

Effect of Three Different Nitrogen Rates and Three Rhizosphere N₂- Fixing Bacteria on Growth, Yield and Quality of Peanuts

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Abstract

Raising prices of organic fertilizers, environmental relation of their usage and need for global foodstuff, placed a topic of universal interest in cultivation of legume plants for human nutrients and soil fertility supplementation. Three rates of N fertilizer were studied in the field: 0, 20 and 40 kg N/ha. Three bacterial strains were identified and inoculated in the experiment: *Enterobacter asburiae* (*E. asburiae*), *Klebsiella quasipneumoniae* (*K. quasipneumoniae*) and *Enterobacter cloacae* (*E. cloacae*). The soil samples were taken before and after experiment for chemical attributes analysis. The effect of N₂ fertilizer and 3 bacterial strains was on soil chemical properties, yield components and yield of peanut plant. The results of present study showed that application of different N₂ fertilizer rates was a little influence on the N₂-fixing ability of rhizosphere bacteria, but high effect was significant at the peanut nodule number. In the 3 N fertilizer rates and 3 bacteria, which only had N₂ fertilizer rate of 40 kg N/ha and *E. asburiae* significantly raised the maximum yield component and yield of peanut, while there was the lowest yield component and yield of peanut was observed in N fertilizer rate application of 0 kg N/ha and *E. cloacae* inoculation. The differences in rhizosphere bacterium strains between different N fertilizer rates was better than that caused by the nitrogen fertilizer amount. This could be due to the substantial improvement in soil chemical attributes at different soils, especially in, pH, OM and total N, as these significantly impacted the soil structure.

Keywords: *E. asburiae*, *K. quasipneumoniae*, *E. cloacae*, N fertilizer, Peanut, Soil property, Yield

Introduction

Peanut (*Arachis hypogaea* L.) that is a key role for supplying the essentially nutrient resource for human, stands thirteenth in kinds of food crops, 4th in ranks oilseed and 3rd in ranks of protein resources for humans around the world [1]. Peanut gives greater productivity and quality when suitable cultivars are planted on soils with a good soil nutrients management system [2]. Nitrogen is the most important nutrient in all plant, due to its important roles in different biochemical and physiological processes of the crops [3]. According to prior study of Holl and Vose [4], presented protein concentration of low pea seed from N deficient soils. Tiller with poor soil N content (< 10 kg/ha) before planting, needed addition application of supplementary starter N for better pea productivity and quality [5]. McKenzie *et al.* [6], showed that when soil N content was lower than 20 kg N per ha, needed addition application of starter N fertilizer improved pea productivity. Conversely, the application of a high dose of N fertilizer could lead to the development of ineffective nodules [7]. In general, higher rate of N fertilizer negatively impacts nodulation of peanut crops [5,8-10].

The correlation between plants and other microorganism in the agricultural soil environment, which has composed a flora and soil fauna. These correlations determine the multiform characteristic of the soil microorganism, although these relationships are all not benefit for the crops. Rhizosphere N₂-fixing bacteria (RNFB), which have significantly found in the rhizosphere of crops colonize and correlated with plant roots. Although species of rhizobia exist, the term "rhizobacteria" has used only in the condition of benefit correlation and symbiotic N fixation in which the bacteria help in the growth of plant. This is reason why these RNFB are named plant growth promoting rhizobia (PGPR) [11,12]. PGPR are popularly used for poor nutrient and contamination soils [5]. Their benefit impacts in growth promoting have been researched during a variety of stress conditions such as salinity [12,13], drought [14], heat stress [15], metal toxicity [16]. From these studied results, PGPR are now used as bio-fertilizers, soil amendment, and rhizoremediators [17]. However, positive effects of PGPR on the crop growth and yield have been discovered for a long time, but the depth study of enhance mechanisms of plant growth have not been determined [18]. RNFB isolated from rhizosphere soil in Korea [19], India [20] and Tibet [21]. The plant growth promoting activity (PGPA) of RNFB was firstly discovered by Lee *et al.* [19], who showed that RNFB raised the *Xanthium italicum* growth [19], some trains, which have been discovered zinc solubility, raised the soybean yield by raising the mobilization and bio-fortification of zinc. Some RNFB strains have also been found for other positive abilities, such as the phosphatase solubility [22]. RNFB take N₂ and use atmospheric N₂ by means of biological N₂ fixation mechanism to alter N₂ from the air into inorganic

