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PELLETIZED PLANT GROWTH-PROMOTING BACTERIA DERIVED FROM FERMENTED RICE WATER AND ITS INFLUENCE IN PAKCHOY GROWTH ENHANCEMENT

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Graphical abstract

Abstract

The usage of inorganic fertilizer has been shown to have a negative impact. However, most farmers tend to use chemical fertilizers due to its significant useful effects on plants. This study aims to determine the influence of pelletized plant growth-promoting microbial consortium (PGPR) derived from fermented rice water in Pakchoy plants *(Brassica rapa subsp.chinensis)* growth enhancement. PGPR are characterized by tests for nitrogen fixation, phosphate solubilization and potassium solubilization, and indole compound productions which resulted all positive. The most effective isolates are chosen and was encapsulated into pellets form using bentonite powder. It was then applied to Pakchoy plants for four weeks based on four different treatments: control (T0), 0.5 g NPK fertilizer (T1), 0.1 g PGPR (T2), 0.5 g PGPR (T3) and 1.0 g PGPR (T4). The result significantly improved roots length (in cm), number of leaves and dry weight (in g). In future, the sustainable friendly approach like this can be potentially applied to the other crops like chili, rice, pineapple and vegetables.

Keywords: Plant Growth-Promoting Rhizobacteria, *Pakchoy,* Pellet, Biofertilizer, Fermented rice water

Abstrak

Penggunaan baja kimia telah menunjukkan kesan negatif. Kebanyakan petani cenderung menggunakan baja kimia disebabkan oleh kesan yang ketara dan pantas terhadap tanaman. Kajian ini dilakukan dengan tujuan untuk membangunkan konsortium mikrob perangsang-pertumbuhan tumbuhan (PGPR) berbentuk pelet daripada air beras yang diperam dan membandingkan keberkesanannya dengan baja kimia terhadap peningkatan pertumbuhan tanaman Pakchoy (*Brassica rapa* subsp. chinensis). PGPR dicirikan dengan ujian pengikatan nitrogen, pelarutan fosfat dan kalium, serta pengeluaran sebatian indol yang menjukkan keputusan positif. Isolat yang paling berkesan dipilih dan dikapsul dalam bentuk pelet menggunakan serbuk tanah liat bentonit. Ia kemudian digunakan pada tanaman Pakchoi selama empat minggu berdasarkan empat rawatan berbeza: kawalan (T0), 0.5g baja NPK (T1), 0.1g PGPR (T2), 0.5g PGPR (T3)

dan 1.0g PGPR (T4). Hasilnya menunjukkan peningkatan yang ketara pada panjang akar (dalam cm), bilangan daun dan berat kering (dalam g). Pada masa akan datang, pendekatan mesra alam seperti ini berpotensi untuk digunakan pada tanaman lain.

Kata kunci: Mikrob Penggalak-Pertumbuhan Pokok, Pakchoi, *Pelet*, Bajabio, Air Beras Yang Diperam

1.0 INTRODUCTION

Biofertilizer refers to a living assemblage of beneficial microorganisms that enhance soil health and organic content, while also aiding in nutrient accessibility through their biological processes. The main component of the biofertilizer is PGPM, which can be categorized into three groups: Arbuscular Mycorrhizal Fungi (AMF), Plant Growth-Promoting Rhizospheric Bacteria (PGPR), and Nitrogen-Fixing Rhizobia [1]. This study's main concern is chemical fertilizer dependence in agriculture [2]. This can be attributed to the preference for chemical fertilizers over biofertilizers. Chemical fertilizers offer significant advantages, as they do not require direct decomposition and contain high levels of nutrients in mineral form. Additionally, the release of these nutrients is rapid [3]. Overuse of chemical fertilizers in agriculture to ensure global food security has resulted in numerous issues, including health problems and irreversible environmental pollution. Chemical substances are recognized for their lethal properties and have the potential to significantly impact our ecosystem over time [4].

