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The impact of bacillus sp. NTLG2-20 and reduced nitrogen fertilization on soil properties and peanut yield

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Abstract

The excessive use of nitrogen (N) fertilizers has led to farmland degradation and reduced crop yields. To address this drawback, reducing the amount of nitrogen fertilizer and Bacillus sp. NTLG2-20 inoculant are the optimal cultivation method. The impact of different N rates (0, 20, and 40 kg ha-1) combined with the Bacillus sp. NTLG2-20 inoculant on soil chemical properties, growth, development, and peanut yield was designed in the field in Phuoc Hung commune, An Phu district from May to August 2023. The field experiment was designed with 6 treatments and 4 replications. The research results showed that different N rates adequately augmented soil chemical traits such as pH, cation exchange capacity (CEC), soil organic matter (SOM), total N, available phosphorous (AP), and exchangeable potassium (EK). Furthermore, different N fertilizers rates combined with Bacillus sp. NTLG2-20 inoculant adequately augmented plant height, number of leaves, total chlorophyll, nodulous number and weight per groundnut plant. Reducing N fertilizer application by 50% (20 kg N ha-1) was the optimal N reduction rate when combined with the Bacillus sp. NTLG2-20, which resulted in 17.6% higher peanut yield compared to no N application and no difference compared to 100% of recommended N application ($P<0.01$)). Bacillus sp. NTLG2-20 inoculant increased peanut yield by 19.6% when compared to no Bacillus sp. NTLG2-20 inoculant ($P<0.01$). Nitrogen – fixing ability of Bacillus sp. NTLG2-20 promoted peanut yield and reduced fifty percentage of the N fertilizer application. Bacillus sp. NTLG2-20 is the promising species for the production of biological fertilizer in the future.

Keywords: Bacillus sp. NTLG2-20 inoculant, nitrogen fertilizer, peanut, YEMA

1. Introduction

Nitrogen (N), which is one of the essential elements, is an important component for development of plants such as peanut. Unavailing N in farmland, which cannot provide nutrients for the plant development, can make the availability of both N insufficient for plant growth and nutrient limited for plant yield [1]. In peanut cultivation, the urea is widely used and converted to $NH4+$ by the activity of soil microorganisms for the nutrient uptake of plants [2]. $NH4+$ is then absorbed by plants for the purpose of peanut quality and productivity [3]. Soil microorganisms are the factor as they have a relationship between crop and farmland. The soil microorganism community of rhizosphere is found higher than compared to other soils [4]. The density of rhizosphere N-fixing microorganisms is greatly determined by various environmental factors. Different soil kinds and their physic, chemistry, and nutrition of farmland have major impacts on the N-fixing microorganisms of rhizosphere soils [5]. The soil traits such as soil pH, SOM, N, P, and K are significantly correlated with the beneficial microbial communities in the farmland [6,7]. The species of microorganisms in the soil, growth stage, and plant type will determine the type of microorganisms that can enrich soil nutrients in the crop root area [8,9]. Bacillus sp. strains, considered as the naturally sufficient team of bacterium for the bio-fertilizer production and pesticides for crops, lessen the application of N fertilizers in agricultural cultivation [10-12].

Bacillus sp. involved in promoting plant growth and development as well as reducing environmental factors can cause stress to plants such as prolonged drought, salinity, high temperature, and metal pollution, toxic and flood. Some species of the genus Bacillus, such as B. megaterium, B. circlens, B. coagulans, B. subtilis, and B. azotofixans, are known as the plant growth-promoting bacteria [13-15]. Applying 6.0 t ha-1 of chicken manure associated with the Rhizobium sp. strains could increase peanut yield by up to 20.5% compared to control. The study showed that the nitrogen fixation ability of Rhizobium sp. increased peanut growth, soil fertility, yield and seed quality. The inoculation of N-fixing bacteria has significantly proven to be an excellent approach, which increases soil nutrients for subsequent crops and enhances the N uptake from the air into agricultural soil [14,15].

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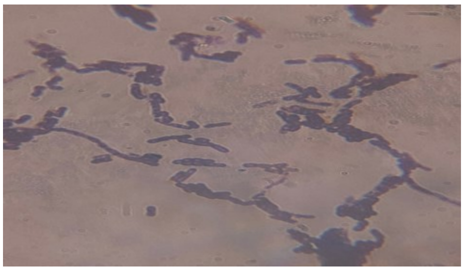
The inoculation of indigenous nitrogen-fixing microorganisms for peanuts, which remarkably reduces inorganic fertilizer application, promotes the peanut growth with natural nitrogen, enhances soil fertility in sustainable agriculture, and increases farmers' profits. The overuse of inorganic fertilizer application, However, has led to a significant decline in the population of beneficial microorganisms in agricultural soils [16]. All agronomic traits, yield components, and yield of peanuts significantly inoculated by Rhizobium strains significantly increased compared to inoculated plots. Notably, peanut pod yield of treatments, applied by the 4.0, 6.0, and 8.0 t chicken manures per ha and Rhizobium sp. inoculation increased by 8.76, 11.6, and 20.5%, respectively, compared to the control [16,14].

