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Combination effect of NPK and plant growth promoting rhizobacteria on edamame soybean growth and yield

Hutabarat, F., Sudjatmiko, S.* and Pujiwati, H.

Department of Crop Production, Faculty of Agriculture, University of Bengkulu, Jl. W.R. Supratman, Kandang Limun, Bengkulu 38121, Indonesia.

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Abstract The results showed that the combination of treatments had a significant effect on plant height and shoot fresh weight, total number of pods, number of pithy pods, and their weight. NPK application alone (300 kg ha^{-1}) resulted in greater growth than PGPR alone (30 mL L^{-1}) and control plants. Plant height was highest at 300 kg ha^{-1} NPK but did not differ significantly from 225 kg ha^{-1} NPK+7,5 mL L^{-1} PGPR and other combination. The shoot fresh weight was highest at 300 kg ha^{-1} NPK but did not differ significantly from 225 kg ha^{-1} NPK+7,5 mL L^{-1} PGPR and 150 kg ha^{-1} NPK+15 mL L^{-1} PGPR. The absence of fertilizer and the use of exclusive application of biofertilizer PGPR led to edamame soybeans with inferior yield. The highest yield was expressed by total number of pods, number of pithy pods, and their weight was produced by 300 kg ha^{-1} NPK alone and 225 kg ha^{-1} NPK+7.5 mL L^{-1} PGPR, but the latter did not differ from 150 kg ha^{-1} NPK+15 mL L^{-1} PGPR; and 75 kg ha^{-1} NPK+22.5 mL L^{-1} PGPR. The maintenance of edamame soybean growth and yield, enhancement of soil quality, and reduction of chemical residues are shown as all potential outcomes of the incorporation of 7.5 mL L^{-1} PGPR with 225 kg ha^{-1} NPK compound fertilizer. These outcomes have the potential to improve soil conditions.

Keywords: Promoting bacteria, Edamame soybean, Combination effect

Introduction

Edamame soybean (*Glycine max* (L. Merril) is a nutritious plant that is harvested while the pods are still green and has the potential to be a profitable crop in Indonesia. The nutritional value of 100 g of edamame is 582 kcal; 6.6 g of fat; 11.4 g of protein; 100 mg of vitamin A; 7.4 g of carbohydrates; B1 0.27 mg; B2 0.14 mg; B3 1 mg; vitamin C 27; calcium 70 mg; iron 1.7 mg; and phosphorus 140 mg (Johnson *et al*., 1999). Edamame soybeans are typically harvested 65-75 days after planting (Umami *et al.*, 2014), and seed is harvested 100 days after planting (BBPPMBTPH, 2020). Despite the fact that edamame

* **Corresponding Author:** Sudjatmiko, S.; **Email:** sigitsudjatmiko@unib.ac.id

has a potential market, its yield in Indonesia is still around 7.5 tons ha^{-1}, which is still less than its potential of 10 tons ha^{-1} (BPPSDMP, 2014).

Plant productivity can be increased by using inorganic fertilizers such as NPK compound fertilizers (Fahmi *et al*., 2014). Application rates ranging from 150 to 300 kg ha^{-1} significantly affected plant height, number of leaves, number of branches, and number of pods in soybean (Nurheliani *et al*., 2019; and Hapsoh *et al.,* 2019). Combining inorganic fertilizers with biological fertilizers such as Plant Growth Promoting Rhizobacteria (PGPR), which regenerates soil from inorganic fertilizer residues, can improve the effectiveness of inorganic fertilizers (Adesemoye, 2008; Phat *et al.*, 2019; and Lobo *et al*., 2019). PGPR are soil bacteria that live around/on the root surface and promote plant growth and development by releasing phytohormones or other biologically active substances, altering endogenous phytohormone levels, or enhancing nutrient availability and uptake through fixation and mobilization (Prasad *et al*., 2019). Furthermore, PGPR, a biological fertilizer based on the collection of bacteria in plant roots, can help plants with immunity, growth, and development (LIPI, 2017).

