Engenharia Agrícola

ISSN: 1809-4430 (on-line)

www.engenhariaagricola.org.br

Scientific Paper
Doi: http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v42n5e20200209/2022

POTENTIAL OF *Rhizobium sp.*I_3 AND *Agrobacterium sp.*I_{37} IN FORMATION OF ROOT NODULES AND STIMULATING GROWTH OF *Arachis hypogaea* L.

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KEYWORDS

biofertilizer, PGPR, Rhizobacteria, nitrogen-fixing bacteria.

ABSTRACT

Rhizobium sp.I_3 and *Agrobacterium* sp.I_{37} bacteria can increase plant growth and have the potential to become a member of the plant growth-promoting rhizobacteria (PGPR). *Rhizobium* bacteria can increase nitrogen availability for legume plants by forming nodules. *Rhizobium* sp. and *Agrobacterium* sp. are closely related; therefore, we speculated that *Agrobacterium* sp. can also form nodules. The study was based on completely randomized design and conducted from July 2019 to December 2019 in laboratories of the Faculty of Agriculture, Universitas Sebelas Maret, Central Java, Indonesia. The study included 11 different treatments of biofertilizer: P0, P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, and P11 with each treatment in triplicate to obtain 36 experimental units. The results showed that the control treatment (P0) had 0 nodules, 8.26 g biomass, and 3.54% tissue nitrogen uptake. The three best treatments were in the order: P4 (*Rhizobium* biofertilizer) with 174.3 root nodules, 14.77% tissue nitrogen (four times higher than that of control), and 24.05 g biomass; P8 (*Rhizobium* biofertilizer) with 167 nodules, 12.42% tissue nitrogen (three times higher than that of control), and 19.26 g biomass; P5 (*Agrobacterium* biofertilizer) with 146.6 root nodules, 13.15% tissue nitrogen (three times higher than that of control), and 16 g biomass.

INTRODUCTION

Rhizobacteria have a symbiotic relationship with plant roots, particularly bacteria get nutrients provided by the plants, and rhizobacteria are capable of nitrogen fixation, phosphate solubilization, decomposition, bioremediation, and overcoming pathogenic stress. These bacteria are commonly grouped as plant growth-promoting rhizobacteria (PGPR) (Zafar-Ul-Hye et al., 2019). *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} bacteria can stimulate plant growth and are potential members of PGPR group (Rosariastuti et al., 2013). *Rhizobium* sp. bacteria can increase nitrogen availability for legumes by forming nodules (Damanhuri et al., 2020). As *Rhizobium* sp. and *Agrobacterium* sp. are closely related (Mousavi et al., 2014), *Agrobacterium sp.* bacteria are also expected to have the ability to form nodules. Based on their potential, *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} can be used as functional bacteria for biological fertilizers. Biofertilizers contain live microbes that colonize the rhizosphere or parts of plants and increase plant growth (Kour et al., 2020). Biofertilizer should have a good carrier material that act as a medium for microbes to survive. Good carrier materials increase microbe viability and increase plant growth when biological fertilizers are used (Paungfoo-Lonhienne et al., 2019). One of the carrier materials used is peat, which has sufficient moisture for the growth of microbes and sufficient permeability for air and water exchange (Prihastuti, 2013). Peat can be combined with modified cassava flour (mocaf) to provide good nutrition to microbes. Mocaf is tapioca flour that is processed by fermentation. The solid and liquid waste of mocaf flour industry, which also contain high nutrition, was used as a bacterial carrier in this study. Moreover, using mocaf

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Area Editor: Edna Maria Bonfim-Silva
Received in: 12-11-2020
Accepted in: 9-16-2022

waste overcomes waste disposal problem of tapioca flour industry (Rosariastuti et al., 2017). Manure and artificial media that have the potential to support the growth of live bacteria can also be used for carrier combination (Rosariastuti et al., 2018). This study aims to evaluate the potential of *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} in producing nodules to stimulate the growth of peanut plant.