Peanut plants, which contain many available nutrients for human, have the impacts of promoting cell growth, improving intelligence, and preventing cancers [17,18]. Therefore, they have popularly been planted around the world [17]. The application of N fertilizer and N-fixing microorganisms is mainly correlated to the peanut yield and quality. However, previous studies have shown that yield as well as oleic acid and linoleic acid content of peanuts are completely related by N fertilizer application [19]. The research's objectives include: (i) to evaluate the natural N-fixing ability of Bacillus sp. NTLG2-20 to reduce the inorganic N fertilizers use in peanut cultivation and (ii) to develop peanut cultivation practices towards sustainable agriculture that is safe for both environment and humans, increases peanut yield, quality and provides high profitability for farmers.

2. Materials and Methods

2.1. Isolation and identification of Bacillus NTLG2-20 from peanut root nodules

(a)



(b)

Fig. 1. (a) *Bacillus* spp. NTLG2-20 colony was isolated from YEMA medium; (b) Gram staining: Gram-negative, short rod-like bacteria

2.2. Experimental location

Table 1 shows the location of the field experiment conducted in fixing Commune, An Phu district from May to August 2023. The climate in terms of study region was grouped into two seasons: a rainy and dry season, dry and windy in spring and autumn, and hot and rainy season. The annual average temperature varied between 22°C and 37°C with the highest temperature at 37°C and the lowest one at 22°C. Table 1 shows SOM, soil pH, irrigation pH, CEC, total N, exchangeable K and available P in the 0-20 cm soil layer at 2.47, 6.20, 6.71 and 6.02 Cmol+ kg-1, 0.140%, 96.2 mg kg-1, and 20.7 mg kg-1, respectively. The analysis of soil chemical soil traits included pH, total N, available P, and exchangeable K. Cation exchange capacity determines the exchangeable cation number of soil as well as the ability nutrient exchange between negative and positive charges in the farmland [8, 9,14]. Soil organic matter improves soil structure, increases water holding capacity, provides readily available nutrients for soil microorganisms, and enhances root development. This leads to healthier plants and provides more carbons and nutrients for nitrogen-fixing bacteria to thrive [14,16]. Soil and water pH was determined by using a pH meter (soil/water 1:2.5) and total N was determined by using the Kjeldahl digestion method [20]. Available P was determined by using the alkaline hydrolysis method. Exchangeable P was extracted with ammonium acetate and measured by using flame photometry [21]. CEC was determined by NH4CH3CO2, and percentage of SOM was analyzed by Brady, (1990). Soil texture was determined by method of Yuan and Zhou, (2014) [9].

Table 1. Physic and chemistry of farmland and irrigation water before the experiment

Property	Result	Property	Result
Sand (%)	61.0	Total N (%)	0.14
Silt (%)	35.0	AP (mg kg^{-1})	20.7
Clay (%)	4.00	EK (mg kg^{-1})	96.2
pH soil	6.20	SOM (%)	2.47
pH water	6.71	CEC (Cmol kg^{-1})	6.02

2.3. Experimental design

Table 2 shows field experiment that consisted of two factors (i. inoculation and non-inoculation of *Bacillu*s sp. NTLG2-20; ii. three NF raters) and had six treatments from 0 to 5 with four replications. Six treatments included: 0 (control) non N application and 60.0 kg P ha^{-1}+ 60.0 kg K ha^{-1} application only; 1: *Bacillu*s sp. NTLG2-20 inoculum + (non N_2 fertilizer) + and 60.0 kg P ha^{-1}+ 60.0 kg K ha^{-1} application; 2: applied 20.0 kg N ha^{-1} and 60.0 kg P ha^{-1}+ 60.0 kg K ha^{-1} application (reduced 50% N2 fertilizer); 3: applied 20.0 kg N ha^{-1} + and 60 kg P ha^{-1}+ 60.0 kg K ha^{-1} application + *Bacillus* sp. NTLG2-20 inoculum + PK; 4 (100% N_2 fertilizer application): applied 40kgN ha^{-1}+ and 60 kg P ha^{-1}+ 60 kg K ha^{-1} application; 5 (100% N fertilizer application): *Bacillus* sp. NTLG2-20 inoculum + applied 40kgN ha^{-1}+ and 60 kg P ha^{-1}+ 60 kg K ha^{-1} application. The chemical nitrogen, phosphorous and potassium fertilizers,used by the urea (46.2% of N), the superphosphate (16% of P_2O_5) and potassium chloride (61% of K), collected from Phu My company, Vietnam, were converted into weight of N, P, and K.