nitrogen of soil compounds, such as NH_3 , and promote the growth and yield of crops [23], sorghum [24], maize, wheat, cucumber [25]; switchgrass [26], oil palm [27] and others [28-30]. The main aim of this study could select the best train of 4 RNFB trains, which were isolated and identified to base their impacts on the growth and yield of peanut in order to use for the next studies.

Materials and methods

Bacteria

The root and nodule samples of peanut plants were collected from local farms of Phuoc Hung commune, An Phu, An Giang province, Vietnam. *E. asburiae*, *K. quasipneumoniae* and *E. cloacae* that were isolated and identified by genotypes of 3 RNFB from roots and nodules of groundnut Nodules in the central laboratory of An Giang University, used during this research. Three bacteria, which were identified through sequencing technology of 16S rRNA and phylogenetic position of *K. quasipneumoniae*, *E. cloacae* and *E. asburiae*, were determined by blasting the 16S rRNA sequence on NCBI. The similar rates of the 16S rRNA sequence of our target bacterium valued from 99.65 to 99.93 % of the 16S rRNA sequence of our target bacterium to these bacteria [31].

Table 1 Homology percent of 3 strains and accession number [31].

| Strains | References strains | Homology (%) | Accession number |
|---------|---------------------------|--------------|------------------|
| V1 | <i>E. asburiae</i> | 99.74 | 210223-230214291 |
| V2 | <i>K. quasipneumoniae</i> | 99.65 | 210223-230214292 |
| V3 | <i>E. cloacae</i> | 99.93 | 210223-230214293 |

Design and location of experiment

The experimental location was carried out in the research center of An Giang University from January to May of 2023. The studied soil was the poor nutrients (**Table 1**). Ground seeds were incubated by *E. asburiae*, *K. quasipneumoniae* and *E. cloacae* 1 day before sowing. Three N fertilizer rates and bacterial population of *E. asburiae*, *K. quasipneumoniae* and *E. cloacae* were presented in **Table 3**. This experiment was designed by 2 factors (3 bacterial strains and 3 N fertilizer rates), 9 treatments and 4 replications (**Table 3**). This experimentally main goal was to find the best effect of these bacteria and N fertilizer ratio on the soil nutrients and yield of peanut when planning in the nutrient poorness of crop soil (**Table 2**). NPK fertilizers were used by urea, di-ammonium phosphate and potassium chloride were shown in **Table 3**. The total area of field study was 72 m² (1×2 m²×04 repeats×09 treatments).

Early and last samples of experimental soil were collected 0 - 20 cm in the soil depth to analyze the soil chemical composition. Soil samples were used by methods of Carter and Gregoric, [32] to determine the physical - chemical composition of soil such as texture, total N, available P, exchangeable K, CEC and pH. Component yield, which were counted by growth time of groundnut had height and shoot number of each plant, No. of pods per plant, number of biomass, number of nodule per plant, weight of fresh nodule, fresh weight of fill and empty pods per plant (g). The fresh yield was recorded by tons/ha for fresh pods. The soil texture was the silt sandy, soil pH (6.39), CEC (1.09 cmol⁺/kg), total N (0.07 %), the available phosphorus was quite poor (2.84 mg/100 g). The available P, which is very essential element of *Rhizobia* and groundnuts, needs for the plant development and *Rhizobia* life. Especially, exchangeable K of experimental soil was undetected at all collected samples. In generally, experimental soil had such a poor nutrient composition and lacked potassium (**Table 2**). The peanut seeds, which bought from Loc Troi company, had good disease resistance and high yield. The tillable technology was followed by local farmers.