MATERIAL AND METHODS

Study area

This study was conducted from July 2019 to December 2019 in Screen House, Universitas Sebelas Maret. Analysis of soil samples and plant samples were carried out in the Laboratory of Soil Biology and Biotechnology, Laboratory of Chemistry and Soil Fertility, Universitas Sebelas Maret.

Experimental design

The study was based on completely randomized design (CRD), in which each of 11 treatments was applied in triplicates to obtain 36 experimental units.

Treatments	Description
P0	Soil + without inoculum and carrier
P1	Soil + *Rhizobium* sp.I3 with no carrier
P2	Soil + *Agrobacterium* sp.I37 with no carrier
P3	Soil + without inoculum with peat 75% + mocaf 25% + 70 g wheat flour per kg carrier
P4	Soil + *Rhizobium* sp.I3 with peat 75% + mocaf 25% + 70 g wheat flour per kg carrier
P5	Soil + *Agrobacterium* sp.I37 with peat 75% + mocaf 25% + 70 g wheat flour per kg carrier
P6	Soil + without inoculum with peat 75% + manure 25% + 70 g wheat flour per kg carrier
P7	Soil + *Rhizobium* sp.I3 with peat 75% + manure 25% + 70 g wheat flour per kg carrier
P8	Soil + *Agrobacterium* sp.I37 with peat 75% + manure 25% + 70 g wheat flour per kg carrier
P9	Soil + without inoculum with peat 50% + 50% Luria-Bertani liquid + 70 g wheat flour per kg carrier
P10	Soil + *Rhizobium* sp.I3 with peat 50% + 50% Luria-Bertani liquid + 70 g wheat flour per kg carrier
P11	Soil + *Agrobacterium* sp.I37 with peat 50% + 50% Luria-Bertani liquid + 70 g wheat flour per kg carrier

Procedure

1. Propagation of bacterial isolates

Pure isolates of *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} were inoculated into two test tubes containing Luria-Bertani (LB) agar. The isolates were then incubated for three days and then transferred to liquid LB medium.

2. Production of bacterial inoculum

Each of the two test tubes containing LB agar medium was inoculated into 300 ml of liquid LB, and then agitated using shaker at a speed of 55 rpm for five days to achieve a bacterial density of 10^{11} cells.ml^{-1}, as analyzed using hemocytometer. Bacterial inoculum at a density of 10^{11} cells.ml^{-1} was added to the carrier. There should be at least 10^7 live bacterial cells.ml^{-1} in the carrier when applied to plants according to the quality standards established in the Regulation of the Minister of Agriculture Number 70 of 2011 related to Organic Fertilizers, Biological Fertilizers, and Soil Repairers.

3. Carrier preparation and sterilization process

Peat, manure, and mocaf were air-dried using a 0.05-mm soil sieve. LB was used as the liquid carrier. The ingredients were mixed according to different treatment combinations and put into plastic bags that were tightly closed using rubber to minimize contamination. Sterilization of the carrier was carried out using an autoclave at a temperature of 121 °C for 60 min.

4. Carrier inoculation process

A total of 5 ml of inoculant (10^{11} cells.ml^{-1}) was put into a plastic bag containing 50 g of carrier material for the treatments containing *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} bacteria. The carrier material was mixed until aseptically homogeneous and tightly closed using a rubber band to minimize contamination and labeled. The carrier was stored in a box at room temperature (25 °C) and incubated for approximately one week for bacterial adaptation.

5. Preparation and sterilization of planting media

The soil Alfisol (360 kg) used in this study was obtained from the laboratory of the Faculty of Agriculture Jumantono 07 ° 37 '50.2 "LS and 110 ° 56' 53.8" East Longitude. The soil was drained and sieved through a 0.05-mm sieve. Five kilogram of soil was put into a plastic bag and then sterilized using an autoclave at a temperature of 121 °C for 60 min.