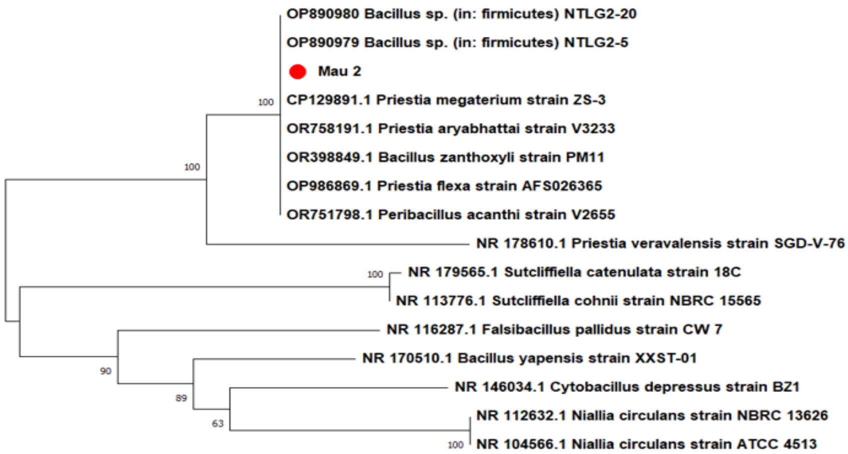


Fig. 2. Phylogenetic tree for *Bacillus* spp. NTLG2-20

Table 2. Effects of N_2 rates and *Bacillus sp.* NTLG2-20 on soil chemical properties

Factor	pH	CEC (Cmol kg^{-1})	OM (%)
Nitrogen (N) kg ha^{-1} (A)			
0.00	7.14^a	6.09^c	1.18^c
20.0	7.03b	6.22^b	1.35^b
40.0	6.74^c	6.61^a	2.10^a
Bacillus sp. NTLG2-20 (B)			
1	7.37^a	7.56^a	1.59^a
0	7.14^b	5.05^b	1.50^b
F (A)	**	**	**
F (B)	**	**	**
F (AxB)	**	**	**
CV (%)	11.8	11.1	12.5

Bacillus sp. NTLG2-20*: (0) non-inoculation; (1): inoculation. ** $p \leq 0.01$.

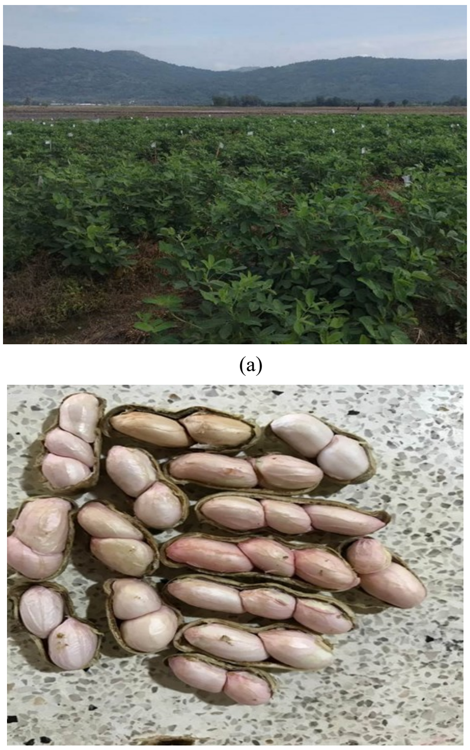


Fig. 3. (a) Growth period of 65 DAS; (b) Filled pods

2.4. Increase and inoculation of Bacillus sp. NTLG2-20

Bacillus sp. NTLG2-20 was cultured in a diluted YMA medium after five days at 27°C. Number of Bacillus sp. NTLG2-20 spores was raised in flask, which was contained with 500 ml flash of diluted YMA solution and was shaken at 150 rpm for 24 hours at 27°C. The spores of Bacillus sp. NTLG2-20 were centrifuged at 10000 rpm for ninety seconds at 3°C. All spores were then cleaned by distilled water. Meanwhile, the genera of Bacillus sp. NTLG2-20 were collected and diluted by distilled water and seed inoculant. All seeds were well soaked before Bacillus sp. NTLG2-20 inoculation (10^8CFU mL^{-1}).

The treated seeds were then taken at dark room for eight hours before sowing. The colony number of Bacillus sp. NTLG2-20 reached 10^8 CFU per seed. Groundnuts were planted in single-row strips at the test site with 20 cm space between plants, distance of 30 cm in small rows, and 50 cm between large rows. Here, peanut seeds (L14) were used during the field experiment.

