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Formulation of Biofertilizer Using Locally Isolated *Methylorubrum thiocyanatum* UMK-PM2 and *Methylorubrum salsuginis* UMK-PM3 and Their Effect on The Paddy Growth

Kuiek Siew Chin

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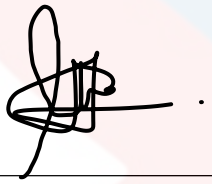
DECLARATION

I declare that this thesis entitled formulation of biofertilizer using locally isolated *Methylorubrum thiocyanatum* UMK-PM2, *Methylorubrum salsuginis* UMK-PM3 and their effect on paddy growth is the results of my own research except as cited in the references.



Signature : _____

Student's Name : Kuiek Siew Chin

Date : _____

Verified by:

Signature : _____

Supervisor's Name : Ts. Dr. Ainihayati binti Abdul Rahim

Stamp : _____

Date : _____

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Formulasi Baja Bio Menggunakan *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 Tempatan dan Keberkesanan Terhadap Pertumbuhan Pokok Padi

ABSTRAK

Pemerhatian baja bio dengan menggunakan bakteria penggalak pertumbuhan tumbuhan (PGPB) mempunyai potensi besar yang menyumbang kepada pertumbuhan tumbuhan yang lebih baik dan yang mampan kepada pertanian semasa. Metilotrof fakultatif berpigmen merah jambu (PPFM) merupakan organisma yang penting dalam kandungan baja bio kerana ia dapat meningkatkan pertumbuhan dan kualiti tanaman dengan mengurangkan kesan negatif kemarau. Mikroorganisma yang digunakan dalam projek penyelidikan ini ialah *Methylorubrum* sp sebagai bakteria penggalak pertumbuhan tumbuhan (PGPB) untuk meningkatkan kesuburan tanah dan membantu dalam penyerapan nutrien seperti nitrogen dan fosforus dalam tumbuhan. Mikroorganisma tersebut juga dapat meningkatkan produktiviti tanah melalui penetapan nitrogen atmosfera, melarutkan fosforus tanah, dan menggalakkan pembangunan tumbuhan tanpa menjejaskan tanah dan alam sekitar. Tujuan kajian ini dijalankan adalah menghasilkan formulasi baja bio dengan menggunakan inokulan *Methylorubrum thiocyanatum* UMK-PM2 dan *M. salsuginis* UMK-PM3 yang disediakan sehari sebelum menyemai melalui cara penanaman padi dalam kajian pasu untuk memastikan mikroorganisma menyesuaikan diri dalam keadaan optimum dan membuat penilaian pertumbuhan pokok. Kajian ini berjaya dilaksanakan dan keputusan yang diperoleh menunjukkan prestasi pertumbuhan tumbuhan meningkat selepas penggunaan *M. thiocyanatum UMK*-PM2 dan *M. salsuginis UMK-PM3* dalam medium formulasi. Berdasarkan keputusan yang diperolehi daripada penyelidikan ini juga dapa membuktikan bahawa medium formulasi tanah adalah lebih baik daripada tanah dengan kokopit dan *M. salsuginis* UMK-PM3 ($p<0.05$) adalah strain PGPB yang lebih berkesan berbanding *M. thiocyanatum* UMK-PM2 dalam menggalakkan pertumbuhan tumbuhan kerana tahap signifikan adalah lebih yakin.

Kata kunci: Bakteria Penggalak Pertumbuhan Tumbuhan (PGPB), Metilotrof fakultatif berpigmen merah jambu (PPFMs), formulasi, Prestasi pertumbuhan tumbuhan.

Formulation of Biofertilizer Using Locally Isolated *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 and Their Effect on The Paddy Growth

ABSTRACT

The observation of biofertilizer by using plant growth-promoting bacteria (PGPB) have great potential to provide better growth of plant and sustainable solutions to current agricultural challenges. The pink-pigmented facultative methylotrophs (PPFM) is one such organism that extremely important in biofertilizer due to it can improve crop growth and quality by reducing the negative impacts of drought. The microorganisms used in this research project was *Methylorubrum* sp as plant growth promoting bacteria (PGPB) which increasing soil fertility and nutrient uptake such as nitrogen and phosphorus in plants. They increase the soil productivity through fixing atmospheric nitrogen, solubilizing soil phosphorus, and promoting plant development without impact the soil and environment. The study was carried out by formulate the biofertilizer with locally isolated *Methylorubrum* sp in pot study one day before sowing to ensure that the microorganisms were well adapt to their optimal conditions for paddy plant growth. The microbial inoculants employed in this study were *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 that been evaluate for their ability in promoting paddy plant growth performance by pot study. The results were successfully obtained showed the plant growth performance had increased after *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatment. According to the result obtained from this research, it can conclude that the formulation medium of soil are better than soil with cocopeat and it can also indicate that *M. salsuginis* UMK-PM3 ($p<0.05$) is effective PGPB strain than *M. thiocyanatum* UMK-PM2 to promote the plant growth due to its significant level was more confident.

Keywords: Plant Growth Promoting Bacteria (PGPB), Biofertilizer, Pink-Pigmented Facultative Methylotrophs (PPFMs), Formulation, Plant growth performance.

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LIST OF ABBREVIATIONS

NH_4Cl	Ammonium Chloride
PGPB	Plant Growth Promoting Bacteria
CO_2	Carbon Dioxide
AMS	Ammonium Mineral Salts
sp	Species
N_2	Nitrogen Gas
cfu/ml	Colony Forming Unit Per Milliliter

LIST OF SYMBOLS

µL	Microliter
%	Percentage
°C	Degree Celsius
mL	Mililitre
g	Gram
cm	Centimetre
nm	Nanometre
v/v	Volume per volume
mg	Milligram
mg/g	Milligram per gram
L	Litre
g/L	Grams per liter
lbs	Pounds

CHAPTER 1

INTRODUCTION

1.1 Research Background

The rising use of chemical fertilisers in agriculture has a negative effect on ecosystems. Chemical fertilisers are not properly absorbed by plants, causing them to eventually reach water bodies through leaching and causing eutrophication and greenhouse effect (Liu et al., 2021). Furthermore, because crop plants are under increasing abiotic and biotic stress as a result of imbalance in soil fertility, climate change, yields may become unpredictable(Pirttilä et al., 2021). Chemical fertilisers have a negative impact on the soil's biodiversity through altering its chemical composition, microbialflora, and ecology (Ranjan et al., 2020). Majority of farmers utilise chemical fertilisers to boost crop yields on a large scale today (Denaya et al., 2021). The study demonstrated that biological fertilisers that use microbes as fertilisers have the potential to replace chemical fertilizer due to they are most affordable source of plant nutrients, as well as important sources of micronutrients, organic matter, and growth hormones that help offset the harmful effects of chemical fertilisers (Denaya et al., 2021).

Biofertilizers are aggressively colonise the root systems of plants, improving nutrient uptake, productivity, crop yield, stress tolerance, pathogen resistance, and plant growth through the mobilisation of nutrients and plant growth hormones processes. The long period time use of biofertilizers can improves crop production by up to roughly 10–40%, soil fertility and environmentally benign via increasing the amounts of protein, vital amino acids, and vitamins, as well as via nitrogen fixation. As a result, the microbes that are used to formulate the biofertilizer are crucial for sustainable crop production and stimulating plant growth as well as other creatures that are beneficial for accelerating plant growth (Daniel et al., 2022).

In addition, the term "plant growth promoting bacteria" (PGPB) refers to these microbes that function as biofertilizers. By aiding the process of nutrient uptake, PGPB indirectly

improves plant development. Because of its capacity to promote plant development, PGPB can be utilised in agriculture as a sustainable alternative to artificial fertilisers. Chemical fertilisers have the potential to harm soil structures and lower soil organic matter levels. Thus,the bacterial isolates as biological fertiliser agents are more efficient than chemical fertilisers (Chen et al., 2018; Wang et al., 2018).

Nevertheless, the PGPB is also described as having the potential to promote nitrogen fixation, phosphorus solubilization, and phytohormone production, as well as protect plants from disease and abiotic stressors, through a variety of processes (Daniel et al., 2022). The PGPB enhance to promote the plant growth which get the food sources from the root exudation to plays the role as plant growth regulators like phytohormone indole acetic acid (IAA), biological nitrogen fixation, and the solubilization of phosphate and potassium (Daniel et al., 2022).

Lastly, the pink-pigmented facultative methylotrophs (PPFM) is one of organism that important in biofertilizer due it can enhance plant growth through a variety of mechanisms, including nitrogen fixation, phosphate solubilization, the production of plant growth regulators that directly improve crop growth and quality. Methylotrophic bacteria can also produce biofilm, aggregation, and chemicals that defend against ultraviolet light in order to adapt to stressful environments like inadequate nutrition, drought, and high temperatures on plants (Immanuel & Sasikumar, 2023).

1.2 Problem Statement

The demand for food in the current generation is directly correlated with population growth. Therefore, as the human population grows, so does the need for agricultural products to sustain life (Daniel et al, 2022). As a result, farmers who frequently use chemical fertilisers to boost agricultural yields without understanding the adverse impacts directly contribute risks to both human and animal health. It is difficult to increase crop productivity while maintaining environmental safety.

In addition, the chemical fertilizers are less fertile, more resistant to the environment and actually degrade soil and land quite a bit (Chen et al, 2018). Nevertheless, the high concentrations of chemicalfertilizers might instantly harm, which is similar to root burn. Long term chemical fertilizer canchange the pH of soil balance and result in hazardous buildup of some nutrients (Wang et al, 2018).

One of the alternatives is the use of biofertilizers as a substitute for chemical fertilizers and pesticides. Microorganisms that support and encourage plant growth are known as biofertilisers. It is economical, non-toxic, and environmentally responsible. By boosting plant resistance to diseases, increasing nutrient intake, and mobilising essential elements in soil, appropriate biofertilizer formulations can aid in promoting plant development and enhancing soil fertility (Tariq et al., 2022). The microorganism that used in this research was *Methylorubrum* sp which enhance the plant growth and develop bioinoculant formulations to increase rice productivity of rice (Green & Ardley, 2018).

1.3 Objectives

a) To formulate biofertilizer using locally isolated *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3.
b) To compare plant growth performance activites of *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 on paddy growth by pot study.

1.4 Scope of Study

To achieve the objectives of the research study, a biofertilizer was formulated using *M. thiocyanatum UMK-PM2 and M. salsuginis UMK-PM3* strain. These isolates were be obtained from the paddy's rhizosphere by previous study (Kamaruzaman, 2023). Then, to study the effectiveness of formulated biofertilizer, the growth of paddy plants based on plant's height, weight, chlorophyll content, and moisture content were evaluated compared between two formulation.

1.5 Significance of Study

The result and data obtained from this study should be able to verify that *Methylorubrum* sp. as a plant growth promoting bacteria is important for the plant to improve or promote its growing performance and can be utilized as biofertilizer. The PGPB biofertilizer has many potentials and benefits to environmental and plant which as microbial inoculant for increased the plant growing performance.

CHAPTER 2

LITERATURE REVIEW

2.1 Microbial Fertilizer

Use of biofertilizers or plant growth boosters in place of synthetic or chemical fertilisers, insecticides, and herbicides that pose several risks to human and animal health is one of the key approaches. There are several types of biofertilizer which including nitrogen fixing biofertilizer, phosphorus-solubilizing biofertilizer, phosphate-mobilizing biofertilizer and plant growth promoting biofertilizers. These biofertilizers aggressively colonise the root systems of plants, improving nutrient uptake, productivity, crop yield, stress tolerance, pathogen resistance, and plant growth through processes like the mobilisation of vital elements, nutrients, and plant growth hormones. The regular use of biofertilizers improves soil fertility and is both economical and environmentally benign via increasing the amounts of protein, vital amino acids, and vitamins, as well as via nitrogen fixation, they also raise crop production by up to roughly 10–40%. As a result, these microorganisms are crucial for sustainable crop production and stimulating plant growth as well as other creatures thatare beneficial for accelerating plant growth (Wanget al., 2018; Daniel et al., 2022; Cao et al., 2023).

