Journal of Tropical Crop Science Vol. 7 No. 3, October 2020
www.j-tropical-crops.com

Enrichment of Organic Manure with Plant Growth Promoting Rhizobacteria Improved the Root and Shoot Growth of Okra (*Abelmoschus esculentus* L.) Moench.)

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Abstract

A well-structured root system is essential to ensure optimal plant growth and yield. Two experiments were conducted to determine the effects of plant growth promoting rhizobacteria (PGPR) on the root system of okra plant. These experiments were arranged with a completely randomized design. The first experiment was conducted in the growth chamber with 8 different bacterial isolates consisting of *Methylobacterium* sp., *Bacillus* sp., *Bacillus methylotrophicus*, *Flavobacterium tirrenicum*, *Providencia stuartii*, *Azotobacter vinelandii*, *Methylocystis parvus* and PGPR consortium. The second experiment was conducted in the greenhouse and examined the effects of four poultry manure rates, i.e. 0, 6, 12 and 18 ton.ha^{-1}, or equivalent to 0, 75, 150 and 225% of recommended rates and how these are altered with the presence or absence of PGPR. The results of the experiments showed that, PGPR significantly improved root architecture; the number and length of lateral roots was increased by 242.86% and 777.79% respectively, as well as the dry weight of the roots and shoots of okra plant by 236.36% and 333.33%, respectively. Moreover applying 150% (12 t.ha^{-1}) of the recommended rate of poultry manure enriched with PGPR was found to be most effective in terms of improving the growth and root attributes of okra plants.

Keywords: PGPR consortium, poultry manure, root architecture

Introduction

Okra (*Abelmoschus esculentus*) is a vegetable crop grown in subtropical and tropical regions of the world (Adetuyi et al., 2011, Camciuc et al., 1998). It is widely known for its medicinal properties as well as high nutrient content. Okra is used to treat several gastric diseases, prevents cancer (Islam et al*.,* 2018), and exhibits antioxidant and anti-diabetic properties (Muneerappa, 2018). Okra fruit has been reported to be a source of vitamins, minerals, carbohydrates and dietary fibers (Petropoulos et al., 2018). It is known that, okra yield and quality is determined by the amount and uptake of nutrients from the soil. One of the most effective ways to improve plant nutrient uptake is by improving plant root structure. As inorganic fertilizers are currently facing some criticism from environmentalists, organic manure as well as plant growth promoting rhizobacteria (PGPR) have become a promising alternative for improving the structure and functioning of plant roots.

PGPR in particular, promote plant nutrition and modify root structure as well as root architecture (Vacheron et al., 2013). They do so by colonize the surface or inner tissues of root systems leading to enhanced growth and development of the plant. Several PGPR strains that belongs to various genera has been reported to promote plant growth. Some of these PGPR strains include *Methylobacterium* sp., *Bacillus* sp., *Bacillus methylotrophicus*, *Flavobacterium tirrenicum*, *Providencia stuartii*, *Azotobacter vinelandii* and *Methylocystis parvus.*

Methylobacterium sp is known to interact symbiotically with crops and brings about many desirable plant growth and disease resistance (Vadivukkarasi and Bhai, 2020). Generally, *Methylobacterium sp*. use methanol (a by product associated with plant metabolism) as a sole carbon and energy source. These bacteria are able to promote growth by enhancing the production of plant hormones such as auxin and cytokinin, and through the activity of 1-aminocyclopropane- 1-carboxylate deaminase, they lower ethylene levels in plants (Mizuno et al., 2013). Similarly, bacteria such as *Methylocystis parvus* (a gram negative bacteria) as well as *Bacillus methylotrophicus* have also been reported to grow in members of C1 compounds (methane and methanol) as their sole source of carbon and energy (Lindner et al., 2007, Madhaiyan et al., 2010). *Bacillus methylotrophicus* in particular, interact with plant roots and bring about beneficial effects to the growth of plants. Results obtained by Vicente-Hernández et al. (2019) revealed that, *Bacillus methylotrophicus* interaction improved the growth characteristics of strawberry and induced resistace against Botrytis sinerea. *Bacillus spp*. in the other side, include gram positive type of bacteria which can almost be found everywhere (ubiquitous) in nature. These bacterial usually associate with the plant roots or rhizosphere and produce biofilm for the plant growth improvement. They are also able to convert the unavailable form of N and P and make them available to the plant. Furthermore these bacteria are able to control plant diseases as well as improve the availability of Fe to the plants by solubilizing Fe from minerals and organic compunds using siderophore. (Radhakrishnan et al., 2017). *Azotobacter vinelandii* has also been described as a free living Nitrogen fixing bacteria that was first isolated in Vineland, New Jersey. It belongs to azotobacter; a genus which is commonly known as a nitrogen fixer . Studies have shown that, *Azotobacter vinelandii* have an ablity to produce siderophore called azotobactin which enhance the availability of Fe to the plants in iron limited condition (Noar and Bruno-Bárcena, 2018). Moreover research done by Bellenger et al., (2008) has shown that, *A. vinelandii* have an ability to bind metals other than Fe (e.g Molybdenum) as long as they contain three versions of nitrogenase enzyme with different metals in their cofactors. These enzymes are known to substitute one another to allow growth in the absence of one important metal. Generally nitrogen fixation reaction is mediated by nitrogenase enzyme and requires Molybdenum as one of important metal during the reaction. *Providencia stuarti*, a gram negative bacteria that is commonly found in soil, water and sewage has also been reported ot be able to solubilize phosphate (Rodríguez and Fraga 1999), act as a bio control (Rana et al., 2011) as well as produce IAA and improve plant growth.

