was calculated as follows:

$$\text{Leaf Length Grown (cm)} = \text{Initial Height} - \text{Height after 18 Days}$$

Equation 3.1 Leaf Length Grown

3.2.3.3 Moisture Content of Paddy Plant (%)

3.2.3.3.i Plant Fresh Weight (g)

The plant's initial weight was recorded. After 18 days, the plants were gently lifted from the soil without causing damage to the root system and then rinsed with tap water to eliminate any remaining dirt particles as shown in Figure 3.2. Individual roots and shoots were weighed using an analytical balance.

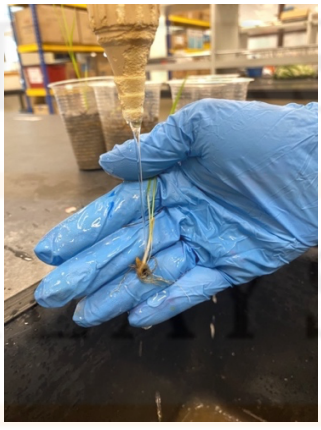


Figure 3.2 Cleaning the soil from the root plant

Figure 3.3 After cleaning

3.2.3.3.ii Plant Dry Weight (g)

The plants were then dried at 60 °C in an oven overnight as shown in Figure 3.4, the dry matter yield was determined after weighing the dried sample.

Figure 3.4 The sample was kept in the oven overnight for drying

3.2.3.3.iii Determination of Moisture Content

To measure the moisture content of the paddy plant, the plant's fresh weight and dry weight were recorded. Using the data from plant fresh weight and plant dry weight, the moisture content was determined as follows:

$$Moisture\ (\%) = \frac{plant\ fresh\ weight - plant\ dry\ weight}{plant\ fresh\ weight}\ x\ 100$$

Equation 3.2 Moisture Content Percentage

3.2.4 Determination of the Antimicrobial Activity of *Bacillus zanthoxyli* UMK-PNF6 Against Gram-positive and Gram-negative Bacteria

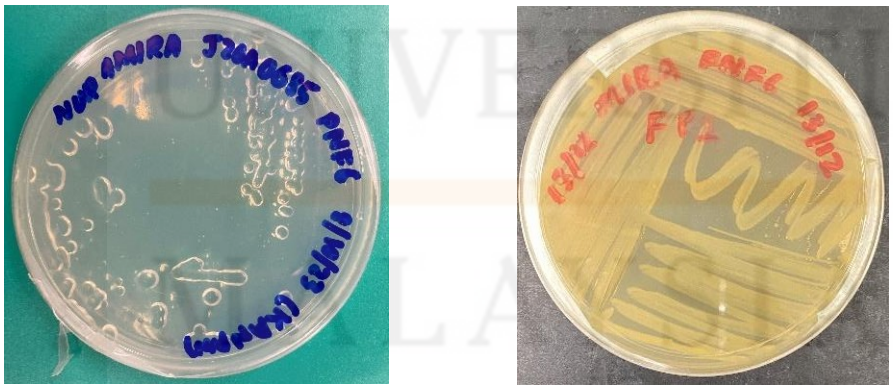
The antimicrobial test was conducted with two bacteria strains, Gram-negative *Escherichia coli* and Gram-positive *Bacillus subtilis*. Both of the bacteria strains were cultured into 10 mL of nutrient broth media. Firstly, 4 sterile paper discs were placed on the agar plate. Next, a sterile cotton swab was dipped into the *E. coli* culture and then evenly swabbed on the nutrient agar plate. Then, 4 sterile paper discs were placed on the media with 10 μl of 0.025g/mL of chloramphenicol as positive control meanwhile sterile distilled water as negative control in this process. The other two sterile filter papers were for 10 μl of *Bacillus zanthoxyli* UMK-PNF6 broth culture. The plates were incubated at 37 °C overnight. The result was collected by observing the zone of inhibition along with the diameter. The same procedure was repeated for the antimicrobial test using *Bacillus subtilis.*

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Bacterial Strain

Firstly, Burk's media was chosen because it was commonly used for the cultivation of nitrogen fixation bacteria such as Azotobacter species from soil including *Bacillus zanthoxyli* UMK-PNF6. Secondly, because *Bacillus zanthoxyli* UMK-PNF6 was initially isolated from nitrogen-fixing media. However, the nutrient medium was selected because it is a rich media that allows *Bacillus zanthoxyli* UMK-PNF6 to have a shorter lag phase and enter the exponential phase more quickly (Kram & Finkel, 2015) which is suitable to use for pot study. Figure 4.1(a) shows *Bacillus zanthoxyli* UMK-PNF6 grown on a Burk's agar plate while Figure 4.1(b) shows thebacteria grown on a nutrient agar plate.

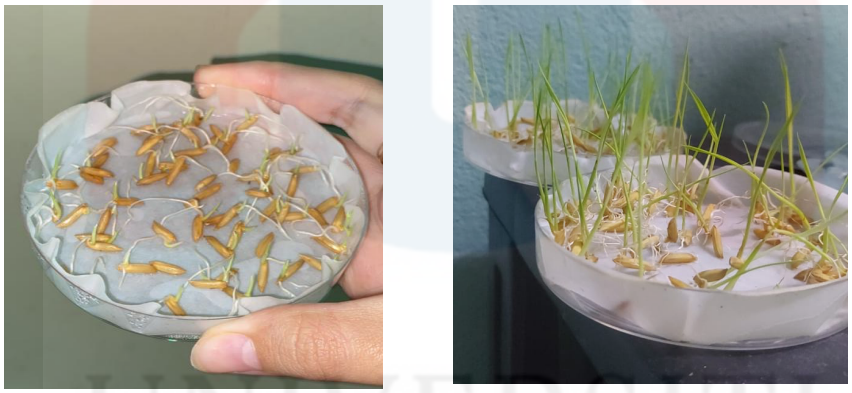


(a) **(b)**

Figure 4.1 *B. zanthoxyli* UMK-PNF6 strain on **(a)** Burk's Medium and **(b)** nutrient medium

4.2 Germination Process

Germination is the process by which a seed begins to sprout and grow into a new plant. It typically involves the activation of dormant seeds, triggering metabolic processes that lead to the emergence of a seedling. The key factor to ensure successful seedling establishment is environmental conditions. This includes light and moisture management (Wolny et al., 2018). Therefore, the paddy seeds were maintained under consistent moisture levels as overwatering, which can lead to rotting, or underwatering, which can inhibit germination. During the germination process, the filter paper was observed consistently and sprayed with sterile distilled water to prevent it from drying. Figure 4.2 below shows the growth of paddy seed during the germination process.



(a) **(b)**

Figure 4.2 Growth of paddy seed during germination process **(a)** after 3 Days and **(b)** after 10 Days

4.3 Pot Study Process

When the pot study was run, we controlled the environmental conditions such as growing media selection, soil composition, moisture levels, temperature, and light exposure. Firstly, topsoil was chosen because it is nutrient-rich, it is rich in organic matter and nutrients essential for plant growth. It contains a variety of minerals, organic compounds, and

microorganisms that support healthy plant development (Paluch & Gruba, 2012). Secondly, cocopeat was chosen due to its excellent water retention properties. It can hold moisture well which helps in maintaining consistent moisture levels around the plant roots, which is crucial for healthy plant growth (Jana & Boxi, 2020). For the soil composition, we used 2 ratios 1 of topsoil and cocopeat into 3/4 of a plastic cup. Next, for moisture content, each cup was added with the same amount of water throughout 18 Days of pot study. Moving on to light exposure, all the cups were placed in the same location with the same light exposure which was in the nursery. Other than that, throughout the 8 Days, the plants were regularly monitored with proper watering to avoid drought stress. Figure 4.3 below shows the growth of paddy during the pot study process.