Nitrogen-fixing bacteria such as *Rhizobium*, *Azotobacter*, and *Azospirillum* are found in PGPR, as are phosphate-solubilizing bacteria such as *Bacillus* and *Pseudomonas*, *Arthrobacter*, *Bacterium*, and *Mycobacterium* (Biswas *et al*., 2000). Biofertilizers accelerate nutrient absorption, bio-stimulants produce phytohormones to ensure proper nutrient absorption, and biopectans protect plants from various pathogens (Rai, 2006; and Saharan and Nehra, 2011). Application of PGPR at concentrations ranging from 10 to 20 ml L^{-1} improves plant height, number of leaves, leaf area, dry weight, number of flowers, plant pods, dry weight, filled pods, and yields of black soybean (Ramlah and Guritno, 2019; and Arfandi, 2019), as well as yields of leavy vegetables (Tabriji *et al*., 2016).

Despite lower yields from primary cultivation in less fertile soil, edamame production in Indonesia is increasing. As a result, improving soil quality and fertility is expected to increase edamame yield more. NPK compound fertilizers itself, which contain essential nutrients, can improve soil nutrition. Nonetheless, using NPK alone may have a negative impact on the soil's physical and chemical properties. The combination of NPK compound fertilizers and biological fertilizers (PGPR) is expected to stabilize fertilizer requirements while improving soil quality. Few studies have been conducted to evaluate the combination treatment of compound NPK with PGPR for Fabaceae family plants. The present study was conducted to examine the combining effect of NPK fertilizer with PGPR on edamame soybean growth and yield.

Materials and methods

Location and research materials

The research was conducted at the Agronomy Experimental Unit of the University of Bengkulu in Indonesia, at 3°45'20.6" S and 102°16'18.8" E, at a height of 5 m above sea level. NPK fertilizer (16:16:16) in combination with different concentrations of Plant Growth Promoting Rhizobacteria (PGPR) containing 576 x 10^7 colony g^{-1} was applied to the Ryokoshoh variety of Edamame soybean.

Experimental setup

A completely randomized block design was used to set up a single factor treatment of NPK compound fertilizer combined with PGPR. The treatment includes P_0: Without Fertilizer (control); P_1: 300 kg ha^{-1} NPK; P_2: 225 kg ha^{-1} NPK+7.5 mL L^{-1} PGPR concentration; P_3: 150 kg ha^{-1} NPK+15 mL L^{-1} PGPR concentration; P_4: 75 kg ha^{-1} NPK+22.5 mL L^{-1} PGPR concentration; and P_5: 30 mL L^{-1} PGPR. The treatment was divided into six experimental units, each of which was replicated three times; each unit contained 144 plants with a plant spacing of 25 cm x 25 cm. Crop management adopted the common cultivation method of soybean. Before being used for current research, the soil was planted with sweet corn and remained unplanted for 6 months. Prior to treatment, the soil was assessed for C-Organic content, pH, total N, available P, and exchangeable K. Following soil testing, the pH of the soil was raised to 6 by applying 3 tons ha^{-1} of dolomite.

The rhizobacteria in the study were obtained from a dry inoculant. The PGPR was applied to the plant by dissolving the inoculated bacteria in one litre of water and pouring it on the soil near the plant's roots. PGPR is applied at 7-day intervals beginning 2-6 weeks after planting, depending on the treatment concentration. NPK fertilizer was applied twice, once at planting and again three weeks later. Following harvesting, soil samples were collected to determine the number of bacteria. The total number of bacteria was identified after serial dilution and nutrient agar plate dilution. In this method, 1 g of soil samples were diluted 10 times to obtain a 10^{-5} dilution, which was then spread in triplicate on the surface of nutrient agar. The samples were left at room temperature for 48 hours before being tested for bacteria using the Gram staining method (Lee, 2021). *Bacillus aureus*, *Rhizobium*, and *Pseudomonas* sp. were isolated as total counts from soil samples. The soil chemicals were analyzed for N content using the Kjeldahl method, P content using the Bray P1 method, K content using an atomic absorption spectrophotometer set on emission mode at 776 mm, C-organic content using the Walkley-Black titration method, and pH using a Beckman pH meter.

Data collection and analysis

The variable measurements were based on ten randomly selected plant samples from each plot and included plant height, number of leaves, shoot fresh weight, number of nodules, number of productive branches per plant, total number of pods, number of pithy pods, weight of pithy pod and total pod weight. Multiple comparisons on the combined effects of NPK compounds and PGPR on the measured parameters were conducted after an analysis of variance (ANOVA). Using Fisher's protected test, means were divided based on the least significant difference with a 5% probability.