2.5. Sample collection and analysis

Sample collection was performed on May 1, 2023 (before the experiment) and September 15, 2023 at harvest. The samples of farmland were well collected, mixed and taken to the laboratory to be dried naturally and sieved through a 1 mm mesh to determine the chemical properties of the soil. Soil organic matter, pH, total N, CEC, available P, and exchangeable K were analyzed by the Department of Central Laboratories, An Giang University

2.6. Peanut productivity traits and productivity

The groundnut productivity traits and productivity were monitored from the mature period to harvest including plant biomass, number and weight of pods per plant, weight of fill pods and weight of 1,000 seeds. Fresh pod yield was determined by ton per ha per treatment.

2.7. Datum calculation

Statistical calculation was conducted by means of

Statgraphic XV software and Microsoft Office Excel 2010. A randomized completely block design was used for all experiments with 4 replications for each treatment. Data were presented from representative experiments with 4 repetitions showing similar results. Methods were compared via ANOVA using the significantly statistical difference ($p \leq 0.05$).

3. Results and Discussion

3.1. Effect of N_2 rates and Bacillus sp. NTLG2-20 on soil attributes

Table 2 and Table 3 show the positive effect of N fertilizer application combined with *Bacillus sp.* NTLG2-20 inoculant on soil fertility. The results showed that reducing N fertilizer by 50% (20 kg N ha^{-1}) positively affected soil chemical properties such as soil pH, OM, CEC, total N, Available P and exchangeable. Similarly, there were some significant differences at $p \leq 0.01$ on these chemical properties in the treatments with *Bacillus* sp. NTLG2-20 inoculant compared to non-*Bacillus sp.* NTLG2-20 inoculant. This study confirmed that reducing N fertilizer by 50% combined with *Bacillus* sp. NTLG2-20 inoculant significantly affected the chemical properties of peanut soil. There were also some significant differences between the rates of N-fertilizer application rate and *Bacillus* sp. NTLG2-20 inoculum on the chemical traits of soil at harvest season at $P<0.01$ (Table 2 and Table 3). The interactions were between N rates (A) *Bacillus* sp. NTLG2-20 (B) (Except total N and exchangeable K). The results of this study demonstrated that reducing N fertilizer by 50% combined with VKA application could significantly increase available P while maintaining total N and exchangeable K. In the experiments with nitrogen fertilization and *Bacillus* sp. inoculum, an augmentation in the content of total N and available P was observed (Table 2 and Table 3).

Table 3. Effects of N rates and Bacillus sp. NTLG2-20 on soil nutrient attributes

Factor	Total N	Available P ($mg\ kg^{-1}$)	Exchangeable K
Nitrogen (N) kg ha^{-1} (A)			
0.00	90.0^{c}	20.8^{c}	76.4
20.0	100^{b}	29.6^{b}	74.3
40.0	140^{a}	32.9^{a}	73.1
Bacillus sp. NTLG2-20 (B)			
1	110	29.6^{a}	74.1^{b}
0	100	25.9^{b}	78.5^{a}
F (A)	**	**	ns
F (B)	ns	**	*
F (AxB)	ns	**	ns
CV (%)	23.1	11.6	24.6

Note: Bacillus sp. NTLG2-20*: (0) non-inoculation; (1): inoculation. $^{ns}p>0.05$; $*p < 0.05$; $**p < 0.01$.

In the experiments with nitrogen fertilization and Bacillus sp. inoculum, an augmentation in the content of total N and available P was observed. The N application and Bacillus sp. strains, regardless of the dose, had in a remarkably augmentation in soil pH, organic matter, and CEC compared to the control [22-24]. This was related by the bacterial decomposition process of OM, which could oxidize a variety of organic compositions and ferments [25]. Rhizobacteria that promotes plant growth can help to promote the decomposition process of OM as in mineralization [26]. Organic matter contributes to high carbon and energy resources for microorganisms as high OM content is associated with greater biodiversity [27]. Studies conducted with Bacillus sp. combined with nitrogen fertilization showed a higher nutrient concentration in farmland, indicating that plants inoculated with rhizosphere N2-fixing bacteria in the seed absorbed more P and N [28]. The increase could also be related to the no-till method due to less soil mobility, in addition to the formation of phosphorus complexes with organic matter, greater biological activity, and crop nutrient recycling.