Table 2 Soil chemical properties before the experimental design (n = 5, 0 - 20 in soil depth).

| pH _{H2O} | Soil properties | | | | | Soil texture (%) | | |
|-------------------|-----------------|-----------------------------|-------------|------------------------|--|------------------|------|------|
| | OM (%) | CEC (cmol ⁺ /kg) | Total N (%) | Available P (mg/100 g) | Exchangeable K (Cmol ⁺ /kg) | Sand | silt | clay |
| 6.39 | 1.79 | 1.09 | 0.07 | 2.84 | undetected | 80.1 | 18.6 | 1.3 |

Table 3 experimental treatments.

| N fertilizers (kg/ha) | Three rhizosphere N ₂ -Fixing Bacteria (10 ⁸ CFU/mL) | | | P, K fertilizers (kg/ha) |
|-----------------------|--|---------------------------|-------------------|--------------------------|
| | <i>E. asburiae</i> | <i>K. quasipneumoniae</i> | <i>E. cloacae</i> | |
| 0 | inoculated | uninoculated | uninoculated | 60 P - 60 K |
| 20 | inoculated | uninoculated | uninoculated | |
| 40 | inoculated | uninoculated | uninoculated | |
| 0 | uninoculated | inoculated | uninoculated | |
| 20 | uninoculated | inoculated | uninoculated | |
| 40 | uninoculated | inoculated | uninoculated | |
| 0 | uninoculated | uninoculated | inoculated | |
| 20 | uninoculated | uninoculated | inoculated | |
| 40 | uninoculated | uninoculated | inoculated | |

Statistical analysis

The statistical data were analyzed by using Statgraphics software version XVIII. The 1-way and Multifactor ANOVA analysis of variance and was used to determine the data and the significance and compare LSD (standard deviation). A statistical data of $p < 0.05$ was considered significantly different value.

Results and discussion

The results of **Table 4** showed that average soil pH valued from 6.57 to 6.60 before the experiment and 6.68 to 7.07 after the experiment in 3 N₂ fertilizer rates and 3 bacterium strains. However, there were insignificant differences among treatments before and after the experiment at level 5 %. Furthermore, there were not an interaction between nitrogen and bacterium in all treatments. The unclear effects of soil pH on rhizosphere N₂-fixation by bacteria were studied [33]. This studied results showed that the N₂-fixing ability of rhizosphere bacteria was not governed by fluctuations oxidation-reduction of the soil, pH and organic matter [34].

Table 4 Soil pH before and after of experiment.

| Factors | Soil pH | |
|--|-----------------------|----------------------|
| | Before the experiment | After the experiment |
| Nitrogen (kg/ha) (A) | | |
| 0.00 | 6.59 | 6.87 |
| 20.0 | 6.58 | 6.68 |
| 40.0 | 6.59 | 7.13 |
| Rhizosphere N₂-fixing microbiome (B) | | |
| <i>E. asburiae</i> | 6.60 | 6.74 |
| <i>K. quasipneumoniae</i> | 6.57 | 6.86 |
| <i>E. cloacae</i> | 6.60 | 7.07 |
| F (A) | ns | ns |
| F (B) | ns | ns |

| Factors | Soil pH | |
|---------|-----------------------|----------------------|
| | Before the experiment | After the experiment |
| F (A×B) | ns | ns |
| CV (%) | 9.82 | 15.4 |

Note; ns is insignificant difference at p -value ≤ 0.05 .

Organic matter (OM) of soil

The results of **Table 5** presented that the pre-experimental organic matter of soil ranged from 1.45 to 1.50 %, and the statistically insignificant differences at level 5 %. However, the content of organic matter in the experimental soil after 3 months of N fertilizer application, and RNFB inoculant, which improved the soil OM content in soil. Further, the treatments level of 40 kg N fertilizer per ha and *E. asburiae* and *K. quasipneumoniae* inoculation reached the maximum level and OM values ranged from 0.49 to 1.74 %. On contrary, the lowest OM value observed without N₂ application and *E. cloacae* inoculum, significantly different at level 1 %. Research results of Leu [35] and Monaco *et al.* [36] showed that interactions between rhizosphere bacteria and plant may release up to 50 % of the total CO₂ released from terrestrial ecosystems. The soil carbon convertibility in large quantities has been extensively explored in ecosystems between rhizosphere microorganisms and terrestrial plants. The rhizosphere priming effect (RPE) is the promotion or redundant of SOM convertibility by roots and rhizosphere organisms [37] and is one of the most key properties of rhizosphere bacterial and root interactions [38]. Prior researches under *in vitro* conditions were shown that the soil OM was remarkably different between plant species and soil variables [39].