In addition to the effects of PGPR, organic manure has shown an ability to improve soil structure (Afe and Oluleye 2017), and potentially improving the environmental condition for the growth and multiplication of PGPR. Among the available types of organic manure, poultry manure is preferred for promoting the growth of crops (Amanullah et al., 2010). Research conducted by Fagwalawa and Yahaya (2016) showed that the addition of poultry manure can result in improved growth performance of okra compared to sheep and cow manure. Similar results were obtained in a study by Khandaker et al. (2017), in which poultry manure elicited a positive response in terms of okra growth compared to rat, goat and rabbit manures

The application of organic manure for the growth promotion of okra is widely practiced in rural areas especially by smallholder farmers. In spite of its advantages regarding environmental sustainability, organic manure has to be applied in large quantity in order to provde the nutrients required for plant growth. For example, research done by Gashua et al. (2014) suggested that, in order to obtain a high yield of okra fruit, about 50 tons of organic manure (cow dung) per hectare of the land are required. Applying such large amounts of manure may be both time consuming as well as economically unfeasible for farmers. Furthermore, Rizk et al. (2007) revealed that, the use of *Azospirillum* combined with 50% nitrogen inorganic fertilizer gives better results in okra compared to 100% nitrogen fertilizer by itself. Similarly, Bhushan et al. (2013) found that, okra seeds treated with *Azobacter* required less nitrogen from inorganic sources. Despite this, there is little information on how and at what quantities organic manure can be influenced by PGPR when they are applied in combination. Moreover, Anisa et al. (2016) reported an increase in microbe populations in the rhizosphere of okra plant following the application of PGPR even though no information was provided on the influence of these PGPR on the root system of okra plants. Consequently, this study aimed to investigate the effect of several PGPR strains and combination applications of PGPR and poultry manure on the growth of okra seedlings and young plants to find the optimal rates for PGPR and manure filed application.

Materials and Methods

Study Area and Soil Preparation

This study was conducted at the Biofertilizer Pilot Plant and Greenhouse of the Indonesian Soil Research Institute, Bogor, from October to December 2019 to determine the effects of PGPR and various application rates of poultry manure on the roots and shoots of okra plants. This area is located at latitude -6.5758 and longitude 106.7544 and an altitude of 218.79 m above sea level. Two experiments were conducted as part of this research, namely (1) the effects of PGPR strains on shoot and root characteristics of okra seedlings and (2) the effects of poultry manure and PGPR consortium on the growth of young okra plants.

First Experiment

The first experiment was conducted in a growth chamber and arranged in a completely randomized design with 8 bacterial strains and a control. The control contained distilled water as a medium with no bacterial strains. These treatments were then replicated three times, so as to obtain a total number of 27 treatments.

