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Effect on the inoculation of *Paraburkholderia kururiensis* UMK PPS-6 towards plant's and it antimicrobial activity

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DECLARATION

I declare that this thesis entitled Investigation of *Paraburkholderia kururiensis* UMK-PPS6 bacterial 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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Acknowledgment

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Effect on the inoculation of *Paraburkholderia kururiensis* UMK PPS-6 towards plant's and it antimicrobial activity

ABSTRACT

Paraburkholderia kururiensis UMK-PPS6 is one of the PGPB strain bacteria that can help the growth of agricultural trees for the production of agricultural products. It has been identified for its ability to establish a symbiotic relationship with plants, especially in the Rhizosphere in soil areas directly affected by root secretions. Through this study, the ability of *Paraburkholderia kururiensis* UMK-PPS6 to help the growth of paddy plants based on the parameters of chlorophyll content, growing leaf length and moisture content was explored. Then, this research investigates the antimicrobial activity of *Paraburkholderia kururiensis* UMK-PPS6 and explores its potential effects on improving salinity stress tolerance in plants. Paddy plants inoculated with *Paraburkholderia kururiensis* UMK-PPS6 were subjected to different salinity levels (50mM, 100mM and 150mM), and their growth and physiological responses were carefully analyzed. In the antimicrobial activity segment, research investigated the inhibitory effect of *Paraburkholderia kururiensis* UMK-PPS6 on both gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*) bacteria. The experimental setup involves culturing the bacteria and evaluating its effect on bacterial growth, providing insight into its potential as an antimicrobial agent. Overall, this investigation contributes to the understanding of *Paraburkholderia kururiensis* UMK-PPS6 as a multifaceted microorganism with promising applications in antimicrobial strategies, biocontrol practices, and improving plant stress tolerance. These findings offer valuable insights into the potential use of these bacteria for sustainable and resilient agricultural practices.

Kesan ke atas inokulasi Paraburkholderia kuuriensis UMK PPS-6 pada tumbuhan dan aktiviti antimikrobnya

ABSTRAK

Paraburkholderia kururiensis UMK-PPS6 merupakan salah satu bakteria terikan PGPB yang boleh membantu pertumbuhan pokok pertanian untuk penghasilan hasil pertanian. Ia telah dikenal pasti kerana keupayaannya untuk mewujudkan hubungan simbiotik dengan tumbuhan, terutamanya di Rhizosphere di kawasan tanah yang terjejas secara langsung oleh rembesan akar. Melalui kajian ini, kebolehan Paraburkholderia kururiensis UMK-PPS6 membantu pertumbuhan tanaman padi berdasarkan parameter kandungan klorofil, panjang daun dan kandungan lembapan telah diterokai. Kemudian, penyelidikan ini menyiasat aktiviti antimikrob Paraburkholderia kururiensis UMK-PPS6 dan meneroka kesan potensinya terhadap meningkatkan toleransi tekanan kemasinan dalam tumbuhan. Tumbuhan padi yang disuntik dengan Paraburkholderia kururiensis UMK-PPS6 tertakluk kepada tahap kemasinan yang berbeza (50mM, 100mM dan 150mM), dan pertumbuhan serta tindak balas fisiologinya dianalisis dengan teliti. Dalam segmen aktiviti antimikrob, penyelidikan menyiasat kesan perencatan Paraburkholderia kururiensis UMK-PPS6 pada kedua-dua bakteria gram-positif (Bacillus subtilis) dan gram-negatif (Escherichia coli). Persediaan percubaan melibatkan pengkulturan bakteria dan menilai kesannya terhadap pertumbuhan bakteria, memberikan gambaran tentang potensinya sebagai agen antimikrob. Secara keseluruhannya, penyiasatan ini menyumbang kepada pemahaman Paraburkholderia kururiensis UMK-PPS6 sebagai mikroorganisma pelbagai rupa dengan aplikasi yang menjanjikan dalam strategi antimikrob, amalan biokawalan dan meningkatkan toleransi tekanan tumbuhan. Penemuan ini menawarkan pandangan berharga tentang potensi penggunaan bakteria ini untuk amalan pertanian yang mampan dan berdaya tahan.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Agricultural production has started to decline in recent years. This is due to the number of issues such as the lack of agricultural land, the threat of pests and pathogens as well as the high salinity of agricultural land which hinders plant growth and affects agricultural yields. Recent socioeconomic developments have exacerbated the loss of farmland and the dispersion of farm labor, while the fundamental driver of agricultural expansion has changed from an increase in labor to an expansion in capital expenditures such as fertilizers and machinery (G Yuan et al., 2022). This scarcity of agricultural land necessitates innovative approaches to maximize productivity and address the challenges associated with limited resources. Researchers have studied the factors that contribute to the loss of paddy fields, and they concur as the expansion of development land and the rise in the extent of dry land are the primary causes (X Li et al., 2021). Additionally, climate change has a variety of consequences on agriculture, which vary depending on the place, the time period, and the kinds of crops under consideration. There has been a significant and detrimental effect on agriculture as a result of climate change effects, particularly rainfall and temperature alterations (Mahli el at. 2021).

Next, the breeding of pests and pathogens that seriously harm crops, resulting in lower yields and financial losses, is a factor that interferes with agriculture. All of these which are pathogen and pests include insects, fungi, bacteria, and viruses, which can spread quickly and infect cultivated plants. The presence of weeds and other pests, including as viruses, bacteria,

fungi, and insects, can cause significant harm to plants, resulting in reduced crop output and, in some instances, entire crop destruction (Kubiak el at., 2022). Especially in densely populated areas with intense agricultural practises, their presence is a persistent threat to crop output. Salt is a significant abiotic factor that exerts pressure on plant expansion and growth globally. Consequently, it poses a crucial constraint on agricultural production in many places worldwide.. Plant development and yield are severely stunted by salinity (Etesami et al., 2019).

Paraburkholderia kururiensis is a gram-negative bacterium with potential use in agriculture its potential as a tool for biocontrol because of its produce various secondary metabolites that have antimicrobial activity. Along with fostering plant development, PGPB as the species may additionally enhance the absorption of three essential nutrients: tolerance for stress, resistance system induction, and defense against disease in plants (Souza et al, 2015). It was demonstrated that the bacterium produces a variety of bioactive substances, such as volatile organic, *phenazines*, and *lipopeptides* compounds, it possesses the ability to impede the proliferation of several kinds of fungi and bacteria that pose a threat to plants (M Ayaz el at., 2023).

1.2 Problem Statement

Recently, most crop plants are vulnerable to salinity triggered by excessive levels of salt in the soil, making salinity one of the harshest environmental variables affecting crop plant yield. The percentage of land influenced by salinity is growing steadily everyday. (Shrivastava el at., 2015). Competent inspection of salt-affected zones can aid in the fight against global environmental change as well as the efficient management and use for scarce land and water resources, making salinity a pressing issue on a worldwide scale. (Kayode el at., 2021). Various factors cause plant soil salinity to be high. High osmotic tension, nutrient problems, toxicities, poor soil quality, and lower yields are some of the issues that affect crops cultivated in saline soils. (Shrivastava el at., 2015). Then, the other problem related is plant pathogens. The presence of pathogens, likely as fungi, viruses, and microbes can result in devastating diseases that can lead to substantial yield losses in paddy production. Pathogens including microorganisms, viruses, fungi, even protozoa, alongside arthropods including parasitical plants, are typically to blame for infectious plant illnesses. (P Nazarov el at., 2020). Symptoms of infection from such pathogens include dying out, turning yellow, tissue necrosis, lesions, and poor quality of grain, and they can manifest themselves wherever on the rice crop, from the roots to the stems to the leaves to the grains themselves.. The diseases caused by plant pathogens not only reduce the yield of rice crops but also compromise their nutritional value and marketability.