Table 5 Organic matter of soil before and after the experiment.

| Factors | Organic matter (%) | |
|--|-----------------------|----------------------|
| | Before the experiment | After the experiment |
| Nitrogen (kg/ha) (A) | | |
| 0.00 | 1.50 | 0.89 ^c |
| 20.0 | 1.45 | 1.14 ^b |
| 40.0 | 1.45 | 1.74 ^a |
| Rhizosphere N₂-fixing microbiome (B) | | |
| <i>E. asburiae</i> | 1.48 | 1.43 ^a |
| <i>K. quasipneumoniae</i> | 1.46 | 1.30 ^a |
| <i>E. cloacae</i> | 1.46 | 1.05 ^b |
| F (A) | ns | ** |
| F (B) | ns | ** |
| F (A×B) | ns | ** |
| CV (%) | 10.1 | 14.4 |

Note; ns is insignificant difference at p -value ≤ 0.05 and ** is significant difference at p -value ≤ 0.01 .

Total nitrogen (N) of soil

The total N of the soil before the experiment ranged from 0.85 to 0.90 g/kg and the difference was not statistically significant at level 5 %. However, the results of soil N percent after the experiment ranged from 0.53 to 0.75 g/kg and significant differences among treatments. The highest soil N percent was 0.75 g/kg at application of 40 kg N/ha and 0.70 g/kg of *K. quasipneumoniae* inoculation and lowest N percent (0.53 g/kg) in control (without N₂ application) and *E. asburiae* inoculum (0.5 g/kg) (**Table 6**). Total N of experimental soil is a commonly analyzed parameter to assess the potential fertility of soil. Total N content in the soil varies little according to the agricultural system. Therefore, it is not, on the basis of soil total N content, possible to predict the ability to provide useful nitrogen from the soil to N uptake of crops. The key role of the rhizosphere N₂-fixing microbiome, which was discovered by many scientists for promoting and increasing the growth, nutrient and yield of plants in recent years, has been widely recognized and become a hotspot in the field of agriculture [43]. Rhizosphere N₂-fixing microbial play an irreplaceable role in the process of nitrogen convertibility into ammonium and nitrate and have widely involved in 6 distinct N₂ convertibility processes, such as nitrogen fixation [41]. The

rhizosphere is the most combined environment of plants, soil and microbes. Plant roots directly receive N from the rhizosphere soil region in order to provide nutrients for growth and N deficiency has a direct effect on growth, yield and quality of plant. Urea is one of the most popularly used N fertilizers in agricultural application, and it is remarkably manufactured by maintaining global food output and advancing modern agriculture. Urea fertilizers are rapidly converted to ammonium by microbial action in soils. After urea application to the soil, the activity of soil urease can be increased, allowing it to hydrolyze urea and increase $\text{NH}_4^+\text{-N}$ content, promoting nitrification to form $\text{NO}_3\text{-N}$ [43].

Table 6 N of soil before and after the experiment.

| Factors | Total nitrogen (g/kg) | |
|--|-----------------------|----------------------|
| | Before the experiment | After the experiment |
| Nitrogen (kg/ha) (A) | | |
| 0.00 | 0.89 | 0.53 ^c |
| 20.0 | 0.88 | 0.75 ^a |
| 40.0 | 0.87 | 0.64 ^b |
| Rhizosphere N₂-fixing microbiome (B) | | |
| <i>E. asburiae</i> | 0.90 | 0.50 ^b |
| <i>K. quasipneumoniae</i> | 0.89 | 0.70 ^a |
| <i>E. cloacae</i> | 0.85 | 0.72 ^a |
| F (A) | ns | ** |
| F (B) | ns | ** |
| F (A×B) | ns | ** |
| CV (%) | 10.1 | 16.6 |

Note; ns is insignificant difference at p -value ≤ 0.05 and ** is significant difference at p -value ≤ 0.01 .