Bacterial Strains

The bacterial strains used in this study were *Methylobacterium sp.* (PC2T5), *Bacillus sp.* (39), *Bacillus methylotrophicus* (N2P4), *Flavobacterium tirrenicum* (M22), *Providencia stuartii* (M18), *Azotobacter vinelandii* 1CM), *Methylocystis parvus* (BGM3) and *PGPR Consortium* (collection of the Indonesian Soil Research Institute). These bacteria were grown on Nutrient Broth containing 10 $g.L^{-1}$ beef extract, 10 $g.L^{-1}$ peptone and 5 $g.L^{-1}$ sodium chloride. The cultures were incubated in a shaking incubator at 30°C until the optical density at 600 nm (OD_{600}) reached 0.6-0.8. Afterwards, the bacteria were spun at 10,000 rpm for 10 minutes at a temperature of 4°C. The supernatant was subsequently removed and the pellet washed using distilled water. The bacteria were again spun at 10,000 rpm for 10 minutes at a temperature of 4°C. The supernatant was again removed and the pellet resuspended in 1% Carboxymethyl cellulose solution. At this stage, the inoculants were ready to be added to the okra seedlings.

Root and Shoot Elongation Assay

The experiment used “Zahira” variety (red okra) seeds. These seeds were surface sterilized by soaking them in 1.0% sodium hypochlorite for 10 min and then thoroughly rinsed with sterile distilled water. The seeds were then placed in Petri dishes (30 seeds per Petri dish) containing filter paper and incubated in a dark room (28 ± 2°C) for three days. Afterwards, healthy and uniform seedlings were chosen, inoculated with bacterial strains and finally planted in tubes containing a nitrogen-free semi solid Jensen medium (3.5 g agar per L). Each liter of Jensen media contains 20 g sucrose, 1 g Na_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g NaCl, 0.1 g $FeSO_4.7H_2O$, 0.005 g Na_2MoO_4 and 2 g $CaCO_3$ (Jensen 1942). The tubes were kept in the growth chamber for 30 days with a 12 h photoperiod and were maintained at 28 ± 2°C and relative humidity of 76 %. At the end of the incubation, the tubes were opened and the plant root and shoot lengths were measured.

Second Experiment

The second experiment was conducted in the greenhouse. Soil was taken from Cikarawang field located at latitude -6.5507 and longitude 106.7286 (5.9 km from the greenhouse). The soil was air dried, ground and sieved using a 3 mm x 3 mm wire mesh. The Poultry pmanure to be used in the experiment was also air dried and allowed to decompose for four weeks.

The experiment used a completely randomized factorial design with 8 treatments and 3 replicates. The first factor was the application rate of poultry manure, i.e. 0, 6, 12, and 18 $ton.ha^{-1}$, or equivalent to 0, 75, 150, and 225% of recommended rates based on Afandi (2016). The second factor was the PGPR consortium (with or without PGPR). Each treatment consisted of 6 polybags. Firstly, 2 kg of well prepared unsterlilized soil was added in each polybag (12 cm diameter and 10 cm height), followed by the application of the poultry manure.

The variables measured were plant height and number of leaves on the 1st, 2nd, 3rd and 4th week after planting; while fresh weight, root length, lateral root length, dry weight and root structure were observed on the 4th week after planting. Fresh weight was determined by weighing the plants immediately after harvesting, whereas plant biomass was attained by oven-drying the plants at 70^{0}C for 3 days until a constant weight was achieved. Data were analysed by Analysis of variance (ANOVA) and Duncan’s multiple range test (DMRT) was used to assess the significance of differences between mean values of the samples.

Results and Discussion

Experiment 1: The effects of PGPR strains on shoot and root characteristics of okra seedlings

Number of Leaves

The bacteria strains did not significantly affect leaf number at 1 to 3 weeks after planting (WAP), but significant effects were observed at 4 WAP (Figure 1 and 2A). During the fourth week after planting, PGPR consortium and 1CM resulted in the highest number of leaves (4.3 each), 18.0% higher compared to the control. The high number of leaves in plants treated by PGPR consortium may have been due to the additive effect of all bacteria which provided essential phytohormones for the growth of leaves. Similarly, the greater number of leaves in plants treated with *A. vinelandii* (1CM) was likely caused by its ability to fix nitrogen. This was also documented by Rafique et al., (2018) who reported that, the greatest number of okra leaves was recorded following seed inoculation with *Azotobacter* sp.