3.2. Effect of different N rates and Bacillus sp. NTLG2-20 on agronomic traits

Table 4 shows the observation showing that the highest groundnut plant height was found in all three stages of 20 – 45 – 65 DAS when applying 40 kg N ha^{-1} with plant height of 12.7 – 22.8 – 53.2 cm, respectively. In addition, Bacillus sp. NTLG2-20 inoculum also promoted the plant height values of 13.0 – 23.1 – 53.7 cm at the 20 – 45 – 65 DAS stages, respectively. There was a statistically adequately diversification at the nitrogen fertilizer rates ($p<0.01$) and *Bacillus* sp. NTLG2-20 inoculum ($p<0.01$). However, its interaction was an insignificantly statistical diversification between nitrogen fertilizer rates and *Bacillus* sp. NTLG2-20 inoculum at the 20 and 45 DAS (Expect 65 DAS). In particular, the treatment of *Bacillus* sp. NTLG2-20 inoculum combined with 40 kg N ha^{-1} fertilization had higher plant height compared to the treatments with 20 kg N ha^{-1} addition, non N addition, and non-*Bacillus* sp. NTLG2-20 inoculum. *Bacillus* sp. NTLG2-20 inoculum + 40 kg N ha^{-1} fertilization provided the plant with both N and P, which are available nutrition for plant growth, while no N fertilizer and *Bacillus* sp. NTLG2-20 inoculum (control) provided neither N nor P.

Table 4 shows that the number of branches at the 20 DAS was not statistically significant difference among the treatments. At the 45 and 65 DAS, there was a statistically significant difference at $p<0.01$ (except N fertilizer rates). Bacillus sp. NTLG2-20 inoculant had the higher branches in 45 and 65 DAS (5.5 and 6.5 branches) compared to non-*Bacillus* sp. NTLG2-20 inoculant. However, there was no interaction between nitrogen fertilizer and Bacillus sp. NTLG2-20 inoculant in two factors. The total chlorophyll of peanut plants was insignificant difference at two stages 15 and 65 DAS in the application of three N_2 fertilizer ratios (0, 20 and 40 kg N ha^{-1}) However, *Bacillus* sp. NTLG2-20 inoculation, which had higher total chlorophyll index compared to control treatment (non - *Bacillus* sp. NTLG2-20 inoculation), was 41.1, 44.3 and 44.9 at the 20, 45, and 65 DAS, respectively, compared to the non-*Bacillus* sp. NTLG2-20 inoculant (34.8, 40.9 and 42.5, respectively) at the 20, 45, and 65 DAS. There was no interaction between two factors and no statistically significant difference between the nitrogen fertilizer rate and *Bacillus* sp.

NTLG2-20 inoculant.

As shown in Table 4, data presentation indicated that total chlorophyll concentration was insignificantly impacted by N fertilizer ratios from 20 and 65DAS (except 45 DAS). On the contrary, results as shown in Table 4 were proclaimed that *Bacillus* sp. NTLG2-20 inoculation had the leaf chlorophyll concentration that was always higher than the one without *Bacillus* sp. NTLG2-20 inoculation and statistically significant differences at 1%. However, their interaction between N fertilizer ratios and *Bacillus* sp. NTLG2-20 inoculation was not remarkable differences. *Bacillus* sp. NTLG2-20 inoculation raised the total leaf chlorophyll concentration of all treatments during the peanut growth. The research indicated that *Bacillus* sp. NTLG2-20 inoculation significantly impacted the total leaf chlorophyll concentration of peanuts. (Table 4). In peanuts, chemical analysis showed less change in the parameters evaluated with only a decrease in exchangeable K content observed during the inoculation of Bacillus sp. This might be related to rhizobacteria promoting nutrient mineralization, phosphate solubilization, nitrogen fixation, and enhanced root nutrient uptake [29]. Recent study of Lobo *et al.,* (2022) [30] evaluated the mixture use of microorganisms and bio-fertilizers applied to peanuts, which has been proven to improve crop productivity traits and productivity more than the individual strains and applications. The amendment of N fertilizers in combination with the use of formulations containing nitrogen-fixing microbial strains often leads to high efficiency in the crop growth promotion. Therefore, the application of a combination of nitrogen fertilizers and bacillus strains can be a promising approach to expand the scope of biological control activities. The use of the right and sufficient amount of nitrogen and the combination of rhizosphere nitrogen-fixing microorganisms can help to increase the height, number of leaves, and total chlorophyll content of baby corn plants grown on poor nutrient soil compared to no nitrogen application and *Priestia aryabhattai* inoculation [31]. Recent study observed that the usage of N significantly could raise the groundnut photosynthetic efficiency, especially the groundnut photosynthetic rate and respiration ratio in the pod filling stages [31,32].

The inoculation of rhizosphere N-fixing bacteria obtained darker green leaves and increased total chlorophyll level compared to non- rhizosphere N-fixing bacterium inoculation [32]. The prior study of Etesami, (2018) [33] proved that the adequate use of irrigation water and N fertilizer could raise the leaf total chlorophyll concentration of crops. In this research, the total chlorophyll concentration was insignificant effect in all different N fertilizer treatments due to low N levels, which were applied in these treatments (<40 kg ha-1). Rhizobia, a symbiotic nitrogen-fixing strain of peanut plants, could convert atmospheric nitrogen into NO3-, which could absorb from crop roots thank to its naturally nitrogen-fixing microorganisms [33,25]. Therefore, the full enhancement of rhizobia's nitrogen-fixing ability plays a key role in lessening N fertilizer use.