Figure 4.3 The growth of paddy after 10 Days of pot study

4.4 Evaluation of the Effect of the Inoculation of *Bacillus zanthoxyli* UMK-PNF6 in Soil on Paddy's Growth

4.4.1 Results

After 18 Days of pot study, there was growth present in both control and treated paddy plants with *Bacillus zanthoxyli* UMK-PNF6 as shown in Figure 4.4. However, from Figure 4.4 the bar graph compares the growth of paddy inoculated with *Bacillus zanthoxyli* UMK-PNF6

against the control batch, there are increases in the height of the bars for the paddy inoculated with *Bacillus zanthoxyli* UMK-PNF6 demonstrating better growth. The bar graph's increase represents improvements in paddy's growth parameters such as chlorophyll content, leaf length grown (cm), and moisture content (%) which indicates that the bacterial inoculation resulted in enhanced growth.

Following the statistical analysis of two independent experiments from Table 4.2, Using a significance level of $p < 0.05$, the paddy inoculated with *Bacillus zanthoxyli* UMK-PNF6 differs statistically significantly in plant growth performance from the control batch, as proven by the obtained t-test values which are 0.02825, 0.036524 and 0.008515.

Table 4.1 Average Mean And SD From Two Independent Experiments

Sample / Parameters	Chlorophyll Content	Leaf Length Grown (cm)	Moisture Content (%)
Control	11.905±0.276	10.75±1.061	83.915 ± 0.276
Paddy inoculated with UMK-PNF6	16.573±1.0996	15.685±0.87	88.61 ± 0.552

Table 4.2 Statistical Analysis (t-test value) From Two Independent Experiments

Sample / Parameters	Chlorophyll Content	Leaf Length Grown (cm)	Moisture Content (%)
Control	0	0	0
Paddy inoculated with UMK-PNF6	0.02825	0.036524	0.008515

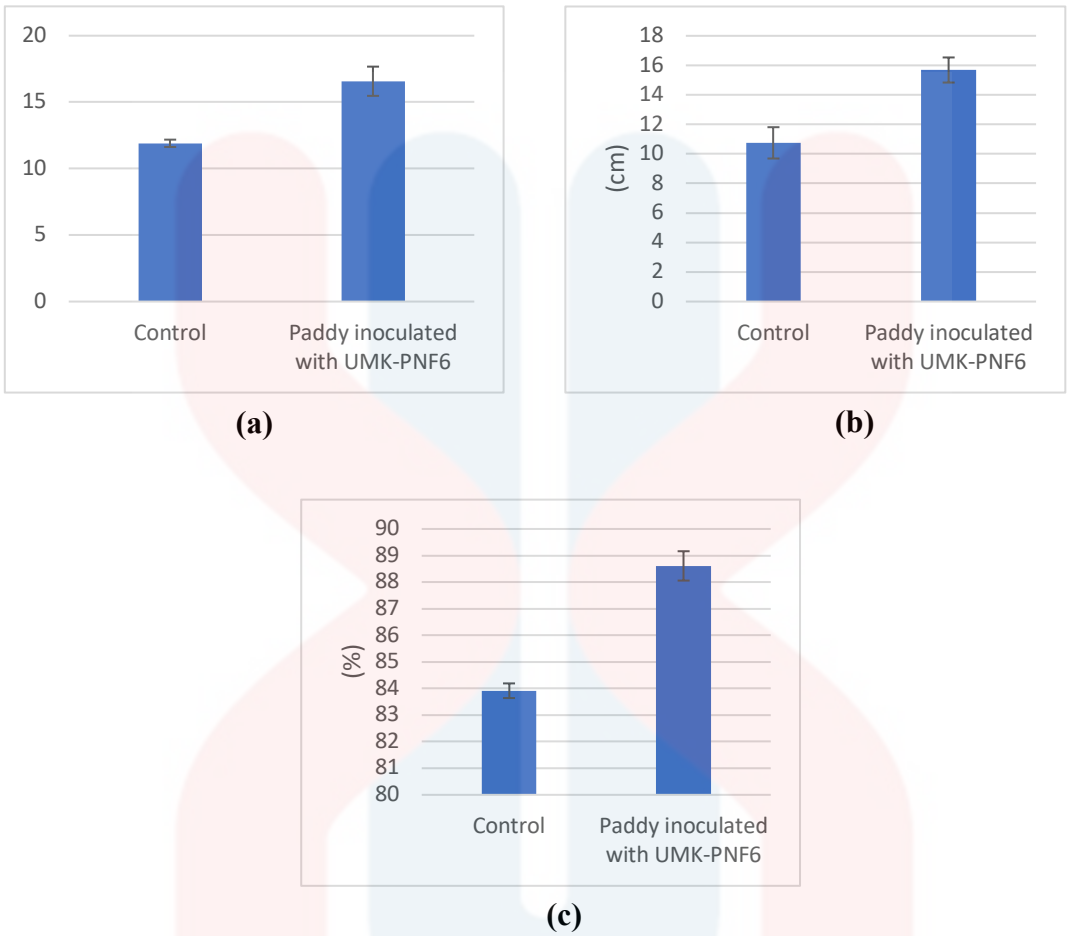


Figure 4.4 The effect of the inoculation of *B. zanthoxyli* UMK-PNF6 in soil on **(a)** chlorophyll content **(b)** leaf length grown, and **(c)** moisture content.

4.4.2 Discussions

Firstly, chlorophyll content. This finding has implications for understanding the effect of *Bacillus zanthoxyli* UMK-PNF6 inoculation on the production of chlorophyll in paddy plants, with a statistically significant threshold set at $p < 0.05$. According to the results from Table 4.2, *Bacillus zanthoxyli* UMK-PNF6 has had a statistically significant impact on the amount of chlorophyll present. According to the previous study, *Catharanthus roseus*'s chlorophyll content increased when it was treated with *Azospirillum, Azotobacter, Pseudomonas*, and *Bacillus* (Lenin & Jayanthi, 2012). Therefore, it is proven that PGPR inoculation increased the growth characteristics, specifically the chlorophyll content and total

biomass (El-Taher et al., 2022). The higher accumulation of plant nourishment and photosynthesis may be the cause of the enhanced chlorophyll content in plant leaves following bacterial co-inoculation (Bashan et al., 1990). This suggests that photosynthetic activity may be improved, which could lead to an increase in total plant growth and production.

Secondly, leaf length grown. From Figure 4.4(b) the UMK-PNF6-inoculated batch's observed increase in leaf length signifies a positive outcome on the paddy plants' vegetative growth. The plant growth-promoting properties of *Bacillus zanthoxyli* UMK-PNF6 may have played a role in this development through various mechanisms. One potential mechanism is the enhancement of nutrient availability facilitated by *Bacillus zanthoxyli* UMK-PNF6. As a plant growth-promoting bacteria, it can solubilize phosphorus and fix atmospheric nitrogen, which improves the soil's nutritional status and encourages plant development. Resulting in greater plant growth, especially improved leaf elongation, is frequently connected to improved nutritional availability (Timofeeva et al., 2023). Subsequently, the observation of a statistically significant increase in leaf length in the paddy batch inoculated with *Bacillus zanthoxyli* UMK-PNF6 indicates that the bacteria have a beneficial effect on the vegetative growth of paddy plants. These findings highlight the function of microbial inoculants in boosting growth parameters and offer insightful information about the possible uses of these agents in sustainable agriculture.