6. Planting

A polybag measuring 20 cm × 40 cm was filled with 5 kg of Alfisol, and then 50 g biological fertilizer with carrier at a bacterial density of 10^{11} cells.g^{-1} was added for each treatment. The soil was left for three days for bacterial adaptation, and groundnut seeds were then planted. Watering was done once a day in the morning or evening; plant height was measured once a week. Planting was carried out for three months.

7. Harvest

Harvesting was done when peanut plant entered the generative phase (3 months). Soil samples were taken before the plants were harvested, and nodules were calculated at harvest time. The plant samples were separated from the roots and shoots. Soil and plant samples were dried and then analyzed in the laboratory.

Observation parameters and laboratory analysis

Observation parameters included cation exchange capacity (CEC) (ammonium treatment method), pH (electrometric method), determination of soil nitrogen (Kjeldahl method), organic carbon (C-organic; Walkley-Black method), total colonies of *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} (Total Plate Count method), and tissue nitrogen (distillation), plant height, and plant biomass.

Data Analysis

Data were statistically analyzed using ANOVA (95% significance level) followed by Duncan's Multiple Range Test (DMRT) (95% significance level). Correlation test was performed to understand the relationship between observed parameters.

RESULTS AND DISCUSSION

Initial soil characteristics

Based on the initial analysis, the content of nutrients were: Total Nitrogen: 0.06%; Total Phosphorus: 6.49 mg.100g^{-1}, and Total Potassium: 9.71 mg.100g^{-1}. Macro-essential nutrients such as N, P and K were observed to have very low values in Alfisol soil, indicating reduced fertility of Alfisol soil, which is also caused by high pH. Alfisol soil is a soil that is generally poor in both macro and micro nutrients (Awanish et al., 2015). Moreover, the soil has a very low organic carbon value (0.15%), which is due to small amount of organic matter in the soil, according to Hati et al. (2008). The initial CEC had a moderate value: 23.05 me.100g^{-1} soil, which is consistent with the findings of Adeyemo et al. (2019), who demonstrated that low CEC value of Alfisol was due to low content of organic matter. The initial pH was slightly alkaline with a value of 8.1. Although Alfisol is good for the growth of food plants including peanut plant, but its high pH value (7.9 to 8.2) resulted in very low N, P, K, Fe, and S contents in Alfisol soil (Chinnadurai et al., 2014).

Plant characteristics

TABLE 1. Plant characteristics (mean value).

Treatment	Tissue nitrogen (%)	Nodule count	Plant height (cm)	Plant biomass (g)
P0	3.54^{a}	0^{a}	24.5^{a}	8.26^{a}
P1	7.58^{bc}	161^{b}	28.00^{b}	12.69^{ab}
P2	5.39^{abc}	80.6^{ab}	28.67^{b}	10.31^{ab}
P3	7.35^{bc}	0^{a}	33.17^{c}	12.26^{ab}
P4	14.77^{e}	174.3^{b}	37.00^{d}	24.05^{d}
P5	13.15^{e}	131.3^{b}	37.17^{d}	22.54^{d}
P6	6.9^{abc}	0^{a}	35.50^{cd}	15.39^{bc}
P7	11.72^{de}	146.6^{b}	43.50^{e}	16^{bc}
P8	12.42^{e}	167^{b}	40.50^{e}	19.26^{cd}
P9	4.25^{ab}	0^{a}	32.00^{c}	12.45^{ab}
P10	8.73^{cd}	148.3^{b}	34.50^{cd}	14.03^{bc}
P11	12.41^{e}	114.3^{b}	33.25^{c}	16.13^{bc}