Other names for Pakchoy plant include Chinese chard, Chinese white cabbage, Bai cai, and Bok choy. The scientific name is *Brassica rapa* subsp. chinensis. Pakchoy comes in a few different variants, the most common of which are Pakchoy which has a green stem, and Bukchoy that has a white stem. Pakchoy does well on soil that is moist and well-drained, as well as full sun to light shade [5]. As leafy vegetables, Pakchoy needs good consistent water to develop good texture taste, and to avoid leaf burn. Additionally, Pakchoy an easily planted plant which it is only takes six to seven weeks to harvest [5], so that results can be seen as soon as possible. It can thus be used to enhance plant growth and to save the environment.

Nutrients, including macronutrients and micronutrients. are needed to ensure the plant growth because they perform several vital functions throughout a plant's life [6]. Nitrogen (N) is an essential component of the chlorophyll molecule and plays a crucial function in facilitating the absorption of solar energy and its subsequent conversion into chemical energy during the process of photosynthesis in plants. One of the observable symptoms associated with nitrogen deficit in plants is the alteration in leaf coloration from green to yellowish. The inclusion of phosphorus (P) within the soil matrix surrounding a plant has the capacity to cause a positive response in root development, thus facilitating the generation of elongated and robust stems. The presence of insufficient phosphorus (P) in plants can be discerned through many indications, such as disturbed plant development. Finally, it is important to highlight that potassium (K) is a vital nutrient for plants due to its significant contribution to enhancing plants productions and promoting floral parts. Nevertheless, it is commonly recognized that the utilization of chemical fertilizers has substantial harmful effects on the environment and health. As a substitute for the use of chemical fertilizers, the researchers seek to encourage the growth of beneficial microbes in the soil. The project was undertaken with the aim of assuring the enduring viability of the land.

Bacillus sp., Pseudomonas sp., and Rhizobium sp. are just a few examples of the beneficial microbes that function to promote plants growth under adverse situations [6]. Beneficial microbes have been used in this study due to their cost-effective and environmentally friendly nature. Microorganisms can be observed thriving either independently or near plants across diverse ecological settings. The term of microbial consortium is defined as the mixture of the beneficial microbes neither by singular nor combined colonies in one agar. A traditional method, rice water also contributes as the organic fertilizers for plants growth, because rice water is rich with essential nutrients. Previous research has revealed that rice water contains an average 7% protein, 35% crude fiber, 25% calcium, 47.5% phosphorus, 11% zinc, 42.5% potassium, and several other minerals [7]. These minerals also promote the growth of good bacteria in fermented rice water. Due to that, this research aims to determine the influence of pelletized plant growth-promoting microbial consortium (PGPR) derived from fermented rice water in Pakchoy plants *(Brassica rapa subsp.chinensis)* growth enhancement

2.0 METHODOLOGY

2.1 Bacterial Isolation

Fermented rice water was prepared for about 2 weeks before microbes isolation using adopted method by [8]. Then, the bacteria from fermented rice water were isolated on a nutrient agar (NA) (Merck, Germany) medium plate. This experiment used the spread plate method to collect bacteria. The bacteria culture was uniformly spread over an agar plate, resulting in isolated colonies distributed evenly across the plate [9]. Colonies started to be seen after a day of incubation, and the single colonized were observed and marked. The marked single colonies were then sub-cultured to other NA plate using the streak plate methods.

2.2 Characterization of Plant-Growth Promoting Rhizobacteria (PGPR)

Single pure colony bacteria went through several characteristic testing cycles to choose the best colony for use in biofertilizers.

2.2.1 Nitrogen Fixation Test

Each of the single colony bacteria chosen were re-isolated on the nitrogen-free malate medium for the nitrogen tests. Nitrogen-free malate plate contained of one L distilled water, five g of malate powder, 0.5 g K_2HPO_4 powder, 0.1 g NaCl powder: 0.2 g $MgSO_4 \cdot 7H_2O$ solution; 0.02 ml $CaCl_2 \cdot 2H_2O$ solution; two ml micronutrient solution, two mL bromothymol blue solution, four ml Fe-EDTA solution; 4.5 g KOH powder and, 15 g agar powder. All the chemicals were from Merck, Germany. The mixed solutions were autoclaved at 151 ^{0}C.

