International Journal of Life Science and Agriculture Research

ISSN (Print): 2833-2091, ISSN (Online): 2833-2105

Volume 02 Issue 12 December 2023

DOI: https://doi.org/10.55677/ijlsar/V02I12Y2023-05

Page No : 505-513

Increasing Forage Yields through Application of Plant Growth Promoting Rhizobacteria (PGPR)

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ABSTRACT: In the last decade, the use of microbial activity has become increasingly widespread with the knowledge of the negative impact of providing inorganic fertilizer inputs on the cultivation of feed crops. A study was carried out to examine the effect of PGPR application from various host plants on increasing the yield of forage *Asystasia gangetica* (L.) *subsp. Micrantha*. The research was a pot experiment designed with a completely randomized design (CRD). The research began with identifying PGPR causes from various host plants, then applied to *A. gangetica*. The variables observed in this study were: the presence and population of PGPR on PGPR leader of *Bambusa vulgaris* Schrad, *Mimosa pudica*, *Paspalum notatum* and *Eleusin indica* grass plants, growth and yield of *A. gangetica* forage plants. The results showed that the highest number of non-symbiotic bacteria was found in PGPR from host plant roots *E. indica* as much as $1.1x10^6$ cfu/ml, while the highest number of symbiotic bacteria was found in *M. pudica* host plant roots. The most TPC was found in the roots of the host *E. indica* as much as $6.4x10^5$ cfu/ml, while the highest P bacteria were found in the roots of the host plants *E. indica* and *M. pudica* each with $3.4x10^5$ cfu/ml. It was concluded that the highest forage yield of *A. gangetica* was obtained from the application of PGPR seeds *E. indica* and *M. pudica* respectively at 21.52 and 21.06 g plant^{-1}.

KEYWORDS: *A. gangetica, yield, TPC, PGPR*

Published Online: 25 December 2023

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INTRODUCTION

The development of feed crop science and technology to increase the availability of sustainable forage is increasingly becoming an urgent need. The need for the development of science and technology is based on various efforts to increase the population and productivity of ruminants that really need the availability of forage for livestock with adequate quantity and quality. The availability of tropical forage can be sought through the establishment of new superior varieties and increasing the development of fodder crop cultivation technology.

Feed crops cultivation technology for sustainable availability of forage for livestock is increasingly stretched with the development of research results in the field of feed plant science and technology. Increased greenhouse gas (GHG) emissions that trigger climate change require all parties to work together to implement mitigation and adaptation to climate change. Of course, the agricultural sector, including the development of tropical feed crops (TFC), must find various alternatives to reduce GHG emissions from the agricultural sector. One of the technologies that can be developed is the utilization of Rhizobacteria services (Suarna *et al*. 2019).

Plant Growth Promoting Rhizobacteria (PGPR) are a group of beneficial soil microorganisms. PGPR is a group of bacteria that live and develop well in soils rich in organic matter (Compant *et al.*, 2005). These bacteria actively colonize the root zone of plants and have three main roles for plants, namely as biofertilizers (PGPR can accelerate the process of plant growth through accelerated nutrient absorption), as biostimulants (PGPR can spur plant growth through the production of phytohormones) and as bioprotectants (PGPR protects plants from pathogens). PGPR watering treatment functions as a supplementary treatment to increase the bacteria in the rhizosphere area and the bacterial population in the rhizosphere area can help absorb nutrients that are useful for plants (Baihaqi *et al*., 2018).

Conventional TFC development with inorganic fertilizer inputs should be reduced and replaced with environmentally friendly inputs. Based on the above thoughts, the utilization of Plant Growth Promoting Rhizobia (PGPR) is one of the research fields of TFC development in order to increase the availability of sustainable and environmentally friendly TFC. The problems that can be

formulated are 1) how is the presence of PGPR bacteria by analyzing its population (population of N-fixing bacteria (synbiotic and non-synbiotic) and phosphate bacteria from the rhizosphere of each plant host? 2) how is the growth and yield of *A. gangetica* plants applied with PGPR? The research objective was to improve the growth and yield of *A. gangetica* (L.) *subsp. Micrantha* through the utilization of PGPR services.

RESEARCH METHOD

The research began with the preparation of PGPR biosolids, with ingredients made from *Bambusa vulgaris* roots, *Mimosa pudica*, *Paspalum notatum*, and *Eleusin indica* obtained on land around Denpasar City and Gianyar. Roots as much as 4 ounces, soaked in 750 ml of water, then placed in a bottle (with lid) and fermented. The research was conducted in the Greenhouse of Sesetan Research Station, Faculty of Animal Husbandry, Udayana University and lasted for 12 weeks (3 months). The study used a univariate randomized complete block design (CRD) experimental design and was repeated 5 times.