Figure 1. Effect of various strains of PGPR on okra leaf number at 30 day after planting; control (1), PC2T5 (2), 39 (3), N2P4 (4), BGM3 (50, M18 (6), PGPR consortium (7), 1CM (8), and M22 (9)

Height of Plants

All treatments with PGPR inoculation showed a significant increase in plant height during all four weeks of observation relative to the control (Figure 2B). During the fourth week after planting PGPR consortium application resulted in the tallest plant height with an increase in height of 44.92% compared to that of control. The increase in the height of plants treated with PGPR consortium may have been due to its high concentration of indole acetic acid along with other phytohormones. Research conducted by Rupaedah et al. (2014) indicated that, among 144 rhizobacteria screened from the rhizosphere of sorghum only 25 increased the growth of sorghum, including which is *M. senegalense* which had the highest concentration of IAA. Generally, PGPR can directly produce auxin through a precursor compound found in root exudates known as tryptophan. In the other hand, PGPR indirectly modify the levels of auxin in the plant by the production of secondary metabolites that interfere with auxin biosynthesis pathway (Vacheron et al., 2013).

Root Structure

Almost all of the treatments showed positive effect in terms of lateral root length upon seed inoculation of okra in comparison to the control. In all of the 8 treatments, PGPR strain N2P4, PC2T5, M22 and PGPR consortium resulted in the greatest lateral root length (Figure 3a). The large total amount of lateral roots in okra plant inoculated with *B. methylotrophcu*s N2P4 (Figure 3B) may have been due to *B. methylotrophcu*s producing high amounts of indole acetic acid (61.99 ppm) which in turn promotes lateral roots growth as also indicated by Pratiwi et al. (2019). This was also documented by Vejan et al. (2016), who suggested that high levels of IAA stimulate lateral root growth and reduce primary root length of a plant. The increased number of lateral roots relative to the control in the other plants could have been due to the effects of PGPR which stimulates the production of different phytohormones (like IAA and cytokinins) as well as metabolic compounds which play an important role in increasing the number of lateral roots.

Experiment II. Effect of poultry manure and PGPR consortium on the root and shoot of okra plants

Number of Leaves

The number of leaves did not differ significantly between the treatments during the first week of observation. However, during the second week after planting, treatment of 12 ton per ha and 18 ton per ha resulted in a greater number of leaves with an 8.33% increase in leaves relative to the control (Table 1).

The results of the analysis showed an interaction between poultry manure and PGPR on number of leaves at 3 WAP (Table 2). The increased number of leaves among treatments with poultry manure enriched with PGPR could have been due to the improved soil structure resulting from the presence of poultry manure which in turn may have boosted the growth improving ability of PGPR. Similar results were previously reported by Tswanya et al. (2017) that poultry manure significantly increased number of leaves in okra.

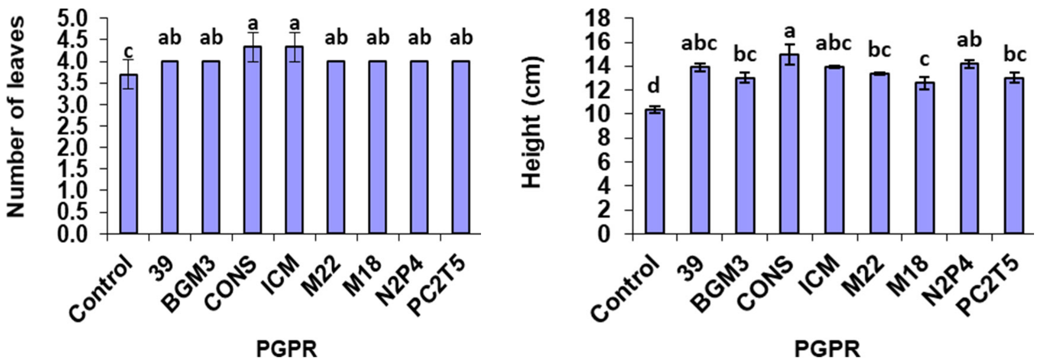
Height of the Plants

The height of plants showed no significant difference between the different poultry manure application rates (6, 12, and 18 $ton.ha^{-1}$) during the first and second week after planting. Noticeable differences were observed during the third and fourth week after planting, where 12 and 18 $ton.ha^{-1}$ resulted in the maximum height (Table 3).