2.2.2 Phosphate-solubilizing Test

Rice fermented water isolates were inoculated into the NBRIP's Phosphate growing medium. The NBRIP plate was prepared by combined one L of distilled water with ten g of glucose powder, one g of $(NH_4)2SO_4$ powder, 0.2 g Merck, Germany KCl powder, five g Merck, Germany $Ca_3(PO_4)_2$, five mL $MgCl_2 \cdot 6H_2O$ solution, 0.25 ml $MgSO_4 \cdot 7H_2O$ solution, and 15 g Merck, Germany agar powder. The pH of the media was adjusted to seven using NaOH solutions prior to autoclaving. The bacteria were subsequently incubated at a temperature of 30°C for a duration of 48 hours.

2.2.3 Potassium Solubilization

PGPR of rice water isolated was grown in NA medium for a day before inoculated into a modified Aleksandrov medium to test for potassium solubilization ability. The modified Aleksandrov medium was prepared used one liter of distilled water mixed with 3.5 g glucose powder, 0.5 mL $MgSO_4 \cdot 7H_2O$ solution, 0.0005 g $FeCl_3$, 0.1 g $CaCO_3$ powder, one g insoluble potassium, two g $Ca_3(PO_4)_2$ powder and 15 g agar [10]. All chemicals were obtained from Merck, Germany.

2.2.4 Indole Compound Production

Indole compounds production was tested for four selected isolates using the colorimetric method [11]. The Salkowski reagent was prepared by combining one ml $FeCl_3$ solution with 50 ml distilled water and 30 ml H_2SO_4. The rice water isolated derived from PGP sources was solubilized in 100 ml nutritional broth (NB) and cultivated for 24 hours at a temperature of 28 °C. Subsequently, a fresh 100 ml nutrient broth (NB) was utilized to introduce one ml aliquot of bacterial culture, along with an additional five ml of L-tryptophan. The mixture then incubated at a temperature of 28 °C for 24 hours. A control was maintained using an untouched broth culture. 1.5 ml of bacterial cultured was thereafter put into a microfuge tube and subjected to centrifugation with a force of 7000 times the acceleration due to gravity (g) for a duration of seven minutes. The cuvette was gradually filled with 1 mL of supernatant. The detection of indole compounds was indicated by the appearance of a pink hue after the introduction of four ml of Salkowski reagent, which was allowed to incubate for a duration of 15 to 30 minutes. The OD530 measurement was conducted with a UV/VIS spectrophotometer (Dynamica Halo, UV VIS 10, Australia).

2.3 Preparation of PGPR Pellet

13 g nutrient broth (NB) powder were added to one L of distilled water. Then, the mixture was mixed completely until it became yellowish. solution was transferred to one L conical flask and autoclaved at 121^{0}C for two and half hours. NB will not be as solid as the other medium, because it does not contain agar. After autoclaving the NB solution, it was left to cool under laminar airflow. Then, all single colonies were placed in the NB solution and incubated for a day to allow it to grow. After a day, the incubated isolated NB was taken for exchanged its form into pellets using bentonite. These pellets were made manually using 625 g bentonite powder. Then, one L nutrient broth (NB) was slowly poured and mixed in the bentonite powder until completely mixed. This was then transferred to a whipping pipe and cut into segments two mm to five mm long and left to dry for one to three days in laminar airflow to completely dry.

2.4 Experimental Design and Pakchoy Cultivation

This experiment was designed by using the Completely Randomized Design (CRD). The treatments were: control (T0), 0.5g NPK fertilizer (T1), 0.1g PGPR (T2),0.5 g PGPR (T3) and 1.0 g PGPR (T4). For each treatment, three replicates were used to increase

the reliability of the findings. The Pakchoy cultivation was done in UITM greenhouse 1, Jasin, Melaka. Firstly, the raw seedling of Pakchoy were germinated and transplanted into polybag. All the plants were watered two times a day. After two weeks, the treatments were applied using pocket application to prevent the pellets from drying due to hot sunny days at that time. The treatments were observed for 28 days after sowing (DAS). The agronomic parameters in this research include plant height (in cm), root length (in cm), number of leaves, fresh weight (in g), dry weight (in g) and chlorophyll content using SPAD-502 meter (Konica Minolta, Japan).

