THE POTENTIAL OF PGPR (PLANT GROWTH PROMOTING RHIZOBACTERIA) TO REDUCE INROGANIC FERTILIZERS NEEDS IN PEANUTS CULTIVATION

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Abstract

Plant growth promotion rhizobacteria (PGPR) can be applied around the roots of plants and function as biofertilizers, biostimulants, and biopectants. When PGPR improves soil fertility in plant area, the dose of inorganic fertilizers can be lowered. On the other hand, it may also affect the growth of weeds around the crops. The objectives of the research were to study the interaction of PGPR with the dose of inorganic fertilizers on the growth and yield of peanuts, as well as the impact of the PGPR application on the appearance of weeds around the planting area. The results showed that there was a significant interaction of PGPR with the doses of fertilizers on peanut yields. The yield of peanuts was not significantly different from 100% recommended dose (RD) of inorganic fertilizers if 1.0% PGPR was combined with 75 and 50% RD of inorganic fertilizers, and 2.0% PGPR was combined with 75, 50 and 25% RD of inorganic fertilizer. The application of PGPR has no effects on the appearance of weeds in peanut fields. It is concluded that applying PGPR at 1.0% or 2.0% in peanut cultivation in coastal lands of ultisol showed the potential in reducing the need for inorganic fertilizers of nitrogen and phosphor down to 50% or 25%, respectively.

Key words: biofertilizers, inorganic fertilizers, coastal areas, peanuts, PGPR, ultisol

1. INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the legumes in Fabaceae family, an important groundnut that ranks second after soybeans (Friend et al., 2010). Peanuts can be used for food or raw materials for the food industry, cooking oil, margarine and soap (Prasad et al., 2010). Peanuts are rich in protein, iron, vitamins E, B complex, A and K, as well as phosphorus, calcium and the amino acid lecithin and choline (Sadras and Calderini. 2021). As an industrial raw material, peanuts have a high oil content, reaching 40 – 48%, followed by protein and carbohydrates of 27 and 18%, respectively (Kumar et al., 2014).

The yield and production of peanuts in Indonesia are still low compared to other countries such as China, India and the USA. According to Sadras and Calderini (2021), China and India are the world's largest peanut producers, accounting for more than 40% and 15% of world production respectively, followed by the USA (7.2%). Now day, crop yields often fluctuate due to changes in global climate, genetic resources, and cultivation aspects (Wei et al., 2022).

Demand for peanuts continues increasing along with the increase in human population. Various efforts continue to be made to increase peanut production, including expanding planting areas and improving production facilities and technology. In Indonesia, expanding planting areas by managing marginal land in coastal areas can be an opportunity to increase crop production including peanuts (Gunadi 2002; Arianto, 2020). Most coastal areas are marginal ultisol which have low productivity and can be used

for crop cultivation by optimizing production facilities, especially fertilization (Ray et al., 2014; Simarmata et al., 2015). The use of inorganic fertilizers in sufficient doses can increase crop yields, but if done continuously it will cause saturation and even damage to the physical, biological and chemical properties of the soil (Mahrous et al., 2015; Ahmed et al., 2017). According to Utami et al. (2018), inorganic fertilizer will be more effective and beneficial if combined with biological fertilizer such as PGPR (plant growth promoting rhizobacteria) because PGPR can regenerate soil from inorganic fertilizer residues.

PGPR is a group of bacteria that can be developed around the roots (rhizosphere) of plants and can colonize aggressively so that PGPR can stimulate the root system architecture (RSA) which supports plant growth (Kalita et al., 2015). Several types of microbes formulated in PGPR are nitrogen fixing bacteria, namely *Rhizobium azobacterer* and *Azospirillium* sp. and phosphate solubilizing bacteria such as the genus *Bacillus pseudomonas, Arthrobacter, Bacterium* and *Mycobacterium* (Munees and Mulugeta, 2014; Biswas et al., 2010). The *Azotobacter* genus is generally a nitrogen-fixing bacteria that is often found in agricultural soil and has various beneficial roles by producing vitamins, amino acids and auxin (Sharma et al., 2009). Azotobacter produces indole acetic acid (IAA), gibberllic acid (GA) which are important growth hormones and these hormones will help in germination and growth (Dodd et al., 2010).