Every day the solution is shaken manually by calculating the shaking time and speed. The purpose of shaking is to increase the microbial population, because microbes multiply by dividing themselves. Bacteria formed in the bottle are characterized by a sour-smelling solution, there is foam above the dough, when shaken out bubbles - air bubbles from the dough. To increase the number of bacteria, a growth medium is made for this PGPR culture. PGPR bacteria are soil bacteria whose life span is not long. Therefore, it is necessary to restore its population every time you spread seeds, by making the growth media so that it stays alive and the bacterial population increases (Ferdiansyah, 2020).

The resulting PGPR powder was then tested including total microbes, total phosphate solubilizing bacteria, total symbiotic N bacteria, total non-symbiotic bacteria, pH and total dissolved solids. Laboratory analysis data obtained were then analysed using descriptive analysis.

Preparation of growth media using ingredients: clean water (without chlorine) 20 liters, shrimp paste without preservatives 100 grams, fine bran 500 grams or rice washing water 1 liter, brown sugar 200 grams, slaked lime (for eating betel nut) 1 teaspoon.
How to make:

1. Heat the water to boiling
2. Combine all ingredients, stir until evenly distributed, let it keep boiling (30 minutes)
3. Remove from furnace and let cool
4. Filter the material so that a ready-to-use liquid is obtained

Preparation of bacterial culture by: taking *Bambusa vulgaris* roots, *Eleusine indica* roots, *Mimosa pudica* roots, *Paspalum notatum* grass roots with a little soil attached, as much as 250 gr. Soak in 2 - 5 liters of cooking water (which has cooled and left for 2 - 5 days) Soaking water is ready to be used for culture.

Mix the two ingredients above in a clean container. The container is closed and a curved pipe/hose is attached to allow the gas to escape. The other end of the hose is placed in a bucket of water so that outside air cannot enter the mixing container. Place in the shade (not exposed to direct sunlight). Stir every day for about 5 - 10 minutes or use a aquarium aerator. Leave for 5 - 7 days, until the aroma changes to tape-like and no foul odor. The mixture is ready to use with a dilution of 50 - 100 times depending on the need. Soak the seeds in the PGPR solution with a mixture of 10 ml of culture per liter of water for 10 minutes to 8 hours depending on the seed Then dry in a shady place before planting.

Mix the two ingredients above in a clean container. The container is closed and a curved pipe/hose is attached to allow the gas to escape. The other end of the hose is placed in a bucket of water so that outside air cannot enter the mixing container. Place in the shade (not exposed to direct sunlight). Stir every day for about 5 - 10 minutes or use a aquarium aerator. Leave for 5 - 7 days, until the aroma changes to tape-like and no foul odor. The mixture is ready to use with a dilution of 50 - 100 times depending on the need. Soak the seeds in the PGPR solution with a mixture of 10 ml of culture per liter of water for 10 minutes to 8 hours depending on the seed then air-dry in a shady place before planting.

Asystasia gangetica (L.) *subsp. Micrantha* seeds used were obtained from the research station of the Faculty of Animal Husbandry Bukit Jimbaran. The soil to be used was obtained from 3 places, namely the Farm of the Faculty of Animal Husbandry, Udayana University, Pengotan Village, Bangli Regency, Faculty of Animal Husbandry, Udayana University. The soil was air dried first, then sieved with a sieve measuring 2 x 2 mm. Weighed as much as 8 kg and put into pots. The soil used was analyzed at the Soil Science Laboratory, Faculty of Agriculture, Udayana University. Observations were made on the variables: plant height, number of branches, number of leaves, leaf area, and forage yield. The data obtained will be analyzed by variance analysis at a real level of 5%. If there are differences between the mean values of the treatments, the test will be continued with the BNT test at the 5% level (Steel and Torrie, 1989).

Laboratory Analysis

The materials used for analysis were host plant roots which are PGPR breeding materials, Buffered Peptone Water, Plate Count Agar (Oxoid), Asbhy Manitol Agar (Oxoid), Yeast Extrac Manitol Agar (YEMA), Pikovskaya, 70% alcohol, acetone, sodium sulphate, hexane, ethanol, iodine, sodium, amylum, folin-ciocalteu, gallic acid solution and $NaSO_4$.

The equipment used were closed bottles/jars, petri dishes (Iwaki CTE33), beakers (Iwaki CTE33), volumetric flasks (Iwaki CTE33), measuring cups (Iwaki CTE33), micropipettes (Socorex Swiss), pH-meter (Beckman), Vortex (Barnstead Thermolyne Type 37600 mixer), digital TDS and glassware, filter paper, funnel, glass plate, object glass, test tube, aluminium foil, Durham tube, autoclave, laminar air flow, incubator, test tube, bent glass rod, (spread), Bunsen, drying oven (Shimidzu), spectrophotometry (Biochrom), and spectrophotometry (thermo scientific), analytical balance (Shimadzu).