A class of microbes known as plant growth promoting rhizobacteria (PGPR) can be used as biofertilizer. When administered to the soil, seed, or surface of the plant,PGPR colonise the rhizosphere and the interior of the plant, fostering plant growth. By supplying nutrients to the soil, they not only increase soil fertility and crop yield but also shield the plant from pests and diseases. In order to boost

the availability of nutrients that plants can readily absorb, biofertilizers are frequently utilised. By fixing atmospheric N_2 and resolving insoluble phosphates in the soil, they boost soil fertilityand produce compounds that promote plant development. As a result, it significantly decreased nitrogen runoff and leaching loss and reduced the formation of nitrate nitrogen (NO_3-N) in soil. In addition, biofertilizer reduced the abundance of the nitrogen-fixing gene by up to 2 times (Sun et al.,2020; Basu et al., 2021; Hang et al., 2022).

They are the most affordable source of plant nutrients, as well as important sources of micronutrients, organic matter, growth hormones, and supplies that help offset the harmful effects of chemical fertilisers. Chemical fertilisers have a negative impact on the soil's biodiversity through altering its chemical composition, microbial flora, and ecology (Ranjan et al., 2020).

2.2 Plant Growth Promoting Bacteria（PGPB）

The PGPB as microbes to promote the plant growth and can be isolates from the rhizosphere. Because of its capacity to promote plant development which are nutrient uptake, root and shoot growth and abiotic stress, PGPB can be utilised in agriculture as a sustainable alternative to artificial fertilisers (Chen et al., 2018; Wang et al., 2018). Many plant species' rhizospheres are colonised by PGPB, which have positive impacts on the host, including improved plant development and decreased susceptibility to illnesses brought on by nematodes, fungus, bacteria, and viruses. Increased seed germination rates, yields, root growth, leaf area, chlorophyll content, nutrient uptake, protein content, hydraulic activity, resistance to abiotic stress, shoot and root weights, and postponed senescence are just a few advantages of PGPB (Chenet al., 2018).

PGPB have their ability to promote nitrogen fixation, phosphorus solubilization,and phytohormone production, as well as protect plants from disease and abiotic stressors, through a variety of processes. Besides, due to the plentiful availability of substrates present in root exudates, microbial isolates from the

rhizosphere of different crops seem to have a larger potential to synthesize and release auxins as secondary metabolites. A PGPB which can also be found in the rhizosphere in the soil. Furthermore, the PGPB also plays their role to the biotic stress with antibiotics production, extracellular lytic enzymes, siderophore, and hydrogen cyanide. The action of PGPB in promoting plant growth by obtained the food sources from the root exudation, such as sugars, vitamins, enzymes, amino acids, and organic acids under drought and salinity stress to act as plant growth regulators likephytohormone indole acetic acid (IAA), biological nitrogen fixation, and the solubilization of phosphate and potassium (Daniel et al., 2022). In addition, there are several types of PGPB which including *Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Serratia, Enterobacter, Klebsiella, Pseudomonas, and Rhizobium* spp (Gouda et al., 2018; Grossi et al., 2020).

2.3 Nitrogen Fixing Bacteria (NFB)

Naturally, nitrogen is present in the atmosphere as nitrogen gas. However, nitrogen cannot be utilised directly by plants. In order to transform nitrogen into ammonia form (NH_3) for plant uptake, a biofertilizer utilises nitrogen-fixing bacteria (NFB) for plant absorption through a process of nitrogen fixation. In this process, nitrogen from the atmosphere enters the soil, and nitrogen fixers fix nitrogen into ammonium ions by releasing nitrogenase enzymes. There are several strains of nitrogen-fixing bacteria such as *Azotobacter* sp., *Rhizobium* sp. and *Cyanobacteria* sp. In addition, *Azotobacter* sp has the capacity to produce antifungal chemicals that are effective against plant diseases and fix a sizeable quantity of nitrogen from theatmosphere. It is crucial to introduce nitrogen-fixing bacteria as biofertilizer to the soil in order to keep the soil fertile and provide plants with enough nutrients (Sarbani & Yahaya, 2022).

The biological nitrogen fixation act to increase nutrient availability. It is also can improve nitrogen use efficiency and reducing the dinitrogen present in the biosphere into suitable forms of nitrogen. Majority of the organisms in soil are difficult to synthesize the nitrogen directly from reservoir. Thus, nitrogen

transported with water through the process of biological nitrogen fixation to improving plant growth (Masood et al., 2020).

2.4 Phosphate Solubilizing Bacterium (PSB)

By using a variety of methods, including the secretion of organic acids, the creation of enzymes, and the excretion of siderophores that can chelate the metal ions and form complexes, these bacteria can solubilize insoluble phosphates in soil and make them available for plant absorption (Rawat et al., 2021). These microorganisms produce auxins, gibberellins, and cytokinins, plant growth-promoting hormones, antibiosis against infections, and other hormones that encourage plant development, crop yield and improves plant resistance to heavy metal toxicity and promotes plant development under stress. Nevertheless, in order to increase P availability and make up for the P shortage on saline land, salinity-tolerant phosphate-solubilizing bacteria (ST-PSB) can be a very successful and affordable method. According to this studies, focuses on the effects of soil salinization on P availability, the mechanisms by which P is solubilized by ST-PSB, the diversity of ST-PSB, their function in reducing salinity stress in plants, possible uses for ST-PSB in the present and the future, and the potential use of this knowledge to manage the sustainable environmental system. Studies show that applying ST-PSB to saline soils may be a substitute for reducing salinity's detrimental effects on plants and may improve a plant'stolerance to salinity (Dey et al., 2021).

2.5 Pink-Pigmented Facultative Methylotrophs (PPFMs)

A class of microorganisms known as pink-pigmented facultative methylotrophic bacteria (PPFMs) are distinguished by their capacity to use one-carbon compounds—especially methylated compounds—as their exclusive source of carbon and energy. The word "facultative" refers to the ability of these bacteria to alternate between growing methylotrophically and non-methylotrophically in response to the availability of various carbon sources. The carotenoid pigments that are present in their cells are frequently the cause of the pink coloration. Because of their significance for crop seed germination, yield, disease resistance, and drought stress tolerance, PPFMs have been extensively researched in agricultural systems (Aswathy et al., 2020).

It has been found and observed to be surviving in a variety of environments, such as the phyllosphere, soil, dust, freshwater, drinking water, root nodules, and lake sediment. PPFMs are rod-shaped, methylotrophic, aerobic, gram-negative bacteria that proliferate either on multicarbon substrates or as the only carbon and energy source on single carbon molecules like methanol, formate and formaldehyde (Bajpai et al., 2022).

Pink-Pigmented Facultative Methylotrophs (PPFMs) are known to enhance plant growth through a variety of mechanisms, including nitrogen fixation and nodule formation, phosphate solubilization, the production of plant growth regulators (auxins, cytokinins, gibberellic acid), the synthesis of siderophores, the production of urease enzyme, and the production of vitamin B12. Methylotrophic bacteria produce biofilm, aggregation, and chemicals that defend against ultraviolet light in order to adapt to stressful environments like inadequate nutrition, drought, and high temperatures (Immanuel & Sasikumar, 2023). A well-known example of a facultative methylotroph with pink pigmentation is Methylobacterium extorquens. It's a bacterium that's frequently researched because of its capacity to use single-carbon molecules as a source of carbon and energy, such methanol.

2.6 *Methylorubrum* sp.

Methylorubrum sp is a Gram-negative pink-pigmented Bacillus which formerly known as *Methylobacterium* sp. The ability of Methylobacterium species to utilise single carbon substrates like methanol and other methylated compounds as their only sources of carbon and energy, which they assimilate via the serine cycle, might be the most notable characteristic of these organisms. This could be as these organisms synthesise biologically active metabolites like cytokinins and auxins. It has been shown that *Methylobacterium* spp. prefer to live in the phyllosphere. In a phyllosphere exposedto direct sunshine, *Methylobacterium* spp. can create UV-absorbing chemicals that might assist the bacteria and plants survive.

There are 52 *Methylobacterium* sp but can no longer be maintained in one genus based on 16S rRNA gene, multi-locus sequence analysis, genomic and phenotypic data. In order to fit the 11 new species of Methylorubrum, the previously *Methylobacterium* spp. are now classified as *Methylorubrum* spp which are including *Methylorubrum aminovorans* sp, *Methylorubrum extorquens* sp, *Methylorubrum podarium* sp, *Methylorubrum populi* sp, *Methylorubrum pseudosasae* sp, *Methylorubrum rhodesianum* sp, *Methylorubrum rhodinum* sp, *Methylorubrum salsuginis* sp, *Methylorubrum suomiense* sp, *Methylorubrum thiocyanatum* sp. *and Methylorubrum zatmanii* sp (Green & Ardley, 2018).

Methylorubrum sp act as PGPB that can enhance the plant growth by having potential to promote bioactive compound including phytohormones Indole-3-Acetic Acid (IAA), nitrogen fixation, phosphate solubilizing and 1-aminocyclopropane-1- carboxylase (ACC) deaminase as well as protect plants from disease and abiotic stressors, through a variety of processes (Green & Ardley, 2018). When seedlings grewfrom endophyte-primed seeds, metabolite profiling revealed the abundance of a few metabolites that are engaged in pathways linked to PGP characteristics. Thus, the *Methylorubrum thiocyanatum* sp can develop bioinoculant formulations using these chosen endophytes to increase rice productivity by modulating certain sets of metabolites in rice plants (Krishnamoorthy et al., 2020).

Moreover, *M. salsuginis* sp capable of utilizing single-carbon compounds such as methanol as a carbon cycle and energy source which frequently found in methanol-containing habitats such soil, water, and plant rhizospheres. This metabolic feature distinguishes it from many other bacteria that require more complex carbon sources. *M. salsuginis* was an excellent organism to investigate microbial life in harsh settings because of its tolerance to salt environments (Green & Ardley, 2018).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The materials used in this research are the paddy seed, topsoil, plastic cup, cocopeat as the purpose of pot study performance have been obtained commercially. *Methylorubrum* sp. strain were used in this study were obtained from previous study while the distilled water, agar powder, chemicals substances such as bleach, NH_4Cl, K_2HPO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$, $FeSO_4$, $ZnSO_4$, $MnCl_2$, H_3BO_4, $CoCl_2$, $CuCl_2$, $NiCl_2$and Na_2MoO_4. The equipment used such as the machines of incubator, autoclave, and centrifuges can be obtained directly from lab assistants.

3.1.1 Bacterial Strain

The *Methylorubrum* sp strain was used in this study are growth promoting rhizobacteria that were obtained from previous study (Kamaruzaman, 2023). There were two bacteria strains of *Methylorubrum* sp used in this study were *M. thiocyanatum* UMK-PM2, and *M. salsuginis* UMK-PM3. The bacterial strain was maintained on medium Ammonium Mineral Salts (AMS) throughout the study.

3.1.2 Paddy Seed

The paddy seed variety MR297 obtained from Kemubu Agriculture Development Agency (KADA) was utilized in this study using pot study.

3.2 Methods

3.2.1 Preparation of AMS Media Agar

The Ammonium mineral salt (AMS) media have been prepared by mixing 1L of distilled water with the composition chemicals as shown in Table 3.1 to ensure the pH value is adjusted to 6.8 with NaOH. Then, 15g agar powder was added into the solution and sterilized at 121˚C for 2 hours by autoclaving. Next, the media were cooled to 50˚C then added 0.5% methanol into media. The solution was shaked a bit to obtain a medium solution mixed well before pouring into sterile petri dish plate.