Table 1. Impact of poultry manure and PGPR consortium on the number of leaves of okra

Treatment	Plant age (WAP)		
	1	2	4
Poultry manure (ton.ha^{-1})			
0	2.9 ± 0.1 a	3.6 ± 0.3 b	4.8 ± 0.3 c
6	3.0 ± 0.0 a	3.8 ± 0.1 ab	5.1 ± 0.3 b
12	3.0 ± 0.0 a	3.9 ± 0.2 a	6.0 ± 0.2 a
18	3.0 ± 0.0 a	3.9 ± 0.2 a	5.8 ± 0.3 a
PGPR consortium			
Without	2.9 ± 0.1 a	3.7± 0.2 a	5.4 ± 0.6a
With	3.0 ± 0.0 a	3.9 ± 0.2 b	5.5 ± 0.5a
Poultry manure	ns	ns	**
PGPR consortium	ns	*	ns
Poultry manure x PGPR consortium	ns	ns	ns

Note: Values followed by different letters in the same column are significantly different according to Duncan's multiple range test (DMRT) at α = 5%, ns = not significantly, * = significant (at α = 5%), ** = very significant (α = 1%) WAP = week after planting



[A] [B]

Figure 2. The effect of different strains of PGPR on okra's number of leaves and plant height during the 4th week after planting

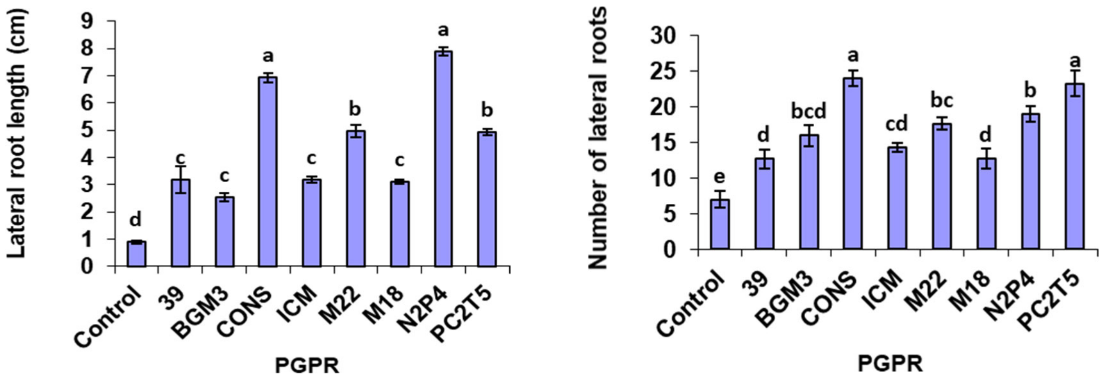


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Figure 3. The effect of different strains of PGPR on okra's root structure during the 4th week after planting

Table 2. The Interaction effect of PGPR and Poultry manure on number of okra leaves at 3 week after planting

Poultry manure rates (ton.ha^{-1})	Without PGPR	With PGPR	Average
0	4.20 ± 0.17 c	4.80 ± 0.50 ab	4.50 ± 0.50
6	4.60 ± 0.23 bc	4.60 ± 0.23 bc	4.60 ± 0.20
12	4.70 ± 0.01 ab	5.00 ± 0.01 a	4.90 ± 0.20
18	4.80 ± 0.17 ab	4.60 ± 0.23 bc	4.70 ± 12.00

Note: Values followed by different letters are significantly different based on DMRT at α 5%

The significant increase of okra plant height due to poultry manure during the third week after planting was similar to the one reported by Ali et al*.,* (2014) which indicated an increased height of okra plants treated with poultry manure compared to other organic manures. Nevertheless, a weekly decrease in the growth rate (15.04%, 10.42%, 7.89%, respectively) in inoculated plants was observed and may have been caused by the competition and predation of other microorganisms such as protozoa and nematodes. According to Martínez-Viveros et al*.* (2010), non-sterile soil will show a weekly decrease in terms of magnitude of growth as a result of a reduced bacterial population caused by competition with other microorganisms. This decrease in population will continue until the bacterial population reaches an equilibrium with the environment. Moreover, our results showed an interaction between poultry manure and PGPR consortium at 4th week after treatment, where application of 18 ton per ha enriched with PGPR resulted in a slightly decreased plant height in comparison to 12 ton per ha (Table 4, Figure 4). The decrease in the height of plants due to the application of 18 ton/ ha together with PGPR consortium was likely due to the availability of large amounts of nutrients which was greater than the optimal level for the normal growth of the okra plants. According to Taiz and Zeiger (2002) the growth or yield of the plant is directly related with the increase in the availability of nutrients. But a point will be reached where a further increase in nutrients will no longer correlate with the growth or yield of the plant but it will only be reflectefd in the rise of nutrient's concentration within the tissues. When the nutrients levels are further increased beyond the critical nutrients concentration of tissues, the decline in yield or growth will occur as a result of toxicity. Similar results were obtained by Gashua et al. (2014) who suggested that, application of 15 t.ha^{-1} of poultry manure was not optimal for the growth of okra.