1.2 Objectives

This research is carried out with the following objectives:

I. To evaluate the effect of the inoculation of *Paraburkholderia kururiensis* UMK-PPS6 on paddy's growth
II. To evaluate paddy inoculated with *Paraburkholderia kururiensis* UMK-PPS6 on plant's tolerance against salinity stress
III. To determine the antimicrobial activities of *Paraburkholderia kururiensis* UMK-PPS6 against gram-positive and gram-negative bacteria

1.3 Scope of Study

The strain of bacteria known as UMK-PPS6 which is *Paraburkholderia kururiensis* was utilized in this research. This particular strain is a *rhizobacterium* that was obtained from paddy fields in the previous research (Kamaruzaman et al., 2022). The performance of paddy's growth after 18 days was examined in order to evaluate the influence of the inoculation of *Paraburkholderia kururiensis* UMK-PPS6 on paddy's growth. This evaluation was carried out in order to determine *Paraburkholderia kururiensis* effect on paddy's growth, tolerance against salinity stress and antimicrobial activities against gram-positive and gram-negative bacteria. Recorded data included the amount of chlorophyll, the length of the leaves that had grown, and the amount of moisture content. A pot study was conducted with three different amounts of sodium chloride in the soil: 50 Mm, 100 Mm, and 150 Mm. Each paddy plant injected with UMK-PPS6 *Paraburkholderia kururiensis* will be sprayed with different salinity concentrations according to batches of 50 Mm, 100 Mm and 150

Mm and then data was taken and compared with control data of paddy plants without injected bacteria.

The salinity stress tolerance of the plant was noticed from the performance of the plant's growth, and this was the basis for the evaluation. As an example, the chlorophyll content of each leaf, the length of the leaf that has grown, and the amount of moisture present. A total of two different microorganisms were utilized in order to conduct the antibacterial activity evaluation. *Bacillus subtilis*, a gram-positive bacteria, was used for the experiment, while *Escherichia coli*, a gram-negative bacterium (abbreviated as *E. coli*), was used for the experiment. A disk susceptibility assay was performed on it for testing.

1.4 Significant of Study

Through this study, the effect of *Paraburkholderia kururiensis* UMK-PPS6 on plant growth was determined. Since it shows positive results, It could be formulated as a highly effective bioinoculant to species of trees to decrease fertilizer consumption, enhance crop yield, and mitigate the emission of greenhouse gases (Shahwar et al., 2023). Moreover, *Paraburkholderia kururiensis* can be extremely efficient as a substitute for conventional fertilizers and chemical substances (Madhaiyan el at., 2021). In addition, based on the results of plant tolerance to salinity stress, *Paraburkholderia kururiensis* also showed positive results which is that paddy plants are able to be independent and have a strong resistance to soil salinity. Based on a previous study, Ruanming Zhu in 2021, the resistance of *Paraburkholderia kururiensis* (rice seedling) to salinity stress was successfully proven by the researcher with positive test results. In addition, the antimicrobial activity of *Paraburkholderia kururiensis* UMK-PPS6 was determined and it showed positive results against microbes. This means that *Paraburkholderia kururiensis* has the potential to be a biological agent to fight plant pathogens

including fungi, bacteria and virus (Dias et al., 2019). Consequently, it can serve as a substitute for pesticides that contain chemicals and mitigate the adverse environmental consequences associated with their use. In summary, The symbiotic relationship between bacteria and plants is crucial for farmland and has the potential to enhance fertility of the soil, increase crop output, and thereby mitigate the adverse effects of fertilization on the environment. This, in turn, can help accomplish the objective of agricultural production that is sustainable (Suman et al., 2022). Overall, this research can provide insight into the potential use of *Paraburkholderia kururiensis* As an antimicrobial substance in the presence of high salt levels, it also makes an important contribution towards the industry of agriculture. Specifically, it can enhance the sustainability of agricultural output, enlarge yield, and strengthen the ability of plants to adapt to environmental conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 Paraburkholderia kururiensis

Paraburkholderia kururiensis, a rhizobacterium isolated from various agricultural environments, has been the subject of extensive research due to its diverse range of plant growth promoting and biocontrol activities. (Elshafie, H. S., & Camele, I.,2021). One of its notable attributes is its ability to solubilize phosphate minerals in the soil. Paraburkholderia species typically possess a robust ability to dissolve inorganic phosphates (Madhaiyan el at.,2021). Phosphorus is a vital element for the growth of plants, yet it is frequently scarce in numerous types of soil. The limited availability of phosphorus in soil, caused by its a fixation, often hinders the optimal development and advancement of plants (Malhotra el at., 2018). *Paraburkholderia kururiensis* produces organic acids and enzymes that break down insoluble phosphate compounds, making phosphorus more accessible to plants. *Paraburkholderia kururiensis* primarily colonized root hairs and then spread into xylem vessels in rice plants (Mattos et al., 2008). This enhances nutrient uptake and promotes healthy root development, ultimately leading to improved plant growth and yield. In addition to phosphate solubilization, *Paraburkholderia kururiensis* is known for its nitrogen-fixing capability. Over the last two decades, *Paraburkholderia kururiensis* gained considerable importance for their ability to fix nitrogen, promote plant growth and degrade recalcitrant chemical compounds (Dias el at., 2019). It forms a symbiotic relationship with leguminous plants, where it converts atmospheric nitrogen into a form that plants can utilize. This reduces the reliance on synthetic nitrogen fertilizers, which can have environmental implications, and promotes sustainable nitrogen management in agriculture.

Another noteworthy aspect of *Paraburkholderia kururiensis* is its ability to produce phytohormones, such as indole-3-acetic acid (IAA). Phytohormones play essential roles in the development and advancement of plants, overseeing processes that include elongation of cells, branching of roots, and reactions to stress (Sabagh et al., 2022). The production of IAA by *Paraburkholderia kururiensis* facilitates root development and increases nutrient assimilation, resulting in enhanced plant vitality and resistance (Singh et al., 2013). According to the previous study by Pandey (2005), The identification of *Burkholderia* was based on several factors, including the generation of aminocyclopropane-1-carboxylic acid deaminase, fixation of nitrogen, phosphate solubility, indol acetic acid (IAA) production, siderophores, and hydrogen cyanide (HCN) production. *Paraburkholderia kururiensis* Additionally, it demonstrates biocontrol properties against plant diseases, encompassing bacteria, fungus, and virus. It generates antimicrobial chemicals that hinder the proliferation of harmful pathogen, safeguarding plants against illnesses (Fernandes et al., 2021). In addition to enhancing plant development, species of Plant development Promoting Bacteria (PGPB) might additionally improve absorption of nutrients, enhance tolerance to stress, induce resistance in the system, and provide defense against plant diseases (Vitorino & Bessa., 2017). Additionally, *Paraburkholderia kururiensis* engages in competition with diseases for resources and territory, hence restricting their ability to establish and propagate. Bacteria can defend plants against infections by reducing the levels of reproductive hormones and stimulating resistance systems in plants (Lakshmanan et al., 2012). This biocontrol activity provides an ecologically sound alternative to pesticides that are chemical-based, diminishing the dependence on artificial substances and advocating for sustainable management of pests methods.

2.2 Antimicrobial Activities

Antimicrobial activities refer to the ability of a substance or organism to inhibit the growth or kill germs, as also as fungi, bacteria, viruses, or other diseases. These acts are able to be demonstrated through several processes, which include the synthesis of antimicrobial substances, enzymes, or the regulation of microbial growth through competitive interactions. The primary classes of antimicrobial compounds can be categorized based on their biological mechanism of antimicrobial activity. Agents that hinder the manufacture of cell walls, disrupt the polarization of cell membranes, impede the production of proteins, hinder the synthesis of nucleic acids, and obstruct metabolic pathways in bacteria (Reygaert et al., 2018). Based on previous study by Caulier, S (2019), The prevailing belief among scientists is that microorganisms generate antibiotic chemicals to regulate their interactions with neighboring microbes. Bacillus is a genus of Gram-positive, rod-shaped bacteria that are found in various settings. They exhibit antibacterial properties against a range of pathogens, such as molds, viruses, bacteria, and parasites.. These antimicrobial activities are of great importance for various applications such as agriculture, biotechnology and medicine (Sumi et al., 2015). The generation of non-peptide metabolites is one of the main ways that Bacillus species show antibacterial action. These non-peptide metabolites, which are structurally diverse and bioactive, have been extensively studied for their biosynthetic pathways, biological activities, and structural characteristics (Tran et al., 2022).

