



## Effect of *Bacillus sonklensis* Associated with Cattle Manure Fertilization on the Farmland Health and Peanut Yield

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### ABSTRACT

A key objective of this research is to determine the overall effectiveness of *Bacillus sonklensis*, chicken manure application (CMA) and nitrogen fertilizer application (NFA) in increasing soil fertility and peanut yield. The nine experimental plots included: NT1-control (without NFA, CMA and *B. sonklensis*); NT2 (*B. sonklensis* inoculation+no NFA+5.0tCMA/ha); NT3 (*B. sonklensis* inoculation+no NFA+10.0tCMA/ha); NT4 (no *Bacillus sonklensis* inoculation+ 20kgNFA/ha+ 0.0tCMA/ha); NT5 (*B. sonklensis* inoculation+20kgNFA/ha+5.0tCMA/ha); NT6 (*B. sonklensis* inoculation+20kgNFA/ha+10.0tCMA/ha); NT7 [*B. sonklensis* inoculation+ 40kgNFA/ha (100% of NFA)+0.0tCMA/ha; NT8 (*B. sonklensis* inoculation+40kgNFA/ha+ 5.0tCMA/ha); and NT9 (*B. sonklensis* inoculation+40kgNFA/ha+10.0tCMA/ha). The study demonstrated that a significant combination of 10tCMA/ha+20kgN/ha, with *B. sonklensis* inoculation remarkably enhanced agronomic characteristics, yield components, pod yield and seed quality of peanut. Furthermore, this approach reduced the need for NFA by 50% while achieving the greatest fruit yield. In contrast, the lowest yield was achieved in the treatments using only phosphorous and potassium fertilization. The yield of the NT6 treatment increased by 41.5 and 18.2% compared to the NT1 treatment and the NT9 treatment, respectively. This then led to the conclusion that the CMA fertilization combined with *B. sonklensis* amendment increased the yield and quality of peanut and reduced the 50% nitrogen fertilizer utilized by 50%.

**Keywords:** Cattle manure, *Bacillus sonklensis*, Inoculation, Nitrogen fertilizer reduction.

### Article History

Article # 24-984

Received: 19-Nov-24

Revised: 19-Feb-25

Accepted: 25-Feb-25

Online First: 02-Mar-25

### INTRODUCTION

In the recent years, peanut (*Arachis hypogaea* L.) production has been declining globally (Brasileiro et al., 2015). This is determined by a combination of factors including: (i) changing climate conditions, frequent hot weather and irrigation water mainly leading to stress on peanut crops. These changes, in turn, can lead to reduced yields, lower quality peanuts, and increased susceptibility to pests and diseases; (ii) declining soil fertility: intensive agricultural practices, such as monoculture and excessive NFA and high pesticides reducing the depletion of essential nutrition in farmland, can make it more disadvantageous for planting and raising peanut's output; (iii) water scarcity: peanut is a relatively drought-tolerant crop, but it still requires adequate water to grow and produce yields. Prolonged droughts has been a common

global phenomenon and severely impact the peanut output (Basu et al., 2008; Prasanna et al., 2022). Peanut productivity has significantly been correlated by annual average precipitation. Drought and highly sandy content in peanut farmland have been identified as the key environmental factors that significantly reduce soil nutrient and peanut yield (Krishna et al., 2015). Prolonged drought stress can cause the loss of cell water, leading to osmotic stress as well as inhibiting cell expansion. Water deficiency has seriously reduced irrigation water, turgor reduction, closed stomata, cell membrane integrity, and denatured proteins. Water deficit causing stomata closure in turn slows down photosynthesis. Therefore, the advanced cultivation technologies are deemed crucial for mitigating the extended negative effects of drought on farmland nutrient availability and crop production. One potential solution is improving soil conditions through a biological

**Cite this Article as:** Chuong NV, Trang NNP, Liem TT and Dang PTH, 2025. Effect of *Bacillus sonklensis* associated with cattle manure fertilization on the farmland health and peanut yield. International Journal of Agriculture and Biosciences 14(4): 629-636. <https://doi.org/10.47278/journal.ijab/2025.030>