PGPR also functions as a control agent for soil pathogens (bioprotectants) by producing antipathogenic metabolites such as siderophore, chitinase, β1,3-glucanase, cyanide, and antibiotics (Riaz et al., 2021). As a biopectan, it can control pathogens and plant pest organisms through the production of compounds that function as anti-fungals and antibiotics (Pineda et al., 2013; Riaz et al., 2019). In more detail we explain the beneficial effects of PGPR as a biopectan against root-dwelling microbial pathogens that have systemic effects on above-ground insect communities at several trophic levels and can trigger physiological changes and induction of defense in host plants.

The practical consideration for using PGPR is to increase the effectiveness of fertilizer doses, especially on ultisol which has never been managed for peanut cultivation. A'yun et al., (2013) reported that applying PGPR at a concentration of 10 ml/L to cayenne pepper could reduce the intensity of TMV (Tobacco Mosaic Virus) attacks by up to 89.92%, increase plant height and increase chili plant production. Munees and Mulugeta (2014), reported that PGPR can increase crop yields by 20-30%, increase nitrogen and phosphate in the soil by 25%, stimulate soil biological activity, and increase plant growth and biomass production.

The application of PGPR will increase soil fertility in the planting area which stimulates the growth of the main crops, therefore it may reduce the need for inorganic fertilizer as source of nitrogen and phosphorous. On the other hand, it is possible that it may stimulate the emergence and growth of weeds and cause competition to crops if it is not controlled as early as possible. The research was conducted aimed to determine the interaction of PGPR with N and P fertilizer doses on the growth and yield of peanuts, as well as analysis of the emergence of weeds around the peanut planting area.

2. MATERIALS AND METHODS

2.1. Site preparations and experimental design

The research was carried out at the Bengkulu University Experimental Station from April to August 2023. The coordinates of the areas are 3.45206^0 LS 102.1618^0 BT with the latitude ± 10 meter above the sea level. Two experimental factors that will be tested are the PGPR concentration and the dose of urea and SP-36 fertilizers, which arranged in completely randomized bloc design with 3 replications. The first factor consisted of 3 PGPR concentrations, namely 1.0, 2.0% v/v, and without PGPR as a control. The second factor consists of 4 doses of fertilizers namely 100% recommended dose (DR) 100 kg/ha of Urea (46% N) and 150 kg/ha SP-36 (36% P_2O_5), 75%, 50%, and 25% DR, while KCl (60% K_2O) fertilizer was 150 kg/ha for each treatment.

The research began by clearing weeds, tilling the soil and creating experimental plots measuring 2m x 1m. Liming is carried out with one ton of dolomite per ha to raise the soil pH to (6 – 7), optimal for peanut plants. Planting is carried out two weeks after liming. Peanuts were planted 2 seeds in a hole with a depth of 3 cm and a spacing of 20 cm x 20 cm or a population of 250,000 plants per ha.

Plant maintenance is carried out according to standards cultivation in the field. Plant were fertilized using Urea, SP-36, and KCl fertilizer to following to the treatment dose. Urea fertilizer is applied 2 times, at planting time and 3 weeks after planting (WAP) at half dose for each, while SP-36 and KCl are applied at planting time. Replanting and thinning were at 7 days old. PGPR was applied 5 times starting at planting

with an interval of 7 days. PGPR application is carried out by watering PGPR solutions at 200 ml/plant according to the treatment concentration. Pest control was carried out by using pesticides that are appropriate to the symptoms. Weed control is carried out manually using kored at the age of 2 and 4 WAP. Harvesting plants was carried out at 95 WAP.

2.2. Variables observed and data analysis

Some of the variables observed include plant height, flowering time, flowering time, plant canopy, greenness leaf, leaf chlorophyll content, number of nodules, number of pithy pods, yields (weight of pods from one plot were converted into yield per hectare, plant stover weight (dry biomass), soil bacterial population, dry weight of weed (harvested from 2 square sample plots in each treatment plot).