Thirdly, moisture content. Numerous potential mechanisms linked to *Bacillus zanthoxyli* UMK-PNF6's capability to promote plant growth can be used to explain the observed increase in moisture content in the inoculated batch. For instance, improved water uptake. It can be achieved for plants to obtain water from the soil more efficiently and have higher moisture content in their tissues because *Bacillus* species release siderophores and exopolysaccharides that facilitate water transport in plant tissues and block the passage of harmful ions while maintaining the ionic equilibrium (Hashem et al., 2019). In the final

analysis, *Bacillus zanthoxyli* UMK-PNF6 appears to have a beneficial impact on the efficiency of water use of paddy plants, as evidenced by the statistically significant increase in moisture content seen in the UMK-PNF6 inoculated paddy batch.

4.5 Evaluation of Paddy's Growth Inoculated with *Bacillus zanthoxyli* UMK-PNF6 in Soil on Its Tolerance Against Salinity Stress

4.5.1 Results

After 18 Days of pot study, there was growth of paddy plant present. The paddy's growth for each different batch was observed and recorded. The data collected was presented through a bar graph as in Figure 4.5. The bar graph provides a thorough overview of the general performance of plants at different NaCl concentrations, both with and without bacterial inoculation, taking into consideration a variety of growth parameters such as chlorophyll content, leaf length grown (cm), and moisture content (%). An overall increase in the height of the bars for treated plants in comparison to untreated plants at each NaCl concentration across a range of parameters indicates that inoculated plants with *Bacillus zanthoxyli* UMK-PNF6 demonstrated better growth as it improves their resistance to salt stress, which promotes healthier growth and greater production.

Following the statistical analysis of two independent experiments from Table 4.4, Using a significance level of $p < 0.05$, the paddy inoculated with *Bacillus zanthoxyli* UMK-PNF6 with different concentrations of NaCl differs statistically significantly in plant growth performance from the control batch, as proven by the obtained t-test values. However, based on the result shown in Table 4.4 *Bacillus zanthoxyli* UMK-PNF6 was the most effective in its growth-promoting performance at 50 mM of NaCl, from its lowest value for the statistical

analysis (t-test value) compared to 100 mM and 150 mM for each parameter (chlorophyll content, leaf length grown and moisture content). Besides, 50mM of NaCl has the highest difference compared to the other concentrations proving its effectiveness. Besides, *Bacillus zanthoxyli* UMK-PNF6 showed greater benefits in terms of plant growth performance at lower salinity levels, 50 mM NaCl than at higher concentrations, 150 mM NaCl.

Table 4.3 Average Mean And SD From Two Independent Experiments

Parameter	Chlorophyll Content		Leaf Length Grown (cm)		Moisture Content (%)	
Sample / NaCl concentration (mM)	Control	Paddy inoculated with UMK-PNF6	Control	Paddy inoculated with UMK-PNF6	Control	Paddy inoculated with UMK-PNF6
50	10.598±0.209	16.736±0.0622	11.2±0.170	15.495±0.403	83.25±0.240	87.509±0.267
100	7.54±0.127	11.58±0.170	8.96±0.141	11.985±0.318	82.385±0.0919	85.43±0.304
150	4.007±0.273	8.615±0.134	5.495±0.375	8.2925±0.117	79.45±0.396	83.4±0.226

Table 4.4 Statistical Analysis (t-test value) From Two Independent Experiments

Parameter	Chlorophyll Content		Leaf Length Grown (cm)		Moisture Content (%)	
Sample / NaCl concentration (mM)	Control	Paddy inoculated with UMK-PNF6	Control	Paddy inoculated with UMK-PNF6	Control	Paddy inoculated with UMK-PNF6
50	0	0.000632	0	0.005144	0	0.003544
100	0	0.001376	0	0.00656	0	0.004714
150	0	0.003536	0	0.0097	0	0.007633

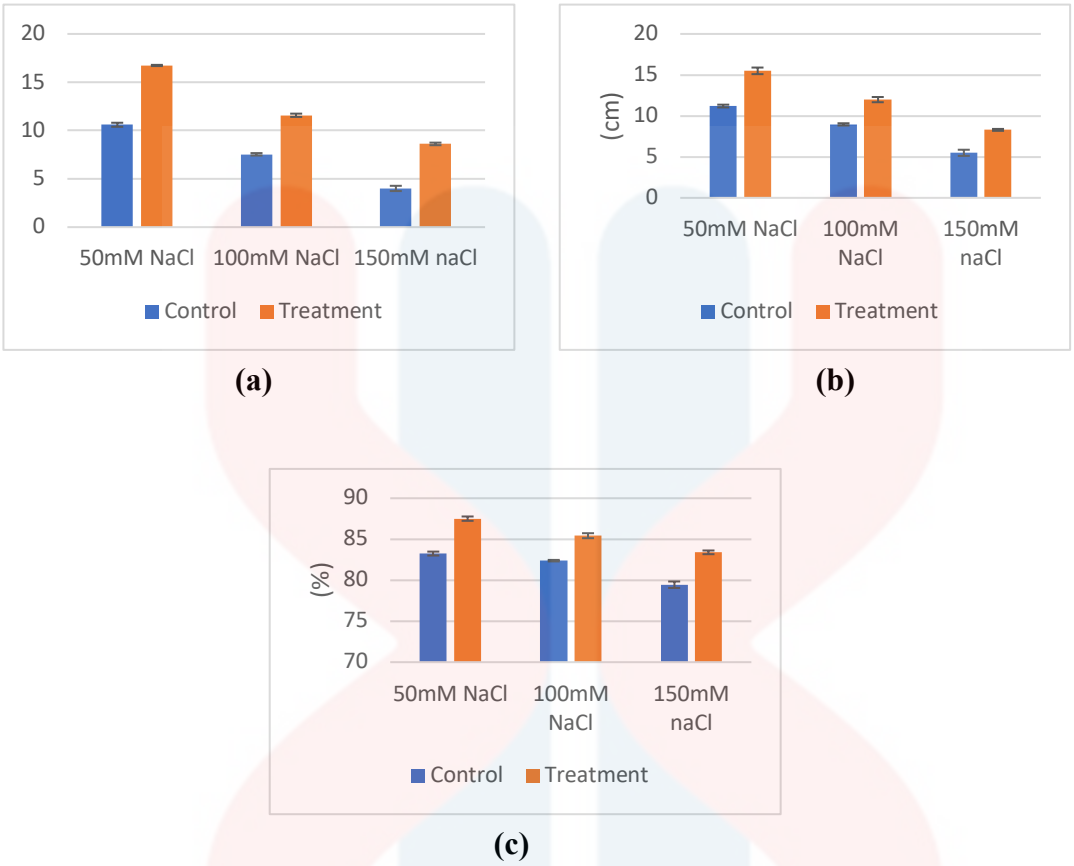


Figure 4.5 The effect of the inoculation of *B. zanthoxyli* UMK-PNF6 on **(a)** chlorophyll content **(b)** leaf length grown, and **(c)** moisture content under salt stress.

4.5.2 Discussions

Based on result showed that *Bacillus zanthoxyli* UMK-PNF6 was the most effective in its growth-promoting performance at 50 mM of NaCl, from its lowest value for the statistical analysis (t-test value) compared to 100 mM and 150 mM in terms of significant value ($p < 0.05$) for each parameter (chlorophyll content, leaf length grown and moisture content). Besides, 50mM of NaCl has the highest difference compared to the other concentrations proving its effectiveness.