Table 5 shows that N fertilization had a sufficient effect on the number and fresh weight of peanut root nodules. Nitrogen addition reduction raised remarkably the nodulous number and fresh weight in the 75 DAS. The nodulous number of peanut roots ($p \leq 0.01$) and fresh weight ($p \leq 0.01$) in peanut roots at 75 DAS decreased by 23.5, 41.0% and 25.3, 43.5%, respectively, at the nitrogen application rates of 20 kg N ha^{-1} and 0.0 kg N ha^{-1} compared to 40 kg N ha^{-1}. Similarly, *Bacillus* sp. NTLG2-20 inoculant increased the nodulous number and fresh weight with by 3.90% and 8.80%, respectively, compared to the non-*Bacillus* sp. NTLG2-20 inoculant and was statistically sufficient differences at 5% level. However, the interaction between the nitrogen fertilizer rates and *Bacillus* sp. NTLG2-20 inoculant were insufficiently differences. According to the research results of Wu *et al.,* (2016), N resources for groundnut throughout the mainly mature period comes from groundnut nodules, farmland, and inorganic fertilizers. The N supply ratio from root nodules, soil, and N fertilizer was 5:3:2. Similarly,

Table 4. Effects of N rates and *Bacillus* sp. NTLG2-20 on agronomic attributes

Factor	Plant height (cm)			branches $plant^{-1}$			Total chlorophyll (µg mL^{-1})		
	Days after sowing (DAS)								
	20	45	65	20	45	65	20	45	65
Nitrogen (N) kg ha^{-1} (A)									
0.00	11.1^{b}	20.4^{b}	49.3^{c}	3.75	4.50^{c}	5.12	16.0	48.3^{b}	99.5
20,0	11.7^{b}	20.9^{b}	50.6^{b}	3.50	5.13^{b}	5.88	16.6	50.6^{b}	101
40.0	12.7^{a}	22.8^{a}	53.2^{a}	3.86	5.75^{a}	6.25	17.4	55.9^{a}	107
Bacillus sp. NTLG2-20 (B)									
	13.0^{a}	23.1^{a}	53.7^{a}	3.67	5.50^{a}	6.50^{a}	41.1^{a}	44.3^{a}	44.9^{a}
	10.6^{b}	19.6^{b}	48.4^{b}	3.50	4.75^{b}	5.50^{b}	34.8^{b}	40.9^{b}	42.5^{b}
F (A)	**	**	**	ns	**	ns	ns	**	ns
F (B)	**	**	**	ns	**	**	**	**	**
F (AxB)	ns	ns	**	ns	ns	ns	ns	ns	ns
CV (%)	16.7	14.7	12.2	16.7	14.7	12.2	13.3	14.4	14.9

DAS: days after sowing; *Bacillus* sp. NTLG2-20*: (0) non-inoculation; (1): inoculum. $^{ns}p>0.05$; $^{**}p \leq 0.01$.

Etesami, (2018) [33] also found that the high nitrogen-fixing ability of rhizobium contributes to promoting the growth and increasing the yield of peanut plants. The overuse of N fertilizer can lead to negative effects on peanut growth, including increased plant height leading to pests and lodging, reduced nitrogen fixation by nitrogen-fixing bacteria, reduced yield, and reduced microbiome [34,35].

Table 5. Effects of N rates and *Bacillus* sp. NTLG2-20 on nodulous number and weight

Factor	75 DAS	
	No. of available nodules (each plant)	Wt. of available nodules (gr plant^{-1})
Nitrogen (N) kg ha^{-1} (A)		
0.00	181^{c}	0.96^{c}
20.0	235^{b}	1.27^{b}
40.0	307^{a}	1.70^{a}
Bacillus sp. NTLG2-20 (B)		
1	257^{a}	1.39^{a}
0	245^{b}	1.22^{b}
F (A)	**	**
F (B)	*	*
F (AxB)	ns	ns
CV (%)	11.6	11.8

Bacillus sp. NTLG2-20*: (0) non-inoculation; (1): inoculum. ^{ns}p>0. 05; *$p \le 0.05$; **$p \le 0.01$.