Chlorophyll content in leaves is carried out during plant growths followed procedures of Rahimi et al. (2019). One gram of peanut leaves is crushed using a mortar in 100 mL of 70% alcohol and filtered with filter paper. The aliquots was put into a cuvette to measure the chlorophyll content using a spectrophotometer (Cray 100 UV-Vis) at wavelengths A662 and A645. The contents of Chlorophyll a, Chlorophyll b, and total Chlorophyll are calculated as follows:

$$\text{Chlorophyll } \alpha = 11{,}24 \times A_{662} - 2{,}04 \times A_{645}$$
$$\text{Chlorophyll b} = 20{,}13 \times A_{645} - 4{,}19 \times A_{662}$$

Soil bacterial was analyzed following the procedures of Simarmata et al. (2018). Inoculation media was prepared by diluting 20 g of NA and 15 g of agar in 1liter distilled H2O, cooked and stirred homogeneously, cooled at room temperature and then stored in a refrigerator at 4 C. The inoculant material collected from the soil around the roots of peanut plants. composite. One gram of soil sample was diluted homogeneously in 9 ml of water (1×10^{-1} g soil suspension). The dilution was repeated to make a 1×10^{-5} soil suspension. The media stock is heated to 50 C and allowed to cool in room temperature. One ml of the inoculant suspension was pipetted into the petridish and media was poured up to two thirds, then shaken so that the inoculant was evenly distributed in the media. The petridish was wrapped and incubated in a room temperature. Bacterial population was observed after 2 x 24 hours of inoculation in Colony Counter and data were presented as CFU (colonies forming unit) in one g of soil calculated according to 5 times dilution

Weeds growing in the plant area were harvested by cutting the weeds above the ground from each treatment unit, put into paper bags to dry at a temperature of 72 C for 72 hours. Data collected were analyzed statistically for ANOVA and separated by Least significant difference (LSD) with confident level at $P < 0.05$.

3. RESULTS AND DISCUSSION

Laboratory analysis of the soil in the field research was observed that C-organic = 2.63%, N-total = 0.22%, P available = 2.01 ppm (very low), K-dd = 0.23 me 100 g-1 (low), and pH = 3.5 (Soil Science Laboratory, University of Bengkulu). These data were indicated the low level of soil fertility but the field can still be used for peanuts cultivation. Climatological data during the study from January to April 2023 including the average rainfall were 114, 116, 147, and 200 mm, relative humidity were 98.53, 82.96, 83.19, and 84.26%; light intensity were 51.19, 57, 64, 65.41, and 70.9%, respectively. According to Friend et al., (2010) the climatological conditions are suitable to grow peanuts. To raise the soil pH, the field was applied with agricultural limestone at 1 ton/Ha. Statistical analysis showed significant interaction between PGPR and the dose of inorganic fertilizers of urea (N) and SP36 (P) on the yield and number of pithy pods (Table 1). PGPR had a significant effect on the number of root nodules, shoot biomass weight, number of pithy pods, the weight of 100 seeds and peanut yield. The doses of inorganic fertilizer had a significant effect on the plant canopy, number of pithy pods, weight of 100 seeds and peanut yield.

3.1. Interaction of PGPR with inorganic fertilizer

Further tests of the interaction between PGPR treatment and inorganic fertilizer doses on yield and number of pithy pods are shown in Tables 2 and 3. Without applying PGPR, peanut yields decreased significantly with reductions of inorganic fertilizer doses to 75, 50 and 25% of recommended doses (RD). If 1.0% PGPR is applied, plant yields are not significantly different even though the fertilizer dose were reduced to 75 and 50% RD, but yields decrease significantly with the 25% RD of inorganic fertilizer. With applying PGPR of 2.0%, there was no significant difference in crop yields in all level of inorganic fertilizer

doses, namely 100, 75, 50, and 25% RD (Table 2). The similar pattern was observed on the number of pithy pods when using PGPR 10 and 20% combined with inorganic fertilizers at 100, 75, 50 and 25% of RD (Table 3).

Table 1. The summary of analysis of variance (ANOVA) on variables observed.