Tools for microbial analysis such as petri dishes before use were sterilized at 160 $^oC \pm 2$ hours in the oven. Test tubes, plastic tips sterilized with otoklap at 121 $^oC \pm 30$ minutes. The microbes analyzed were bacterial groups, because this was the initial stage before identifying microbes in PGPR biosolids. For this reason, this analysis was carried out to determine the number of microbes contained in PGPR fermented bamboo roots, mimosa pudica, paspalum and *padang belulang*.

a. Dilution

Buffered Peptone Water was weighed as much as 1 g and dissolved into 1000 ml of distilled water, then stirred until completely dissolved. Then put into a schoot duran tube as much as 180 ml and then closed. After that, it was sterilized with an autoclave at 121°C for 15 minutes. After the BPW (Buffered Peptone Water) solution is made then do the dilution process of the liquid sample (10^{-1}). Then after diluted, diluted by taking 1 ml of the dilution sample results with a volumetric pipette and poured into a test tube containing 9 ml of distilled water (dilution 10^{-2}) do the same treatment until (dilution 10^{-3}, 10^{-4}, etc.).

The purpose of multilevel dilution is to minimize or reduce the number of microbes suspended in the liquid. Determination of the amount or number of dilution levels depends on the estimated number of microbes in the sample. A ratio of 1: 9 for the sample and the first and subsequent dilutions, so that the next dilution contains 1/10 microorganism cells from the previous dilution.

b. Measurement of pH and Temperature of Cultures

Measurements of pH and temperature of the culture were carried out before analysing the variables in this study.

c. Bacterial Population (TPC)

Each 0.1 ml of solution for dilutions 10^{-4} to 10^{-9} was poured onto PCA media using an Eppendorf of sterile tips. Next, the solution was spread with a spreader that had been dipped in alcohol and heated. Then incubated at room temperature for 48 hours. Colonies were counted only those that amounted to 30 - 300 colonies.

Preparation of PCA media by dissolving 15 g agar, 1 g dextrose, 5 tripton, 1.5 g yeast into 1000 ml of distilled water. The solution was heated while stirring with a magnetic stirrer so that it was homogeneous. Next, the solution was sterilized in an autoclave at 121^{o}C for 15 minutes. After slightly cooled poured into sterile petri dishes ± 15 as much as 20 ml and cooled. After the media has solidified, the Petri dish is closed and placed in an inverted position.

d. Population of Symbiotic Nitrogen Fixing Bacteria

Population analysis of N-fixing bacteria followed the Standard Plate Count method (Swansonn, 1982) with the scatter method by pipetting 0.1 ml of inoculant from 10^{-3}-10^{-8} dilution. Then it was put into a Petri dish that already contained solid YEMA media (for symbiotic) and levelled with a bent rod. Then incubated in an incubator at $37^0C \pm 48$ hours in an inverted position. Bacterial growth is characterized by white to pink coloration.

e. Population of Non Symbiotic Nitrogen Fixing Bacteria

Bacteria from the PGPR group are the genus *Rhizobium, Azotobacter, Azospirillum* and phosphate solubilizing bacteria such as the genus *Bacillus, Pseudomonas, Arthrobacter, Bacterium,* and *Mycobacterium* (Biswas et al., 2000). *Rhizobium, Azotobacter, Azospirillum* and phosphate solubilizing bacteria have important functions such as decomposition of organic matter such as proteins, carbohydrates, microorganisms, mineralization of organic compounds, nutrient fixation, nutrient solubilization, nitrification and denitrification. In addition, PGPR also contains *Bacillus* spp. and *Pseudomonas fluorescens* bacteria that are able to synthesize growth hormones IAA, Cytokinin, and Gibberellin which are plant growth hormones, so that they can increase plant growth.

Population analysis of non-symbiotic N-fixing bacteria followed the Standard Plate Count method with the scatter method by pipetting 0.1 ml of inoculant from a dilution of 10^{-3}-10^{-8}. Then it was put into a Petri dish that already contained solid mannitol agar and NFB (for non-symbiotic) media and leveled with a bent rod. Then incubated in an incubator at 37 $^0C \pm 48$ hours in an inverted position.

f. Pospatial Bacteria Population

Phosphate in the soil is a nutrient that plays an important role in the process of plant growth. The availability of phosphate is assisted by phosphate solubilizing bacteria that are commonly found in the rhizosphere (Marista *et al*., 2013). Phosphate-solubilizing bacteria are decomposer bacteria that play a role in soil fertilization because they are able to carry out phosphate dissolution mechanisms by excreting a number of low molecular weight organic acids. Bacteria utilize simple carbon compounds (exudates from plant roots and plant residues) (Ilham *et al*., 2014).