Bacillus species engage in competition with other microbes by synthesizing an extensive variety of antibiotic chemicals. Several strains of *Bacillus*, or the antimicrobial substances extracted from them, have the potential to effectively manage phytopathogenic bacteria (Wan et al., 2022). This could result in reduced dependency on chemical pesticides, which often have negative effects on the environment. Soil-borne pathogens have the ability to persist for extended periods of time in many sources such as host plant fragments, soil organic

matter, free-living organisms, or soil structures. This particular species poses a challenge for control since it has a wide range of hosts and can persist in the soil. As a result, substantial dosages of chemical fungicides are necessary to manage it effectively (Dutta et al., 2023).

2.3 Importance of Antimicrobial Activities

The production of antimicrobial chemicals by microorganisms has important ramifications for a variety of industries, including environmental protection, agriculture, and medicine. Bioactive chemicals possessing anti-fungal, antibacterial, and cytotoxic properties are identified as being produced by microorganisms (Elissawy et al., 2021). Among the importance of antimicrobial activity characterized in this literature review, there are 3 which are plant disease management, environmental protection, and sustainable agriculture. Firstly, an important threat to the world's food supplies is posed by plant pathogens that cause plant diseases. Since their numbers fluctuate across time, geography, and their genotype, plant diseases are challenging to regulate (RN Strange et al., 2005). Effective control of these pathogens is essential to ensure sustainable crop production. Bacterial species such as *Rhizobacteria* have successfully protected plants from plant pathogens and this has been proven through the studies of previous researchers. *Rhizobia are microorganisms that live together present in the roots of leguminous crops. They have a crucial role in nitrogen fixation and also contribute to biocontrol and plant growth promotion. They achieve this by producing mycolytic enzyme, antibiotic, siderophore, and the acid hydrocyanic (HCN), which restrict the development of pathogenic fungi like Macrophomina, Rhizoctonia, Fusarium and Sclerotium* (MA Pandit et al., 2022). The antimicrobial activity of *Paraburkholderia kururiensis* will offer promising prospects for biocontrol strategies.

2.4 Salinity Stress Tolerance

Salinity stress poses a significant limitation on agricultural productivity, especially in regions characterized by elevated soil salt levels. The phenomenon presents substantial obstacles to the growth and development of plants, resulting in diminished agricultural output and financial setbacks. The presence of salt in plants leads to the activation of ion tension and osmotic pressure, resulting in a disturbance in metabolism as well as the harmful buildup of reactive oxygen species (ROS), which in turn causes oxidative harm to the plant (Majeed et al., 2019). Salinity-induced osmotic stress induces physiological alterations in plants, such as nutritional imbalance, hindered detoxification of reactive oxygen species (ROS), membrane deterioration, and decreased photosynthetic activity (Chourasia et al., 2021). Therefore, it is imperative to devise solutions that can improve the ability of plants to withstand salinity stress in order to promote sustainable agriculture.

Plants have developed resistance to stress mechanisms that are regulated by phytohormones to withstand the constantly changing stressful circumstances of their environment. Phytohormones are essential for plants to adapt and respond to salt stress by controlling their growth and development (Zhao et al., 2021). An analysis of salt stress indicators and the modulation for the immune system is significantly influenced by phytohormones. Based on a previous study by Pal G (2022), *Burkholderia* species have been discovered to enhance plant functioning and increase nutrient availability, particularly in stressful situations. *Burkholderia spp.* enhance plant survival against lethal diseases through processes like competitors, spontaneous systemic resistance, as well as antibiosis. Moreover, it has been claimed that they enhance plant resilience against many environmental stresses, specifically drought, salinity, and cold.

Moreover, studies have shown that *Paraburkholderia kururiensis* can improve the ability of plants to tolerate high levels of salt stress by aiding in the regulation of ions and

maintaining balance inside the plant by regulating the uptake and efflux of ions, maintaining a balanced ion ratio, and preventing toxic ion accumulation. The primary methods of adaptation to salinity overload are maintaining ion homeostasis, accumulating osmolytes, and producing universal proteins associated with salt stress resistance (Goyal et al., 2019). It also stimulates antioxidant defense systems in plants, reducing oxidative stress caused by salinity stress. Additionally, *Paraburkholderia kururiensis* promotes osmotic adjustment by facilitating the accumulation of compatible solutes, may aid in preserving cellular hydration and rigidity in the presence of high salt concentrations. Recently published studies have shown that Plant Growth Promoting Bacteria (PGPB) can induce salt tolerance in plants and enhance their growth (Hou et al., 2023).

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

3.1.1 Bacterial Strain

Paraburkholderia kururiensis UMK-PPS6 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 Pikovskaya'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 the inoculation of *Paraburkholderia kururiensis* UMK-PPS6 on paddy's growth

3.2.1.1 Preparation of Pikovskaya Media and Nutrient Agar

For Pikovskaya's Media, all of the composition 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 35g of the nutrient agar powder in 1000 mL distilled water. Then, autoclave for sterilization 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 35g of the nutrient agar powder in 1000 mL of distilled water.

Composition of Pikovskaya's Media	Concentration (g/L)
Glucose	10.000
Tricalcium Phosphate	5.000
Ammonium Sulphate	0.500
Sodium Chloride	0.200
Potassium Chloride	0.200
Magnesium Sulphate	0.100
Yeast Extract	0.500
Manganese Sulphate	0.002
Ferrous Sulphate	0.002

Table 3.1 Composition of Pikovskaya's Media

3.1.1.2 Cultivation of *Paraburkholderia kururiensis* UMK-PPS6 Bacteria on Pikovskaya's Medium Agar

Bacterial strain UMK-PPS6 was inoculated on the Pikovskaya's media agar plate. Then, left it in the incubator at 30°C for 3 days.

3.2.1.3 Preparation of Nutrient Medium Broth

15.75 grams 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 out 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 UMK-PPS6 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.

3.2.1.5 Preparation of seed

Seeds were added into the stock bottle together with 200ml of sterile distilled water with bleach 4% from the total content which was 8mL which later was shaken and soaked for 10 minutes. After 10 minutes, floating seeds indicate bad seeds were thrown away. The 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 it for 5 to 10 Days. The paper was sprayed constantly to avoid drying out.

Figure 3.1 Germination Process

3.2.1.7 Soil Media Preparation

Soil media was prepared with 2 top soil:1 cocopeat ratio. Soil was added into a plastic cup until it reached 3/4 of the cup, Moisture content tests were run to determine the volume of water needed to add into soil media to achieve 50%. Sterile distilled water was added a day prior pot study and left overnight. 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.

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 The evaluation of paddy inoculated with *Paraburkholderia kururiensis* UMK-PPS6 on plant's tolerance against salinity stress

3.2.2.1 Salt stress evaluation

The pot study for salinity stress is similar to the first objective methodology mentioned above which are in section 3.2.1.4 to 3.2.1.8 however with the addition of NaOH solution. Different concentrations of NaOH solutions were prepared (50Mm, 100Mm, 150Mm) with 1M of NaOH of stock solution through the dilution method. Every 4 days, the plants were sprayed with 10ml of NaOH solution.

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. Calibration was done before introducing plant tissue by pressing on the fingerrest 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.

Figure 3.2 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:

$$LEAF\ LENGTH\ GROWN = INITIAL\ HEIGHT - HEIGHT\ AFTER\ 18Days$$

Equation 3.1 Leaf Length Grown formula

3.2.3.3 Moisture Content (%)

3.2.3.3.i Plant Fresh Weight (g)

The plant's initial weight was recorded. After 25 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. Individual roots and shoots were weighed using an analytical balance.

Figure 3.3 Cleaning the soil from the root plant

Figure 3.4 After cleaning process

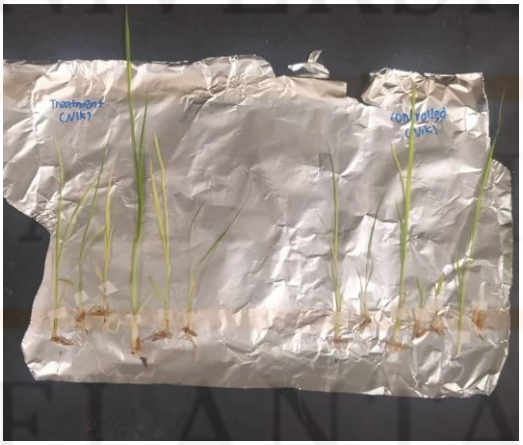

Figure 3.5 After cleaning process

3.2.3.3.ii Plant dry weight

The plants were then dried at 60 °C in an oven overnight, the dry matter yield will be determined after weighing the dried sample.