2.5 Statistical Data Analysis

The experiment was designed as a completely randomized design with three replications. The SPSS Statistical software was used for data analysis using one-way ANOVA and means of treatments were compared using Tukey's test.

3.0 RESULTS AND DISCUSSION

3.1 Bacterial Isolation

Pure bacterial cultures were obtained through isolation [12]. Based on Figure 1, after a day of incubation in an incubator, the colonized culture was observed, and different morphology of colony were marked as different isolates. The single colony in this experiment are indicated as clear colour, which the unmarked white spotted coloured colonies were considered mixed colonies. The streak plate method is a commonly used for microbiological laboratory approach that involves the isolation of pure cultures and the acquisition of well-isolated colonies of bacteria from a mixed population. This technique is usually applied to obtain sterile cultures of bacteria but can also be used to isolate yeast. Aseptic procedures are commonly used in microbiology for bacterial isolation and propagation [13].

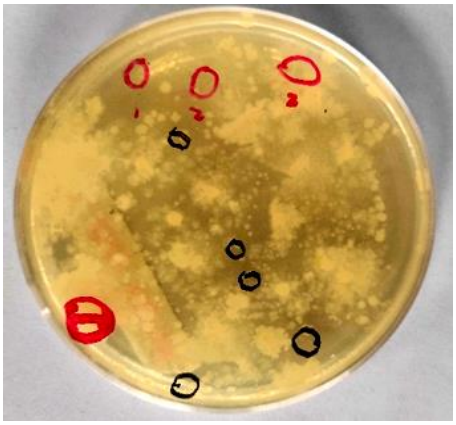

Figure 1 Observation and marking of microbial isolates

3.2 Characterization of Plant Growth Promoting Rhizobacteria (PGPR)

3.2.1 Nitrogen Fixation

Figure 2 shows that PGP rice water's nitrogen fixation activity caused the plate's green colour to turn blue. This indicates that the bacteria isolated contained of all the macronutrients needed by the plant, as identified by the characterization test of PGPR. The results from figure 2 show that all of the nitrogen fixation test are positive, which means all of the bacterial isolates have ability to fix nitrogen. Nitrogen fixation and phytohormone production by these bacteria are essential for plant growth [14]. Nitrogen-fixing bacteria possess the genetic information necessary to produce nitrogenase, an enzyme that facilitates the process of biological nitrogen fixation (BNF). Through BNF, these bacteria can absorb atmospheric nitrogen and convert it into inorganic nitrogen-containing compounds, including ammonia (NH_3). This metabolic activity of nitrogen-fixing bacteria contributes to the enhancement of plant development and productivity [15].

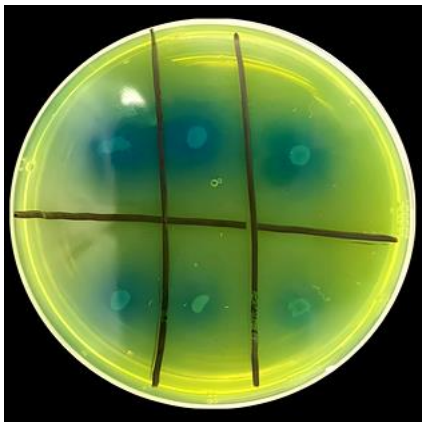

Figure 1 Result of Nitrogen fixation on Nitrogen-free solid malate medium

3.2.2 Phosphate Solubilization

After 24 hours of incubation, bacterial isolation on National Botanical Research Institute's Phosphate growth medium (NBRIP) start to form halozone which indicated as positive results. Three isolates show formation of halozone which are AFV1, AFV2 and AFV3. Phosphate-solubilizing bacteria (PSB) shown could improve plant growth possibilities, reduce the impact of overused fertilizers in farmland, and protect the soil stated by Wang *et al.* [16].