Nodulous number and weight of peanut

The results of **Table 7** showed that *E. asburiae* inoculant was the highest number of nodules (257) and nodule weight (1.39 gr) per plant and significant difference at $\text{LSD} \leq 0.01$. The application of 40 kg N/ha also gave the lowest number of nodules (181) and the weight of nodules (0.96 gr/plant) and a significant difference at level 1 %. In contrast, the treatment without nitrogen application (control) achieved the highest number and weight of nodules (307 and 1.70 g per plant, respectively). The symbiotic relationship between the rhizosphere N₂-fixing microbiome and the legume as a major source of fixed nitrogen. The bacteria colonize legume roots and form nodules [44]. According to recent study of Yan *et al.* [45], proved that the effects of nitrogen fertilizer application on the different structures and forms of peanut rhizosphere microorganism during different growth period was discovered in the southern paddy soil. Applying more nitrogen fertilizer for peanut plants, the number of nodules decreased. This showed that when there is a N redundant in the soil, rhizosphere bacteria could not need to take N from the air because there is enough N in the soil for them to live and grow. Contrary, number of peanut nodules raised significantly at the control (without N application), which had the maximum number nodule to compare with 2 other treatments (2 different N rates) [45]. The recent study of Mbah and Dakora, [46] showed that applying a lot of N fertilizer to legume can lessen the N₂ fixation of RNFB. For this reason, the number of peanut nodules can be produced less than where N fertilizer is lacked of soil nitrogen.

Table 7 Effect of N and bacteria on nodulous number and weight of peanut of peanuts.

| Factors | 75 DAS | |
|-----------------------------|-----------------------|----------------------------|
| | Nodulous number/plant | Nodulous weight (gr/plant) |
| Nitrogen (kg/ha) (A) | | |
| N1:0 | 307 ^a | 1.70 ^a |
| N2:20 | 235 ^b | 1.27 ^b |
| N3:40 | 181 ^c | 0.96 ^c |

| Factors | 75 DAS | |
|--|-----------------------|----------------------------|
| | Nodulous number/plant | Nodulous weight (gr/plant) |
| Rhizosphere N₂-fixing microbiome (B) | | |
| <i>E. asburiae</i> | 257 ^a | 1.39 ^a |
| <i>K. quasipneumoniae</i> | 255 ^b | 1.22 ^b |
| <i>E. cloacae</i> | 215 ^c | 1.12 ^c |
| F (A) | ** | ** |
| F (B) | * | * |
| F (A×B) | ** | ** |
| CV (%) | 11.6 | 11.8 |

Note; * and ** are significant difference at p -value ≤ 0.05 and 0.01 %.

Yield attributes of peanuts

Results in **Table 8** presented that the biomass and full pods number per plant of peanut, which was not impacted by applying different N fertilizer rates, was statistically insignificant differences at level 5 %. However, there were statistically significant differences at level 1 % among 3 inoculated treatments of 3 bacteria. Three RNFB that was inoculated by *E. asburiae*, was the highest biomass and full pods number per plant (296 gr/plant and 73.2 pods/plant, respectively). Contrariwise, the lowest values of the peanut biomass and full pods number per plant observed at treatments of *E. cloacae* inoculum (208 gr/plant and 45.5 pods/plant, respectively). The values of biomass and full pods number per plant of peanut ranged from 208 to 296 gr/plant and 45.5 to 73.2 pods/plant, respectively. Furthermore, weight of full pods and 100 seeds were significantly various among all treatments at level 5 and 1 % in 3 different N rates (0, 20 and 40 kg/ha). The maximum weight of full pods and 100 seeds (176 gr/plant and 105 gr, respectively) in application of 40 kg N/ha. Contrariwise, the minimum values of weight of full pods and 100 seeds, which observed at treatments of control (P and K application alone), had 131 gr/plant and 86.0 gr, respectively (**Table 8**).