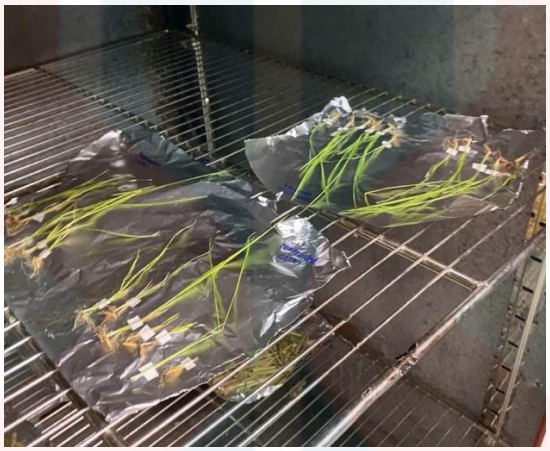

Figure 3.6 The sample was kept in the oven overnight for drying

3.2.3.3.iii Determination of moisture content

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 formula

3.2.4 Determination of the antimicrobial activity of *Paraburkholderia kururiensis* UMK-PPS6 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 10mL of nutrient broth media. For the first one, divide 4 parts of the inhibition zone on the agar container by marker and put 4 paper disc onto the agar plate. Next, dip the sterile cotton swab into *E.coli* culture and swabbed evenly on nutrient agar. Then, put 0.025g/mL of chloramphenicol on the paper disc in the positive control area, and put the same amount of sterile distilled water on the paper disc in the negative zone. The remaining 2 parts of the zone are 10*µl* of *Paraburkholderia kururiensis*. The plates were incubated at 30℃ overnight and the same procedure was repeated for antimicrobial test using *Bacillus subtillis*.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Bacterial Strain

Figure 4.1 for UMK-PPS6 strain on Pikovskaya media, Figure 4.2 for UMK-PPS6 strain on Nutrient Agar show the ability of bacteria to grow on the Pikovskaya media and Nutrient Agar. The selection of Pikovskaya as a media initially because Pikovskaya contains calcium triphosphate which is able to help the growth of bacteria dissolve insoluble phosphate. Phosphorus microorganisms play an important role in making phosphorus available to plants. They convert the insoluble form of phosphate in the soil into a soluble form, which can be absorbed by plant roots (Heba Adel el at., 2021). Solubilization of phosphate in agar plays a role in helping to increase nutrient phosphorus for crop plants (Sharma el at., 2013). However, because it took Pikovskaya quite a while to grow the bacteria *Paraburkholderia kururiensis* UMK-PPS6, Nutrient Agar was selected because it rich with nutrients that can help the bacteria grow rapidly in a shorter time in lag phase (Dhiraj el at., 2022).

Figure 4.1 : UMK-PPS6 strain on Pikovskaya media

Figure 4.2 : *Paraburkholderia kururiensis* UMK-PPS6 strain on Nutrient Agar

4.2 Germination Process

The germination process as Figure 4.3 below shows the grown paddy seed after 3 days of germination and Figure 4.4 growth of paddy after 10 days of germination.

Figure 4.3 Growth of paddy seed after 3 days

Figure 4.4 Growth of paddy seed after 10 days

4.3 Pot Study Process

The figure below shows the pot study process for the control batch after 10 Days.

Figure 4.5 The growth of paddy after 10 Days of pot study

4.4 Evaluation performance of *Paraburkholderia kururiensis* UMK-PPS6 on paddy's growth

The bacterial strain, *Paraburkholderia kururiensis* UMK-PPS6 that was injected into the paddy plant was compared with the paddy plant without the bacterial strain and its growth was observed for 18 days and the average mean and standard deviation were taken from the range (Table 4.1). Chlorophyll content, leaf length grown and moisture content were taken as measurements for paddy growth performance. The data taken after 18 days were ranged and the average mean and standard deviation (SD) were taken into account and statistical analysis t-test value statistically significant was set at $p < .05$ (Table 4.2). As shown in graph Figure 4.1, plant inoculated with *Paraburkholderia kururiensis* UMK-PPS6 shows better growth compared to control (without bacteria strain).

Table 4.1 Average Mean And SD From Two Independent Experiments

Sample / Parameters	Chlorophyll Content	Leaf Length Grown (cm)	Moisture Content (%)
Control	11.5±0.477	11.09±0.823	87.04±0.269
Paddy inoculated withUMK-PPS6	12.238±1.443	12.89±1.462	89.548±0.615

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-PPS6	0.003429	0.0309	0.00643

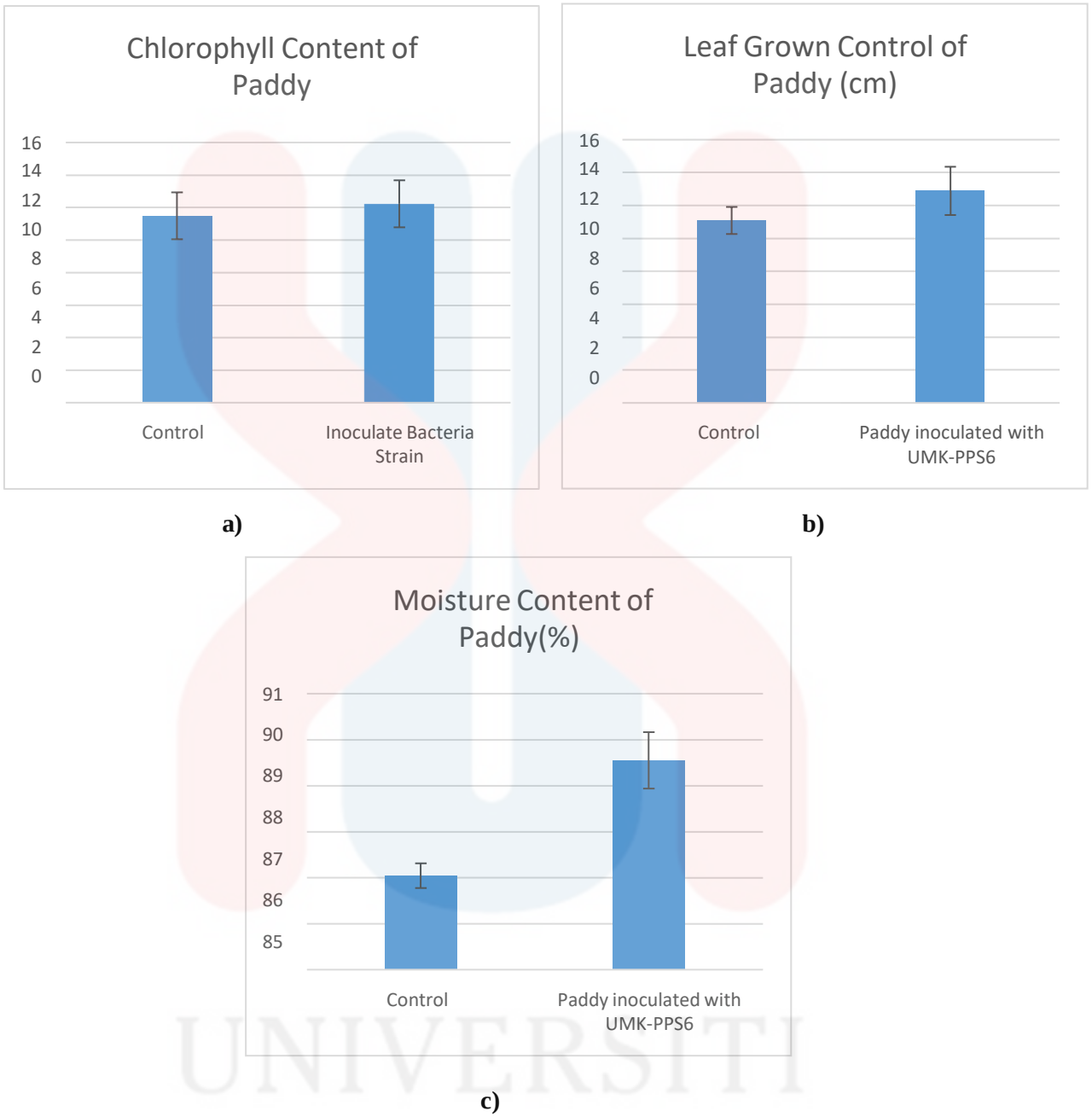

Figure 4.1 The effect of the inoculation *Paraburkholderia kururiensis* UMK-PPS6 on **(a)** Chlorophyll Content **(b)** Leaf Length Grown **(c)** Moisture Content