Table 3.1: Composition of ammonium mineral salt medium agar

Composition	**Concentration (g/L)**
Ammonium Chloride, NH_4Cl	0.50000
Dipotassium Phosphate, K_2HPO_4	0.70000
Monopotassium Phosphate, KH_2PO_4	0.54000
Magnesium Sulphate, $MgSO_4 \cdot 7H_2O$	1.00000
Calcium Chloride, $CaCl_2 \cdot 2H_2O$	0.20000
Iron Sulphate, $FeSO_4 \cdot 7H_2O$	0.00400
Zinc Sulphate, $ZnSO_4 \cdot 7H_2O$	0.00010
Magnesium Chloride, $MnCl_2 \cdot 7H_2O$	0.00003
Boric acid, H_3BO_4	0.00030
Cobalt Dichloride, $CoCl_2 \cdot 6H_2O$	0.00020
Copper Chloride, $CuCl_2 \cdot 2H_2O$	0.00001
Nickel Chloride, $NiCl_2 \cdot 6H_2O$	0.00002
Sodium Molybdate Dihydrate, $Na_2MoO_4 \cdot 2H_2O$	0.00006

Distilled water, dH_2O	1000.00
Agar	15.0000

3.2.2 Inoculation of *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 on AMS Medium Agar

The *Methylorubrum* sp strains were inoculated into AMS growth media plate and incubated the culture at 30˚C in an IB-15G incubator for 4-5 days of growth. These colony bacterial strains were used to formulate the biofertilizer in different medium formulation which including soil and soil with cocopeat.

3.2.3 Preparation of AMS Medium Broth

The agar powder was omitted for the preparation of AMS broth compare with AMS medium agar. The Ammonium mineral salt (AMS) media broth have been prepared by mixing 1L of distilled water with the composition chemicals as in Table 3.1 in Schott bottle and to ensure the pH value of medium solution is adjusted to 6.8 with NaOH. After that, 50mL from the mixture were poured into 250mL Erlenmeyer flasks for cultivation. Then, the medium solution was sterilized at 121˚C for 2 hours by autoclaving. Next, the broth was cooled to 50˚C then added 0.5% methanol into each Erlenmeyer flasks. The broth was shaked a bit to obtain a medium solution was mixed well before cultivation of both *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3. Figure 3.1 showed the sterile AMS medium broth were prepared in Erlenmeyer flasks.

Figure 3.1: Sterile AMS medium broth was prepared in Erlenmeyer flasks.

3.2.4 Preparation of Inoculum for Pot Study

After 4-5 days, the bacterial strains from AMS agar media was inoculated under aseptic conditions into sterile AMS broth media and incubated at 30˚C in Orioner Incubator Shaker (Vis180).

Then, left it to propagate to approximately 5.0 x 10^7 cfu/ml for 5-6 days until it reached OD_{600} at 0.5 OD value to obtain the fresh *Methylorubrum* sp culture at its log phase growing phase for pot study preparation purpose. Figure 3.2 showed the inoculum after incubated in Incubator Shaker.

Figure 3.2: The inoculum in medium broth was incubated at 30˚C in Orioner Incubator Shaker (Vis180).

3.2.5 Sterilization of Paddy Seed

4% of bleach solution was used for sterilizing the paddy seeds. The 4% of bleach solution was added with 200mL sterile distilled water and mixed well. Then, the paddy seeds were poured into the Schott bottle. Then, the 200mL of 4% mixture of bleach solution was poured into the Schott bottle that contain paddy seeds and soaked for 10 minutes. There were some paddy seeds floats on surface of solution which are the bad seed that should discarded and good seeds were used for pot study purpose. After 10 minutes soaked, the bleach solution was discards then rinse the paddy seeds with 200mL of sterile distilled water. This rinsing process were repeated in 3 times. Once the rinsing process finished, the paddy seeds were soaked in Schott bottle overnight. Thus, the paddy seeds were surface sterilized prior to germination process. The overall sterilization step was conducted in laminar flow hood to prevent contamination occurs.

Figure 3.3: The paddy seeds were soaked overnight in Schott bottle for germination purpose.

3.2.6 Germination of Paddy Seed

After the paddy seeds were soaked for overnight, the excess sterile distilled water was removed or poured out from Schott bottle. Next, the paddy seeds were germinated on the sterile filter paper in sterile petri dish for 5 days.

Figure 3.4: The paddy seeds were germinated on the sterile filter paper in sterile petri dish for 5 days.

3.2.7 Formulation of Biofertilizer

The ratio of isolated *Methylorubrum* sp were put into the pot medium for each plastic cup with the amount of *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 were showed in Table 3.2 and Table 3.3. A total of 120g of soil and cocopeat medium, and 250 g of moist silty loam soil medium (72% silt, 2% clay, 26% sand; pH6) amended with inoculated *M.thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 were filled in a plastic cup (7 cm diameter by 12 cm height) with holes at the sides and bottom. The moisture content was adjusted at 50% before planting step conducted. The pot medium was prepared one day before planting to allowing the medium to well adapted with its optimal conditions as showed in Figure 3.5.

Table 3.2: The medium formulation with *M. thiocyanatum* UMK-PM2.

	Formulation 1		Formulation 2	
Sample / Parameter	Control (Soil)	Treatment (Soil + *M.thiocyanatum* UMK-PM2)	Control (Soil + Cocopeat)	Treatment (Soil + Cocopeat + *M.thiocyanatum* UMK-PM2)
Medium Weight (g)	250	250	120	120
M. thiocyanatum UMK-PM2 amount (mL)	-	2.5	-	1.2

Table 3.3: The medium formulation with *M. salsuginis* UMK-PM3.

Sample / Parameter	Formulation 1		Formulation 2	
	Control (Soil)	Treatment (Soil + *M. salsuginis* UMK-PM3)	Control (Soil + Cocopeat)	Treatment (Soil + Cocopeat + *M. salsuginis* UMK-PM3)
Medium Weight (g)	250	250	120	120
M. salsuginis UMK-PM3 amount (mL)	-	2.5	-	1.2

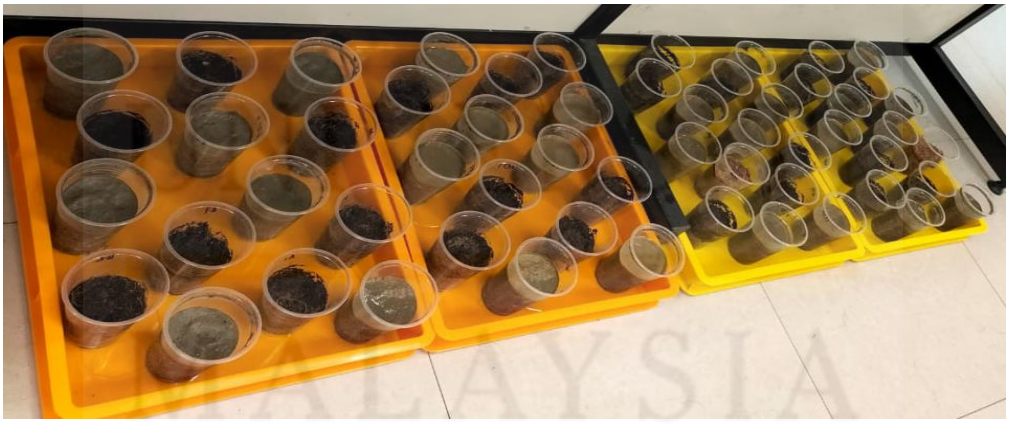


Figure 3.5: The pot medium was prepared in plastic cup on the trays 1 day before sown germinated paddy seeds.

3.2.8 Preparation of Pot Study

The performance of the formulated biofertilizer was tested on paddy (Oryza sativa) growth. The trays were placed at nursery for 14 DAS of plant's growing as showed in Figure 3.6 and

Figure 3.7. Through direct planting after 1 day of preparation pot medium, the germinated paddy seed was buried in each formulation 1 and formulation 2 uniformly at a depth of 1 cm for each cup. Water are applied from the bottom of the cups to ensure the moisture and humidity were maintained for the proper growth of paddy seedlings.

Figure 3.6: The paddy plant after sowing germinated seeds in pot medium on trays for *M.thiocyanatum* UMK-PM2 treatment.

Figure 3.7: The paddy plant after sowing germinated seeds in pot medium on trays for *M. salsuginis* UMK-PM3 treatment.

3.2.8 Evaluation of Paddy Growth

Paddy growth performance was evaluated based on the plant's height, plant's weight, chlorophyll content, plant's moisture content after 14 DAS as in Figure 3.8. The term of DAS was defined as Days After Sowing of paddy plant.

Figure 3.8: The paddy plants in pot medium on trays after 14 DAS.

3.2.8.1 Plant's Height (cm)

The plants were removed carefully from the medium after 14 DAS without disturbing the root system, and the roots and shoots were then rinsed with tap water to removes any remaining dirt as showed in figure 3.9. The height of fresh plants was measured with the ruler.

Figure 3.9: The roots and shoots of paddy plants were rinsed with tap water to removes any remaining dirt.

3.2.8.2 Plant's Weight (g)

The plants were removed carefully from the medium after 14 DAS without disturbing the root system, and the plants were then rinsed with tap water to remove any remaining dirt. Before

the fresh plants being weighed, the plants were dried by using tissue to obtain the accurate data of weight.

3.2.8.3 Chlorophyll Content of Leaves

Chlorophyll content was measured by using SPAD meter. Chlorophyll meter must be calibrated before used it. Calibration was done before introducing plant tissue by pressing on the finger rest to seal the head. A display appears after the metre beeps, indicating that the metre is ready for the first sample. The reading has been monitored by the 'N=' displayed at the top of the screen on the SPAD meter. Thus, the chlorophyll content of paddy plant for each leaf were measured by using the Chlorophyll SPAD Meter as showed in figure 3.10.

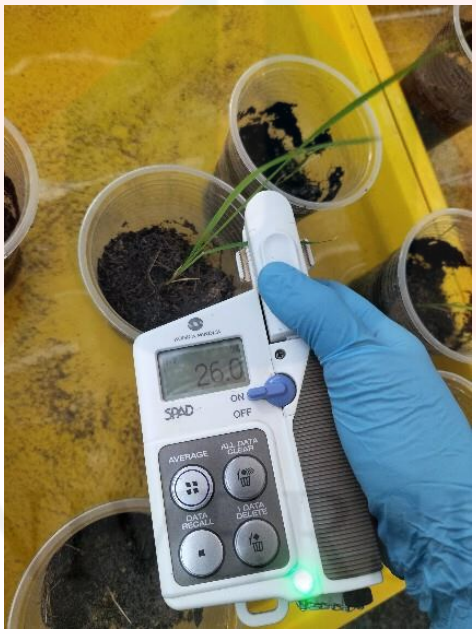


Figure 3.10: The chlorophyll content of plant leaves was measured by using Chlorophyll SPAD Meter after 14 DAS.

3.2.8.4 Plant's Moisture Content

The plants were removed carefully from the medium after 14 DAS without disturbing the root system, and the plants were then rinsed with tap water to remove any remaining dirt. Before the fresh plants being weighed, the plants were dried to obtain the accurate data of fresh weight. Then, before being weighed for dry weight, the plants were dried by using tissue to obtain the

accurate data of fresh weight. The, the fresh plants are dried in an oven at 70°C for 24 hours. Next, the plant's dry weight was obtained and calculated the moisture content as the formula below:

$$\text{Moisture content (\%)} = \frac{\text{Plant's fresh weight - Plant's dry weight}}{\text{Plant's fresh weight}} \times 100$$

3.2.9 Statistical Analysis

The pot trials were carried out in triplicate and set up in a totally random design. One-way ANOVA was applied to the percentage data. Next, to compare the mean among the treatments at 5% of significant level by excluding percentage data of control, the Tukey test was conducted.