3.3. Effect of different N_2 rates and Bacillus sp. NTLG2-20 on yield traits and yield

Table 6 shows that application of three N fertilizer rates did not affect the peanut biomass. However, Bacillus sp. NTLG2-20 inoculant, which was impacted on groundnut biomass at p≤0.01, was 23% higher than that of treatments of non-Bacillus sp. NTLG2-20 inoculant. Meanwhile, there was no interaction between nitrogen fertilizer and Bacillus sp. NTLG2-20. The number of peanuts, filled peanuts, and weight of peanuts were insufficient differences in the N fertilizer ratios [except the weight of filled peanuts (p<0.05)]. The highest weight of filled peanuts was 176 g per plant in application of 40 kg N ha^{-1}, following 20 kg N ha^{-1} (158 gr plant^{-1}) and lowest without N application (131 gr plant^{-1}). Contrariwise, Bacillus sp. NTLG2-20 inoculant were 80.8 peanuts plant^{-1}, 73.2 filled peanuts plant^{-1}, 183g plant^{-1} (weight of peanuts) and 175g plant^{-1} (weight of filled peanuts), higher than those of non - Bacillus sp. NTLG2-20 inoculation 64.9 peanuts plant^{-1}, 55.8 filled peanuts plant^{-1}, 146g plant^{-1} (weight of peanuts) and 135g plant^{-1} (weight of filled peanuts). 1,000 seed weight had sufficient differences (p<0.01) in both nitrogen fertilizer rates and Bacillus sp. NTLG2-20 inoculant. The 40 kg N ha^{-1} application gave the highest weight of 1,000 seeds (1,050g), lowest in the one without N2 application (860g). Reducing N fertilizer by 50%, with the peanut fresh yield showed insufficient differences (p>0.05) compared to 100% N fertilizer application higher than no N2 application by 17.6%. Bacillus sp. NTLG2-20 inoculation had the higher fresh peanut yield (8.07 t ha^{-1}) compared to the treatments of non-Bacillus sp. NTLG2-20 inoculation (6.49 t ha^{-1}) with sufficient differences at p<0.01. The fresh peanut yield of Bacillus sp. NTLG2-20 inoculation treatments was 19.6% higher than that of the non- *Bacillus* sp. NTLG2-20 inoculation. The interaction between factor (A) (N kg ha^{-1}) and factor B (*Bacillus* sp. NTLG2-20) had no significant differences. Excessive nitrogen fertilization did not increase both peanut yield and production costs. The results of this experiment showed that 50% N reduction showed the insignificant differences in yield compared to 100% recommended application and animal manure supplementation increased leaf photosynthesis, and increased nutrient traits and peanut yield. The maximum yield was raised by combining N fertilization and inoculation with nitrogen-fixing bacteria. This results showed that (i) N contents of N fertilizer could be quickly affected and added directly to the farmland; (ii) N contents of inorganic fertilizers needs to be converted by microbial processes before planting; and (iii) groundnuts have the ability to absorb N from both N fixation and chemical fertilizer; therefore, (iv) peanut physiological traits decrease rapidly when the treatments of N application were reduced, and peanut photosynthetic efficiency and yield were improved when treated with additional nitrogen-fixing bacteria at harvest [36,24].

Table 6. Effects of N rates and *Bacillus* sp. NTLG2-20 on peanut yield attributes and yield

Factor	Biomass (gr plant^{-1})	No. of pods (per plant)	No. of fill pods (per plant)	Wt. of pods (gr plant^{-1})	Wt. of fill pods (gr plant^{-1})	Wt. of 1,000 seeds (gr)	Fresh pod yield (t ha^{-1})
Nitrogen (N) kg ha^{-1} (A)							
0.00	244	63.8	54.9	142	131^{b}	860^{c}	6.38^{b}
20.0	260	78.3	69.4	170	158^{ab}	925^{b}	7.74^{a}
40.0	282	76.5	69.1	181	176^{a}	1,05^{a}	7.84^{a}
Bacillus sp. NTLG2-20 (B)							
1	296^{a}	80.8^{a}	73.2^{a}	183^{a}	175^{a}	1,050a	8.07^{a}
0	228^{b}	64.9^{b}	55.8^{b}	146^{b}	135^{b}	840b	6.49^{b}
F (A)	ns	ns	ns	ns	*	**	**
F (B)	**	**	**	**	**	**	**
F (AxB)	ns	ns	ns	ns	ns	ns	ns
CV (%)	12.5	18.2	20.6	19.6	21.9	13.3	18.2

Note: Bacillus sp. NTLG2-20*: (0) non-inoculation; (1): inoculum. ^{ns}p>0. 05; *$p \le 0.05$; **$p \le 0.01$.