Variables observed	Source of variations			CV (%)
	Interaction	PGPR	Inorganic Fertilizer	
Plant height	0.53 ^{ns}	1.83 ^{ns}	1.12 ^{ns}	6.92
Plant canopy	0.94 ^{ns}	2.88 ^{ns}	3.81*	9.07
Flowering time	1.74 ^{ns}	4.73^{ns}	2.71^{ns}	2.21
The number of nodules	0.33^{ns}	38.00*	0.10 ^{ns}	16.46
Chlorophyll content	1.04 ^{ns}	2.37 ^{ns}	0.56 ^{ns}	23.31
Plant stover weight	1.78 ^{ns}	6.17 *	1.04 ^{ns}	8.82
Number pods	3.91*	6.60 *	3.14*	20.60
Weight of 100 seeds	1.55 ns	7.01*	3.43*	3.82
Yield	3.21 *	6.91 *	3.46 *	19.23
Weed dry weight $^{(T)}$	0.46 ^{ns}	0.86 ^{ns}	0.07 ^{ns}	28.33
Soil bacterial population	0.58 ^{ns}	1.35 ^{ns}	0.79 ^{ns}	18.61

ns = not significant, * = significant at P<0.05, T = data transformed by ($\sqrt{x + 0.5}$).

Table 2. The interaction of PGPR with the dose of inorganic fertilizers on peanut yields (ton ha^{-1})

PGPR (% v/v)	Recommended dose of inorganic fertilizers			
	100%	75%	50%	25%
0	4.24 Aa	3.48 Bb	3.46 Bb	3.00 Bb
1.0	4.60 Aa	4.92 Aa	4.82 Aa	2.80 Bb
2.0	4.98 Aa	4.66 Aa	4.78 Aa	4.58 Aa

The means were separated by LSD test (P < 0.05), capital letters valid in a column and lowercase letters valid in a row; Recommended doses of Urea and SP-36 are 100 and 150 kg ha^{-1}, respectively.

Table 3. The interaction of PGPR with dose of inorganic fertilizers on the number of pithy pods per plants (pods plant^{-1})

PGPR (% v/v)	Recommended dose of inorganic fertilizers			
	100%	75%	50%	25%
0	16,1 Aa	13,6 Bb	10,4 Bc	10,1 Bc
1.0	16,8 Aa	18,4 Aa	17,0 Aa	10,3 Bb
2.0	16,6 Aa	17,6 Aa	16,6 Aa	17,8 Aa

The means were separated by LSD test (P < 0.05), capital letters valid in a column and lowercase letters valid in a row; Recommended doses of Urea and SP-36) are 100 and 150 kg ha-1, respectively.

PGPR is a microorganism that can live and develop around plant roots and functions as a biofertilizer by binding nitrogen from the air and dissolving phosphorus to make them available to plants (Alexander et al., 2019; Aquilanti et al., 2004). Another role of PGPR is as a biostimulant and biopectan. As a biostimulant, namely by synthesizing various growth regulators (phytohormones) such as IAA, gibberellins, cytokinins, ethylene in the rhizosphere. Indole Acetic Acid (IAA) plays a role in cell division so that it can increase the quality and quantity of harvest results. Basically, plants are unable to produce sufficient IAA for plant growth and development, but PGPR contains the microbes *Pseudomonas flourescens*, *Azospirillum* and *Azotobacter* which can synthesize IAA (Alexander et al., 2019; Nazir et al., 2019).

The benefit of using PGPR is that it can reduce the need for N and P nutrients which are usually provided through urea and phosphate fertilizers (Mahrous et al., 2015). The results of this study illustrate that with 1.0% PGPR application, the application of N and P nutrients via urea and SP-36 can be reduced to half or 50% of the recommended dose and if the PGPR application is increased to 2.0% then the application of peanut plant fertilizer can be reduced to a quarter. or 25% of the recommended dose.

3.2. The effects of PGPR

Further test of PGPR effects showed that there were no significant differences due to the PGPR dose treatment on flowering age, plant height, plant stand canopy and chlorophyll content, but the number of root nodules, shoot biomass weight and weight of 100 peanut seeds increased significantly with PGPR doses of 1.0 and 2.0% (Table 4).

Table 4. The effect of PGPR doses on the growth and yield components of peanuts.