CHAPTER 4

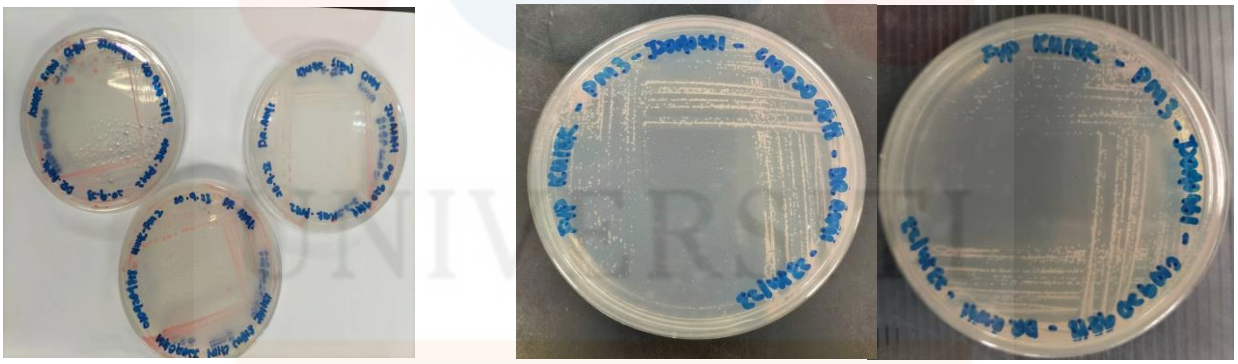
RESULTS AND DISCUSSION

4.1 Results and Discussion

4.1.1 Bacterial Strain

AMS media are nutrients that have different anions mixed with the ammonium cation ($NH3_4^+$). These salts can have a variety of uses, based on the application and circumstances. These are a few typical uses for mineral salts containing ammonium

Figure 4.1 showed below the ability of each bacterial strain grow on the AMS agar media which (a) *Methylorubrum thiocyanatum* UMK-PM2 and (b) *M. salsuginis* UMK-PM3.



(a) (b)

Figure 4.1: (a) *M. thiocyanatum* UMK-PM2 strain and (b) *M. salsuginis* UMK-PM3 strain on AMS agar media.

There are 2 figures showed below the ability of each bacterial strain grow in the AMS broth media which *Methylorubrum thiocyanatum* UMK-PM2 in Figure 4.3 and *Methylorubrum salsuginis* UMK-PM3 in Figure 4.4.

Figure 4.3: The *Methylorubrum thiocyanatum* UMK-PM2 strain in AMS broth.

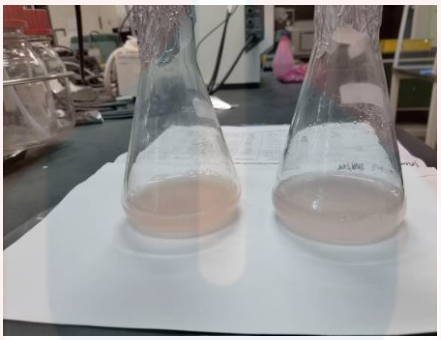


Figure 4.4: The *Methylorubrum salsuginis* UMK-PM3 strain in AMS broth.

4.1.2 The Growth of Paddy Plants

The plant material used in this experiment was the paddy plant. Before beginning the research project, MR297 paddy seeds were acquired from Kemubu Agriculture Development Agency (KADA). The paddy plant measured three to five centimetres in height at the time of the first inspection. There were two planting batches that needed to be watched for this study. For every batch, a total of 48 average-height and average-weight paddy plants were measured. The observation period for this investigation was around thirty days. Only water was provided to the paddy plants in the pot research during the observation period, along with the medium.

There were 48 plants were sown into each of four parameter categories which include control, inoculated with bacterial isolates in formulation 1 and formulation 2 in order to collect

data. While the control group received no treatment, the paddy plants were seeded in two distinct medium categories containing isolated *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 cultures. This study set out to determine how well-formulated biofertilizer affected the growth performance of paddy plants in a pot experiment. Following 14 DAS of growth, assessments of plant growth performance were carried out. The performance was assessed using measures including plant weight, plant height, moisture content and chlorophyll content, which were recorded after the plant was harvested. The Figure 4.5 and Figure 4.6 showed below are the growth of paddy plants after 14 DAS.

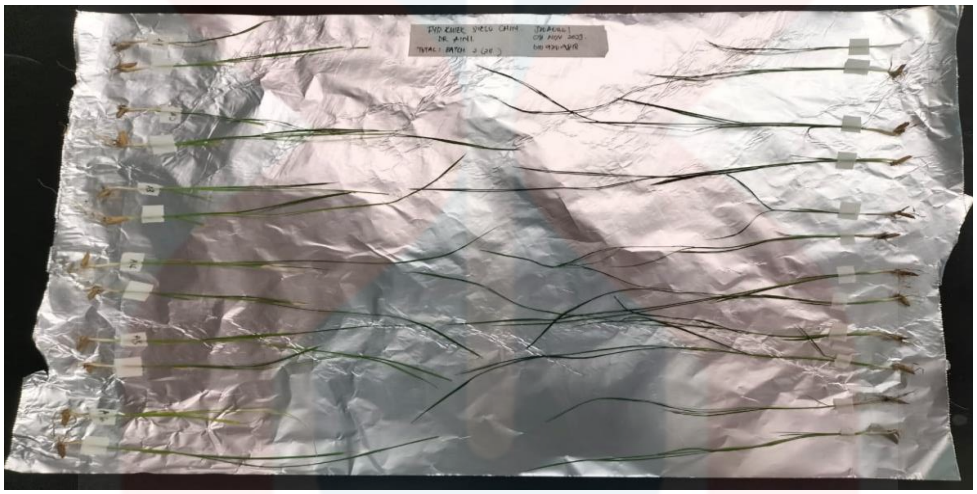


Figure 4.5: The paddy plants growth for *Methylorubrum thiocyanatum* UMK-PM2 treatment after 14 DAS.

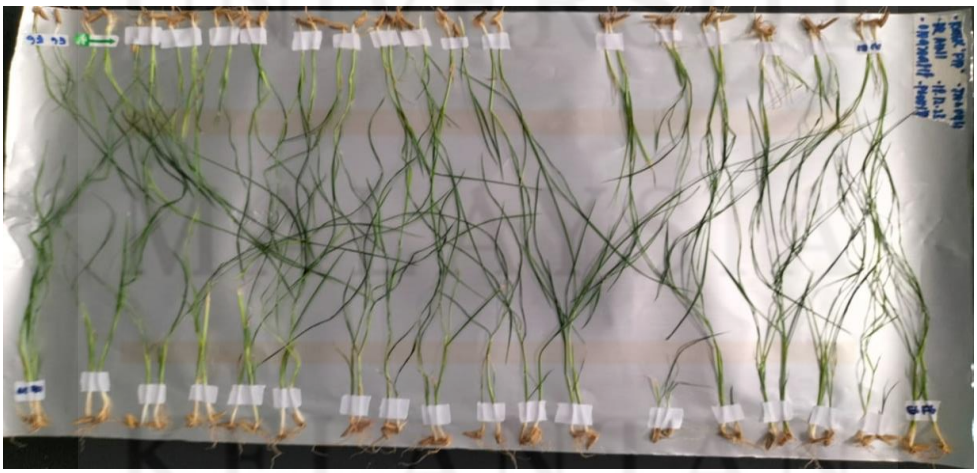


Figure 4.6: The paddy plants growth for *M. salsuginis* UMK-PM3 treatment after 14 DAS.

4.1.3 The Performance of Paddy Plants Growth

4.1.3 (a) The performance of paddy isolated with *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 in Biofertilizer Formulation 1 and Formulation 2 after 14 DAS

The plants were evaluated based on difference parameters which are including weight, height, moisture content and chlorophyll content of each paddy plant and recorded after 14 DAS.

Table 4.1 showed the mean and standard deviation of paddy plant results in formulation 1 medium with *M. thiocyanatum* UMK-PM2 treatment. However, the Figure 4.7 showed the graph mean of plant while the Table 4.2 showed the T-test results of paddy plant in formulation 1 with *M. thiocyanatum* UMK-PM2 treatment after 14 DAS.

Formulation 1:

Table 4.1: The mean and standard deviation of paddy plant performance for *M. thiocyanatum* UMK-PM2 treatment in Formulation 1 pot medium after 14 DAS.

Parameter / Sample Medium	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
Control (Soil)	0.0253 ± 0.0159	12.87 ± 1.730	72.55 ± 4.00	14.8 ± 1.8
Treatment (Soil + UMK-PM2)	0.0488 ± 0.0116*	18.85 ± 3.306*	77.99 ± 2.82*	18.6 ± 2.2*

Note: Mean ± Standard deviation

*Significant at $p<0.05$ (Independent T-test); compared to each treatment.

The data shown is the representative from two independent experiments which each experiment conducted in triplicate.

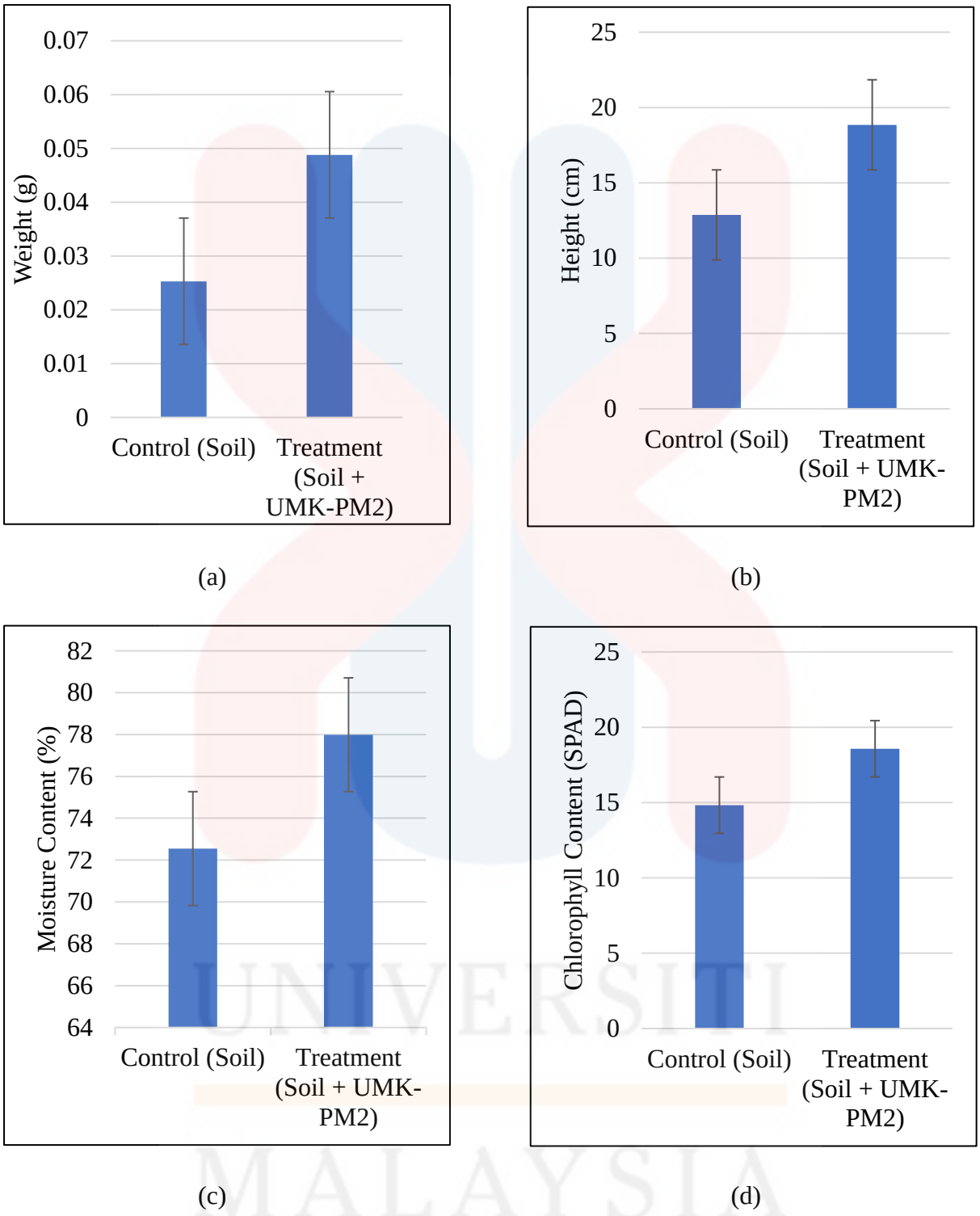


Figure 4.7: The paddy plant evaluation for *M.thiocyanatum* UMK-PM2 treatment in formulation 1 after 14 DAS on :

(a) Weight of Plant

(b) Height of Plant

(c) Moisture Content of Plant

(d) Chlorophyll Content of Leaves

Figure 4.7 showed the *Methylorubrum thiocyanatum* UMK-PM2 treatment in soil formulation for 4 parameters in each replicate. It was a graphical representation the soil of the control and treated with the change in value of total mean.