As that have been shown from Table 4.1, average mean for paddy inoculated with UMK-PPS6 for each measurement (chlorophyll content, leaf length grown and moisture content) is higher than control. Average data mean for paddy inoculated chlorophyll content UMK-PPS6 is 12.238 higher than control (11.5) while the value of leaf length grown obtained by paddy inoculated with UMK-PPS6 is 12.89 compared to control (11.09). In the moisture content section, the average mean value for paddy inoculated with UMK-PPS6 is 89.548 against the control value 87.04 Next, the **statistical graphbar** sketch (Figure 4.1) overall have shown a comparison between the control and paddy inoculated with UMK-PPS6 (chlorophyll content, leaf length grown and moisture content). This shows that Paraburkholderia kururiensis UMK-PPS6 clearly helps the growth of paddy in all aspects. By colonizing the rhizosphere of paddy plants, Paraburkholderia kururiensis can solubilize insoluble phosphorus in the soil, making it more accessible to the plants (Coutinho el at., 2015).

Next, based from Table 4.2 and statistical graphbar in Figure 4.1, the paddy batch inoculated with UMK-PPS6 distinguishes significantly from the control batch in terms of chlorophyll content, as shown from the obtained t-test value of 0.003429. This finding has implications for understanding the effect of *Paraburkholderia kururiensis* UMK-PPS6 inoculation on the production of chlorophyll in paddy plants, with a statistically significant threshold set at $p < .05$. This suggests that photosynthetic activity may be improved, which could lead to an increase in total plant growth and production. To sum up, the notable difference in chlorophyll content between the paddy batch that was inoculated with UMK-PPS6 and the control batch illustrates the capacity of this bacterium to promote plant growth by improving photosynthetic activity. Evidence that these phytohormones produced by bacteria can cause plant growth promotion has been demonstrated in some species of genus *Burkholderia* and *Paraburkholderia*, such as *P. phytofirmans* and *P. kururiensis* (Dias et al., 2019).

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As shown by the t-test value of 0.0309 obtained after statistical analysis of two independent experiment (Table 4.2) and statistical graphbar (Figure 4.1), the paddy batch inoculated with *Paraburkholderia kururiensis* UMK-PPS6 differs statistically substantially in leaf length grown from the control batch. This data indicates that UMK-PPS6 has a significant effect on paddy leaf growth at a significance level of $p<0.05$. A positive impact on the growth rate of the paddy plants is indicated by the observed increase in leaf length in the UMK-PPS6 inoculation batch. This process may have been influenced by *Paraburkholderia kururiensis* UMK-PPS6's plant growth-promoting qualities through a number of different mechanisms. The enhancement of nutrient availability facilitated by *Paraburkholderia kururiensis* UMK-PPS6 is one potential mechanism. As a plant growth-promoting bacteria, it can promote plant growth and use their own metabolism to solubilize phosphates, produce hormones and fix nitrogen, and they can directly affect plant metabolism. Besides, it also increase plant absorption of water and nutrients, and improving root development (Rigobelo, 2020). After that, the observation of a statistically significant increase in leaf length in the paddy group inoculated with *Paraburkholderia kururiensis* UMK-PPS6 showed that the bacteria had a positive effect on the vegetative growth of paddy plants. These findings highlight the function of microbial inoculants in improving growth parameters and offer insightful information on the possible use of these agents in sustainable agriculture.

The calculated t-test value of 0.00643 shown a statistically significant difference in moisture content between the control group and the paddy group inoculated with *Paraburkholderia kururiensis* UMK-PPS6, with the significance level set at $p < 0.05$. The observation that the moisture content was higher in the paddy batch treated with *Paraburkholderia kururiensis* UMK-PPS6 indicates a direct effect of the microbial inoculant on the water status of the paddy plant. The action of *Paraburkholderia kururiensis* as a plant growth promoting mechanism helps increase soil moisture and is more efficient in regulating water use for plant independence. *Paraburkholderia kururiensis* can improve nutrient absorption, increase tolerance to stress, induce systemic resistance (Fernandes el at., 2021) . In the final analysis, *Paraburkholderia kururiensis* UMK-PPS6 appears to have a favorable impact on the water use efficiency of paddy plants, as evidenced by the statistically significant increase in moisture content seen in the UMK-PPS6 inoculated paddy group.

4.5 Evaluation performance of *Paraburkholderia kururiensis* UMK-PPS6 on planttolerance against salinity stress

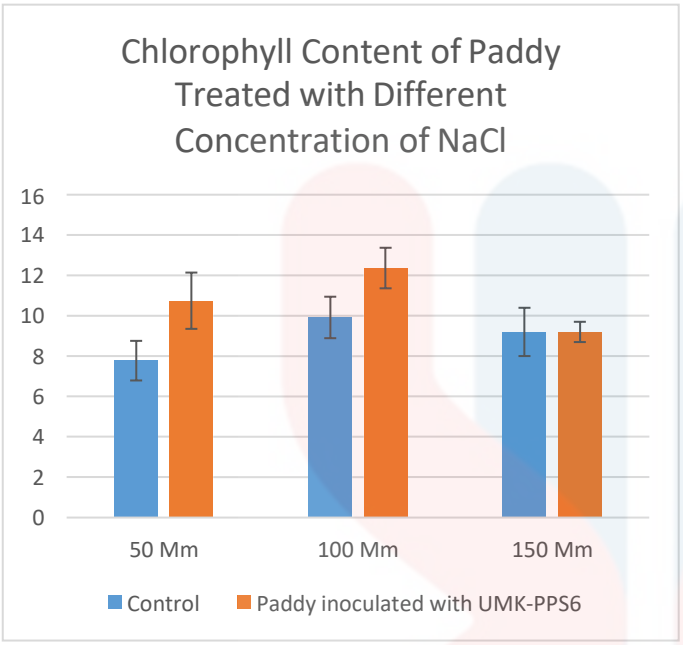
Salinity stress was introduced to the plants by spraying different concentrations of NaCL every four days. As shown in Figure 4.2, the graphbar statistically displays the average mean value for each different concentration for the measurement of chlorophyll content, leaf length grown and moisture content. Based on the data that has been displayed, paddy inoculatedwith UMK-PPS6 gave higher data than the control for the results of salinity stress tolerance.

Table 4.3 Average Mean And SD From Two Independent Experiments

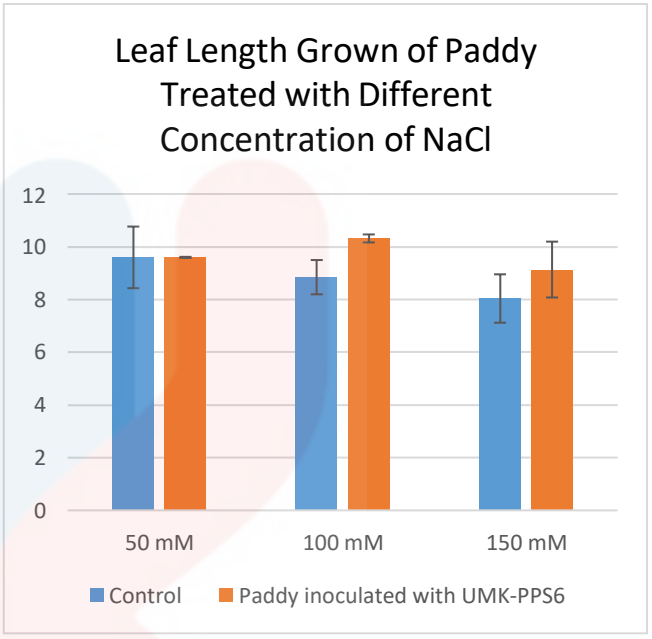
Parameter	Chlorophyll Content		Leaf Length Grown (cm)		Moisture Content (%)	
Sample / NaCl concentration (mM)	Control	Paddy inoculated with UMK-PPS6	Control	Paddy inoculated with UMK-PPS6	Control	Paddy inoculated withUMK-PPS6
50	7.775±0.983	10.743±1.394	9.6±1.168	11.6±0.02	80.382±0.308	82.428±0.932
100	9.915±1.025	12.365±1.005	8.85±0.65	10.32±0.15	81.003±0.636	81.671±0.9925
150	9.189±1.195	11.605±0.502	8.04±0.92	9.14±1.06	80.72±0.284	79.04±1.175