Similar to the study of stress treatment on cabbage plants treated with *Bacillus* exhibited higher levels of chlorophyll than plants treated with control-treated plants. After 50 mM of NaCl treatment, the carotenoid level in the *Bacillus*-treated plants remained

comparatively higher than in control-treated plants (Usmonov et al., 2021). A vital indicator for tracking plant growth and senescence is the level of chlorophyll, a photosynthetic pigment, which decreases and is directly correlated with the degree of environmental stressors (Kalaji et al., 2016). Given that salinity stress increases chlorophyllase activity and makes pigment-protein complexes unstable, it can lower the level of chlorophyll (Jamil et al., 2007). Based on the result, *Bacillus zanthoxyli* UMK-PNF6 treatment prevents the degradation of the pigments of the paddy plant as in the different concentrations of NaCl, every treated batch has visibly higher chlorophyll content compared to the control batch. It aligns with the result *of B. zanthoxyli* HS1 as it also has distinct impacts on the pigment stability of cucumber and cabbage plants and can stop salinity stress from destroying the pigments in cabbage plants (Usmonov et al., 2021). In summary, a positive impact of the microbial inoculant on the abilities of the plant to tolerate and adapt to changes in salinity is suggested by the statistically significant increase in chlorophyll content in paddy plants infected with *Bacillus zanthoxyli* UMK-PNF6 at varied salinity stress levels.

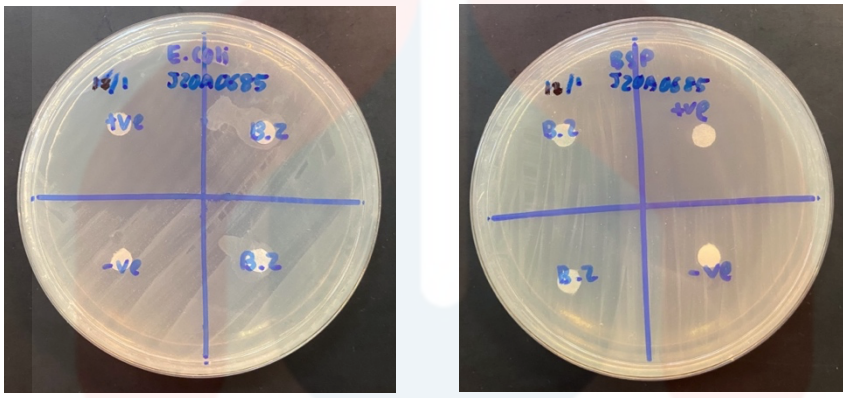
Salt stress reduced the length of the leaf elongating zone as well as the growth intensity in its central and distal areas, slowing the rate of leaf growth. This can be seen as the control batch leaf length grown is gradually decreasing however treatment batch is visibly higher due to the inoculation of *Bacillus zanthoxyli* UMK-PNF6. According to previous study, it showed that *Bacillus* strains NMCN1 and LLCG23 demonstrated their key function in promoting wheat growth under salt stress conditions due to their capacity to control phytohormones and salt resistance genes that lowered the damaging impact of salinity stress in wheat (Ayaz et al., 2022). This is because *Bacillus* species can secrete metabolites that promote plant growth and create long-lasting, stress-tolerant spores making *Bacillus* genus members able to endure in hostile environments for extended periods as endospores. (Radhakrishnan et al., 2017). Therefore, adding bacteria to the root system is a way to increase resistance to abiotic stress,

particularly the environmental factors including salinity stress. For the final analysis, the beneficial impact of the microbial inoculant on plant development is shown by the statistically significant increase in leaf length grown in paddy plants treated with *Bacillus zanthoxyli* UMK-PNF6 under varied salinity stress levels.

Bacillus zanthoxyli UMK-PNF6 applied a few mechanisms to achieve this result even under salt stress. Firstly, the systemic agent's induction in plant roots. Plant roots and other residing microbes compete with bacteria for nutrition. The interactions that occur between plants and rhizosphere microorganisms are therefore very important. The exchange of carbon compounds between resident microorganisms and their plant hosts, as well as the plant host's increased intake of nutrients and water as a result of the activity of beneficial microbes, are examples of the evolution of mutually beneficial relationships (Gouda et al., 2018). In addition, *Bacillus zanthoxyli* UMK-PNF6 enhances the absorption of water. *Bacillus* species release exopolysaccharides and siderophores which promote water transport in plant tissues and shut the passage of damaging ions thereby preserving the ionic equilibrium, permitting plants to draw water from the soil more effectively and to retain higher moisture content in their tissues (Hashem et al., 2019). In summary, the observed substantial rise in moisture content through statistical analysis among paddy plants treated with *Bacillus zanthoxyli* UMK-PNF6 across diverse salinity stress levels indicates a beneficial influence of the microbial inoculant on the overall water status of the plants.

4.6 Determination of The Antimicrobial Activity of *Bacillus zanthoxyli* UMK-PNF6 Against Gram-positive and Gram-negative Bacteria

The antimicrobial test of *Bacillus zanthoxyl*i UMK-PNF6 was run against two bacteria strains which were *E. coli* and *Bacillus subtilis strain.* The antimicrobial test agar plates were left in the incubator at 30 °C overnight. The results of the antimicrobial tests were observed on the next day. The presence of clear zones was observed and the diameter of it was measured using a ruler.



(a) **(b)**

Figure 4.6 The antimicrobial test of *Bacillus zanthoxyli* UMK-PNF6 on **(a)** *E. coli* strain and **(b)** *Bacillus subtilis* strain

Table 4.5 Antimicrobial test of *Bacillus zanthoxyli* UMK-PNF6

Sample	Zone of inhibition (cm)	
	E. coli	*Bacillus subtilis*
Positive Control	4.0	3.6
Bacillus zanthoxyli UMK-PNF6	-	-

From the result Table 4.5, it is shown that *Bacillus zanthoxyli* UMK-PNF6 showed negative results towards the antimicrobial essay. Notably, *Bacillus zanthoxyli* UMK-PNF6 does

not exhibit any antimicrobial activity, as evidenced by the absence of inhibition zones against *E. coli* and *Bacillus subtilis.* However, no other studies are being done on this to validate this result. There are a few factors that might affect bacteria's antimicrobial activity. Firstly, the bacteria growth phase. Bacteria may have reached the phase known as the cell death phase. The capacity to divide is lost in bacteria, therefore there are more dead cells than active ones. It is believed that the appearance of dead cells results from inadequate nutrients and the buildup of waste products, harmful metabolites, and inhibitory substances. This could be the possible factor contributing to *Bacillus zanthoxyli* UMK-PNF6 antimicrobial activity.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study's purpose is to evaluate *Bacillus zanthoxyli* UMK-PNF6 bacterial inoculation effect on paddy growth, plant salinity stress tolerance, and antimicrobial activities. Based on this study, it was found that paddy treated with *Bacillus zanthoxyli* UMK-PNF6 had a positive and good effect on growth performance. It was evaluated through the collection of chlorophyll content, leaf length grown, and moisture content from two independent experiments after 18 days. Other than that, after performing a statistical analysis, all the data were significant. The experiment's findings showed that *Bacillus zanthoxyli* UMK-PNF6 positively influenced indicators related to paddy growth. Key growth indices, including increased chlorophyll content, leaf length, and moisture content, were all significantly improved in the paddy treated with *Bacillus zanthoxyli* UMK-PNF6. Thus, it can be concluded that *Bacillus zanthoxyli* UMK-PNF6 has a positive effect on paddy's growth.