According to the Figure 4.7, we can observe that all parameter tabulated in height, weight, moisture content and chlorophyll content of paddy plants treated with *Methlorubrum thiocyanatum* UMK-PM2 showed better growth of paddy performance than control.

Table 4.2: The T-test results of paddy plant for *Methylorubrum thiocyanatum* UMK-PM2 treatment formulation 1 after 14 DAS.

Parameter	Formulation 1			
	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
p-value	0.0368	0.0450	0.0395	0.0480

Based on table 4.2 was showed the T-test value tabulated for *Methylorubrum thiocyanatum* UMK-PM2 treatment in formulation 1 showed a significant value ($p<0.05$) in all parameter which proved that *M. thiocyanatum* UMK-PM2 strain was efficiency improved the paddy growth better than in formulation 2.

The plants were evaluated based on difference parameter which are including weight, height, moisture content and chlorophyll content of each paddy plant and recorded after 14 DAS.

Table 4.3 showed the mean and standard deviation of paddy plant results in soil with cocopeat formulation medium with *Methylorubrum thiocyanatum* UMK-PM2 treatment. However, the figure 4.8 showed the graph mean of plant while the table 4.4 showed the T-test results of paddy plant in soil with cocopeat formulation with *Methylorubrum thiocyanatum* UMK-PM2 treatment after 14 DAS.

Formulation 2:

Table 4.3: The mean and standard deviation of paddy plant performance for *M. thiocyanatum* UMK-PM2 treatment in formulation 2 after 14 DAS.

Parameter / Sample Medium	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
Control (Soil + Cocopeat)	0.0154 ± 0.0069	10.02 ± 0.850	73.35 ± 0.13	9.0 ± 1.9
Treatment (Soil + Cocopeat + UMK-PM2)	0.0340 ± 0.0058*	12.87 ± 1.704*	78.35 ± 2.93*	14.9 ± 3.5*

Note: Mean ± Standard deviation

*Significant at $p<0.05$ (Independent T-test); compared to each treatment.

The data shown is the representative from two independent experiments which each experiment conducted in triplicate.

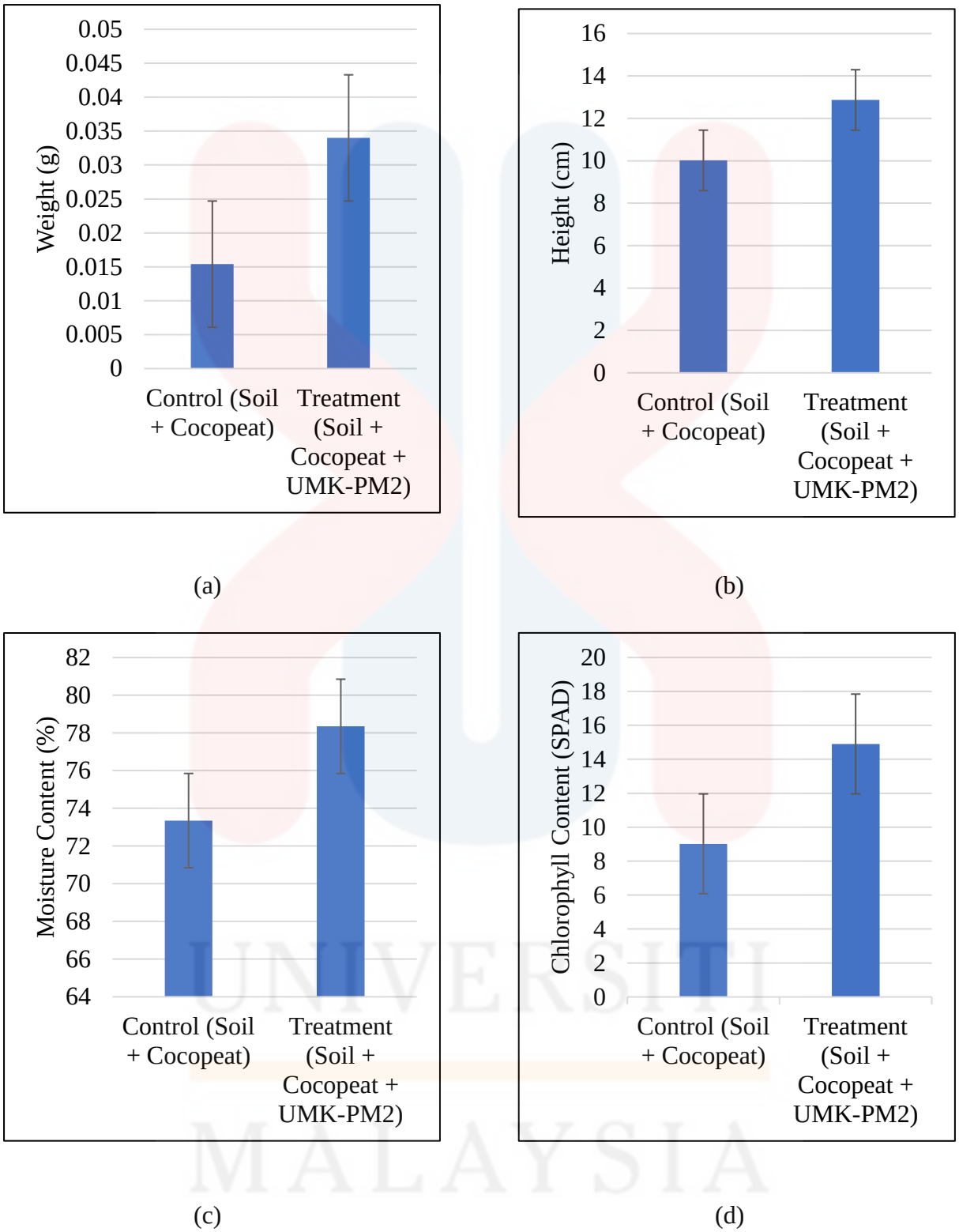


Figure 4.8: The paddy plant evaluation for *M. thiocyanatum* UMK-PM2 treatment in soil with cocopeat formulation after 14 DAS on:

(a) Weight of Plant

(b) Height of Plant

(c) Moisture Content of Plant

(d) Chlorophyll Content of Leaves

Figure 4.8 showed the *Methylorubrum thiocyanatum* UMK-PM2 treatment in soil with cocopeat formulation for 4 parameters in each replicate. It was a graphical representation the formulation 2 of control and isolated medium with the change in increasing value of total mean.

According to the figure 4.8, we can observe that all parameter tabulated in height, weight, moisture content and chlorophyll content of paddy plants treated with *Methylorubrum thiocyanatum* UMK-PM2 showed better growth performance than control.

Table 4.4: The T-test results of paddy plant for *Methylorubrum thiocyanatum* UMK-PM2 treatment in formulation 2 after 14 DAS.

Parameter	Formulation 2			
	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
p-value	0.0154	0.0507	0.0490	0.0502

Based on table 4.4 was showed the T-test value tabulated for *Methylorubrum thiocyanatum* UMK-PM2 treatment in soil with cocopeat formulation was also showed a significant value ($p<0.05$) in all parameter which proved that *M. thiocyanatum* UMK-PM2 strain was improved the paddy growth.

The plants were evaluated based on difference parameter which are including weight, height, moisture content and chlorophyll content of each paddy plant and recorded after 14 DAS.

Table 4.5 showed the mean and standard deviation of paddy plant results in formulation 1 medium with *Methylorubrum salsuginis* UMK-PM3 treatment. However, the Figure 4.9 showed the graph mean of plant while the Table 4.6 showed the T-test results of paddy plant in formulation 1 with *Methylorubrum salsuginis* UMK-PM3 treatment after 14 DAS.

Formulation 1:

Table 4.5: The mean and standard deviation of paddy plant performance for *M. salsuginis* UMK-PM3 treatment formulation 1 after 14 DAS.

Parameter / Sample Medium	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
Control (Soil)	0.0512 ± 0.0369	13.8 ± 4.594	78.77 ± 7.55	48.9 ± 21.5
Treatment (Soil + UMK-PM3)	0.0902 ± 0.0190*	16.68 ± 2.212*	85.73 ± 3.52*	59.2 ± 12.4

Note: Mean ± Standard deviation

*Significant at $p<0.05$ (Independent T-test); compared to each treatment.

The data shown is the representative from two independent experiments which each experiment conducted in triplicate.

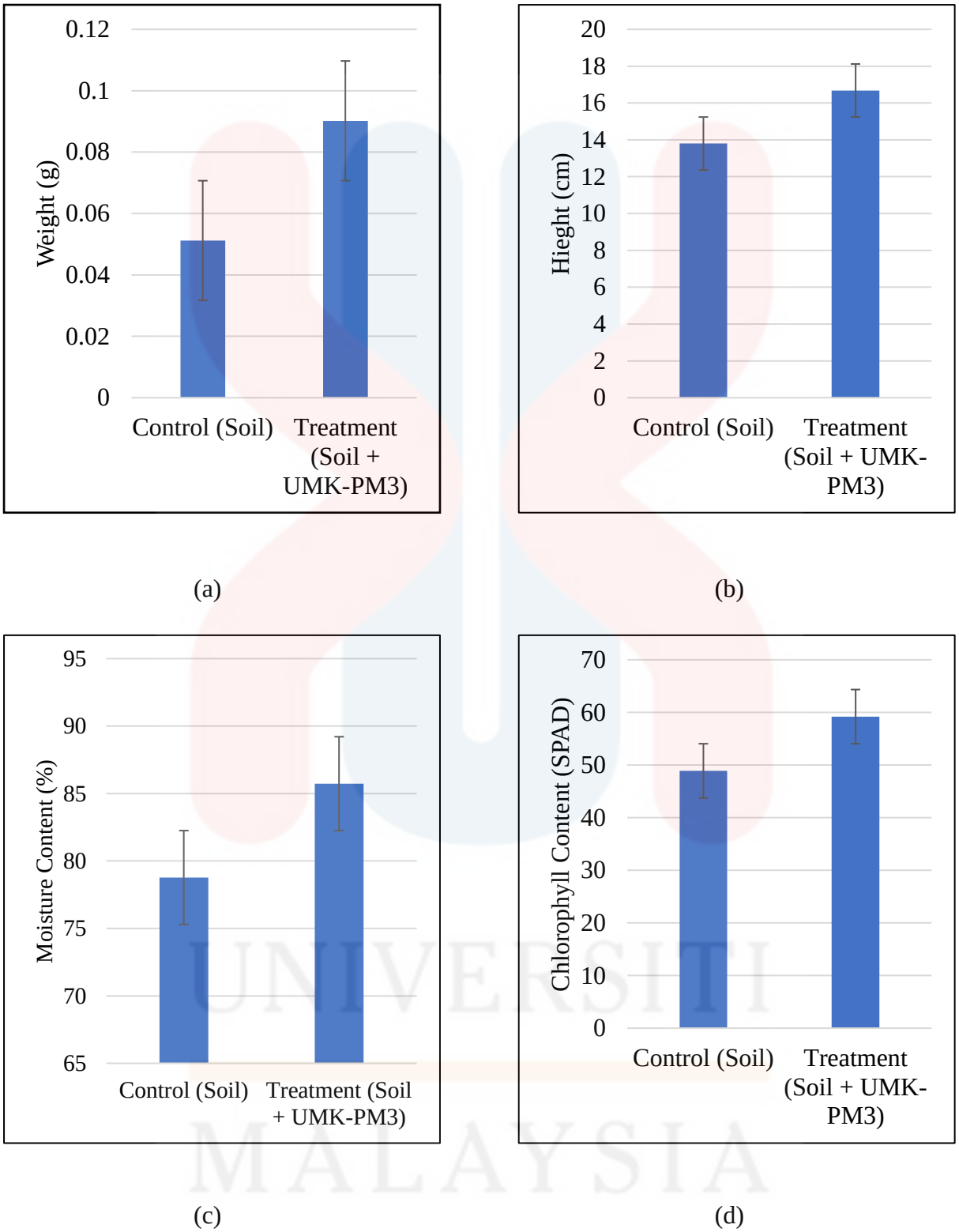


Figure 4.9: The graph of mean of paddy plant evaluation for *Methylorubrum salsuginis* UMK-PM3 treatment formulation 1 after 14 DAS on:

(a) Weight of Plant

(b) Height of Plant

(c) Moisture Content of Plant

(d) Chlorophyll Content of Leaves

Figure 4.9 showed the *Methylorubrum salsuginis* UMK-PM3 treatment in soil formulation for 4 parameters in each replicate. It was a graphical representation the formulation 1 of control and treated with the change in increasing value of total mean.