PGPR (% v/v)	**Flowering time** (days)	**Plant height** (cm)	**Plant canopy** (%)	**Number of nodules** (nodules plant^{-1})	**Chlorophyll content** (mg g^{-1})	**Weight of plant stover** (g plant^{-1})	**Weight of 100 seeds** (g)
0	31	50.98	79.33	138 b	2.915	35.77 b	40.33 b
1.0	30	51.23	81.92	236 a	2.950	39.21 a	42.55 a
2.0	30	53.53	86.58	251 a	2.429	40.05 a	42.75 a
ANOVA (P,0.05)	ns	ns	ns	*	ns	*	*

The means were separated by LSD test ($P \leq 0.05$) valid in one column; ns = not significant, * = significant

The application of PGPR will further stimulate the formation of root nodules. It is suspected that PGPR can be functioned as a biostimulant around the rhizosphere which can be observed by increasing the number of root nodules. As the impact of PGPR which fixes N from the air and dissolves phosphate around the rhizosphere, there is an increase in plant biomass weight and an increase in peanut seed size which is visible in the variable weight of 100 seeds. The same thing has been reported in previous research on pea plants (Marom et al., 2017). PGPR application will help in cultivating legumes in planting areas that have never been cultivated with legumes (Dey et al., 2004).

3.3. The effect of inorganic fertilizer (urea and SP36)

Further tests on treatment doses of inorganic fertilizer, namely urea (N) and SP36 (P) showed significant differences in plant stand canopy, plant stover weight, and weight of 100 seeds, but there were no significant differences in flowering age, plant height, number of root nodules, and plant chlorophyll content (Table 5). The dominant canopy growth of peanut stands was seen in the 100% DR treatment and decreased significantly when the fertilizer dose was reduced to 75, 50 and 25% DR. The similar pattern was happened to the weight of shoot biomass and the weight of 100 seeds.

Table 5. The effect of the doses of inorganic fertilizers nitrogen and phosphate doses on the growth and yield components of peanuts.

RD of inorganic fertilizers	**Flowering age** (days)	**Plant height** (cm)	**Plant canopy** (%)	**The number of nodules** (nodules plant^{-1})	**Chlorophyll content** (mg/g)	**Weight of plant stover** (g plant$^{-1)}$	**Weight of 100 seeds** (g)
100%	30	52,84	89,40 a	212	2.924	39.91 a	42,44 a
75%	30	52,00	82,40 b	206	2.690	38.43 a	41,89 a
50%	30	52,11	80,30 b	205	2.835	38.61 a	40,86 b
25%	31	52,71	78,20 b	212	2.554	36.52 b	40,56 b

ANOVA (P<0.05)	ns	ns	*	ns	ns	*	*

The means were separated by LSD test ($P \leq 0.05$) valid in one column; ns = not significant, * = significant; RD=Recommended doses of Urea and SP-36 are 100 and 150 kg ha^{-1}, respectively.

N fertilizer functions for vegetative plant growth while P fertilizer functions for seed formation of plant generative (Silitonga et al., 2018). Reducing N fertilizer will reduce total growth which is visible in plant biomass weight (Mahrous et al., 2015; Shi-cheng et al., 2021). In this study, plant height was not affected by the dose of N & P fertilizer, so it is possible that differences in canopy biomass occurred in other growth components such as stem diameter, number of branches, and number of plant leaves observed as a stand canopy. Reducing the nutrient N to 25% DR reduced the stand canopy from 89.40 to 78.20% (11.20%), stover weight from 39.91 to 36.52 grams (3.39 grams). Likewise, the size of peanut seeds was observed at the weight of 100 seeds which was reduced significantly by reducing the phosphate dose to 25% DR which reduced the weight of 100 seeds from 42.44 to 40.56 grams (1.88 grams).

4. CONCLUSIONS

The research results showed that there was a real interaction between PGPR and the dose of nitrogen and phosphate fertilizer on peanut crop yields. With the application of 1.0% PGPR, it can reduce the need for nitrogen and phosphate fertilizers by 50% of the recommended dose (DR) to obtain yields and number of peanut pods that are not significantly different from the DR standard. If the PGPR dose is increased to 2.0%, the nitrogen and phosphate fertilizer dose can be reduced to 25% DR to achieve results that are not significantly different from the DR. The results of this research recommend the use of PGPR up to 2.0% in peanut cultivation on coastal land with ultisol soil types. The novelty obtained is the reduction or efficiency of the application of nitrogen and phosphate inorganic fertilizers by using the PGPR biofertilizer

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