The population analysis of phosphate bacteria followed the Standard Plate Count method using the scatter method by pipetting 0.1 ml of inoculant from dilutions of 10^{-3}-10^{-8}. Then put into a petri dish that already contains solid Pikovskaya media and levelled with a bent rod. Furthermore, it was incubated in an inverted position at 37 ^{0}C ± 48 hours in an inverted position.

RESULTS AND DISCUSSION

PGPR is a group of bacteria that live around the rhizosphere and colonize with plant roots and support plant growth, development and immunity thanks to their ability to produce growth regulators (ZPT) and bind or fix free nitrogen from nature. The bacteria live around the roots, both on the surface of the roots and the soil that is still affected by plant root activity, by utilizing the exudates released by the plants concerned. However, these bacteria do not damage/disturb plant life, but interact mutually.

The fixated free nitrogen is then converted into ammonia and then channeled to plants. Sulfur cannot be directly absorbed by plants without going through a process of transformation/oxidation of bacteria Soil fertility is not only determined by the nutrient content in the soil, but also its availability.

The empirical phenomenon of the ability of the rhizosphere of fodder plants to support the growth of cultivated plants to increase their productivity is an important target in this study. The ability or carrying capacity of the rhizosphere is mainly examined from the carrying capacity of PGPR which is currently a strategic need in the development of fodder crop cultivation. The results of PGPR Microbial analysis (TPC, total phosphate solubilizing bacteria, total symbiotic and non-symbiotic N bacteria) of forage can be seen in Table 1.

Table1. PGPR microbes (TPC, non-symbiotic bacteria, symbiotic bacteria and phosphate bacteria) of host plants

Variable	PGPR Host Plants			
	Bambusa vulgaris	*Eleusin indica*	*Paspalum notatum*	*Mimosa pudica*
	..... cfu/ml			
TPC	$6,5x10^4$	$6,4x10^5$	$4,3x10^4$	$1,9x10^5$
Non Symbiotic Bacteria	$4,7x10^4$	$1,1x10^6$	$8,3x10^4$	$8,9x10^5$
Symbiotic Bacteria	$5,2x10^4$	$6,2x10^5$	$4,9x10^4$	$1,1x10^6$
Phosphate Bacteria	$2,0x10^4$	$3,4x10^5$	$2,8x10^4$	$3,4x10^5$

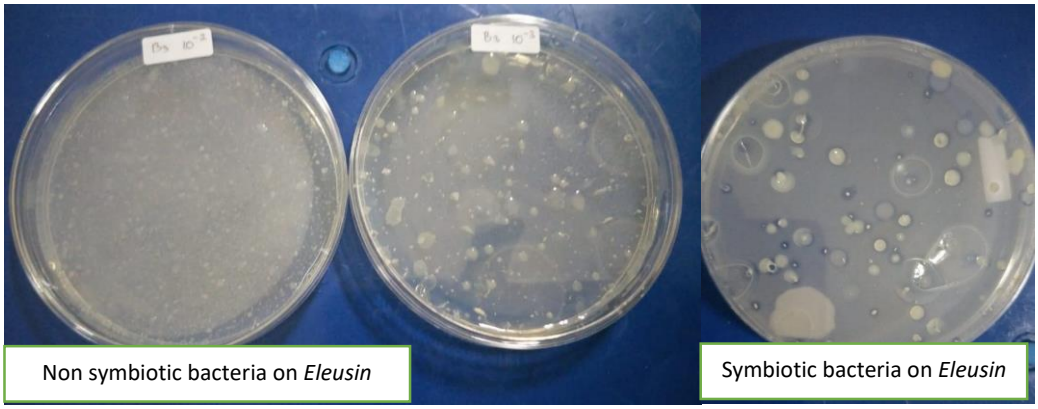

Figure 1. Bacteria from the roots of the *Eleusin indica* (Padang Belulang)