According to the Figure 4.9, we can observe that all parameter tabulated in height, weight, moisture content and chlorophyll content of paddy plants treated with *M. salsuginis* UMK-PM3 showed better growth performance than control.

Table 4.6: The T-test results of paddy plant for *Methylorubrum salsuginis* UMK-PM3 treatment in formulation 1 after 14 DAS.

Parameter	Formulation 1			
	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
p-value	0.0353	0.0198	0.0377	0.0651

Based on Table 4.6 was showed the T-test value tabulated for *Methylorubrum salsuginis* UMK-PM3 treatment in formulation 1 showed a significant value ($p<0.05$) in all parameter except chlorophyll content and was improved the paddy growth performance.

The plants were evaluated based on difference parameter which are including weight, height, moisture content and chlorophyll content of each paddy plant and recorded after 14 DAS.

Table 4.7 showed the mean and standard deviation of paddy plant results in formulation 2 medium with *Methylorubrum salsuginis* UMK-PM3 treatment. However, the Figure 4.10 showed the graph mean of plant while the table 4.8 showed the T-test results of paddy plant in formulation 2 isolated with *Methylorubrum salsuginis* UMK-PM3 treatment after 14 DAS.

Formulation 2:

Table 4.7: The mean and standard deviation of paddy plant performance for *Methylorubrum salsuginis* UMK-PM3 treatment in formulation 2 after 14 DAS.

Parameter / Sample Medium	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
Control (Soil + Cocopeat)	0.0723 ± 0.0173	11.32 ± 3.922	85.01 ± 3.56	44.7 ± 7.6
Treatment (Soil + Cocopeat + UMK-PM3)	0.0896 ± 0.0155	15.18 ± 2.829	85.94 ± 0.59	61.6 ± 61.6

Note: Mean ± Standard deviation

*Significant at $p<0.05$ (Independent T-test); compared to each treatment.

The data shown is the representative from two independent experiments which each experiment conducted in triplicate.

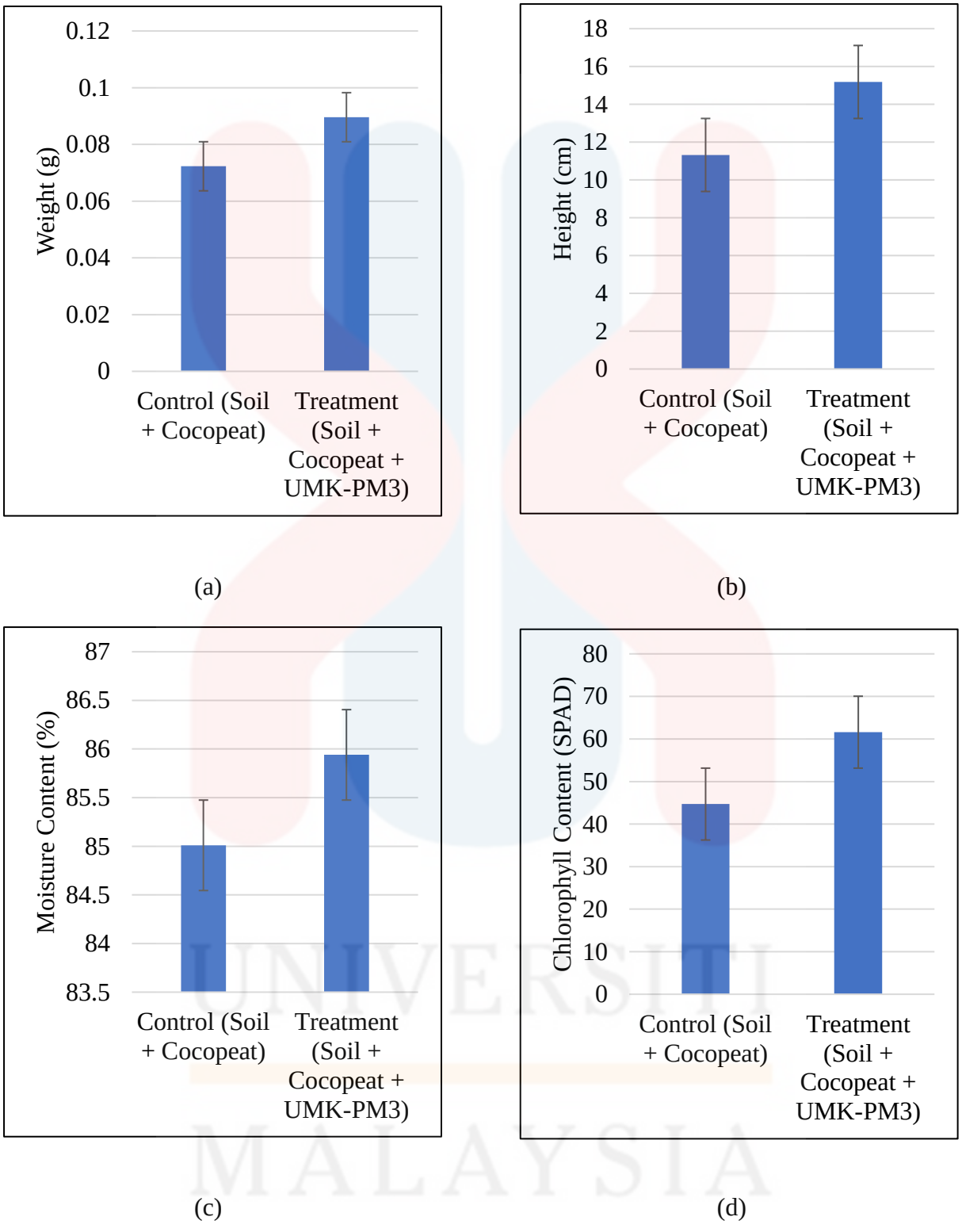


Figure 4.10: The paddy plant evaluation for *Methylorubrum salsuginis* UMK-PM3 treatment in formulation 2 after 14 DAS on:

(a) Weight of Plant

(b) Height of Plant

(c) Moisture Content of Plant

(d) Chlorophyll Content of Leaves

Figure 4.10 showed the *M. salsuginis* UMK-PM3 treatment in soil with cocopeat formulation for 4 parameters in each replicate. It was a graphical representation the formulation 2 of control and treated with the change in increasing value of total mean.

According to the Figure 4.10, we can observe that all parameter tabulated in height, weight, moisture content and chlorophyll content of paddy plants treated with *M. salsuginis* UMK-PM3 showed better growth performance than control after 14 DAS.

Table 4.8: The T-test results of paddy plant for *Methylorubrum salsuginis* UMK-PM3 treatment in formulation 2 after 14 DAS.

Parameter	Formulation 2			
	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
p-value	0.0966	0.0708	0.5000	0.0711

Based on table 4.18 was showed the T-test value tabulated for *Methylorubrum salsuginis* UMK-PM3 treatment in formulation 2 showed not a significant value ($p<0.05$) in all parameter of paddy growth performance evaluation.

From this research, the isolated *Methylorubrum* sp strain that inoculated from previous study had been used as a biofertilizer to evaluate the growth performance of paddy plants. The result of the experiment was proof that both *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatments showed positive effects on paddy growth in terms of plant's height, weight, chlorophyll content production, and moisture content of plants.

The assessment of paddy plant growth is the most evident metric for assessing *Methylorubrum* sp effects. The strains of *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 had promoted the paddy plant growth compared to control by improving the plant height, weight, chlorophyll content and moisture content in this research work based on the

results obtained. This research was conducted triplicate in every treatment and the results was successfully obtained. In overall, the results were obtained that the medium isolated with *Methylorubrum* sp in formulation 1 was showed better growth performance than the formulation 2 due to the pH of soil was tends to alkaline while cocopeat was tends to acid. It was also observed that the soil containing high nutrition retention than cocopeat while cocopeat only have a low nutrient content (Sharda, Singh, & Pandey, 2023).

4.1.3 (b) Evaluation of plant growth performance for paddy inoculated with *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3

The plants were compared between the *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 strain treatments in formulation 1 based on difference parameter which are including weight, height, moisture content and chlorophyll content of each paddy plant after 14 DAS that showed in Table 4.9 with the mean and standard deviation of paddy plant.

However, the Figure 4.11 showed the graph mean of plant while the Table 4.10 showed the T-test results of paddy plant in formulation 1 with both *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatments after 14 DAS.

Table 4.9: The mean and standard deviation of paddy plant for *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatments in formulation 1 after 14 DAS.

Parameter / Sample Medium	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
Soil + UMK-PM2	0.0488 ± 0.0116	18.85 ± 3.306	77.99 ± 2.82	18.6 ± 2.2
Soil + UMK-PM3	0.0902 ± 0.0190*	16.68 ± 2.212	85.73 ± 3.52*	59.2 ± 12.4*

Note: Mean ± Standard deviation

*Significant at $p<0.05$ (Independent T-test); compared to each treatment.

The data shown is the representative from two independent experiments which each experiment conducted in triplicate.