3.2.3 Potassium Solubilization

Isolated microbes showed a positive reaction on for potassium solubilization on Alexandrov agar. The

clear ringspot in Figure 3 demonstrates the solubilization of potassium in agar. The agar were classified into six grids, consisting of all the varieties, named AFV1, AFV2, AFV3, AFV4, AFV5 and AFV6. The function of potassium is to increased availability of potassium from soil potassium-solubilizing bacteria and may encourage plant invasion in some invaded habitats [17].

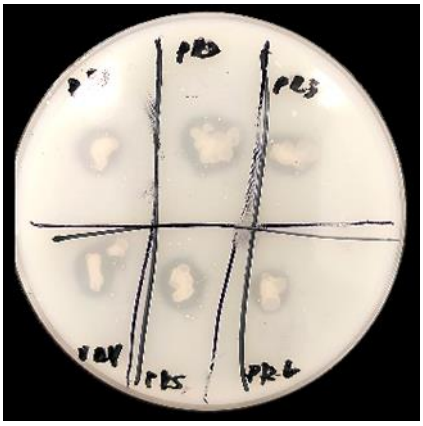

Figure 3 Potassium solubilization effect on modified aleksandrov medium. AFV stands for Afiq Variety representing the isolates' name

3.2.4 Indole Compound Production

Table 1 shows the indole compound production based on OD reading for AFV 1, AFV 2 and AFV 3. The results are significantly different compared to control. Figure 4 shows that the pink color shade from the indole compound of the isolates that had different OD reading indicated different amount of indole compound. A darker shade of pink color indicates a higher amount of indole compound.

Table 1 Results of OD reading for indole compound production. AFV stands for Afiq Variety representing the isolates' name

Isolate	OD530 Reading
Control	0.099b
AFV 1	0.571a
AFV 2	0.596a
AFV 3	0.579a

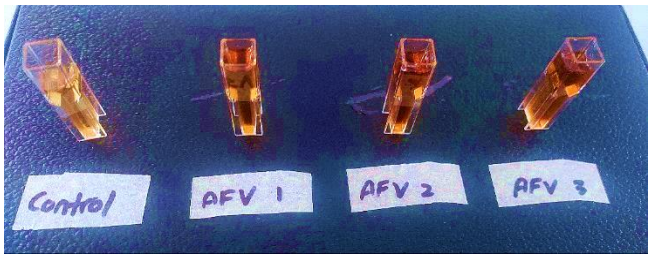

Figure 4 Color comparison of pink shade due to the production of indole compound

3.3 Pelletized Plant Growth-Promoting Rhizobacteria

Figure 5 shows the successfully pelletized PGPR. Some studies have shown that the encapsulation of bentonite powder into hydrogel network enhances its structure and improves environmental applications [18]. One L of nutrient broth was then slowly poured into the bentonite powder and slowly stirred until completely mixed (Figure 6). Nutrient broth is a liquid medium for growing several organisms from laboratory specimens and other materials [19]. Then, the mixture was transferred into a customized pipe and the pipe was filled with bentonite. Then, the mixture which popped out from the piping pipe tip was cut to about 2mm until 5mm long, laid on aluminum foil and left to dry for a day in laminar air flow.

Figure 5 Pellet form of PGPR biofertilizer

3.4 Growth Performance of Pakchoy Cultivation

3.4.1 Plant Height

Based on Figure 6, in week 0 and week 1 there were no significance differences between all the treatments. All the treatments increased plant height uniformly. However, in week 2 and week 3, the treatments started to show significance differences. 1.0 g PGPR fertilizer (T4) showed the highest plant growth at 4.83 cm, Followed by the treatment 0.5 g NPK fertilizer (T1), which had a mean plant height of 4.80 cm. However, control treatments (T0) showed a decrease in plant height in week 3 with a mean plant height of 1.17 cm, because most of the control treatment plants died. Based on these results, biofertilizer incorporating PGPR could be used to enhance plant growth, and could be exchanged for the chemical fertilizer used today. Additionally, some research has shown that beneficial microbes would have a slightly good impact on plant growth. *Bacillus* in biological fertilizer helps beneficial microbes recover after 1,3-D fumigation, reduces pathogenic bacteria, and boosts tomato yield [20]. Which its proven that PGPR also functioned to added nutrients in plants to enhance the plants growth.