Furthermore, this study evaluated the role of *Bacillus zanthoxyli* UMK-PNF6 in enhancing plant salinity stress tolerance. Compared with the control group, paddy treated with *Bacillus zanthoxyli* UMK-PNF6 showed greater tolerance to salinity stress. However, the highest level of the effectiveness of *Bacillus zanthoxyli* UMK-PNF6 was portrayed at 50mM of NaCl. It has shown higher change in growth performance compared to 100mM and 150mM of NaCl.

In addition, for *Bacillus zanthoxyli* UMK-PNF6 antimicrobial activities. The result collected showed that *Bacillus zanthoxyli* UMK-PNF6 did not have any antimicrobial activities towards *E. coli* and *Bacillus subtilis.*

In summary, the experiment indicates that *Bacillus zanthoxyli* UMK-PNF6 has the capability as a helpful inoculant for improving paddy development and providing tolerance to saline stress.

5.2 Recommendations

Firstly, to diverse crop compatibility studies. It is to examine if *Bacillus zanthoxyli* UMK-PNF6 is compatible with and effective in a variety of crops other than paddy. This is to examine the possible advantages of different types of crops to generate a wider range of uses in different types of agricultural systems. Second, conduct a field trial and scale up by expanding the trial to bigger agricultural plots and carrying out field studies to verify *Bacillus zanthoxyli* UMK-PNF6's effectiveness in real-world settings.

REFERENCES

Aktar, W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdisciplinary Toxicology*, *2*(1), 1. https://doi.org/10.2478/V10102-009-0001-7

Al-Ani, L. K. T., & Furtado, E. L. (2020a). The effect of incompatible plant pathogens on the host plant. *Molecular Aspects of Plant Beneficial Microbes in Agriculture*, 47–57. https://doi.org/10.1016/B978-0-12-818469-1.00004-3

Ali, Q., Ayaz, M., Mu, G., Hussain, A., Yuanyuan, Q., Yu, C., Xu, Y., Manghwar, H., Gu, Q., Wu, H., & Gao, X. (2022). Revealing plant growth-promoting mechanisms of *Bacillus* strains in elevating rice growth and its interaction with salt stress. *Frontiers in Plant Science*, *13*, 3190. https://doi.org/10.3389/FPLS.2022.994902/BIBTEX

Allard, S., Enurah, A., Strain, E., Millner, P., Rideout, S. L., Brown, E. W., & Zheng, J. (2014). In situ evaluation of *Paenibacillus Alvei* in reducing carriage of *Salmonella Enterica* serovar newport on whole tomato plants. *Applied and Environmental Microbiology*, *80*(13), 3842–3849. https://doi.org/10.1128/AEM.00835-14/ASSET/47FDFF14-F372-409F-90A5-7C87AE2B815A/ASSETS/GRAPHIC/ZAM9991054360005.JPEG

Andrews, S. C., Robinson, A. K., & Rodríguez-Quiñones, F. (2003). Bacterial iron homeostasis. *FEMS Microbiology Reviews*, *27*(2–3), 215–237. https://doi.org/10.1016/S0168-6445(03)00055-X

Ash, C., Priest, F. G., & Collins, M. D. (1993). Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks, and Collins) using a PCR probe test - Proposal for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek*, *64*(3–4), 253–260. https://doi.org/10.1007/BF00873085/METRICS

Ayaz, M., Ali, Q., Farzand, A., Khan, A. R., Ling, H., & Gao, X. (2021). Nematicidal volatiles from *bacillus atrophaeus* gbsc56 promote growth and stimulate induced systemic resistance in tomatoes against *meloidogyne incognita*. *International Journal of Molecular Sciences*, *22*(9), 5049. https://doi.org/10.3390/IJMS22095049/S1

Ayaz, M., Ali, Q., Jiang, Q., Wang, R., Wang, Z., Mu, G., Khan, S. A., Khan, A. R., Manghwar, H., Wu, H., Gao, X., & Gu, Q. (2022). Salt Tolerant *Bacillus* Strains Improve Plant Growth Traits and Regulation of Phytohormones in Wheat under Salinity Stress. *Plants*, *11*(20), 2769. https://doi.org/10.3390/PLANTS11202769/S1

Barberán, A., Caceres Velazquez, H., Jones, S., & Fierer, N. (2017). Hiding in Plain Sight: Mining Bacterial Species Records for Phenotypic Trait Information. *MSphere*, *2*(4). https://doi.org/10.1128/MSPHERE.00237-17

Bashan, Y., Harrison, S. K., & Whitmoyer, R. E. (1990). Enhanced Growth of Wheat and Soybean Plants Inoculated with *Azospirillum brasilense* Is Not Necessarily Due to General Enhancement of Mineral Uptake †. *Applied And Environmental Microbiology*, *56*(3), 769–775.

Berg, G., Grube, M., Schloter, M., & Smalla, K. (2014). Unraveling the plant microbiome: looking back and future perspectives. *Frontiers in Microbiology*, *5*(JUN). https://doi.org/10.3389/FMICB.2014.00148

Bistgani, Z. E., Hashemi, M., DaCosta, M., Craker, L., Maggi, F., & Morshedloo, M. R. (2019). Effect of salinity stress on the physiological characteristics, phenolic compounds, and antioxidant activity of *Thymus vulgaris L.* and *Thymus daenensis Celak*. *Industrial Crops and Products*, *135*, 311–320. https://doi.org/10.1016/J.INDCROP.2019.04.055

Boukhalfa, H., & Crumbliss, A. L. (2002). Chemical aspects of siderophore mediated iron transport. *BioMetals*, *15*(4), 325–339. https://doi.org/10.1023/A:1020218608266/METRICS

Cui, P., Liu, H., Islam, F., Li, L., Farooq, M. A., Ruan, S., & Zhou, W. (2016). OsPEX11, a peroxisomal biogenesis factor 11, contributes to salt stress tolerance in *Oryza sativa*. *Frontiers in Plant Science*, *7*(9), 1357. https://doi.org/10.3389/FPLS.2016.01357/BIBTEX

El-Taher, A. M., Abd El-Raouf, H. S., Osman, N. A., Azoz, S. N., Omar, M. A., Elkelish, A., & Abd El-Hady, M. A. M. (2022). Effect of Salt Stress and Foliar Application of Salicylic Acid on Morphological, Biochemical, Anatomical, and Productivity Characteristics of Cowpea (*Vigna unguiculata L.*) Plants. *Plants*, *11*(1). https://doi.org/10.3390/PLANTS11010115

Fahad, S., Hussain, S., Bano, A., Saud, S., Hassan, S., Shan, D., Khan, F. A., Khan, F., Chen, Y., Wu, C., Tabassum, M. A., Chun, M. X., Afzal, M., Jan, A., Jan, M. T., & Huang, J. (2015). Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. *Environmental Science and Pollution Research*, *22*(7), 4907–4921. https://doi.org/10.1007/S11356-014-3754-2/METRICS

Fazelian, N., & Yousefzadi, M. (2022). Nano-biofertilizers for enhanced nutrient use efficiency. *Nano-Enabled Agrochemicals in Agriculture*, 145–158. https://doi.org/10.1016/B978-0-323-91009-5.00023-9

Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., & Patra, J. K. (2018). Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiological Research*, *206*, 131–140. https://doi.org/10.1016/J.MICRES.2017.08.016

Gupta, G., Parihar, S. S., Ahirwar, N. K., Snehi, S. K., & Singh, V. (2015). Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of

Sustainable Agriculture. *Article in Journal of Microbial & Biochemical Technology*, *7*(2), 96–102. https://doi.org/10.4172/1948-5948.1000188

Hashem, A., Tabassum, B., & Fathi Abd_Allah, E. (2019a). *Bacillus subtilis*: A plant-growth-promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences*, *26*(6), 1291–1297. https://doi.org/10.1016/J.SJBS.2019.05.004