Tissue Nitrogen

According to ANOVA results, biofertilizer treatment had a significant effect on tissue nitrogen levels (Table 1). DMRT results revealed that the highest value of tissue nitrogen (14.77%) was in the treatment P4, which was higher by 317% than that in the treatment P0 (control; 3.54%) with the lowest level of tissue nitrogen. Moreover, DMRT results revealed that tissue nitrogen in the treatment P4 was not significantly different from that in the treatments P5, P7, P8, and P11. The high level of tissue nitrogen was due to sufficient availability of soil nitrogen to meet plant requirements (Agus et al., 2019). *Rhizobium* bacteria improve plant growth and development through physiological and morphological changes in roots via formation of root nodules (Prell & Poole, 2006). Increase in the number of active nodules increases the amount of nitrogen available for plants and thus increases plant growth. According to Hauggaard-Nielsen & Jensen (2001) soil nitrogen can produce more protein, the higher the availability of nitrogen the faster the conversion of carbohydrates into proteins, and increase in vegetable proteins in the protoplasm increases nitrogen levels in plant tissues.

Tissue nitrogen parameter was positively and closely related ($p < 0.05$, $r \geq 0.5$) with total colonies of *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37}, root nodules, dry weight, plant height, soil nitrogen, and CEC, but negatively correlated with pH. Nitrogen level in plant tissue was influenced by the availability of nitrogen in the soil. *Rhizobium* bacteria are able to provide nitrogen through fixation of nitrogen from the air. Increase in the available nitrogen content in the soil can lead to increase in the plant height and plant biomass (Alam et al., 2015).

Nodule formation

According to ANOVA results, biofertilizer treatment had a significant effect on the number of nodules (Table 1). DMRT results revealed that the highest count (174.3) was in the treatment P4. Furthermore, nodule count in the treatment P4 was not significantly different from that in the treatments P1, P2, P5, P7, P8, P10, and P11. The highest number of root nodules in the treatment P4 was due to incorporation of mocaf waste material as the basic ingredient in biofertilizers, which has high nutrition to support the growth of *Rhizobium* bacteria in the soil. Nodule count in the treatment P8 was lower than that in P4 because it was composed of manure as the carrier material,

which has lower nutrition than that of mocaf. The lowest values were observed in the treatments P0, P3, P6, and P9 because they lacked bacterial inoculation.

Rhizobium sp.I_3 and *Agrobacterium* sp.I_{37} have been shown to form nodules on peanut roots. Cummings et al. (2009) demonstrated that the IRGB74 bacteria *Rhizobium* (*Agrobacterium*) can effectively form root nodules. This is due to the 99% similarity in 16S rRNA gene sequences for *Rhizobium* and *Agrobacterium* bacteria. Kondorosi et al. (2013) showed that the increase in root nodules is caused by the use of legin to increase *Rhizobium* bacteria in the soil and compost functions to provide environmental conditions supportive to the growth of *Rhizobium* bacteria. *Rhizobium* bacteria form effective symbiosis with plant roots.

Root nodule parameter was positively correlated ($p < 0.05$, $r \geq 0.5$) with the total colonies of *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37}, plant biomass, and soil nitrogen. Root nodules are formed by symbiosis between plants and bacteria. Addition of *Rhizobium* bacteria inoculum increased the number of root nodules in peanut plant. According to Masciarelli et al. (2014), the use of legume nodule bacteria inoculum during planting greatly increased the number of nodules on soybean plants. Saharan & Nehra (2011) demonstrated that bacterial activity in biological fertilizers resulted in the formation of nodules on plant roots, which can affect the nitrogen content in the soil and plants, resulting in increase in plant growth.

Plant height

According to ANOVA results, biological fertilizer treatment had a significant effect on plant height (Table 1). Plants exposed to the treatment P7 exhibited the highest plant height of 43.5 cm, which was 77% more than that of the control. DMRT results revealed that the treatment P7 was not significantly different from that of the treatment P8 (*Agrobacterium* sp.I_{37} with peat 75% + manure 25% + 70 g wheat flour per kg of carrier), but significantly different from those exposed to other treatments. The lowest plant height was in the control treatment (P0). These results showed that cow manure helped in the growth of peanut plant; manure acts as an organic fertilizer and improves physical properties of the soil to hold water longer and retain soil moisture and support the initial growth phase of plants, particularly plant height (Cai et al., 2019). The interaction between composting and *Rhizobium* has a significant effect on increasing the average plant height. *Rhizobium* inoculants significantly increased the growth and production of legumes compared with legumes without being provided *Rhizobium* bacteria (Tan et al., 2014).