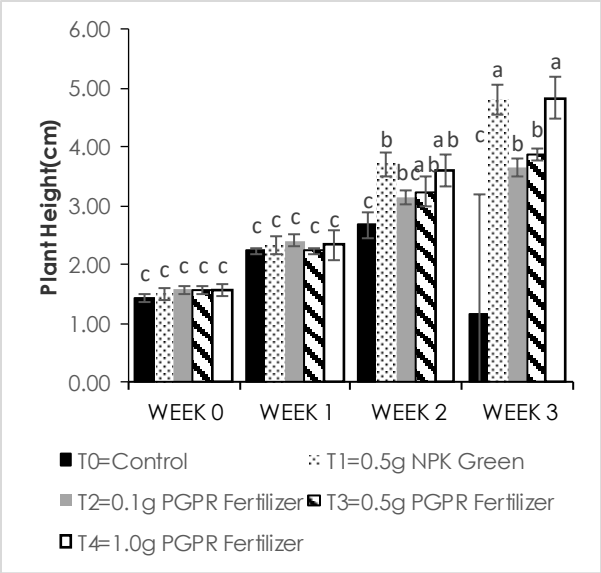

Figure 6 Mean plant height affected by the treatments for four weeks interval at P<0.05

3.4.2 Root Length

The root length means across treatments are shown in Figure 7. Treatment T1 (0.5 g NPK Green) exhibited the highest mean root length of 5.07 cm, followed by T4 (1.0 g PGPR fertilizer) at 4.83 cm, T3 (0.5 g PGPR Fertilizer) at 3.10 cm, T2 (0.1 g PGPR Fertilizer) at 1.67 cm, and the control T0 with the lowest at 0.1 cm. One-way ANOVA revealed a statistically significant difference in root lengths among the treatments (p = 0.000). Post-hoc tests indicated that the PGPR fertilizer treatments (T2, T3, T4) differed significantly from the control (T0). While T2 showed less significance compared to T0, T1 and T4 exhibited highly significant differences from the control. Similar studies have shown that potassium humate from bacteria improves root length, soil fertility, plant nutrient metabolism, and plant resilience [21].

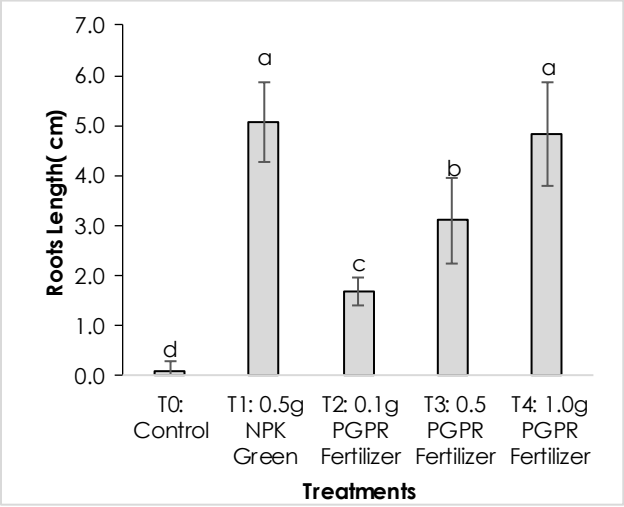

Figure 7 Mean value for different treatments on root length of Pakchoy Plant at P<0.05

3.4.3 Number of Leaves

The mean numbers of leaves across treatments are shown in Figure 8. Treatment T4 (1.0 g PGPR Fertilizer) exhibited the highest mean number of leaves of four leaves, followed by T1 (0.5 g NPK Green) at two leaves, T3 (0.5 g PGPR Fertilizer) and T2 (0.1 g PGPR Fertilizer) at one leaf, and the control T0 with the lowest mean of 0 leaves. One-way ANOVA revealed a statistically significant difference in the number of leaves among the treatments (p = 0.000). Post-hoc tests indicated that the PGPR fertilizer treatments (T4 and T1) differed significantly from the control (T0), while T2 and T3 showed less significance compared to T0, T1 and T4 and exhibited highly significant differences from the control. Similar study shows by Lin *et al*. [22]. The author reported that organic silicon fertilizers function to enhance number of leaves, soil profile, and encounter pathogenic bacteria, which resulted increases crop yield and quality [23].