Dry Weight and Fresh Weight

The results of the analysis of variance indicated an interaction between poultry manure and PGPR consortium in terms of the dry weight of roots and shoots of okra even though no interaction was observed in terms of fresh weight of the roots and shoots (Table 5 and 6). The use of poultry manure in combination with PGPR consortium increased the

Table 3. Impact of poultry manure and PGPR consortium on the height of okra plant

Treatment	Plant age (WAP)		
	1	2	3
Poultry manure (ton.ha^{-1})			
0	10.10 ± 2.20 b	12.20 ± 1.90 b	16.20 ± 2.60 c
6	12.50 ± 1.40 a	15.60 ± 1.30 a	19.60 ± 1.00 b
12	13.10 ± 0.90 a	16.40 ± 1.10 a	21.60 ± 1.00 a
18	12.90 ± 0.8 0a	16.40 ± 1.20 a	21.60 ± 1.30 a
PGPR consortium			
Without	11.30 ± 1.80 b	14.40 ± 2.20 b	19.00 ± 2.80 b
With	13.00 ± 1.50 a	15.90 ± 2.10 a	20.50 ± 2.60 a
Poultry manure	**	**	**
PGPR consortium	**	**	*
Poultry manure x PGPR consortium	ns	ns	ns

Note: Values followed by different letters in the same column are significantly different according to Duncan's multiple range test (DMRT) at α = 5%, ns = not significantly, * = significant (at α = 5%), ** = very significant (α = 1%) WAP = Week after planting root and shoot dry weights of okra by 236.36% and 333.33% relative to the control respectively (Table 6). Increased dry weight in inoculated plants could have been due to the easy solubilization and availability of the nutrients. Similarly, Rafique et al. (2018) reported a significant increase in root dry weight of okra inoculated with various PGPR compared to un-inoculated okra plants

Root Structure

Primary root length was improved *via* the application of poultry manure enriched with PGPR (Figure 5). Regarding the lateral root length, inoculated plants had more lateral roots with a long length compared to uninoculated plants. Importantly, the impact of poultry manure on the number and length of lateral roots increased as the rate of manure increased.

Generally, the root structure of inoculated okra exhibited a long primary root with numerous lateral roots, while the uninoculated okra showed a reduced primary root a lower number of lateral roots. We posit that these effects were due to PGPR interacting positively with poultry manure, which provided essential nutrients and different phytohormones for the growth and shaping of okra roots (Bhattacharyya and Jha, 2012).

Conclusions

Inoculation of okra with plant growth promoting rhizobacteria was shown to have an impact on root structure and growth of okra. Inoculation of okra with PGPR improved the number of lateral roots by 242.86% as well as lateral root length by 777.79%. Moreover, our study indicated that all of the PGPR strains used exhibited some positive effects relative to the control, but inoculation with *Bacillus methylotrophicus* and PGPR consortium showed the most positive effects. Interestingly, the use of 150% the recommended rate of poultry manure (12 ton.ha^{-1}) enriched with PGPR increased the root and shoot dry weight values of okra plant by 236.36% and 333.33% respectively. This could be very useful to

Table 4. The interaction effects of PGPR and poultry manure on okra plant height at 4 weeks after planting

Poultry manure rates (ton.ha^{-1})	Without PGPR	With PGPR	Average
0	18.50 ± 1.01 e	20.67 ± 3.36 de	19.6 ± 2.50
6	22.93 ± 0.84 cd	24.63 ± 0.93 bc	23.8 ± 1.20
12	25.80 ± 0.56 b	32.77 ± 2.22 a	29.3 ± 1.60
18	25.83 ± 1.40 b	26.33 ± 0.90 b	26.1 ± 1.10

Note: Values followed by different alphabets are significantly different based on DMRT