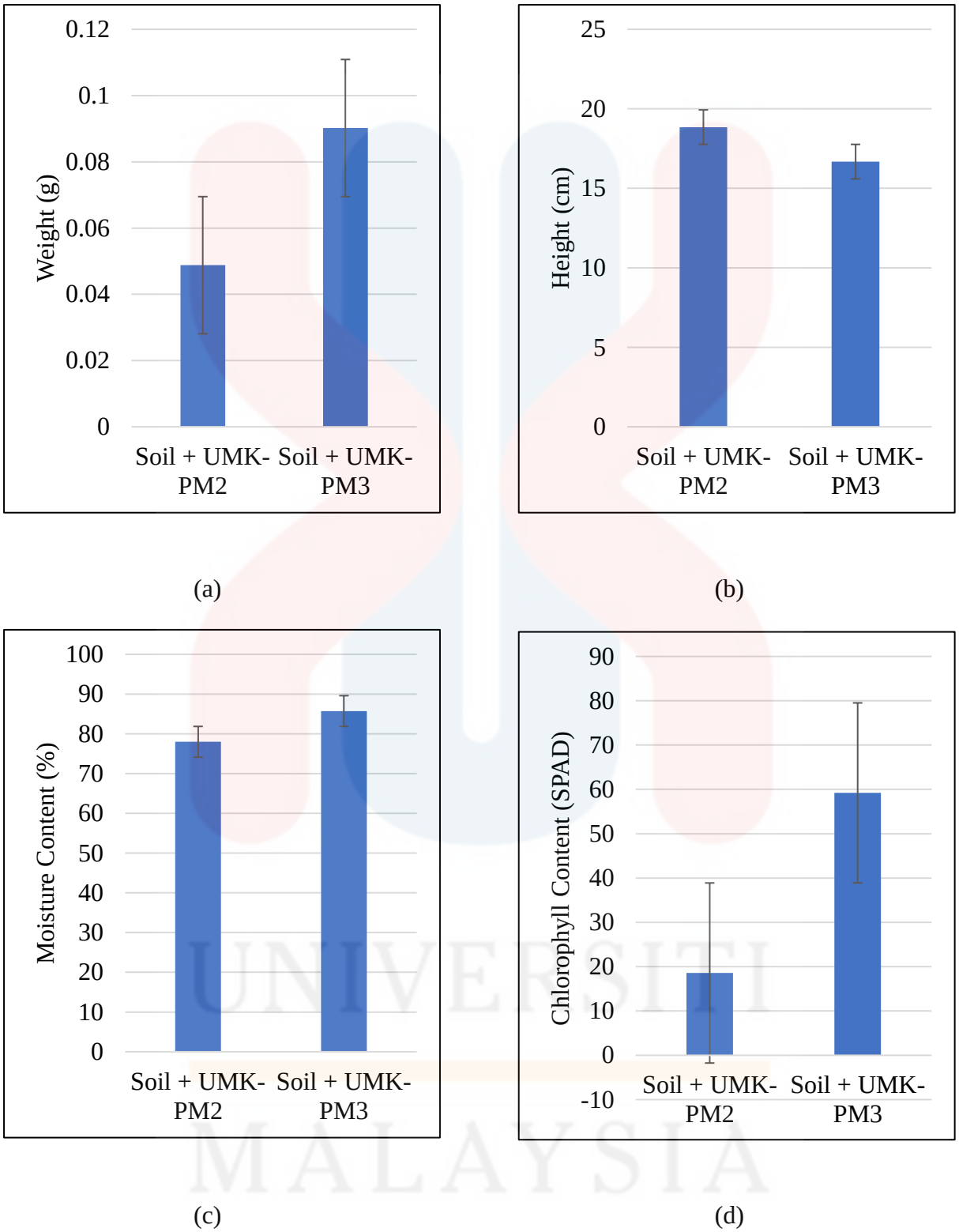


Figure 4.11: The paddy plant evaluation for *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatments in formulation 1 after 14 DAS on:

(a) Weight of Plant

(b) Height of Plant

(c) Moisture Content of Plant

(d) Chlorophyll Content of Leaves

Figure 4.11 showed the *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatment in formulation 1 for 4 parameters in each replicate. It was an increasing value of total mean between these both strains. According to the Figure 4.11, we can observe that the plants treated with *M. salsuginis* UMK-PM3 showed better growth performance than *M. thiocyanatum* UMK-PM2 in formulation 1.

Table 4.10: The T-test results of paddy plant for *Methylorubrum thiocyanatum* UMK-PM2 and *M. thiocyanatum* UMK-PM3 treatment in formulation 1 after 14 DAS.

Parameter	Formulation 1			
	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
p-value	0.0300	0.4600	0.0312	0.0100

Based on Table 4.10 was showed the T-test value tabulated for formulation 1 in both treatment showed a significant value ($p<0.05$) in an weight, moisture content and chlorophyll content which are 0.0300, 0.0312 and 0.0100 while height showed not a significant value due to 0.4600 was more than 5% significant level.

The plants were compared between the *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 strain treatments in formulation 2 based on difference parameter which are including weight, height, moisture content and chlorophyll content of each paddy plant after 14 DAS that showed in Table 4.11 with the mean and standard deviation of paddy plant.

However, the Figure 4.12 showed the graph mean of plant while the Table 4.12 showed the T-test results of paddy plant in formulation 2 with both *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatments after 14 DAS.

Table 4.11: The mean and standard deviation of paddy plant evaluation results for *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatments formulation 2 pot medium after 14 DAS.

Parameter / Sample Medium	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
Soil + Cocopeat + UMK-PM2	0.0340 ± 0.0058	12.87 ± 1.704	78.35 ± 2.93	59.2 ± 12.4
Soil + Cocopeat + UMK-PM3	0.0896 ± 0.0155	15.18 ± 2.829*	85.94 ± 0.59*	61.6 ± 61.6*

Note: Mean ± Standard deviation

*Significant at $p<0.05$ (Independent T-test); compared to each treatment.

The data shown is the representative from two independent experiments which each experiment conducted in triplicate.

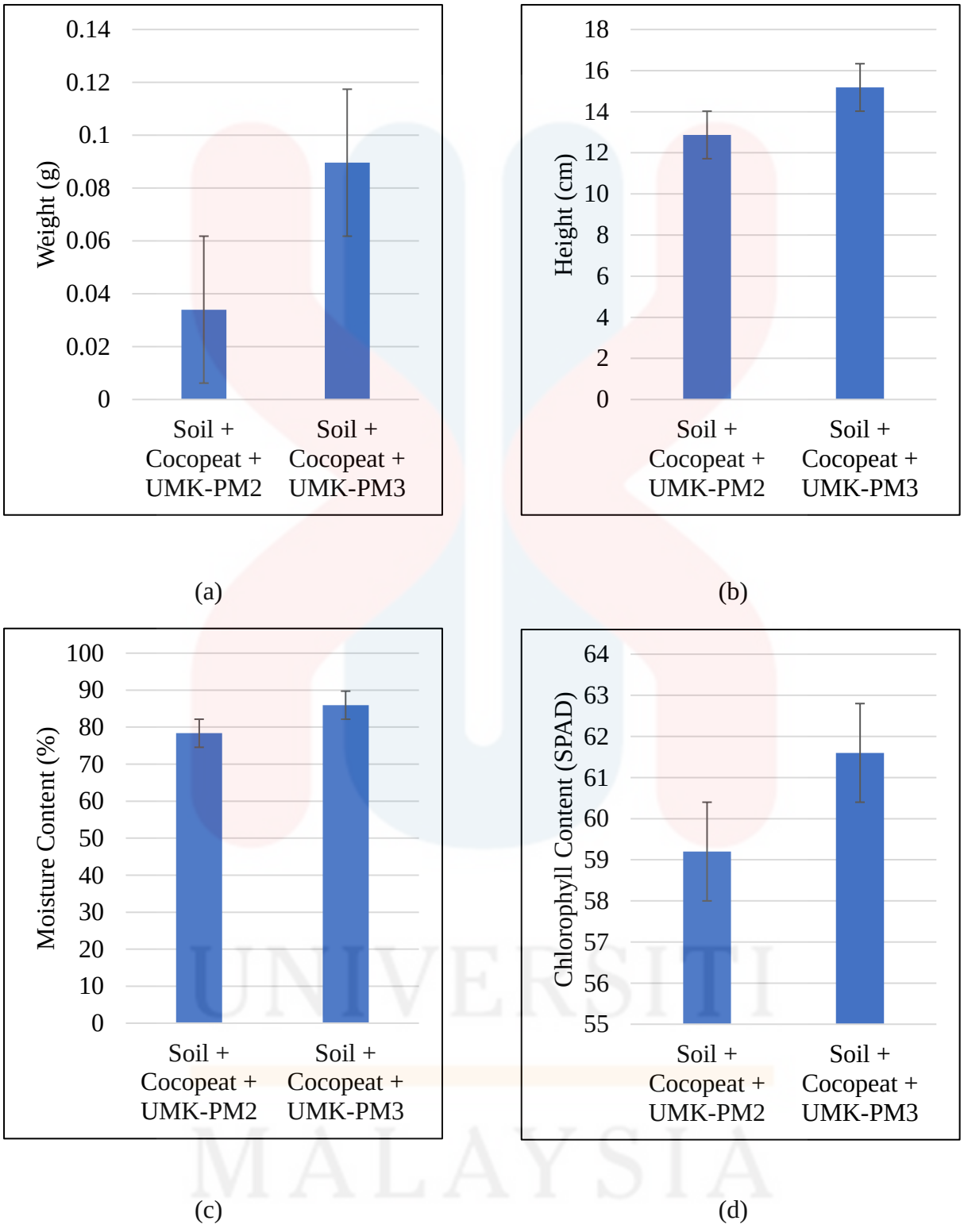


Figure 4.12: The paddy plant evaluation for *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatments in formulation 2 after 14 DAS on:

(a) Weight of Plant

(b) Height of Plant

(c) Moisture Content of Plant

(d) Chlorophyll Content of Leaves

Figure 4.12 showed the *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatment in formulation 2 for 4 parameters in each replicate. It was an increasing value of total mean between these both strains in all parameter.

According to the Figure 4.12, we can observe that the plants isolated with *Methlorubrum salsuginis* UMK-PM3 showed better growth performance than *Methylorubrum thiocyanatum* UMK-PM2 in formulation 2.

Table 4.12: The T-test results of paddy plant for *Methylorubrum thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatment in formulation 2 after 14 DAS.

Parameter	Formulation 2			
	Weight (g)	Height (cm)	Moisture Content (%)	Chlorophyll Content
p-value	0.1500	0.0100	0.0010	0.0100

Based on Table 4.12 was showed the T-test value tabulated for formulation 2 in both treatment was showed a statistically significant value ($p<0.05$) in height, moisture content and chlorophyll content which are 0.0100, 0.0010 and 0.0100 while weight of paddy plant showed not a significant value due to 0.1500 was more than 5% significant level.

Next, according to the total data results in T-Test analysis, the treatment with *Methylorubrum salsuginis* UMK-PM3 in formulation 1 and formulation 2 were showed better than the plants treated with *Methylorubrum thiocyanatum* UMK-PM2. They resulted in the increasing of the value of increment plant height and weight, and moisture content and chlorophyll content which a statistically significant at $p<0.05$. This could be due to the strain of *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 being originally obtained from paddy fields that might be capable of fixing atmospheric nitrogen, converting it into forms that plants can utilize. Nitrogen fixation is crucial for plant growth as it provides an essential

nutrient necessary for various physiological processes, including protein synthesis and overall growth (Grossi et al., 2020). Thus, it directly improves the number of leaves, chlorophyll content and moisture content of paddy plants.

Moreover, it was also induced that the optimum growth condition was effect on the paddy growth performance. Based on previous study, the optimum growth for *Methylorubrum* sp bacteria strain were in pH range of 7.7 at 37˚C (Green & Ardley, 2018). It was proved that the pH of soil was tends to alkaline while cocopeat was tends to acid. It was also observed that the soil containing high nutrition retention than cocopeat while cocopeat only have a low nutrient content (Sharda et al., 2023).

Besides, the *Methylorubrum* sp are able to solubilize phosphate in the soil compared to cocopeat medium that helps it become more available to plant itself. In addition, phosphorus is an essential nutrient required for plant growth to contribute to processes such as photosynthesis capacity, energy transfer, and root development (Gouda et al., 2018; Grossi et al., 2020). Moreover, the *Methylorubrum* sp might enhance the plants' tolerance with various stresses which include drought, salinity, and diseases (Daniel et al., 2022).

From this research, according to t-test analysis in table 4.10 and table 4.12, the paddy plant isolated with *M. salsuginis* UMK-PM3 has the highest chlorophyll content with significant value ($p<0.05$), which induce that *M. salsuginis* UMK-PM3 culture promote the chlorophyll production in leaf compared *M. thiocyanatum* UMK-PM2 culture. This is due to the moisture content in the plant which is resulting the humidity of the plant. Thus, the moisture content of pot medium is one of the most important aspects to achieved at 50%. The moisture content of medium enables the seeds to imbibe water, initiating the germination process. For rice seeds to germinate, a moisture content of about 50% is thought to be ideal as it provides the seeds with enough moisture to begin sprouting (Zareabyaneh et al., 2023).