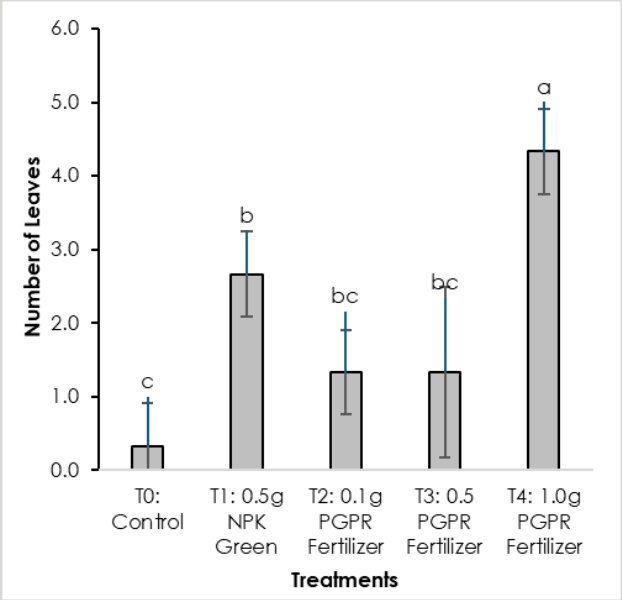

Figure 8 Mean value for different treatments on number of leaves of Pakchoy Plant at P<0.05

3.4.4 Dry Weight

The dry weight means across treatments are shown in Figure 9. Treatment T4 (1.0 g PGPR Fertilizer) exhibited the highest mean dry weight of 0.020 g, followed by T1 (0.5 g NPK Green) at 0.004, T3 (0.5 g PGPR Fertilizer) at 0.004 g, T2 (0.1 g PGPR Fertilizer) at 0.002 g, and the control T0 had the lowest mean of 0 g. One-way ANOVA revealed a statistically significant difference in dry weight among the treatments (p = 0.000). Post-hoc tests indicated that the PGPR fertilizer treatments (T4) differed significantly from the control (T0). Conversely, (T1,T2,T3) showed less significance compared to T4 while exhibiting highly significant differences from the control. In a similar study, maize treated with PGPR showed higher weight compared to NPK treated maize [24].

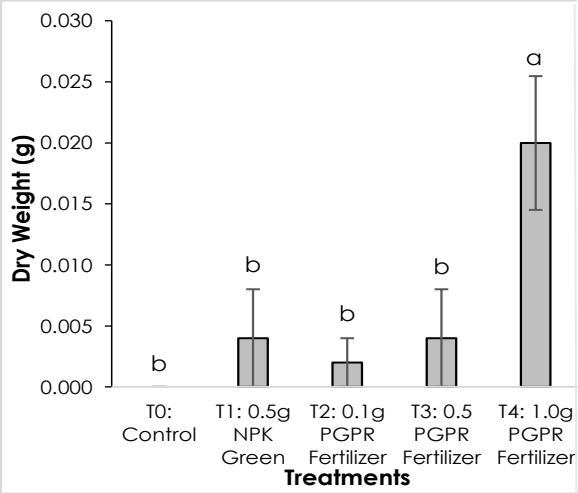

Figure 9 Mean value for different treatments on dry weight of Pakchoy Plant at P<0.05

3.4.5 Fresh Weight

The fresh weight means across treatments are shown in Figure 10. Treatment T4 (1.0 g PGPR Fertilizer) exhibited the highest mean fresh weight of 0.20 g, followed by T3 (0.5 g PGPR Fertilizer) at 0.16 g, T3 (0.5 g NPK Green) at 0.15 g, T2 (0.1 g PGPR Fertilizer) at 0.08 g, and the control T0 had the lowest mean of 0.03 g. One-way ANOVA revealed a statistically significant difference in fresh weight among the treatments (p = 0.000). Post-hoc tests indicated that the PGPR fertilizer treatments (T4,T1,T3) differed significantly from the control (T0). While (T2) showed less significance compared to T4 exhibited highly significant differences from the control.