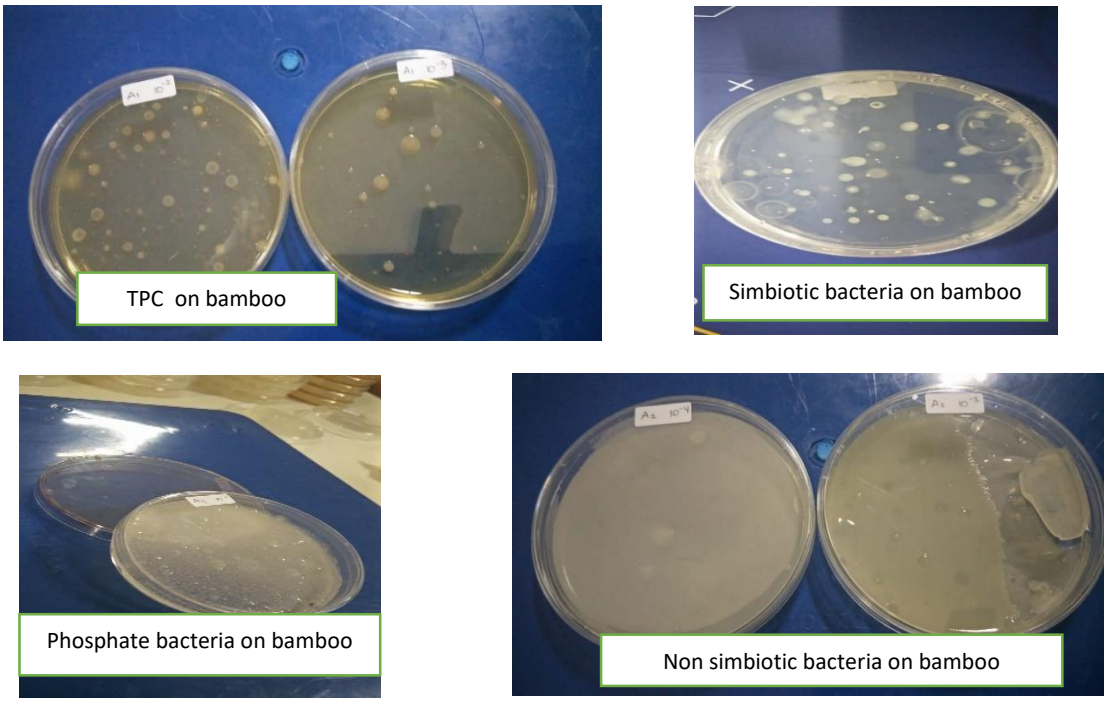

Figure 2. Bacteria from Bamboo plant roots

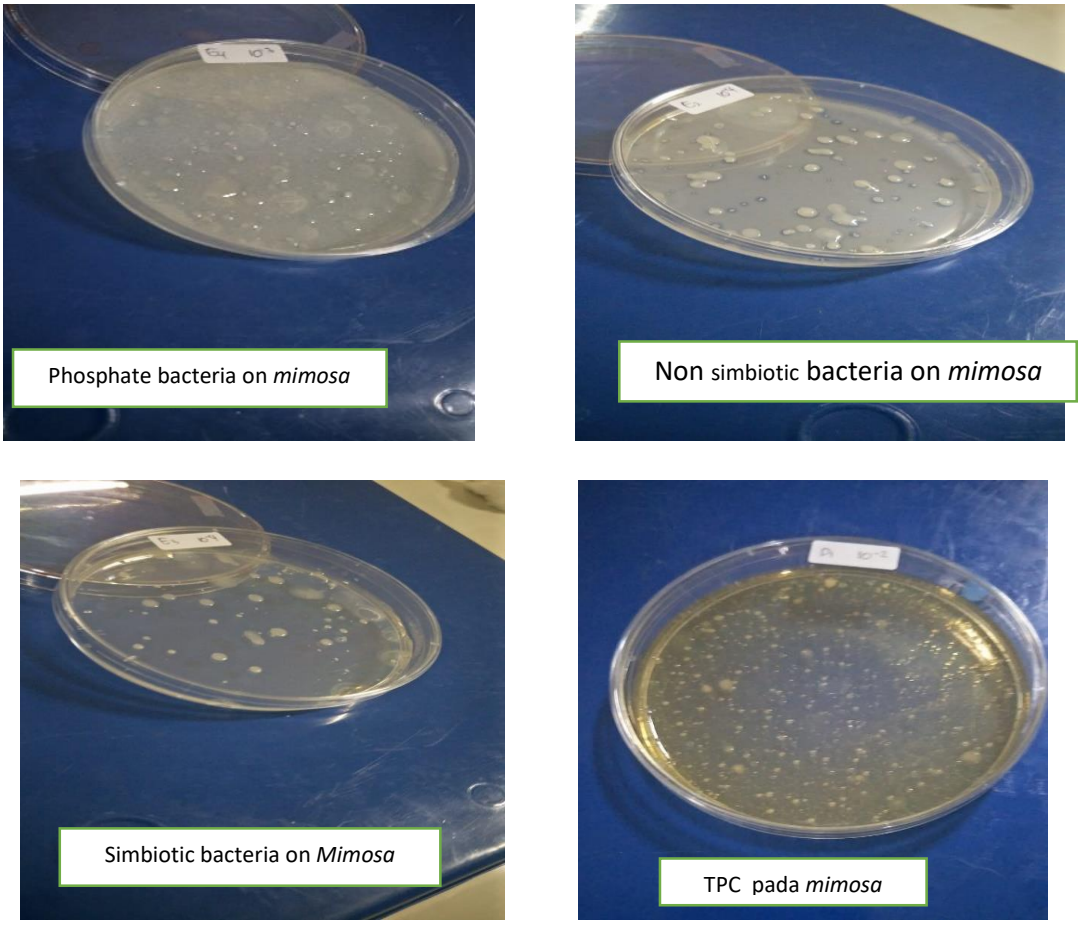

Figure 3. Bacteria from the roots of *Mimosa pudica* (Si Kejut/ Putri Malu)