Plant height parameter was positively correlated and closely related ($p < 0.05$, $r \geq 0.5$) with plant biomass, tissue nitrogen, and CEC but negatively correlated with soil pH. Organic matter and nitrogen-fixing bacteria affect plant growth (Widawati & Suliasih, 2019). In addition, according to a study by Rosariastuti et al. (2018) an increase in plant height followed by an increase in plant dry weight indicates good photosynthesis. Therefore, the higher the plant growth/height, the higher the dry weight or biomass of the plant.

Plant biomass

According to ANOVA results, biological fertilizer treatment had a significant effect on plant biomass (Table 1). DMRT results revealed that the highest plant biomass (24.05 g) was in the treatment P4 (*Rhizobium* sp.I_3 with peat 75% + mocaf 25% + 70 g wheat flour per kg of carrier), which was 191% more than that of the control. Furthermore, plant biomass after treatment P4 was not significantly different from that after treatments P5 (*Agrobacterium* sp.I_{37} with peat 75% + mocaf 25% + 70 g wheat flour per kg of carrier) and P8 (*Agrobacterium* sp.I_{37} with peat 75% + manure 25% + 70 g wheat flour per kg of carrier). The lowest biomass value was observed in the control treatment (P0). *Rhizobium* can form symbiosis with nut plants by fixing nitrogen from air. In plants, nitrogen increases the leaf size and protein content, leading to increase in dry weight of the plant (Colla et al., 2017).

Plant biomass parameter was positively correlated ($p < 0.05$, $r \geq 0.5$) with nodule count, plant height, tissue nitrogen, and CEC. According to Korir et al. (2017), *Rhizobium* treatment could lead to increase in root dry weight, thus increase in plant biomass. Purwaningsih et al., (2019) also stated that *Rhizobium japonicum* could cause increase in dry weight of soybean plant and formation of effective nodules. This showed that the formation of effective nodules contributed to the growth of soybean plant through nitrogen fixation by *Rhizobium japonicum*.

Final soil characteristics

TABLE 2. Soil characteristics (mean value).

No.	Treatment	Soil nitrogen (%)	Organic carbon (%)	CEC (me.100g^{-1} soil)	pH	Total bacterial colonies (CFU.ml^{-1})
1	P0	0.07^{a}	0.6^{ab}	27.92^{a}	7.9^{d}	0^{a}
2	P1	0.16^{de}	0.57^{a}	28.7^{a}	7.65^{d}	8.304^{d}
3	P2	0.13^{cd}	0.56^{a}	29.76^{a}	7.75^{d}	7.509^{b}
4	P3	0.12^{bc}	0.76^{bc}	38.6^{b}	7.08^{c}	0^{a}
5	P4	0.22^{f}	0.73^{abc}	46.25^{e}	6.93^{bc}	8.615^{e}
6	P5	0.15^{de}	0.71^{abc}	39.17^{bc}	7.04^{bc}	10.406^{g}
7	P6	0.1^{ab}	0.79^{c}	39.06^{bc}	7.01^{bc}	0^{a}
8	P7	0.17^{e}	0.63^{abc}	42.24^{cd}	7^{bc}	12.433^{i}
9	P8	0.21^{f}	0.65^{abc}	51.89^{f}	6.88^{bc}	7.901^{c}
10	P9	0.14^{cde}	0.77^{bc}	40.64^{bc}	7.06^{bc}	0^{a}
11	P10	0.22^{f}	0.62^{abc}	43.89^{de}	6.71^{ab}	12.243^{h}
12	P11	0.21^{f}	0.69^{abc}	46^{e}	6.51^{a}	9.001^{f}
Dignity*		Very low to low	Very low	Very high to high	Medium to alkaline	-

Note: *Dignity according to Balai Penelitian Tanah (2009).