Results

The average monthly rainfall, air temperature, and relative humidity (RH) during the study period (October 2021 to January 2022) were 327 mm, 27^{o}C, and 84%, respectively. Soil analysis prior to the experiment revealed that the pH was very low, the C-organic content was very high, the N-total was moderate, the available P was very low, and the exchangeable K was low (Table 1). The microbial population in the PGPR biofertilizer solution was estimated to be 576 x 10^{7} cfu g^{-1} prior to application. The microbial population was measured after harvest and found to be 85.6 x 10^{4} cfu g^{-1} on the untreated (control) soil, 76.8 x 10^{4} cfu g^{-1}on the 300 kg ha^{-1} NPK soil and 102.7 x 10^{4} cfu g^{-1}on the 30 mL L^{-1} PGPR soil.

Table 1. Soil characteristics prior to the experiment

Soil characteristics	Value	Criteria
N total (%)	0,27	Moderate
P–Bray 1 (ppm)	3,39	low
Exchangeable K (cme/100 g)	0,37	Very low
C-Organic (%)	5,68	High
pH H_2O	4,50	Very acid

Edamame growth

The edamame plant growth increased uniformly from 14 to 35 DAP (Figures 1 and 2). Each treatment's plant grew at a different rate between the ages of 14 and 35 DAP. Furthermore, the plants with treatment 300 kg ha^{-1}

NPK were the tallest and had the most leaves 35 days after planting. Plant height and leaf number were reduced in the controlled plants and PGPR alone (30 mL L^{-1}).

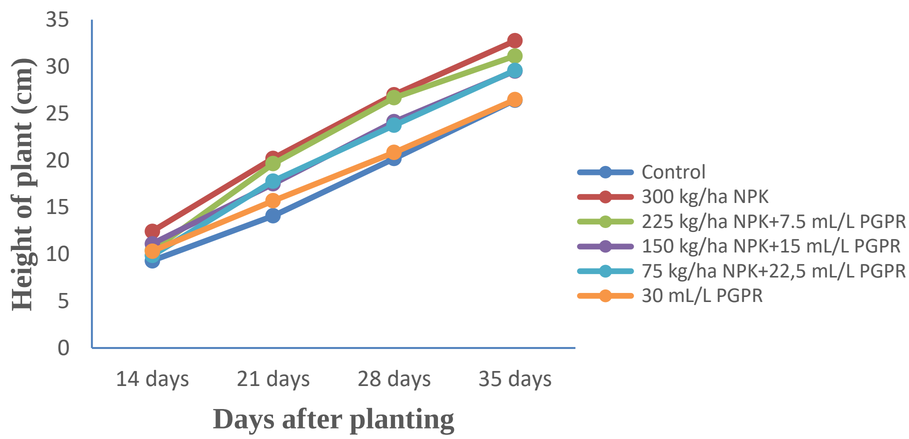

Figure 1. Height of Edamame grown on combinations of NPK compound fertilizers and PGPR at a time interval of 14 to 35 DAP

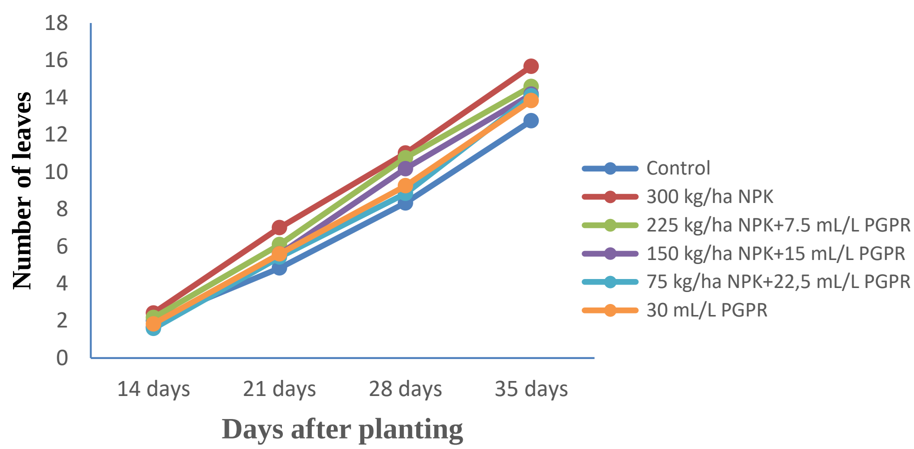

Figure 2. Number of leaves of Edamame grown on combinations of NPK compound fertilizers and PGPR at a time interval of 14 to 35 DAP