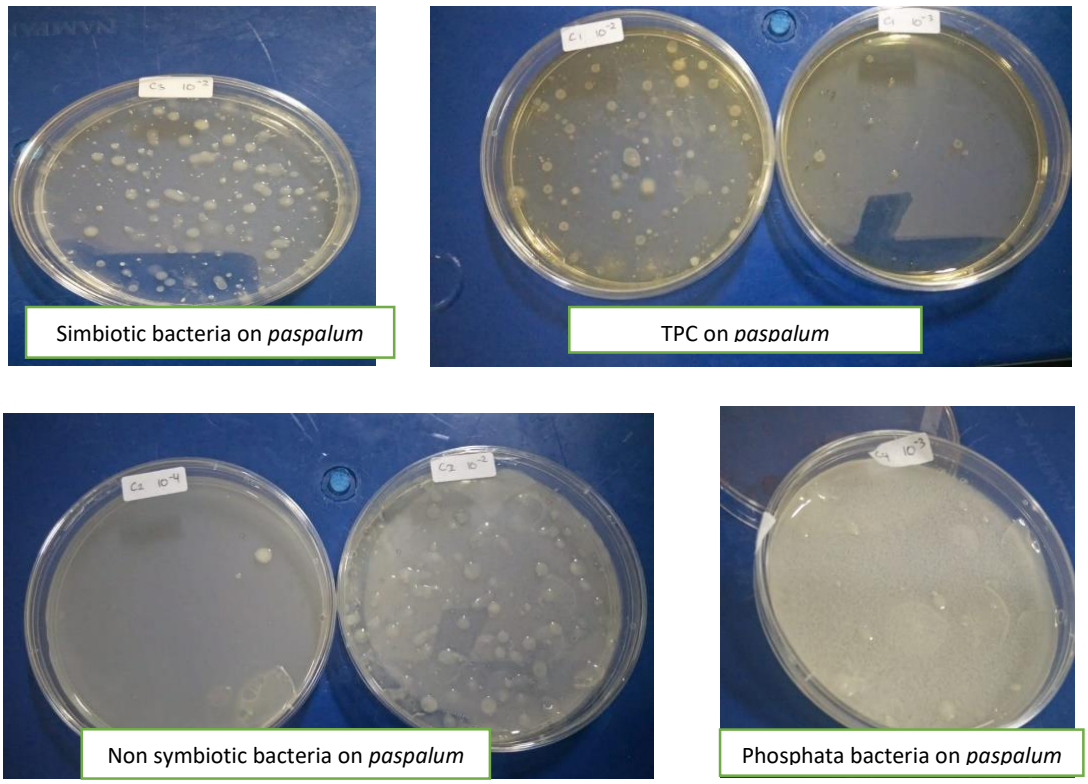

Figure 4. Bacteria from the roots of *Paspalum notatum* cv. Competidor

After obtaining PGPR and TPC of each microbe from various plant roots, preparations for the application of PGPR on *A. gangetica* plants were made. Preparation for the implementation of research in the greenhouse which begins with seeding in the try before being transferred to the experimental pot.

Figure 5. Transplanting of *A. gangetica*

Table 1 shows that PGPR culture from the roots of *E. indica* plants has the highest TPC and non-symbiotic bacteria compared to PGPR culture from other host plants. Phosphate-solubilizing bacteria in the PGPR culture of *E. indica* and *Mimosa pudica* were the same, while symbiotic bacteria were found to be most abundant in the culture of *Mimosa pudica* as much as $1.1x10^6$ cfu/ml *Mimosa pudica* are legumes that already have a good symbiotic cooperation mechanism with nitrogen-fixing bacteria. This condition is possible due to differences in the pattern and distribution of plant roots, although *B. vulgaris, E. indica, and P. notatum* are all three graminae families, the development of the root system seems to affect the existence of rhizosphere microbial colonies.