The results of **Table 5** observed that there was significant difference at $LSD \leq 0.01$ among treatments of 3 N fertilizer rates (0, 20 and 40 kg/ha) and 3 RNFB (*E. asburiae*, *K. quasipneumoniae* and *E. cloacae*). The maximum peanut yield of 40 kg/ha application was 7.84 t/ha and minimum yield of control was 6.38 t/ha. The yield value range of 0, 20 and 40 kg/ha application followed 6.38, 7.64 and 7.84 t/ha, respectively. Similarly, the highest yield of peanut (8.07 t/ha) observed at treatments of *E. asburiae* inoculation. The *E. cloacae* inoculation had the lowest yield (6.49 t/ha) and a significantly different at $LSD \leq 0.01$. There was the symbiotic relationship between the rhizosphere N₂-fixing microbiome and the legume as a major source of fixed N, and was significantly different at level 1 % (P -value < 0.01) among treatments of all yield and yield components of peanut. Yang *et al.* [47] proved that nitrogen application remarkably promoted peanut component and productivity. Nevertheless, the studied results showed that N₂ application was little impact on RNFB, and the impact was really only at the peanut nodules. This was though the significant impacts of N fertilizer on the soil nutrients, and the great correlation between soil nutrient and the rhizosphere N₂-fixing bacterium population. Results of these research showed that the different rhizosphere bacteria inoculation for peanuts could be impacted by soil properties and/or peanut discharges, which has not been a detection. Further, the RNFB could be applied as a potential positive bacterium to promote peanut development and yield [47].

Table 8 Effect of N and bacteria on yield attributes of peanuts.

| Factors | Biomass (g/plant) | No. of full pods (pods/plant) | Wt. of full pods (gr/plant) | Wt. of 100 seeds (gr) | Fresh yield (t/ha) |
|--|-------------------|-------------------------------|-----------------------------|-----------------------|--------------------|
| Nitrogen (kg/ha) (A) | | | | | |
| N1: 0 | 244 | 54.9 | 131 ^b | 86.0 ^c | 6.38 |
| N2: 20 | 260 | 69.4 | 158 ^{ab} | 92.5 ^b | 7.64 |
| N3: 40 | 282 | 69.1 | 176 ^a | 105 ^a | 7.84 |
| Rhizosphere N₂-fixing microbiome (B) | | | | | |
| <i>E. asburiae</i> | 296 ^a | 73.2 ^a | 175 ^a | 105 ^a | 8.07 ^a |

| Factors | Biomass (g/plant) | No. of full pods (pods/plant) | Wt. of full pods (gr/plant) | Wt. of 100 seeds (gr) | Fresh yield (t/ha) |
|--------------------------|-------------------|-------------------------------|-----------------------------|-----------------------|--------------------|
| <i>K quasipneumoniae</i> | 228 ^b | 55.8 ^b | 135 ^b | 84.9 ^b | 7.09 ^b |
| <i>E. cloacae</i> | 208 ^c | 45.8 ^c | 115 ^c | 83.1 ^b | 6.49 ^c |
| F (A) | ns | ns | * | ** | ** |
| F (B) | ** | ** | ** | ** | ** |
| F (A×B) | ns | ns | ns | ns | ** |
| CV (%) | 12.5 | 20.6 | 21.9 | 13.3 | 12.1 |

Note; ns is insignificant difference at p -value ≤ 0.05 and *, ** are significant difference at p -value ≤ 0.05 and 0.01 %.

Conclusions

The studied results presented that application of different N fertilizer rates was a bit influence on the N₂-fixing ability of rhizosphere bacteria, and this influence was significantly observed at the number of peanut nodules. In the 3 N fertilizer rates and 3 RNFB, which only had 1 N fertilizer rate of 40 kg N/ha and *E. asburiae* inoculation significantly raised in relational abundance with the maximum yield component and yield at the N fertilizer and rhizosphere bacterium, while there was the lowest N fertilizer rate of 0 kg N/ha and *E. cloacae* inoculation in yield component and yield. The variousness in rhizosphere bacterium strains between different N fertilizer rates was better than that caused by the nitrogen fertilizer amount. This could be due to the substantial improvement in soil chemical attributes at different soils, especially in, pH, OM and total N, as these significantly impacted the soil structure.

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