The combinations of NPK compound fertilizers and PGPR had significantly affected on edamame soybean growth as measured by plant height and shoot fresh weight (Figure 1 and 2). At 35 DAP, statistical analysis showed that the treatment had a significant impact on plant height and shoot fresh weight, but not on leaf number, root nodule number, or productive branch number (Figure 3 and 4 and Table 2). Based on edamame soybean growth variables, NPK application alone (300 kg ha^{-1}) resulted in greater growth than PGPR alone (30 mL L^{-1}) and even without fertilizer (control). Result showed that plant was highest at 300 kg ha^{-1} NPK but did not differ significantly from 225 kg ha^{-1} NPK+7,5 mL L^{-1} PGPR; 150 kg ha^{-1} NPK+15 mL L^{-1} PGPR; and 75 kg ha^{-1} NPK+22,5 mL L^{-1} PGPR (Figures 3 and 4). Meanwhile, the shoot fresh weight was highest at 300 kg ha^{-1} NPK but did not differ significantly from 225 kg ha^{-1} NPK+7,5 mL L^{-1} PGPR and 150 kg ha^{-1} NPK+15 mL L^{-1} PGPR.

Table 2. Mean performance growth of edamame soybean as affected by NPK compound and PGPR combination

Treatment	Number of leaves	Number of nodules	Number of productive branches/plant
P0	13,0	87,33	5,3
P1	16,0	124,00	8,0
P2	14,7	104,16	7,3
P3	14,3	112,50	6,7
P4	14,3	99,33	5,7
P5	13,7	113,33	6,3

Note: At the LSD 5% level, the average followed by the same letter in the same column shows no significant difference

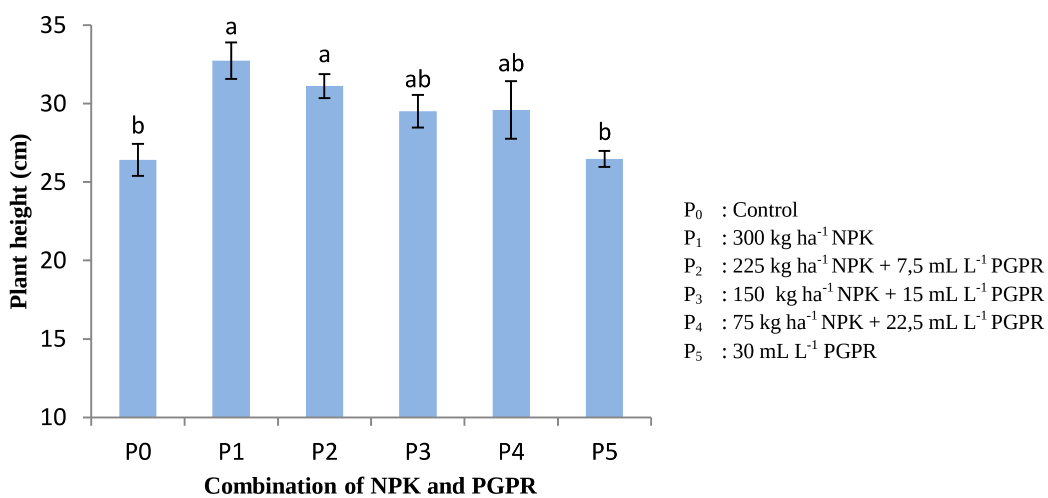

Figure 3. Plant height of Edamame grown on various combinations of NPK compound fertilizers and PGPR