Figure 6. *A. gangetica* 2 weeks after transplanting

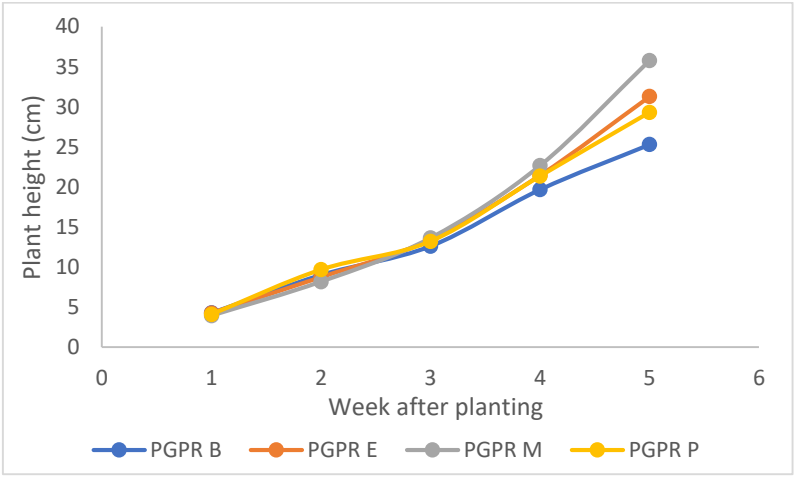

Figure 7. Effect of different PGPR agents on plant height growth of *A. gangetica*.

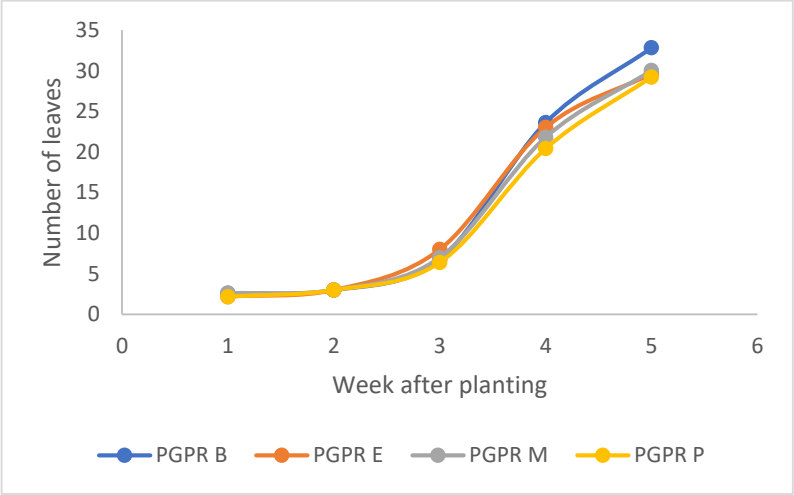

Figure 8. Effect of different PGPR agents on leaf number growth of *A. gangetica*.

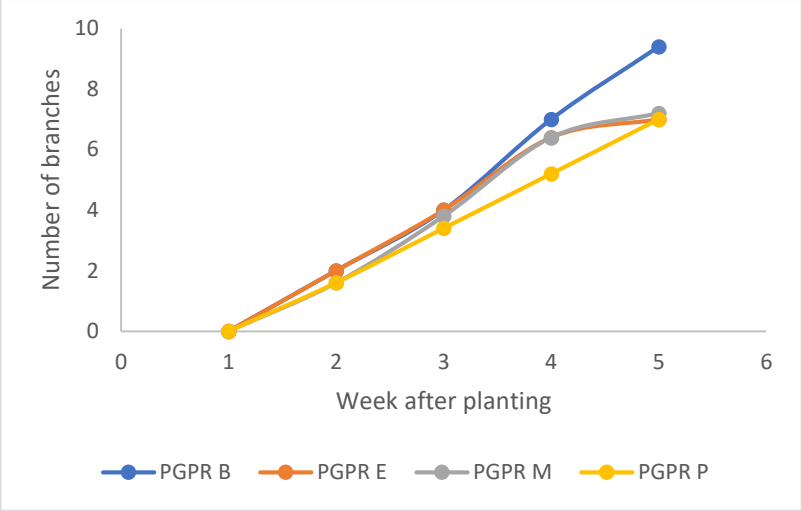

Figure 9. Effect of different PGPR biosols on the growth of the number of branches of *A. gangetica.*

In Figure 7, it can be seen that the best growth of *A. gangetica* plant height was found in the utilization of PGPR from the roots of *Mimosa pudica*. The highest number of leaves and number of branches occurred in *A. gangetica* plants that received PGPR input from the roots of bamboo plants, as shown in Figure 8 and Figure 9. Based on the results of the growth of *A. gangetica* plants above, it appears that PGPR can minimize the negative impact of the use of chemicals in agriculture on the environment, so that environmentally friendly cultivation innovations in the development of animal feed crops are expected to reduce the negative impact of using inorganic fertilizers. The utilization of bacteria that live around the roots of putri malu (*M. pudica* L), bamboo (*B. vulgaris*), padang belulang (*E. indica*), and *P. notatum* has the potential to increase plant growth and ultimately increase forage yields. These bacteria live in colonies covering the roots of plants. These bacteria are summarized in Plant Growth Promoting Rhizobacteria (PGPR). PGPR can be one solution to the dependence on synthetic chemical fertilizer products, so as to maintain sustainable agricultural growth and support the preservation of the environment that has already been damaged by the application of synthetic chemicals. In Figure 7, it can be seen that *A. gangetica* plants have the highest plant height, this is made possible by the large number of symbiotic bacteria of PGPR from the host *M. pudica*, while in Figures 8 and 9, the highest number of leaves and number of branches occurred in the application of PGPR from the host bamboo plant (*B. vulgaris*).