Soil nitrogen

The total nitrogen content of soil in the control (P0) increased by 16% compared with that at the initial soil analysis. Based on ANOVA results, the treatment had a significant effect ($p < 0.01$) on the total nitrogen content of the soil (Table 2). DMRT results showed that the highest total soil nitrogen (0.22%) was in treatments P4 and P10, which was 214% more than that of the control and was not significantly different from that of treatments P8 and P11. The lowest value was found in the treatment control (P0), which was not significantly different from that of treatment P6.

Inoculation of *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} in peanut plant increases soil nitrogen content. According to Zhang et al. (2010) *Rhizobium* inoculation in legumes can increase total soil nitrogen and crop yields and improve seed quality. Rhizobacteria may induce root nodule formation, however, nodule effectiveness is dependent on multiple biotic and abiotic factors such as soil acidity, moisture, and temperature, availability of organic and inorganic compounds as nutrient source, and cell density of *Rhizobium* bacteria in the soil. Koten et al. (2012) demonstrated that the higher the nitrogen fixation activity by *Rhizobium*, the more the available soil nitrogen to be absorbed by plants. Nitrogen fixation are used by the host legume plants for growth and biomass.

Total soil nitrogen parameter was positively and closely related ($p < 0.05$, $r \geq 0.5$) with the total colonies of *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37}, root nodules, plant fresh weight, tissue nitrogen, and CEC, but negatively correlated with soil pH. According to Mengel et al. (2001) low pH inhibits multiplication of bacteria, development of root nodules, and process of nitrogen fixation. This is because intake of mineral nitrogen is inhibited, thus nitrogen activity and formation of root nodules are disrupted.

Organic carbon

Soil organic carbon content in the control (P0) increased by 300% compared with that at the initial soil analysis. According to ANOVA results, soil treatment had a significant effect on soil organic carbon content (Table 2). DMRT results revealed that the highest organic carbon content (0.79%) was in treatment P6, which was 31% more than that of the control, and the effect of treatment P6 on organic carbon content was significantly different from that of treatments P1, P2, and P3. The highest soil organic carbon was observed in treatments P4 and P5 for *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37}, respectively. The lowest value was found in treatment P2. Organic carbon content is influenced by incorporation of organic matter such as manure in the soil. According to Sharma & Bhushan (2001) addition of organic matter in the form of manure can increase soil organic carbon content. Cow manure had a significant effect on improvement of organic carbon in the soil (Darusman et al., 2019), which is due to decomposition of cow manure, which releases a number of carbon compounds. Carbon is the main constituent of the organic materials, therefore addition of cow manure increases organic carbon content of the soil. No parameter having close correlation ($p > 0.05$, $r \leq 0.5$) with soil organic carbon was identified.

Cation exchange capacity

Soil CEC in the control (P0) increased by 21% compared with that at the initial analysis. Based on ANOVA results, biofertilizer treatment had a significant effect on soil CEC levels (Table 2). DMRT results revealed that the highest CEC (51.89 $me.100g^{-1}$) was in the P8 treatment, which was 85% more than that of the control. Furthermore, CEC value after P8 treatment was significantly different from that after other treatments. The highest value of CEC was observed in treatments P4 and P8 for *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37}, respectively. The lowest CEC value (27.92 $me.100g^{-1}$) was observed in the control treatment P0, which was not significantly different from that of treatment P1, but significantly different from that of other treatments. Organic matter can increase soil CEC. In P8 treatment, organic matter was added in the form of peat and manure. According to a study by Baghaie et al., (2011) adding cow manure increases soil CEC. Addition of organic matter in the form of manure can increase organic carbon content in the soil, followed by an increase in soil CEC.