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

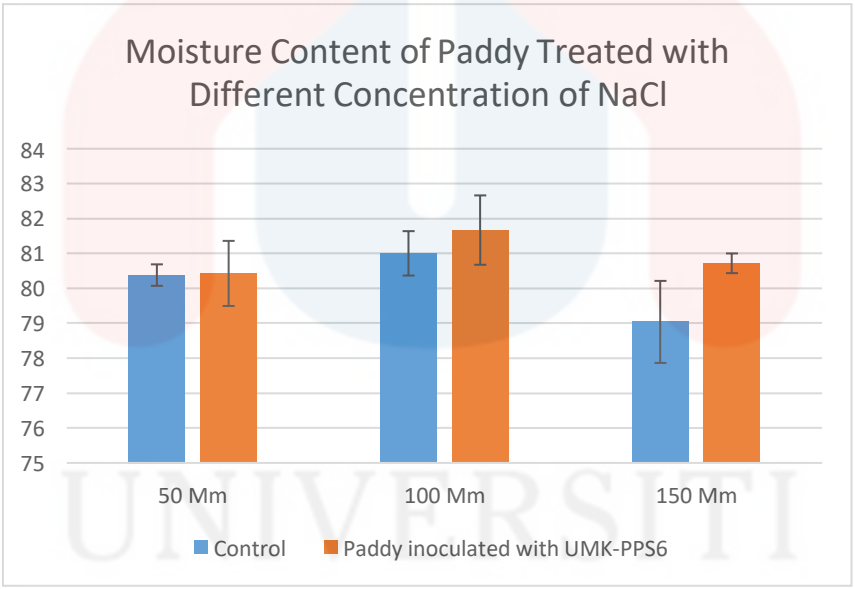
Parameter	Chlorophyll Content		Leaf Length Grown (cm)		Moisture Content (%)	
Sample / NaCl concentration (mM)	Control	Paddy inoculated with UMK-PPS6	Control	Paddy inoculated with UMK-PPS6	Control	Paddy inoculated with UMK-PPS6
50	0	0.001714	0	0.20808	0	0.02204
100	0	0.004474	0	0.033544	0	0.03503
150	0	0.01293	0	0.037143	0	0.0106



a)



b)



c)

Figure 4.2 The effect of the inoculation *Paraburkholderia kururiensis* UMK-PPS6 on **(a)** Chlorophyll Content **(b)** Leaf Length Grown **(c)** Moisture Content under salt stress

Based on chlorophyll content treated with different concentration of salinity, the results shown that *Paraburkholderia kururiensis* UMK-PPS6 is the most effective in growth promoting performance at 100mM NaCl, from the midest value for statistical analysis (t-test value) compared to 50mM and 150mM in terms of significant value ($p < 0.05$). In addition, the plants with NaCl concentration (50mM, 100Mm and 150mM) had a difference (higher than) compared to the control plant that was not injected which proved its effectiveness (Ruanming el at., 2021). The diverse response of each plant is also the reason why the concentration of 100mM NaCl obtained the highest value in addition to the factors of light intensity, temperature and carbon dioxide concentration received by each group of plants (Sudhakar el at., 2016). Similar to the study conducted in this journal, (Gu, Anqi & Zhao, 2022) where following stress treatment, tomato plants treated with *Paraburkholderia kururiensis*, *Burkholderia* species are able to colonize the rhizosphere which contributes to high metabolic flexibility to the growth of paddy plants, exhibited higher levels of chlorophyll than treated plants with control-treated plants. Plants inoculated with UMK-PPS6 *Paraburkholderia kururiensis* contributes to reduce posttransplant stress, improving plant organ tissue (Madhaiyan et al., 2021). A crucial marker to determine plant growth and mortality is the amount of pigment produced by photosynthesis called chlorophyll, which declines in direct proportion to the level of environmental stress (Y Li et al., 2018). Chlorophyll content for control paddy growth is affected because salinity is inhibitory to the growth and development of many plants, including most crops which its affects all cellular processes, including disruption of cellular homeostasis, impairment of photosynthesis, mRNA processing, transcription, protein synthesis, disruption of energy metabolisms, amino acid biosynthesis as well as lipid metabolism that can decrease the level of chlorophyll (Hameed et al., 2021).

The results show that *Paraburkholderia kururiensis* UMK-PPS6 is the most effective in growth promoting performance at 50mM NaCl, from the lowest value for statistical analysis (t-test value) compared to 100mM and 150mM in terms of significant value ($p < 0.05$). Moreover, 50mM NaCl has the highest difference compared to other concentrations which proves its effectiveness. Salt stress slows the rate of leaf growth by reducing the length of the leaf elongation zone and the intensity of growth in the central and distal regions. This can be seen when the length of the leaves of the growing control group is decreasing, but the treatment group is clearly higher due to the inoculation of *Paraburkholderia*

kururiensis UMK-PPS6. Based on the journal (Ruanming Zhu et al., 2021) shows that the strain of *Paraburkholderia sp*. GD17 showed their key function in promoting the growth of paddy seeds under salt stress conditions due to their ability to regulate phytohormones and salt resistance genes that reduce the damaging effects of salinity stress in plants. This is because *Burkholderia* species can secrete metabolites that promote plant growth and help in maintaining the ion balance within plant cells under saline conditions (T Ledger el at., 2015). Therefore, adding bacteria to the root system is a way to increase resistance to abiotic stress, especially environmental factors including salinity stress. For the final analysis, the beneficial effect of microbial inoculants on plant development was shown by a statistically significant increase in the length of cultivated leaves in rice plants treated with *Paraburkholderia kururiensis* UMK-PPS6 under varying levels of salinity stress.

The results show that *Paraburkholderia kururiensis* UMK-PPS6 is the most effective in growth promoting performance at 50mM NaCl, from the lowest value for statistical analysis (t-test value) compared to 100mM and 150mM in terms of significant value ($p < 0.05$). Addition of *Paraburkholderia kururiensis* UMK-PPS6 resulted in higher moisture content in each NaCl concentration compared to the control group. *Paraburkholderia kururiensis* UMK-PPS6 uses several mechanisms to achieve this result even under salt stress. First, the induction of systemic agents in plant roots. Plant roots and other remaining microbes compete with bacteria for nutrition. The interaction between plants and rhizosphere microorganisms is very important. Beneficial microbial communities living in plant and soil rhizospheres establish functional bonds with their hosts and express beneficial traits capable of enhancing plant performance (de Souza et al. ., 2020). In addition, *Paraburkholderia kururiensis* UMK-PPS6 can increase water absorption which its decreased the level of ethylene in host plants by producing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Similarly, it was shown that *Paraburkholderia* strains could contribute to plant nutrition by producing plant hormones, indirectly leading to reduced disease susceptibility (Andreolli et al. , 2016). In summary, a significant increase in moisture content through statistical analysis among paddy plants treated with *Paraburkholderia kururiensis* UMK-PPS6 across various salinity stress levels indicates a beneficial influence of the microbial inoculum on the overall plant water status.

Based on the similar research on previous study by Ruanming Zhu in 2021, the study also conducted tolerance to salinity of Paraburkholderia using rice seedling to see the response of Paraburkholderia sp. GD17 against salt stress. The experiment also performed the same method using control and GD17 inoculation with or without NaCl treatment. Based on the results obtained, it also shows a significant result that Paraburkholderia contributes to the strength and growth of rice seeds overcoming the excess of salt. With that, there is no doubt that the discussion results obtained for the study carried out by this bacterium are significant and similar showing that the UMK-PPS6 strain of Paraburkholderia kururiensis contribute to paddy growth and helps paddy growth.