Tabel 2. Forage yield of *A. gangetica* at various PGPR applications

Host Plant	Variable			
	Leaf Area	Leaf DM	Stem DM	Forage DM
	…..dm^2……	…….. g $plant^{-1}$ ……		
Bambusa vulgaris	128,11± 27,39	8,32±1,02	10,38±1,74 ab	18,70±2,63 b
Mimosa pudica	133,19±1808	8,40±0,87	12,66±1,15 a	21,06±1,87 a
Eleusin indica	137,16±17,24	9,20±2,72	12,32±2,86 a	21,52±5,25 a
Paspalum notatum	105,22±4,48	6,84±0,67	8,40±0,79 b	15,24±1,16 c

Notes:
Treatment mean values followed by the same letter in the same column show no significant difference (P>0.05).

Table 2 shows that PGPR Eleusin indica tends to provide higher leaf area, leaf dry weight and forage dry weight of *A. gangetica* than other treatments. PGPR E. indica gave a significantly higher stem dry weight than PGPR *Paspalum notatum* grass. PGPR *E. indica* did not show a different stem dry weight of *A. gangetica* with PGPR *Bambusa vulgaris* and *Mimosa pudica*. PGPR. *Paspalum notatum* gave the lowest growth and forage yield. The highest forage yield of *A. gangetica* was obtained in the application of PGPR *E. indica* and *M. pudica* as much as 21.52 and 21.06 g $plant^{-1}$, respectively. This forage yield is strongly supported by the performance of rhizosphere microbes which can be seen from the TPC and the number of bacteria (Table 1) and the effect of PGPR biosolids application on the growth and leaf area of *A. gangetica* plants. The high dry weight of forage in *E. indica* indicates the high activity of *Rhizobium, Azotobacter, Azospirillum* and phosphate solubilizing bacteria in decomposing organic matter such as proteins, carbohydrates, microorganisms, mineralization of organic compounds, nutrient fixation, nutrient solubilization, nitrification and denitrification (Marista *et al*., 2013). The presence of PGPR containing *Bacillus* spp. and *Pseudomonas fluorescens* bacteria is able to synthesize growth hormones IAA, cytokinin, and gibberellin, so as to increase plant growth and ultimately increase yields (Biswas *et al*., 2000).

The use of *B. vulgaris* root PGPR agent gave lower forage yields than *E. indica* and *M. pudica* root PGPR agents, but was able to provide higher forage yields than *P. notatum* root PGPR agent. The use of B. vulgaris root PGPR is also known to spur root growth of rice sprouts. The provision of PGPR also has an impact on increasing the dry weight and wet weight of roots and crowns of rice sprouts (Hamdayanty *et al*., 2022). Komansilan *et al*. (2023) stated that PGPR treatment at a maximum concentration of 15 ml/liter only had a significant effect on the number of productive tillers in the intercropping system and the number of harvested dry grain in the monoculture system, but did not significantly affect the formation of full grain, and empty grain, so that rice production also did not experience a significant increase in the intercropping system. This is also consistent with the use of PGPR inhibitors from the roots of B. vulgaris plants, which require higher doses on *A. gangetica* to get the optimal dose.

CONCLUSION

Based on the results of the study, it can be concluded that the PGPR culture of *E. indica* and *M. pudica* plant roots has the highest TPC, which is $6.4x10^5$ and $1.9x10^5$ cfu/ml, respectively. Similarly, non-symbiotic and symbiotic bacteria were found more in the PGPR culture of *E. indica* and *M. pudica* roots. Phosphate solubilizing bacteria were present in similar numbers in *E. indica* and *M. pudica* plants and even higher than the PGPR culture found in the roots of bamboo and *Paspalum*. The highest forage yield of *A. gangetica* was obtained in the application of PGPR *E. indica* and *M. pudica* as much as 21.52 and 21.06 g $plant^{-1}$, respectively.

ACKNOWLEDGMENTS

The implementation of this research was funded by DIPA PNBP Udayana University TA-2022 in accordance with the Research Implementation Assignment Agreement Letter Number: B/151-14/UN14.4.A/PT.01.03/2022, dated November 1, 2022. This research is part of the big theme of Research on PGPR which in various research topics has involved 8 students for the preparation of their thesis. The researcher would like to thank the Rector and the Chairperson of LPPM Udayana University for facilitating the implementation of this research which is very beneficial for lecturers and students at Udayana University and the scientific community in general.

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