3.4. Effect of different N_2 rates and Bacillus sp. NTLG2-20 on seed nutrient traits

Table 7. Effects of N rates and *Bacillus* sp. NTLG2-20 on the peanut quality

Factor	Peanut seed nutrient attributes (%)				
	Lipid	Protein	N	P	K
Nitrogen (N) kg ha^{-1} (A)					
0.00	23.6^{b}	15.2^{b}	2.43^{b}	0.24^{b}	0.22^{b}
20.0	25.2^{ab}	17.3^{ab}	2.74^{ab}	0.35^{ab}	0.38^{ab}
40.0	27.5^{a}	18.3^{a}	2.92^{a}	0.43^{a}	0.51^{a}
Bacillus sp. NTLG2-20 (B)					
1	23.4^{b}	15.5^{b}	2.50^{b}	0.25^{b}	0.24^{b}
0	24.5^{a}	16.4^{a}	2.63^{a}	0.33^{a}	0.35^{a}
F (A)	**	**	*	*	*
F (B)	**	**	*	*	*
F (A x B)	*	*	ns	ns	ns
CV (%)	12.4	13.6	16.7	17.8	14.6

Note: Bacillus sp. NTLG2-20*: (0) non-inoculation; (1): inoculum. $^{ns}p>0.05$; $*p \leq 0.05$; $**p \leq 0.01$.

As shown in Table 7, the statistical analysis results showed that the lipid and protein content in peanut seeds in the N fertilizer factor had a statistically significant difference with 1% level, the highest lipid (27.5%) and protein (18.3%) content. While, the non-fertilized N treatment showed the lowest lipid (23.6%) and protein (15.2%) content. In the *Bacillus* sp. NTLG2-20 bacteria factor, there was a statistically significant difference at 1% level. The lipid and protein content in peanut seeds showed that the *Bacillus* sp. NTLG2-20 inoculation treatments had a higher lipid and protein content compared to the non-*Bacillus* sp. NTLG2-20 inoculation treatments. The two factors of N fertilizer and *Bacillus* sp. NTLG2-20 were an interaction with each other at 5% level in which the 40 kg N ha^{-1} application and *Bacillus* sp. NTLG2-20 inoculation provided a higher lipid content (28.4%), protein (18.3%) than the non-N fertilization + non-*Bacillus* sp. NTLG2-20 inoculation. The N, P and K content in peanut seeds of the N fertilizer factor all showed a statistically significant difference at 5% level. The 40kg N ha^{-1} application had the highest N, P and K content of peanut seeds and the lowest one was found in the non-N fertilizer application. Similarly, the bacterial factor also had a statistically significant difference at 5% level in N, P and K content of peanut seeds in which the *Bacillus* sp. NTLG2-20 inoculation had a higher N, P and K content compared to the non-*Bacillus* sp. NTLG2-20 inoculation. The N fertilizer factor had no interaction with the *Bacillus* sp. NTLG2-20 factor the N, P and K content in peanut fruits. According to previous research of Singh et al., (2021) [37], nitrogen-fixing bacterial strains in agricultural soils could promote the growth, yield components, and yield of crops, and improved seed quality and nutrient content. The inoculation of N-fixing bacteria increased the nutrient concentration of peanut seeds such as kernel yield (19.3%), mineral trait, oil content (4.8%), protein concentration (28.2%) and hydration coefficient (11.6%). The increase of N- fixing bacteria population in farmland promoted nitrogen fixation and raised yield and seed quality [38-40]. Many previous studies proved that the N-fixing ability of endophytic bacteria to form nodules on peanuts was inhibited by urea fertilization [41]. The period of 4-5 DAS N-fixing bacteria begins to form nodules on the roots. Therefore, excessive nitrogen application will cause stress on the number and size of root nodules, leading to a decrease in the effectiveness of N fixation and a reduction in the amount of fixed nitrogen [42]. The results of this research is consistent with the previous study of Thuc et al., (2022) [43,44], showing that endophytic N-fixing bacteria reduced 25% of the N fertilizer application for sesame, obtained equivalent of the total N uptake compared to 100% N application. Furthermore, 25-50% reduction of N fertilizer application combined with the N-fixing bacteria achieved a yield equivalent to 100% N application.

4. Conclusion

Bacillus sp. NTLG2-20 was isolated and identified at the molecular level, which is an endogenous nitrogen-fixing bacterium. It stimulated the plant growth and showed high N-fixing ability when combined with N fertilizer. It improved soil fertility and increased peanut yield. *Bacillus* sp. NTLG2-20 inoculation combined with N fertilizer increased the symbiotic ability and yield. Fifty percent reduction of N fertilizer application combined with the *Bacillus* sp. NTLG2-20, raised up to 19.6% the pod yield compared to the no *Bacillus* sp. NTLG2-20 inoculation showing no yield difference compared to the recommended 100% application. The yield components, nodulous number and fresh weight sufficiently increased when *Bacillus* sp. NTLG2-20 inoculation was combined with 20 kg N ha^{-1} amendment. Our new discovery indicated that this *Bacillus* sp. NTLG2-20 has the potential to be applied in the bio-fertilizer production to improve soil fertility, reduce chemical N fertilizers and increase sustainable crop productivity in the future.

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