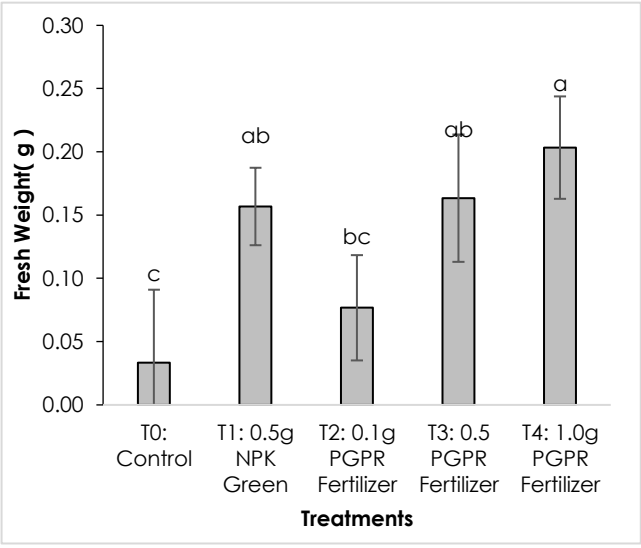

Figure 10 Mean value for different treatments on fresh weight of Pakchoy Plant at P<0.05

3.4.6 Chlorophyll content based on SPAD Value

Based on Figure 11, the data of SPAD value in Figure 26 showed less significant data. But the treatments 1.0 g PGPR fertilizer (T4) showed the highest chlorophyll content which is 17.3. Similar studies were found in [23] However, [24-26] show contradict findings, in which the chlorophyll content increased along with other growth parameters in dill and maize due to ability of PGPR to increase the availability of nutrients like nitrogen to increase chlorophyll synthesis.

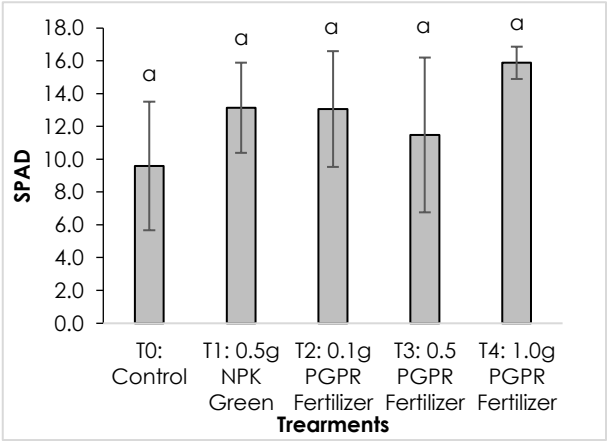

Figure 11 Mean value for different treatments on SPAD value of Pakchoy Plant at P<0.05

4.0 CONCLUSION

In conclusion, plant growth-promoting microbial consortium of fermented rice water isolates has been successfully isolated and encapsulated in pellet form. The bacterial isolate has been shown to positively affect the results of four difference characterizations, including nitrogen fixation, phosphate solubilization and indole compound production. The isolated bacteria were successfully encapsulated in pellet form using bentonite powder, also known as hydrated clay mineral with montmorillonite. The pellets were then applied to the Pakchoy plant to experimentally measure their effects on growth. Plant growth promoted by the microbial consortium was significant as compared to chemical fertilizer in terms of roots length, number of leaves and dry weight. All three growth parameters show highly significant differences between PGPR pellet fertilizer and chemical fertilizer. The findings of this study will help to confirm that natural and ecofriendly materials might function better than chemical fertilizers. This study also shows that PGPR can be pelletized to keep it safe when we apply it to the soil. This technique will help to protect PGPR from competition with other microbes. Thus, this study shows great potential for a new technique to implement biofertilizer as one of sustainable approaches.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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