CEC parameter was positively correlated and closely related ($p < 0.05$, $r \geq 0.5$) with plant biomass, plant height, soil nitrogen, and tissue nitrogen. However, it has a negative correlation with soil pH. Addition of organic fertilizers increases CEC and availability of soil N, P, and K. According to a study by (Rosariastuti et al., 2018), increase in CEC is caused by the addition of soil organic matter. Increased number of microbes capable of forming organic acids can reduce the pH value (Song et al., 2018).

Soil pH

Soil pH in the control (P0) decreased by 2% compared with that at the initial soil analysis. According to ANOVA results, biofertilizer treatment significantly affected the soil pH levels (Table 2). DMRT results revealed that the highest pH value of 7.9 in the control treatment P0, which was not significantly different from that of P1 and P2 treatments, but significantly different from that of other treatments. The lowest pH value of 6.51 was observed in the P11 treatment, which was 17% lower than that of the control. pH of treatment P11 was not significantly different from that of treatment P10 but significantly different from other treatments. The lowest pH value was observed in treatments P10 and P11 for *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37}, respectively.

There was a decrease in pH from the initial value to the final value for most of the treatments, which was due to the addition of Rawa Pening peat that has been air drained and has a pH of approximately 4.8, according to Prihastuti (2013). Peat containing organic matter undergoes aerobic biodegradation process in the soil, forming organic acids such as humic and fulvic acids and thus has a significant impact on soil properties. According to Golabi et al. (2006), the process of biodegradation of organic matter is followed by the formation of organic acids, leading to decrease in the pH of the materials.

pH parameter had a negative correlation and was closely related ($p < 0.05$, $r \geq 0.5$) with plant biomass, plant height, soil nitrogen, tissue nitrogen, and CEC. Addition of organic matter can reduce pH, which may be due to the process of decomposing organic matter to produce organic acids. Addition of organic matter can increase or decrease soil pH, depending on the type of organic matter added.

Total colonies of bacteria

According to ANOVA results, biofertilizers had a significant effect on the number of bacterial colonies in the soil (Table 2). DMRT results revealed that the highest total bacterial colonies (271.10^{10} CFU.ml^{-1}) was observed for treatment P7. The highest total bacterial colonies for *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} was observed after treatments P7 and P5, respectively. According to (Rosariastuti et al., 2020) addition of *Rhizobium* bacterial inoculants can increase the total colonies of *Rhizobium* soil bacteria. In addition, soil organic matter and root exudates are source of nutrition for bacteria. Soil organic matter is the main energy source and has a major influence on the population of soil organisms. Higher content of organic matter leads to higher activity of soil microorganisms, which further increases soil fertility (Cheng et al., 2020). The lowest value (0 CFU.ml^{-1}) was observed in the control treatment (P0), which was not significantly different from that of treatments P3, P6, and P9. The control treatment had the least number of bacterial colonies because it was not provided bacterial inoculants.

Total bacterial colonies parameter for *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} was positively correlated ($p < 0.05$, $r \geq 0.5$) with root nodules, soil nitrogen, and tissue nitrogen. *Rhizobium* sp.I_3 and *Agrobacterium* sp.I_{37} populations affected the number of nodules formed on peanut root. With increase in nodule count, soil nitrogen increases through nitrogen fixation to meet the needs of the plant. According to El-Akhal et al. (2013), nitrogen input from N-fixing legumes, such as peanuts, is very important for agriculture. Peanuts are characterized by their high symbiotic N-binding capacity and the amount of nitrogen accumulated by fixation. Inoculation of peanuts with *Rhizobium* sp. has been shown to increase the growth of peanut plant (*Arachis hypogaea* L.)

CONCLUSIONS

Agrobacterium sp.I_{37} bacteria can form nodules in peanut plant similar to *Rhizobium* sp.I_3 bacteria and can increase plant growth through symbiotic fixation. The best results were observed for the treatment P4 with 174.3 root nodules, 14.77% tissue nitrogen uptake (four times higher than that of the control), and 24.05 g biomass.

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