Hoffmann, T., Boiangiu, C., Moses, S., & Bremer, E. (2008). Responses of *Bacillus subtilis* to hypotonic challenges: Physiological contributions of mechanosensitive channels to cellular survival. *Applied and Environmental Microbiology*, *74*(8), 2454–2460. https://doi.org/10.1128/AEM.01573-07/ASSET/C023A551-3014-4853-8B20-D582E31EA7D1/ASSETS/GRAPHIC/ZAM0080887590005.JPEG

Jamil, M., Rehman, S. U., Kui, J. L., Jeong, M. K., Kim, H. S., & Eui, S. R. (2007). Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. *Scientia Agricola*, *64*(2), 111–118. https://doi.org/10.1590/S0103-90162007000200002

Jana, P., & Boxi, S. S. (2020). Studies on ph, conductivity, and moisture retention capacity of coir pith for its application as the plant growing medium. *Journal of Natural Fibers, 19*(8), 2861–2867. https://doi.org/10.1080/15440478.2020.1827118

Kalaji, H. M., Jajoo, A., Oukarroum, A., Brestic, M., Zivcak, M., Samborska, I. A., Cetner, M. D., Łukasik, I., Goltsev, V., & Ladle, R. J. (2016). Chlorophyll fluorescence as a tool to monitor the physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum*, *38*(4), 1–11. https://doi.org/10.1007/S11738-016-2113-Y/FIGURES/2

Kalev, S. D., & Toor, G. S. (2018). The Composition of Soils and Sediments. *Green Chemistry: An Inclusive Approach*, 339–357. https://doi.org/10.1016/B978-0-12-809270-5.00014-5

Kamaruzaman, N. F. Q. N. M., Ishak, F. N., & Rahim, A. A. (2022). Isolation of plant growth-promoting bacteria from paddy (*Oryza sativa*) rhizosphere. *AIP Conference Proceedings*, *2454*(1). https://doi.org/10.1063/5.0079098/2825155

Khoshru, B., Khoshmanzar, E., Asgari Lajayer, B., & Ghorbanpour, M. (2023). Soil moisture–mediated changes in microorganism biomass and bioavailability of nutrients in paddy soil. *Plant Stress Mitigators*, 479–494. https://doi.org/10.1016/B978-0-323-89871-3.00005-7

Kloepper, J. W., Lifshitz, R., & Zablotowicz, R. M. (1989). Free-living bacterial inocula for enhancing crop productivity. *Trends in Biotechnology*, *7*(2), 39–44. https://doi.org/10.1016/0167-7799(89)90057-7

Kram, K. E., & Finkel, S. E. (2015). Rich Medium Composition Affects *Escherichia coli* Survival, Glycation, and Mutation Frequency during Long-Term Batch Culture. *Applied and Environmental Microbiology*, *81*(13), 4442. https://doi.org/10.1128/AEM.00722-15

Leach, J. E., Leung, H., & Tisserat, N. A. (2014). Plant Disease and Resistance. *Encyclopedia of Agriculture and Food Systems*, 360–374. https://doi.org/10.1016/B978-0-444-52512-3.00165-0

Li, M., Hong, C. Y., Yan, W. X., Chao, Z. S., Gang, Y. C., Ling, D. J., Kui, Z. X., Qin, X. J., Liang, Z. M., & He, M. M. (2017). *Bacillus zanthoxyli* sp. nov., a novel nematicidal bacterium isolated from Chinese red pepper (*Zanthoxylum bungeanum Maxim*) leaves in China. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *110*(9), 1179–1187. https://doi.org/10.1007/S10482-017-0890-Y

Li, Y., You, X., Tang, Z., Zhu, T., Liu, B., Chen, M. X., Xu, Y., & Liu, T. Y. (2022). Isolation and identification of plant growth-promoting rhizobacteria from tall fescue rhizosphere and their functions under salt stress. *Physiologia Plantarum*, *174*(6). https://doi.org/10.1111/PPL.13817

Llorente, B. E., Alasia, M. A., & Larraburu, E. E. (2016). Biofertilization with *Azospirillum brasilense* improves the in vitro culture of *Handroanthus ochraceus*, a forestry, ornamental and medicinal plant. *New Biotechnology*, *33*(1), 32–40. https://doi.org/10.1016/J.NBT.2015.07.006

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., Rio, T. G. Del, Edgar, R. C., Eickhorst, T., Ley, R. E., Hugenholtz, P., Tringe, S. G., & Dangl, J. L. (2012). Defining the core Arabidopsis thaliana root microbiome. *Nature 2012 488:7409*, *488*(7409), 86–90. https://doi.org/10.1038/nature11237

Ma, Y., Zhang, J., & Chen, S. (2007). *Paenibacillus zanthoxyli* sp. nov., a novel nitrogen-fixing species isolated from the rhizosphere of *Zanthoxylum* simulans. *International Journal of Systematic and Evolutionary Microbiology*, *57*(4), 873–877. https://doi.org/10.1099/IJS.0.64652-0/CITE/REFWORKS

Maan, P. K., & Garcha, S. (2021). Production technology, properties, and quality management. *Biofertilizers: Volume 1: Advances in Bio-Inoculants*, 31–43. https://doi.org/10.1016/B978-0-12-821667-5.00013-0

Mahmood-ur-Rahman, Ijaz, M., Qamar, S., Bukhari, S. A., & Malik, K. (2019). Abiotic Stress Signaling in Rice Crop. *Advances in Rice Research for Abiotic Stress Tolerance*, 551–569. https://doi.org/10.1016/B978-0-12-814332-2.00027-7

Makino, Y. (1986). Genus *Bacillus*. *Bergey's Manual of Systematic Bacteriology*, *2*(1), 1105–1139. https://doi.org/10.3136/FSTR.11.115

Mohamed, H. I., Sajyan, T. K., Shaalan, R., Bejjani, R., Sassine, Y. N., & Basit, A. (2022). Plant-mediated copper nanoparticles for agri-ecosystem applications. *Agri-Waste and Microbes for Production of Sustainable Nanomaterials*, 79–120. https://doi.org/10.1016/B978-0-12-823575-1.00025-1

Morath, S. U., Hung, R., & Bennett, J. W. (2012). Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. *Fungal Biology Reviews*, *26*(2–3), 73–83. https://doi.org/10.1016/J.FBR.2012.07.001

Naing, K. W., Anees, M., Kim, S. J., Nam, Y., Kim, Y. C., & Kim, K. Y. (2014). Characterization of antifungal activity of *Paenibacillus ehimensis* KWN38 against soilborne phytopathogenic fungi belonging to various taxonomic groups. *Annals of Microbiology*, *64*(1), 55–63. https://doi.org/10.1007/S13213-013-0632-Y/METRICS

Paluch, J. G., & Gruba, P. (2012). Effect of local species composition on topsoil properties in mixed stands with silver fir (Abies alba mill.). *Forestry, 85*(3), 413–426. https://doi.org/10.1093/forestry/cps040

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., & Crecchio, C. (2015). Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biology and Fertility of Soils*, *51*(4), 403–415. https://doi.org/10.1007/S00374-015-0996-1/METRICS

Podile, A. R., & Kishore, G. K. (2006). Plant growth-promoting rhizobacteria. *Plant-Associated Bacteria*, 195–230. https://doi.org/10.1007/978-1-4020-4538-7_6

Radhakrishnan, R., Hashem, A., & Abd Allah, E. F. (2017). *Bacillus*: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, *8*(9), 293128. https://doi.org/10.3389/FPHYS.2017.00667/BIBTEX

Raza, W., Yuan, J., Ling, N., Huang, Q., & Shen, Q. (2015). Production of volatile organic compounds by an antagonistic strain *Paenibacillus polymyxa* WR-2 in the presence of root exudates and organic fertilizer and their antifungal activity against *Fusarium oxysporum* f. sp. *niveum*. *Biological Control*, *80*, 89–95. https://doi.org/10.1016/J.BIOCONTROL.2014.09.004

Ruzzi, M., & Aroca, R. (2015). Plant growth-promoting rhizobacteria act as biostimulants in horticulture. *Scientia Horticulturae*, *196*, 124–134. https://doi.org/10.1016/J.SCIENTA.2015.08.042

Schasteen, C. S. (2023). Safety of Food and Beverages: Oilseeds, Legumes and Derived Products. *Reference Module in Food Science*. https://doi.org/10.1016/B978-0-12-822521-9.00159-3

Shannon Calvin, M. C. (1984). Benefits and limitations in breeding salt-tolerant crops. *California Agriculture*.