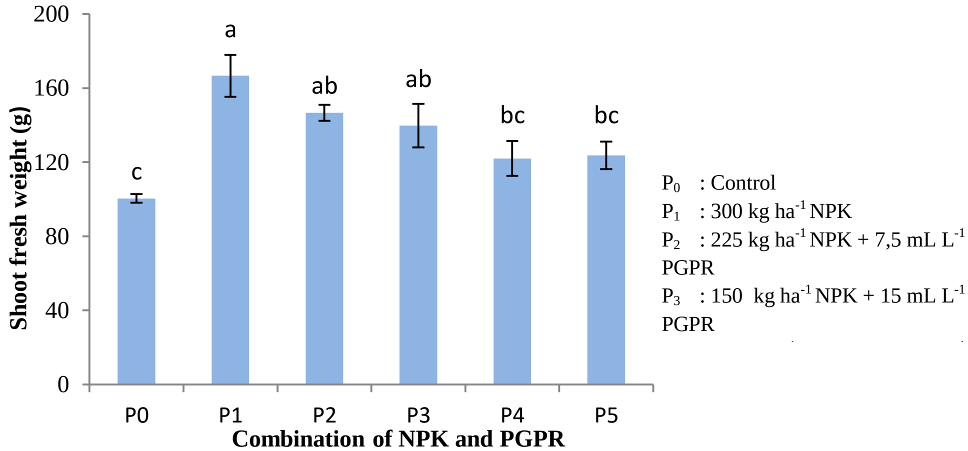

Figure 4. Shoot fresh weight of Edamame grown on various combinations of NPK compound fertilizers and PGPR

Edamame yield

The LSD test of edamame soybean yield variables in response to the combined effect of NPK compound and PGPR was summarized in Table 3. The combination effect of NPK compound fertilizers and PGPR had significantly affected on number of pods, number of pithy pods, weight of pod, weight of pithy pod, and the number of seeds/plant.

Table 3. Mean performances yield of edamame soybean as affected by NPK compound and PGPR combination

Treatment	Total number of pods	Number of pithy pods	Total weight of pod (g)	Weight of pithy pod (g)	Number of seed/plant
P_0	34,33 b	31,00 b	87,07 cd	86,57 cd	64,00 c
P_1	45,33 a	42,33 a	120,41 a	120,37 a	90,00 a
P_2	39,66 ab	36,00 ab	106,80 ab	106,77 ab	86,33 ab
P_3	35,00 b	31,00 b	100,86 bc	100,76 bc	74,66 bc
P_4	35,00 b	31,33 b	83,86 d	83,72 d	68,66 bc
P_5	36,66 b	34,33 b	79,93 d	79,87 d	69,00 c

Note: At the LSD 5% level, the average followed by the same letter in the same column shows no significant difference.

In general, the absence of fertilizer (the control treatment) and the sole use of biofertilizer PGPR resulted in edamame soybean with inferior growth and yield. All findings showed that the 300 kg ha^{-1} NPK alone and 225 kg ha^{-1} NPK+7,5 mL L^{-1} PGPR produced the highest of all variable yield, despite the fact that the latter did not differ from 150 kg ha^{-1} NPK+15 mL L^{-1} PGPR; and 75 kg ha^{-1} NPK+22,5 mL L^{-1} PGPR.

Discussion

Plant height and leaf number are important plant growth variables during vegetative growth because photosynthesis takes place in these organs. When the plant is sufficiently supported by sunlight, the higher of plant is correlated with an increase in the number of leaves (Salisbury and Ross, 1977). Regardless of fertilizer treatment, the consistent increase in plant height and leaf number during vegetative growth indicated that plant nutrients were available for plant growth, even though the best growth was achieved at 300 kg ha^{-1} NPK and combination of 225 kg ha^{-1} NPK+7,5 mL L^{-1} PGPR. This is in agreement with Ahmed *et al.* (2018), Yagoub *et al.* (2012), and Perkasa *et al.* (2016) who found that adding N from Urea or NPK compound fertilizer increased plant height and leaves number of soybean. This finding demonstrates the role of NPK in promoting plant growth, as an increase in nitrogen improves protein formation, and protein constitutes the majority of the tissues of most living organisms. Phosphorus assists plants in utilizing and storing energy, including photosynthesis. Potassium promotes normal plant growth and development while increasing crop yields and quality, also strengthens a plant's roots in dry weather (Salisbury and Ross, 1977). When combined with NPK, the additional PGPR was able to maintain plant growth, particularly plant height and shoot fresh weight. This may be due to the fact that PGPR is a nitrogen and phosphorus-rich biofertilizer. Nitrogen increases photosynthate (fresh weight) by increasing chlorophyll and photosynthetic enzyme content (Salisbury and Ross, 1977). The rhizomicrobiome is a collection of microorganisms that enable plants absorb and use nutrients, improve the texture of the soil, and make hormones, secondary metabolites, antibiotics, and other compounds that benefit plants grow (Backer *et al*., 2018). According to the present research, PGPR was able to reduce the amount of NPK fertilizer in use, thereby encouraging agricultural sustainability.