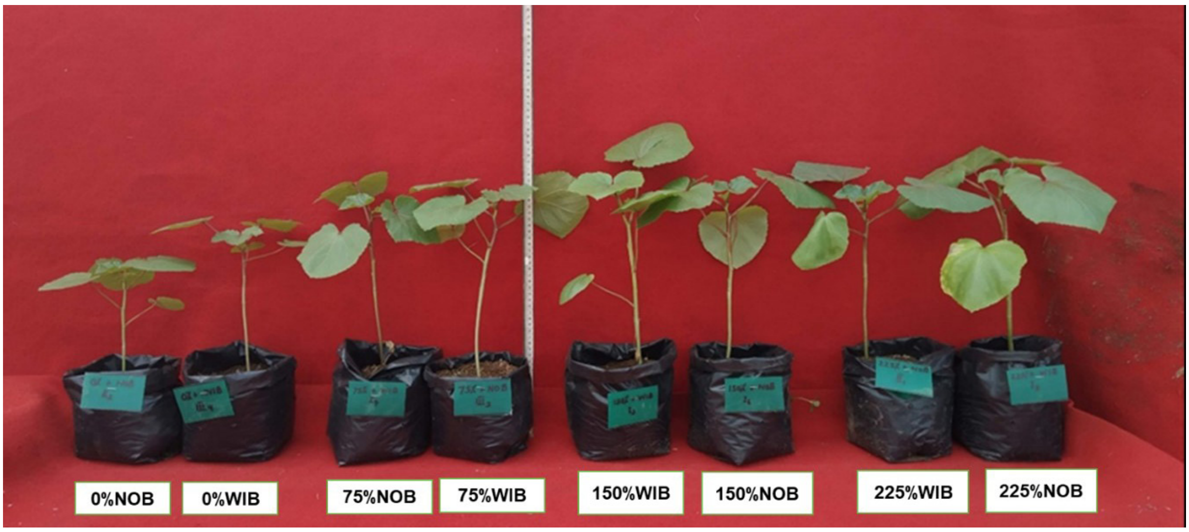

Figure 4. The effect of poultry manure and PGPR on the growth of okra plant

Table 5. Impact of poultry manure and PGPR consortium on the fresh weight of roots and shoots of okra plants

Treatment	Shoot fresh weight (g)	Root fresh weight (g)
Poultry manure (ton.ha^{-1})		
0	6.97 ± 0.94 c	0.50 ± 0.06 c
6	10.74 ± 1.51 b	0.65 ± 0.07 b
12	17.78 ± 2.29 a	1.23 ± 0.14 a
18	17.51 ± 2.75 a	1.18 ± 1.18 a
PGPR consortium		
Without PGPR	12.29 ± 4.64 b	0.80 ± 0.34 b
With PGPR	14.21 ± 5.45 a	0.96 ± 0.37 a
Poultry manure	*	*
PGPR consortium	**	**
Poultry manure x PGPR consortium	ns	ns

Note: Values in the same column followed by different letters are significantly different based on DMRT at α 5%

Table 6. The effect of interaction between poultry manure rates and PGPR on dry weight of shoots and roots of okra plants

Poultry manure rates (ton.ha^{-1})	Without PGPR	With PGPR	Average
	Dry weight of shoot (gram)		
0	1.02 ± 0.09 d	1.13 ± 0.18 d	1.07 ± 0.14
6	1.35 ± 0.09 cd	1.42 ± 0.22 cd	1.39 ± 0.15
12	2.68 ± 0.98 b	4.42 ± 0.02 a	3.55 ± 1.14
18	1.99 ± 0.40 bc	2.25 ± 0.14 b	2.12 ± 0.30
	Dry weight of root (gram)		
0	0.11 ± 0.03 e	0.13 ± 0.04 e	0.12 ± 0.02
6	0.14 ± 0.03 de	0.16 ± 0.07 cde	0.15 ± 0.02
12	0.21 ± 0.03 c	0.37 ± 0.05 a	0.29 ± 0.09
18	0.18 ± 0.06 cd	0.26 ± 0.03 b	0.22 ± 0.06

Note: Means followed by different alphabets are significantly different based on DMRT at α 5%

Figure 5. Effect of poultry manure and PGPR consortium on the root structure of okra plant at 4 weeks after planting.

the farmers as the increased number of roots as well as the biomass of the plant might also improve okra production.

Acknowledgements

The experiments were partly funded by Etty Pratiwi from the Indonesian Soil Research Institute who supplied some PGPR strains and supporting materials for the greenhouse and growth chamber experiment. Moreover, thanks and gratitude are given to all technical staff of Indonesian Soil Research who provided supportive technical assistance in various ways.

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