Simon, J. (2013). Electron Transport in Facultative Anaerobes. *Encyclopedia of Biophysics*, 630–633. https://doi.org/10.1007/978-3-642-16712-6_32

Singh, A. K., & Chhatpar, H. S. (2011). Purification and characterization of chitinase from *Paenibacillus* sp. D1. *Applied Biochemistry and Biotechnology*, *164*(1), 77–88. https://doi.org/10.1007/S12010-010-9116-8/METRICS

Smith, D. L., Subramanian, S., Lamont, J. R., & Bywater-Ekegàrd, M. (2015). Signaling in the phytomicrobiome: Breadth and potential. *Frontiers in Plant Science*, *6*(9), 146627. https://doi.org/10.3389/FPLS.2015.00709/BIBTEX

Timofeeva, A. M., Galyamova, M. R., & Sedykh, S. E. (2023). Plant Growth-Promoting Soil Bacteria: Nitrogen Fixation, Phosphate Solubilization, Siderophore Production, and Other Biological Activities. *Plants*, *12*(24). https://doi.org/10.3390/PLANTS12244074

Usmonov, A., Yoo, S. J., Kim, S. T., Yang, J. S., Sang, M. K., & Jung, H. W. (2021a). The *Bacillus zanthoxyli* HS1 Strain Renders Vegetable Plants Resistant and Tolerant against Pathogen Infection and High Salinity Stress. *The Plant Pathology Journal*, *37*(1), 72–78. https://doi.org/10.5423/PPJ.NT.12.2020.0219

Wang, Q., Dodd, I. C., Belimov, A. A., & Jiang, F. (2016). Rhizosphere bacteria containing 1-aminocyclopropane-1- carboxylate deaminase increase the growth and photosynthesis of

pea plants under salt stress by limiting Na^+ accumulation. *Functional Plant Biology*, *43*(2), 161–172. https://doi.org/10.1071/FP15200

Wolny, E., Betekhtin, A., Rojek, M., Braszewska-Zalewska, A., Lusinska, J., & Hasterok, R. (2018). Germination and the early stages of seedling development in *Brachypodium Distachyon*. *International Journal of Molecular Sciences, 19*(10), 2916. https://doi.org/10.3390/ijms19102916

Yan, F., Yu, Y., Gozzi, K., Chen, Y., Guo, J. H., & Chai, Y. (2017). Genome-wide investigation of biofilm formation in *Bacillus cereus*. *Applied and Environmental Microbiology*, *83*(13). https://doi.org/10.1128/AEM.00561-17/SUPPL_FILE/ZAM999117899SD2.XLSX

Zhao, S., Zhang, Q., Liu, M., Zhou, H., Ma, C., & Wang, P. (2021). Regulation of Plant Responses to Salt Stress. *International Journal of Molecular Sciences 2021, 22*(9), 4609. https://doi.org/10.3390/IJMS22094609

Zhou, X., Wang, J. T., Zhang, Z. F., Li, W., Chen, W., & Cai, L. (2020). Microbiota in the Rhizosphere and Seed of Rice From China, With Reference to Their Transmission and Biogeography. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/FMICB.2020.00995

APPENDIX

A1 chlorophyll content

	Batch	Mean	Average	SD
Control Batch	Batch 1	11.71	11.905	0.276

	Batch 2	12.1		
Paddy inoculated with UMK-PNF6	Batch 1	15.795	16.573	1.0996
	Batch 2	17.35		

A2 leaf length grown

	Batch	Mean	Average	SD
Control Batch	Batch 1	10	10.75	1.061
	Batch 2	11.5		
Paddy inoculated with UMK-PNF6	Batch 1	15.07	15.685	0.870
	Batch 2	16.3		

A3 moisture content

	Batch	Mean	Average	SD
Control Batch	Batch 1	84.11	83.915	0.276
	Batch 2	83.72		
Paddy inoculated with UMK-PNF6	Batch 1	88.22	88.61	0.552
	Batch 2	89.0		

A4 chlorophyll content

	Batch	Mean	Average	SD
	50mM			
Control Batch	Batch 1	10.746	10.598	0.209
	Batch 2	10.45		

Paddy inoculated with UMK-PNF6 with 50mM of NaOH	Batch 1	16.692	16.736	0.0622
	Batch 2	16.78		
	100mM			
Control Batch	Batch 1	7.63	7.54	0.127
	Batch 2	7.45		
Paddy inoculated with UMK-PNF6 with 100mM of NaOH	Batch 1	11.7	11.58	0.170
	Batch 2	11.46		
	150mM			
Control Batch	Batch 1	4.814	5.007	0.273
	Batch 2	5.2		
Paddy inoculated with UMK-PNF6 with 150mM of NaOH	Batch 1	8.52	8.615	0.134
	Batch 2	8.71		

A5 leaf length grown

	Batch	Mean	Average	SD
	50mM			
Control Batch	Batch 1	11.32	11.2	0.170
	Batch 2	11.08		
Paddy inoculated with UMK-PNF6 with 50mM of NaOH	Batch 1	15.78	15.495	0.403
	Batch 2	15.21		
	100mM			
Control Batch	Batch 1	9.06	8.96	0.141

	Batch 2	8.86		
Paddy inoculated with UMK-PNF6 with 100mM of NaOH	Batch 1	12.21	11.985	0.318
	Batch 2	11.76		
150mM				
Control Batch	Batch 1	5.76	5.495	0.375
	Batch 2	5.23		
Paddy inoculated with UMK-PNF6 with 150mM of NaOH	Batch 1	8.21	8.2925	0.117
	Batch 2	8.375		

A6 moisture content

	Batch	Mean	Average	SD
50mM				
Control Batch	Batch 1	83.42	83.25	0.240
	Batch 2	83.08		
Paddy inoculated with UMK-PNF6 with 50mM of NaOH	Batch 1	87.698	87.509	0.267
	Batch 2	87.32		
100mM				
Control Batch	Batch 1	82.45	82.385	0.0919
	Batch 2	82.32		
Paddy inoculated with UMK-PNF6 with 100mM of NaOH	Batch 1	85.43	85.645	0.304
	Batch 2	85.86		
150mM				

Control Batch	Batch 1	79.45	79.73	0.396
	Batch 2	80.01		
Paddy inoculated with UMK-PNF6 with 150mM of NaOH	Batch 1	83.24	83.4	0.226
	Batch 2	83.56		