While insufficient moisture can result in poor nutrient uptake and stunted plant growth, it is crucial for root growth performance to improve the ability of plants to absorb nutrients

found in the soil. Thus, the humidity is one of the factors that is necessary for photosynthesis to occur. The photosynthesis reaction occurs when the stomata of plants is open for nutrient uptake. The stomata close and photosynthesis stops when a plant loses too much water. This process directly impacts the photosynthesis of the plants to produce their own food. By ensuring that the leaves of the plant receive enough water, this response can promote more vigorous plant growth by facilitating efficient photosynthesis (Jin et al., 2019).

It was discovered that the germination process was too slow in comparison to the prior treatment during the research on the phases involved in germination paddy seeds. It can therefore be stated that certain problems with seed storage or environmental factors contributed to it. Thus, the raw paddy seeds must be stored in a zip-lock bag that has been carefully sealed and kept in a freezer at 4˚C before or after usage. It is observed that the ideal temperature range for rice (paddy) seed germination is usually 25 to 35˚C. Paddy seeds can also sprout at temperatures just a little bit outside of this range, but the optimal range promotes faster and more even germination. Lower germination rates or less vigorous seedlings may arise from temperatures below 25˚C or above 35˚C (Kim et al., 2023). The cause of this is because plants show signs of acclimatisation because of their improved capacity to tolerate physicochemical alterations that take place within their cells. Furthermore, plant cells produce osmolytes including soluble sugars and alcohols as well as nitrogenous substances like glycine, betaine, and proline (Fan et al., 2023).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The aim of this study is to formulate the biofertilizer by using locally isolated *Methylorubrum thiocyanatum* UMK-PM2 and *Methylorubrum salsuginis* UMK-PM3 and evaluate their effect on paddy growth performance based on plant height, weight, chlorophyll content, and moisture content by pot study. This study was also studied the comparison between *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 in both formulation 1 and formulation 2 treatment to promote the paddy plant growth.

According to the result obtained in this research was proved that the paddy plant in formulation isolated with *M. thiocyanatum* UMK-PM2 and *M. salsuginis* UMK-PM3 treatment in formulation 1 which is soil formulation as biofertilizer had showed better growth performance compared to formulation 2, soil with cocopeat medium. The treatment with these both bacterial strains was showed a positive effect on plant height and weight, moisture content, and chlorophyll content.

In comparison to *M. thiocyanatum* UMK-PM2, *M. salsuginis* UMK-PM3 was the most effective at promoting the growth of paddy plants. Additionally, *M. salsuginis* UMK-PM3 treatment applied to the paddy plant shown good performance in producing a chlorophyll content on the leaves of paddy plants.

Conversely, *M. thiocyanatum* UMK-PM2 was observed to be less successful across the board, with the treated plant exhibiting only marginal variations across all treatment parameters. In overall, *M. salsuginis* UMK-PM3 treatment had a greater overall impact on

crop growth than *M. thiocyanatum* UMK-PM2 treatment. Thus, plant growth can be assisted by using *M. salsuginis* UMK-PM3 as a biofertilizer using this approach.

5.2 Recommendations

For this research project, several kinds of recommendations are implemented. First and foremost, it should be provided the paddy plants a large study planting space in order to improve their capacity to absorb enough and an enough amount of nutrients and water supply. In addition, the weather also has an impact on the growth of paddy plants since these plants require a certain amount of moisture and humidity to grow in the optimal conditions for growth. Moreover, the suggestion on long term research of project should conducted in order to estimate the duration that the growth-promoting benefits of phosphate-solubilizing bacteria on paddy plants and obtained the accurate results. Besides, the methods of regularly treat the paddy plants with PGPB every 3 days as to ensure it can help better growth for paddy plant in nutrition uptake in time.

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APPENDIX A

All table below showed evaluation four parameters of all results obtained for objective 1:

Formulation 1

		Soil						Soil+Strain					
***M. thiocyanatum* UMK-PM2**	**Chlorophyll Content**	13.0	15.6	15.9	10.8	21.1	12.6	21.4	19.3	12.0	20.3	19.5	19.0
		14.3		13.4		16.8		20.3		16.2		19.2	
	Average	14.8						18.6					
	Standard Deviation	1.8						2.2					
	Moisture Content	63.72	73.88	74.90	78.64	75.03	69.12	79.95	71.97	75.59	78.02	80.30	82.12
		68.80		76.77		72.07		75.96		76.80		81.21	
	Average	72.55						77.99					
	Standard Deviation	4.00						2.82					
	Weight (g)	0.0142	0.0091	0.0490	0.0367	0.0260	0.0173	0.0556	0.0223	0.0407	0.0507	0.0611	0.0621
		0.0117		0.0429		0.0217		0.0390		0.0457		0.0616	
	Average	0.0254						0.0488					
	Standard Deviation	0.0159						0.0116					
	Height (cm)	9.40	13.90	14.30	15.40	12.60	11.62	19.10	16.67	10.20	19.90	19.70	21.50
		11.65		14.85		12.11		17.89		15.05		20.60	
	Average	12.87						17.85					
	Standard Deviation	1.73						2.78					

		Formulation 2											
		Soil+cocopeat						Soil+cocopeat+Strain					
M. thiocyanatum UMK-PM2	**Chlorophyll Content**	7.7	12.6	9.5	4.3	9.3	10.8	19.4	17.1	7.0	15.5	20.5	10.0
		10.2		6.9		10.0		18.2		11.3		15.2	
	Average	9.0						14.9					
	Standard Deviation	1.9						3.5					
	Moisture Content	71.39	75.56	72.16	74.57	71.86	74.57	79.68	82.80	77.79	79.05	74.22	76.55
		73.47		73.36		73.22		81.24		78.42		75.38	
	Average	73.35						78.35					
	Standard Deviation	0.13						2.93					
	Weight (g)	0.0155	0.0307	0.0134	0.0062	0.0070	0.0195	0.0415	0.0387	0.0284	0.0383	0.0373	0.0198
		0.0231		0.0098		0.0133		0.0401		0.0334		0.0286	
	Average	0.0154						0.0340					
	Standard Deviation	0.0069						0.0058					
	Height (cm)	7.10	11.20	12.40	7.70	9.30	12.40	14.10	13.50	7.90	13.90	19.00	8.80
		9.15		10.05		10.85		13.80		10.90		13.90	
	Average	10.02						12.87					
	Standard Deviation	0.85						1.70					

Formulation 1

		Soil						**Soil+Strain**					
***M. salsuginis* UMK-PM3**	**Chlorophyll Content**	62.5	53.2	51.9	6.1	63.6	56.2	64.7	37.2	56.3	70.8	69.4	56.5
		57.9		29.0		59.9		51.0		63.6		63.0	
	Average	48.9						59.2					
	Standard Deviation	21.5						12.4					
	Moisture Content	85.49	85.69	82.65	58.65	77.52	82.60	83.52	79.82	90.29	85.18	87.98	87.59
		85.59		70.65		80.06		81.67		87.73		87.79	
	Average	78.77						85.73					
	Standard Deviation	7.55						3.52					
	Weight (g)	0.0933	0.0491	0.0366	0.0194	0.0705	0.0773	0.0970	0.0404	0.1072	0.0878	0.1031	0.1058
		0.0712		0.0086		0.0739		0.0687		0.0975		0.1045	
	Average	0.0512						0.0902					
	Standard Deviation	0.0369						0.0190					
	Height (cm)	16.90	5.80	0.10	21.80	23.40	14.80	19.00	17.50	17.70	15.00	14.70	23.80
		11.35		10.95		19.10		18.25		16.35		19.25	
	Average	13.80						17.95					
	Standard Deviation	4.59						1.47					

Formulation 2

		Soil+cocopeat						Soil+cocopeat+Strain					
***M. salsuginis* UMK-PM3**	**Chlorophyll Content**	56.7	46.5	47.9	35.7	43.9	37.6	78.7	72.7	70.4	43.3	42.1	62.5
		51.6		41.8		40.8		75.7		56.9		52.3	
	Average	44.7						61.6					
	Standard Deviation	7.6						15.5					
	Moisture Content	87.65	88.94	89.22	81.78	75.67	86.80	85.52	86.31	86.39	84.33	87.07	86.02
		88.30		85.50		81.23		85.91		85.36		86.55	
	Average	85.01						85.94					
	Standard Deviation	3.56						0.59					
	Weight (g)	0.0867	0.0896	0.0794	0.0704	0.0046	0.1031	0.0940	0.1193	0.0976	0.0741	0.0778	0.0748
		0.0882		0.0749		0.0539		0.1067		0.0859		0.0763	
	Average	0.0723						0.0896					
	Standard Deviation	0.0173						0.0155					
	Height (cm)	12.80	3.00	2.40	18.50	16.70	14.50	14.50	16.80	18.30	17.20	7.90	16.40
		7.90		10.45		15.60		15.65		17.75		12.15	
	Average	11.32						15.18					
	Standard Deviation	3.92						2.83					

APPENDIX B

All table below showed T-Test of all results obtained for objective 1:

***M. thiocyanatum* UMK-PM2**

	Formulation 1				Formulation 2			
	SPAD	%	g	cm	SPAD	%	g	cm
Mean	18.57	77.99	0.05	17.85	14.90	78.35	0.034	12.8667
Standard Deviation	2.17	2.82	0.01	2.78	3.50	2.93	0.0058	1.70392
Count	3	3	3	3	3	3	3	3
Standard Error of Mean	1.25	1.63	0.01	1.60	2.02	1.69	0.00335	0.98376
Degrees of Freedom	2	2	2	2	2	2	2	2
Hypothesized Mean	14.83	72.55	0.03	12.87	9.02	73.35	0.01538	10.0167
T-Statistic	2.99	3.35	3.48	3.10	2.91	2.95	5.55721	2.89706
p-Value	0.0480	0.0395	0.0368	0.0450	0.0502	0.0490	0.0154	0.0507

***M. salsuginis* UMK-PM3**

	Formulation 1				Formulation 2			
	SPAD	%	g	cm	SPAD	%	g	cm
Mean	59.15	85.73	0.09	17.95	61.6167	78.7655	0.0896	15.18333333
Standard Deviation	7.11	3.52	0.02	1.47	12.4069	7.5526	0.01551862	2.829016319
Count	3	3	3	3	3	3	3	3
Standard Error of Mean	4.10	2.03	0.01	0.85	7.16312	4.3605	0.00895968	1.633333333
Degrees of Freedom	2	2	2	2	2	2	2	2
Hypothesized Mean	48.92	78.77	0.05	13.80	44.7167	78.7655	0.0723	11.31666667
T-Statistic	2.49	3.43	3.56	4.88	2.35931	0	1.93087292	2.367346939
p-Value	0.0651	0.0377	0.0353	0.0198	0.0711	0.5000	0.0966	0.0708

The table below showed T-Test of all results obtained for objective 2:

	***M. thiocyanatum* UMK-PM2**				***M. salsuginis* UMK-PM3**			
	SPAD	%	g	cm	SPAD	%	g	cm
Mean	59.15	85.73	0.09	17.95	61.6167	85.9394	0.0896	15.18
Standard Deviation	7.11	3.52	0.02	1.47	12.4069	0.59401	0.01552	2.83
Count	3	3	3	3	3	3	3	3
Standard Error of Mean	4.10	2.03	0.01	0.85	7.16312	0.34295	0.00896	1.63
Degrees of Freedom	2	2	2	2	2	2	2	2
Hypothesized Mean	18.57	77.99	0.05	17.85	14.8983	78.3473	0.034	12.87
T-Statistic	9.89	3.81	3.79	0.12	6.52207	22.1372	6.20558	1.42
p-Value	0.01	0.031223	0.03	0.46	0.01136	0.001	0.012	0.15