4.4 Determination of antimicrobial activities *Paraburkholderia kururiensis* against gram-positive and gram-negative bacteria

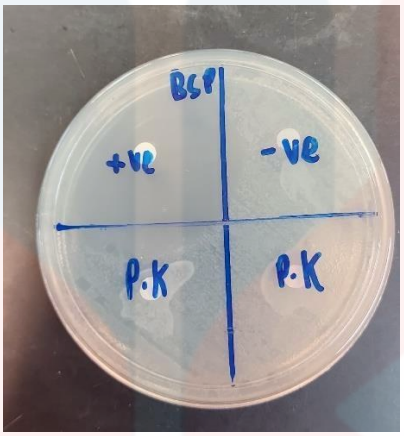


Figure 4.5.1 Antimicrobial Activities of *Paraburkholderia kururiensis* on *Bacillus*

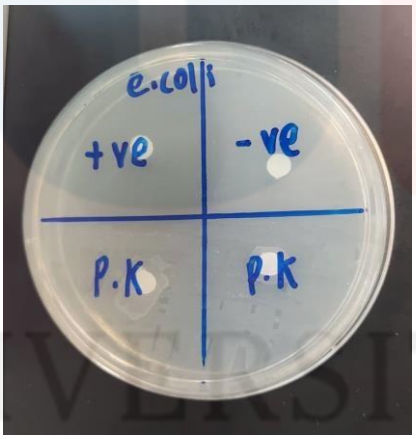


Figure 4.5.1 Antimicrobial Activities of *Paraburkholderia kururiensis* on Es

Sample	Zone of inhibition (cm)	
	E. coli	*Bacillus subtilis*
Positive Control	4.2	2.7
Paraburkholderia kururiensis UMK-PPS6	-	-

From the result, it is shown that *Paraburkholderia kururiensis* UMK-PPS6 showed negative result towards the antimicrobial essay. It is notable that *Paraburkholderia kururiensis* UMK-PPS6 does not exhibit any antimicrobial activity, as evidenced by the absence of inhibition zones against *E. coli* and *Bacillus subtilis.*

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The purpose of this research is to evaluate the effect of *Paraburkholderia kururiensis* UMK-PPS6 inoculation on paddy growth, evaluate paddy inoculated with *Paraburkholderia kururiensis* UMK-PPS6 on plant tolerance to salinity stress and determine the antimicrobial activity of *Paraburkholderia kururiensis* UMK-PPS6 against gram positive and gram negative bacteria. Based on this study, it was found that paddy plants treated with UMK-PPS6 and control paddy plants had significant differences and their growth was seen to have significant differences in species survival.

Based on the plant evaluation data against salinity stress after 18 days, it also shows that UMK-PPS6 helps in the growth of paddy plants and the data obtained clearly shows that the significant data value (t-test value) is likely to be different and affect a statistically significant difference. It explained that *Paraburkholderia kururiensis* is very helpful in paddy growth.

Next, in determining the antimicrobial activity, *Paraburkholderia kururiensis* is seen not to have any clear zone that exists against it. gram-positive and gram-negative bacteria. on the other hand, only antibiotics showed a clear zone reaction in the experiments conducted.

5.2 Recommendations

There are several recommendations for this study. The first is the period of planting paddy crops. The period of paddy cultivation may be increased if there are other researchers who are interested in continuing this study. This is because, the probability of better or different data may be obtained if the period of rice cultivation is carried out longer, and other results may be found. In addition, paddy cultivation needs to be properly examined in terms of care, it needs to be looked at better to ensure that the nutrients of paddy are more intact and suitable for undergoing stress tolerance tests.

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APPENDIX

A1 Chlorophyll Content Using SPADmeter

	Replicate	Mean 1	Total Mean	SD
Controlled Batch	Replicate 1	11.71	11.5	0.477
	Replicate 2	11.29		
Paddy inoculated with UMK-PPS6	Replicate 1	13.22	12.238	1.433
	Replicate 2	11.25		

A2 Leaf Length Grown

	Replicate	Mean 1	Total Mean	SD
Controlled Batch	Replicate 1	10.68	11.09	0.823
	Replicate 2	11.5		
Paddy inoculated with UMK-PPS6	Replicate 1	12.54	12.89	1.462
	Replicate 2	13.24		

A3 Moisture Content

	Replicate	Mean 1	Total Mean	SD
Controlled Batch	Replicate 1	87.658	87.04	0.269
	Replicate 2	86.412		

Paddy inoculated with UMK-PPS6	Replicate 1	89.278	89.548	0.615
	Replicate 2	89.818		

A4 Chlorophyll Content Using SPADmeter (NaCl)

	Replicate	Mean 1	Total Mean	SD
50mM				
Controlled Batch	Replicate 1	10.746	7.775	0.983
	Replicate 2	10.45		
Paddy inoculated with UMK-PPS6 with 50mM of NaOH	Replicate 1	16.692	10.743	1.394
	Replicate 2	16.78		
100mM				
Controlled Batch	Replicate 1	7.63	9.915	1.025
	Replicate 2	7.45		
Paddy inoculated with UMK-PPS6 with 50mM of NaOH	Replicate 1	11.7	12.365	1.005
	Replicate 2	11.46		
150mM				
Controlled Batch	Replicate 1	4.814	9.189	1.195
	Replicate 2	5.2		

Paddy inoculated with UMK-PPS6 with 50mM of NaOH	Replicate 1	8.52	11.605	0.502
	Replicate 2	8.71		

A5 Leaf Length Grown (NaCl)

	Replicate	Mean 1	Total Mean	SD
	50mM			
Controlled Batch	Replicate 1	11.32	9.6	1.168
	Replicate 2	11.08		
Paddy inoculated with UMK-PPS6 with 50mM of NaOH	Replicate 1	15.78	11.6	0.02
	Replicate 2	15.21		
	100mM			
Controlled Batch	Replicate 1	9.06	8.85	0.65
	Replicate 2	8.86		
Paddy inoculated with UMK-PPS6 with 100mM of NaOH	Replicate 1	12.21	10.32	0.15
	Replicate 2	11.76		
	150mM			
Controlled Batch	Replicate 1	5.76	8.04	0.92
	Replicate 2	5.23		

Paddy inoculated with UMK-PPS6 with 150mM of NaOH	Replicate 1	8.21	9.14	1.06
	Replicate 2	8.375		

A6 Moisture Content (NaCl)

	Replicate	Mean 1	Total Mean	SD
	50mM			
Controlled Batch	Replicate 1	83.42	80.382	0.308
	Replicate 2	83.08		
Paddy inoculated with UMK-PPS6 with 50mM of NaOH	Replicate 1	87.698	82.428	0.932
	Replicate 2	87.32		
	100mM			
Controlled Batch	Replicate 1	82.45	81.003	0.0636
	Replicate 2	82.32		
Paddy inoculated with UMK-PPS6 with 100mM of NaOH	Replicate 1	85.43	81.671	0.9925
	Replicate 2	85.86		
	150mM			
Controlled Batch	Replicate 1	79.45	80.72	0.284
	Replicate 2	80.01		

Paddy inoculated with UMK-PPS6 with 150mM of NaOH	Replicate 1	83.24	79.04	1.175
	Replicate 2	83.56		

A7. Controlled Batch

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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 (1 wilted)	13.9, 3.3, 2.3	5.2	18.8	13.6	0.1337	0.019	85.79
2	3 (1 wilted)	20.0, 5.6	4.8	17.3	12.5	0.1158	0.014	87.91
3	4 (1 small)	24.2, 11.2, 15.5	5.4	18.7	13.3	0.2176	0.018	91.73
4	3 (2 wilted)	9.1	4.8	17.0	12.2	0.1129	0.014	87.6
5	3	21.7, 9.6, 7.7	5.5	20.0	14.5	0.1899	0.028	85.26

A8. Controlled Batch (2nd)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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 (1 small)	16.9, 8.3,	5.2	17	11.8	0.1337	0.019	85.79