The insignificance effect of treatment on leaf number, number of nodules, and number of productive branches at 35 days after planting may be due to the fact that the initial soil N content was low at 0.27%, and that it may take time for NPK and PGPR treatments to provide optimal leaf growth nutrients, especially N. Furthermore, Kati *et al.* (2017) discovered that the number of productive branches of soybean was unaffected by the application of NPK fertilizer with Rhizobacteria. Another finding demonstrated that PGPR had no effect because soil bacteria cannot communicate with microorganisms near soybean roots during early development (Pratama and Zakiah, 2017). The high soil organic matter content of the experimental site combined with the addition of N from NPK compound fertilizer and also an increase in pH may have enabled rhizobium growth in all experimental units, regardless of treatment.

The application of NPK or PGPR has increased edamame soybean yield. In fact, N, P, and K have always been present in soil, but they are difficult for plants to utilize in ultisol. The plants will receive the proper amounts of nitrogen, phosphorus, and potassium by using NPK fertilizer (Vacheron *et al*., 2013). This finding is consistent with those of Ratnasari *et al*. (2014), who found that a range of NPK fertilizer dosages ranging from 250 to 300 kg ha^{-1} had a significant effect on soybean and mung bean yield. The fact that the NPK compound fertilizer alone has a large influence by increasing the number of pods, weight of pod and number of seed per plant, but did not differ from 225 kg ha^{-1} NPK+7,5 mL L^{-1} PGPR, indicating that the plant and soil have benefited from the addition of rhizobacteria. Novatriana and Hariyono (2020) also discovered that the higher the PGPR dosage sprayed, the more beneficial microorganisms that optimize agricultural yields. Furthermore, by increasing the number of rhizobacteria and lowering the NPK dosage to 150 kg ha^{-1} NPK+15 mL L^{-1} PGPR, edamame yield can be maintained, though it was 17% lower than 300 kg ha^{-1} NPK alone. Dhiman and Dubey (2017) discovered that combining N fixers and P solubilizers can save up to 25% on wheat and maize production costs. This is because if complex nutrients from the soil, such as N, P, and K, are available for plants, bacteria from PGPR will also play a role in helping plants store seeds, resist drought, and fight disease (Hasanah, 2002). PGPR works by controlling the balance of hormones and nutrients, making plants resistant to pathogens, and making nutrients easier for plants to absorb (Vejan *et al*., 2016; and Ahemad and Kibret, 2014).

After the completion of the research, more than fifty percent of the bacterial population remained on the control soil, soil treated with 300 kg ha^{-1} of NPK, and soil treated with 30 mL L^{-1} of PGPR. The soil treated with 30 mL L^{-1} of PGPR contained twenty-five percent more bacteria than the other treatments combined. Exploiting the bacterial community in PGPR has a high possibility of increasing crop production around the world and minimizing amounts of synthetic fertilizers and agrochemicals. (Barea, 2015; Nehra and Choudhary, 2015; and Smith *et al*., 2015). According to Backer *et al.* (2018), the discovery led to multifunctional PGPR formulations for commercial agriculture in order to reduce synthetic fertilizers and agrochemicals. In conclusion, it is possible to reduce the use of recommended inorganic NPK compound fertilizers to 225 kg ha^{-1} by adding 7.5 mL L^{-1} of PGPR without affecting the growth and yield of edamame soybean, while simultaneously enriching the soil with rhizobacteria to support the growth of subsequent crops. Rhizobacteria should be supported for sustainable agricultural management practices by preserving soil organic matter.

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