A7 control first batch of raw data

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	4 One wilted One small	11.1,12.4	4.2	9.5	5.3	0.1164	0.02	85.4
2	2 One wilted	10.3	4.2	8.0	3.8	0.1085	0.0188	82.57
3	4 One wilted	12.1,5.9,4.8	4.1	18.4	14.3	0.1455	0.022	84.94
4	4 One wilted	19.4,17.5,7.7	3.9	18.1	14.2	0.1827	0.0292	84.01
5	4	13.4,17.2,13.9,11.7	3.5	15.9	12.4	0.1908	0.0313	83.62

A8 control second batch raw data

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	3 One small	16.5, 8.9 11.7	5.0	14.8	9.8	0.1825	0.018	90.14

2	3 One small	15.7, 11.0 13.35	5.2	16.2	11	0.1619	0.018	88.88
3	3 One small	12.3, 10.5 11.4	5.3	17.5	12.2	0.2123	0.037	82.44
4	3 1 wilted	15.2, 7.7 11.45	5.5	17.5	12	0.2481	0.0251	89.82
5	4 2 small	17.9, 7.3 12.6	5.4	17.9	12.5	0.1656	0.021	87.32

A9 treated plant first batch raw data

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	4 One wilted	14.2,19.4,12.84	3.4	17.7	14.3	0.1551	0.022	85.82
2	3	22.5,14.8,7.7	3.4	15.2	11.8	0.1197	0.017	86.07
3	4 One small	21.1,15.8,7.5	3.5	20.3	16.8	0.2199	0.028	87.27
4	4 One wilted One small	19.6,20.0	3.4	19.1	15.7	0.2589	0.022	91.5
5	4	19.1,9.0,13.6	3.4	20.15	16.75	0.2380	0.023	90.34

A10 treated plant second batch raw data

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	4 One wilted	16.9, 18.3, 17.8	5.2	21.0	15.8	0.1337	0.015	88.79

2	3 One small	16.5, 18.8	5.5	21.5	16.0	0.1158	0.0105	90.91
3	4 Two small	20.6, 14.1	4.8	23.7	18.7	0.1129	0.0106	90.6
4	3 One small	20.8, 12.3	4.7	20.5	15.8	0.0761	0.0103	86.44
5	3	16.7, 19.2,16.7	4.8	20.0	15.2	0.1899	0.0233	88.26

A11control for 50mM first batch raw data

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	4 One wilted	13.9, 3.3, 2.3	5.2	17.9	12.7	0.1337	0.0253	81.04
2	3 One small	20.0, 5.6	4.8	17.3	12.5	0.1158	0.0199	82.81
3	4 Two small	24.2, 11.2, 8.35	5.4	15.7	10.3	0.2176	0.0283	86.98
4	3 One small	9.1	4.8	14.6	9.8	0.1129	0.0194	82.85

A12control for 50mM second batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	3 One wilted	14.5,6.2	5.6	12.8	7.2	0.1951	0.0304	84.42

2	4	13.8,16.3,8.7,13.28	5.4	16.92	11.52	0.2863	0.0472	83.51
3	3 One wilted	7.1,2.3	5.4	21.1	15.7	0.1961	0.0351	82.1
4	4	14.2,18.2,14.5,8.02	5.4	15.3	9.9	0.1534	0.0272	82.29

A13treatment for 50mM first btach

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	2 One wilted	20.12	4.5	20.72	16.22	0.1132	0.0123	89.102
2	3 One wilted	20.0,16.3	4.3	23	18.7	0.1593	0.0171	89.27
3	3 One wilted	15.1,11.9	4.6	18.1	13.5	0.1559	0.0193	87.62
4	3	25.5,15.0,4.5	4.4	19.1	14.7	0.1601	0.0243	84.8

A14treatment for 50mM second batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	3	16.2, 17.3,15.1	6.7	21.94	15.24	0.174	0.0218	87.5
2	2	11.02	6.9	21.64	14.74	0.165	0.0203	87.68
3	3 1 wilted	19.3, 20.2	6.8	21.94	15.14	0.147	0.0293	80.07
4	3 1 wilted	18.8, 21.5	6.9	22.6	15.7	0.158	0.0094	94.03

A15control for 100mM first batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)

1	4 One wilted One small	7.5,6.4	4.2	9.5	5.3	0.1164	0.017	85.4
2	2	4.5,10.3	3.4	13.2	9.8	0.1197	0.025	81.57
3	4 One wilted	12.1,5.9,4.8	4.1	9.64	5.54	0.1455	0.019	86.94
4	4 One wilted	11.1,6.9,7.7	3.9	19.5	15.6	0.1827	0.044	75.89

A16control for 100mM second batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	4 One wilted One small	8.5,4.18	4.2	9.5	5.3	0.1164	0.017	85.4
2	3	1.68,14.8,7.7	3.4	13.2	9.8	0.1197	0.025	81.57
3	3 One wilted One small	1.2	3.8	15.0	11.2	0.1204	0.0216	82.06
4	4 2 wilted	11.9, 16.5	6.7	15.84	9.14	0.196	0.037	80.25

A17treatment for 100mM first batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	2	20.8,10.6	4.9	19.22	14.32	0.1772	0.0280	84.18

2	3 One wilted	11.2,5.0	4.8	13.6	8.7	0.1108	0.0179	83.84
3	4 One wilted	19.7,9.3,6.2	4.9	15.4	10.5	0.1622	0.0202	87.55
4	3 One wilted	15.94,6.6	4.8	20.12	15.32	0.1300	0.0188	86.15

A18treatment for 100mM second batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	2 One wilted	11.34	5.8	14.6	8.8	0.179	0.0227	87.3
2	3 One wilted	9.2,12.5	5.8	16.4	10.6	0.121	0.0171	85.87
3	3 One wilted	10.5, 13.1	6.0	16.6	10.6	0.142	0.0207	85.42
4	4 Two wilted	12.5,11.2	5.7	22.74	17.04	0.163	0.0247	84.85

A19control for 150mM first batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	4 Three wilted	8.5	5.7	6.7	1.0	0.145	0.02387	78.65
2	2 One wilted	2.6	5.8	14.6	8.8	0.179	0.0277	79.53
3	3 One wilted	3.12,3.19	6.0	9.37	3.37	0.142	0.0227	84.01
4	2 One wilted	5.0	4.9	14.8	9.9	0.1348	0.0194	75.61

A20control for 150mM second batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	2 One wilted	5.7	5.8	8.51	2.71	0.179	0.0277	75.28
2	4 2 wilted	2.9, 8.5	6.7	9.2	2.5	0.196	0.0387	80.25
3	3 One wilted One small	4.4	6.0	16.6	10.6	0.142	0.0218	84.63
4	3 One wilted One small	5.0	3.8	8.91	5.11	0.1204	0.017	79.88

A21treatment for 150mM first batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	2 One wilted	6.2	5.0	11.3	6.3	0.1476	0.0344	80.69
2	3	15.4,12.3,6.74	5.1	12.5	7.4	0.1354	0.0206	84.82
3	2 One wilted	11.4	5.2	14.07	8.87	0.1144	0.0197	82.78
4	2 One wilted	5.0	5.1	15.4	10.3	0.1346	0.0206	84.67

A22treatment for 150mM second batch

Sample	Number of Leaves	Chlorophyll Content for Each Leaves	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisture Content (%)
1	4 One wilted One small	9.1,14.18	4.2	9.5	5.3	0.1164	0.017	85.4

2	2	11.6,3.4	5.8	14.1	8.3	0.168	0.0327	80.54
3	3 1 wilted	10.1,5.5	6.5	19.8	13.3	0.156	0.0192	87.7
4	3 1 wiled	7.7,8.1	8.0	14.6	6.6	0.165	0.032	80.6