		9.8						
2	3 (1 small)	14.5, 8.8	5.5	19	13.5	0.1158	0.014	87.91
3	4 (2 small)	10.6, 12.0	4.8	19.3	11.7	0.1129	0.014	87.6
4	3 (1 small)	8.8, 12.3	4.7	18.4	11.8	0.0761	0.011	85.55
5	3	16.7, 9.2, 8.7	4.8	17.8	13	0.1899	0.028	85.26

A9. Treatment Batch

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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 (2 wilted)	10.5, 9.9	5.0	16.8	11.8	0.1825	0.018	90.14
2	3 (1 wilted)	13.7, 11.0	4.9	18.6	13.7	0.1619	0.018	88.88
3	5 (1 wilted)	22.0, 10.0, 16.2, 5.7	5.5	20.5	15	0.2481	0.026	89.82
4	4(1 wilted)	8.3, 7.9, 13.3	4.8	14.8	10	0.1740	0.017	90.23

5	4(2 wilted)	13.9, 11.5	4.8	17.0	12.2	0.1656	0.021	87.32

A10. Treatment Batch (2nd)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	Initial Leaf Length (cm)	Leaf Length After 18 Days (cm)	Leaf Length Grown (cm)	Weight Before Drying (g)	Weight After Drying (g)	Moisturecontent (%)
1	3 (1 small)	16.5, 8.9	5.0	14.8	9.8	0.1825	0.018	90.14
2	3 (1 small)	15.7, 11.0	5.2	16.2	11	0.1619	0.018	88.88
3	3 (1 small)	12.3, 10.9	5.2	17.5	12.3	0.2123	0.015	92.93
4	3 (1 wilted)	15.2, 11.7	5.5	19.5	14	0.2481	0.026	89.82
5	4(2 small)	17.9, 7.5	5.4	18.0	12.6	0.1656	0.021	87.32

A11. Controlled Treatment (50Mm)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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, 7.3, 7.1	6.7	16.4	9.7		0.0348	80.0
2	2	9.25	6.9	17.4	10.5	0.165	0.0327	80.18
3	3 (1 wilted)	9.3, 11.2	6.8	18.2	11.4	0.147	0.0293	80.07
4	3 (1 wilted)	8.8, 9.5	6.9	19.4	12.5	0.158	0.0318	79.87
5	4 (2 wilted)	11.9, 8.5	6.7	18	11.3	0.196	0.0387	80.25

A12. Treatment 1 with 50mM

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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 (2 wilted)	6.1	4.9	14.5	9.6	0.149	0.02384	84
2	2 (1 wilted)	5.7	4.5	13.6	9.1	0.166	0.0289	82.59
3	2 (1 wilted)	6.9	4.9	11.5	6.6	0.168	0.0328	80..48
4	3 (2 wilted)	6.5	4.7	16.0	11.3	0.178	0.0335	81.18
5	3 (2wilted)	6.7	4.8	15.8	11	0.175	0.0327	81.31

A13. Control Treatment (100Mm)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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	18.0, 16.0, 8.0	6.0	18.2	12.2	0.174	0.0348	80.0
2	2	16.5, 17.2	6.5	19	12.5	0.165	0.0327	80.18
3	3 (1 small)	14.2, 8.5	6.4	16.2	9.8	0.147	0.0293	80.07
4	3 (1 small)	12.3, 6.5	6.3	15.7	9.4	0.158	0.0318	79.87
5	4 (2 small)	15.4, 11.4	6.3	17.3	11	0.196	0.0387	80.25

A14. Treatment (100Mm)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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 (2 wilted)	13.7,9.9	5.7	13.9	8.2	0.145	0.02387	83.54
2	2 (1 wilted)	11.2	5.8	14.6	8.5	0.179	0.0277	84.53
3	2 (1 wilted)	9.2	5.8	16.4	10.2	0.121	0.0171	85.87
4	3 (2 wilted)	10.5	6.0	16.6	11.3	0.142	0.0207	85.42
5	4 (2 wilted)	12.5,11.2	5.7	11.5	6.8	0.163	0.0247	84.85

A15. Control Treatment (150Mm)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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 (1 wilted)	15.3, 10.3,	6.3	15.6	9.3	0.174	0.0348	80.0
2	3 (1 small and wilted)	14.6, 9.7,	6.4	16.2	9.8	0.165	0.0327	80.18
3	3 (1 small)	8.5, 13.6	6.3	14.7	8.4	0.147	0.0293	80.07
4	4 (2 small)	16.8, 7.9	6.1	13.1	7	0.158	0.0318	79.87
5	4 (2 small)	6.7, 10.0	6.0	12.6	6.6	0.196	0.0387	80.25

A16. Control Treatment 50 mm (2nd)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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	18.2, 14.3, 8.1	6.2	10.2	4.0	0.164	0.0308	81.22
2	2	12.0, 8.1	6.1	10	3.9	0.155	0.0317	79.55
3	4 (2 small)	14.1, 9.2	6.3	8.8	2.5	0.157	0.0333	78.79
4	2 (1 small)	10.4	6.0	9.5	3.5	0.168	0.0358	78.69
5	4 (2 small)	16.7, 8.8	6.1	9.2	3.1	0.183	0.0392	78.58

A17. Treatment 50mM (2ND)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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 (1 wilted)	10.5, 8.7	4.9	15.1	10.2	0.169	0.0258	84.73
2	3 (1 wilted)	9.5, 8.8	4.5	13.6	9.1	0.176	0.0269	84.72
3	2 (1 wilted)	8.4	4.9	14.7	9.8	0.148	0.0228	84.60
4	3 (1 wilted)	11.6, 7.8	4.7	15	10.3	0.178	0.0337	81.07
5	3 (1 small)	10.3, 7.7	4.8	13.8	9.0	0.185	0.0347	81.24

A18. Control Treatment 100Mm (2nd)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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	14.5, 16.1	6.1	15.6	9.5	0.174	0.0348	82.6
2	3	12.0, 15.6, 7.2	6.3	14.4	8.1	0.165	0.0327	83.18
3	3 (1 small)	11.5, 16.0	6.4	14.9	8.5	0.147	0.0293	82.5
4	2	12.3, 13.5	6.2	16.4	10.2	0.158	0.0318	83.87
5	4 (2 small)	12.4, 14.4	6.1	18.1	12	0.196	0.0387	83.25

A19. Treatment 100Mm (2nd)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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 (2 wilted)	11.5, 6.7	5.7	13.9	12.2	0.155	0.0249	83.94
2	3 (1 wilted)	11.2, 6.8	5.8	14.6	7.5	0.169	0.0257	84.79
3	2 (1 wilted)	9.2	5.8	16.4	7.8	0.131	0.0191	85.42
4	3 (2 wilted)	8.5	6.0	16.6	7.9	0.152	0.0227	85.07
5	4 (2 wilted)	8.3, 9.2	5.7	11.5	8.1	0.143	0.0217	84.83

A20. Control Treatment 1500Mm (2ND)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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	12.5, 14.5, 7.8	6.0	16.3	10.3	0.174	0.0348	80.0
2	4 (2 small)	15.6, 7.2	6.5	18.9	12.4	0.165	0.0327	80.18
3	3 (2 small)	14.2	6.4	15.5	9.1	0.147	0.0293	80.07
4	3 (1 small)	14.3, 6.5	6.3	15.1	8.8	0.158	0.0318	79.87
5	3 (2 small)	16.4	6.3	16	9.7	0.196	0.0387	80.25

A21. Treatment 150Mm (2nd)

Sample	Number Of Leaves	Chlorophyll Content for Each Leaf	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 (1 wilted)	12.0, 4.5	6	16.1	10.1	0.171	0.0370	78.36
2	2 (1 wilted)	11.6,	5.8	14.1	8.3	0.188	0.0347	81.54
3	3(1 wilted)	9.2, 8.1	6.5	9.2	2.7	0.163	0.0335	79.45
4	3 (1 wiled)	9.6, 8.3	6.0	14.6	6.6	0.152	0.0323	78.75
5	3 (1 wilted)	10.5, 8.6	6.0	16.2	8.2	0.162	0.0307	81.05