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system purposely to recover from and withstand prolonged drought stress, involving physiological and biochemical changes. Symbiotic rhizosphere bacteria can minimize the impact of water stress on crops (Abuqamar et al., 2009; Chuong, 2024a). The process include the exchanges in phytohormone types, and metabolic productions. Natural organic nitrogen production plays an important role in the development of drought adaptability. Due to their exclusive role in the root zone, their application is limited to aboveground crop parts. Furthermore, endophytic N-fixing bacteria are changeable and development belong to crop tissues. Their simple adaptation effectively improves the growth of the crops under drought stress and N deficiency (Cassan et al., 2009; Nguyen et al., 2024). The parts of peanut plant contain strains of different microorganisms, including seeds, roots, and stems, collectively known as endophytic nitrogen fixing bacteria (ENFB) (Zhang et al., 2017; Mukherjee et al., 2020). The ENFB play a crucial role in the promoting productivity and development of crop through different symbiotic and non-symbiotic mechanisms (Trivedi et al., 2020; Mukherjee et al., 2020). Also called as endophytic microorganisms, they support and promote plant growth. These trains include bacteria, *actinomyces*, and fungi that live all or part of their lives within plant tissues (David et al., 2016; Hassan, 2017; Nguyen et al., 2019; Harrison and Griffin 2020). Native plants in nature maintain their symbiotic relationships with SRB that promote growth and protect against various survival pressures. Symbiotic rhizosphere bacteria enhance nutrient acquisition for plants through various mechanisms, including biological nitrogen fixation in specialized root structures called as nodules (Johnston-Monje et al., 2016). These bacteria also facilitate nutrient uptake, particularly phosphorus, and some produce siderophores that increase iron availability for plants (Coba de la Peña et al., 2018). Symbiotic rhizosphere bacteria enhance plant nutrient acquisition through enzymatic activities mobilizing nutrients from soil, making them readily available for plant uptake (White et al., 2018; Nguyen & Tran, 2024). These bacteria also promote crop development by resisting crop pathogens, which simultaneously contribute both systemic resistibility and safe protection in the host crops, and inhibit the growth and activity of crop pathogens (Irizarry & White, 2017; White et al., 2019). Endophytes can initiate the gene expression of defensibility in the host crops from the baby plant stage to harvest stage.

The ENFB have completely promoted on the plant growth belong to a direct and indirect number of mechanisms. The benefits of ENFB both enhanced soil fertility availability, phytohormone, root and shoot increase, plant pathogen and disease prevent defense. In safe agricultural production, ENFB that aid plants in adapting to biological stresses are becoming increasingly important due to their capacity to lessen the need for synthetic fertilizers and pesticides, encourage plant growth and health, and raise soil quality. Studying on the ENFB use for sustainable production is practical in order to reduce the NFA and improve farmland fertility (De Andrade et al., 2023; Chuong, 2024b). Cattle manure fertilization is one of the revitalizing techniques of organic farming that is

considerably inexpensive for farmers due to its low cost and safety (Chuong & Bush, 2021; Chuong, 2025). Nitrogen loss from organic waste is reduced when cattle manure is used as an organic fertilizer source. The Cattle manure application provided the effectively and economically feasible treatment of animal manure waste (Ali et al., 2018; Aktar et al., 2019). The cattle manure application provides the farmland with a large amount of carbon and easily absorbed nutrients, which is necessary for plant health and endophytic bacteria species (Van, 2024). The cattle manure fertilization is important to improve soil fertility and a stable organic matter (Nguyen, 2024; Van, 2024). Symbiotic rhizosphere bacteria need carbon element for growth and nitrogen fixation processes. Therefore, cattle manure application combined with nitrogen fixing bacteria addition has promoted plant growth and yield (Rafique et al., 2021). Therefore, traits of soil chemical and plant growth involves in organic manures application combined with nitrogen fixing bacteria inoculation to raise peanut quality and yield (Chuong, 2023). This study aimed to evaluate the nitrogen fixation capacity of *Bacillus sonklengsis*, assessed through its ability to reduce nitrogen fertilizer application in combination with cow manure, on peanut yield in sandy soils.

## MATERIALS & METHODS

### Experimental Time and Location

The experiment was performed from August to December of 2023, from Center of Agricultural Research and Experimentation, An Giang University. The peanut variety L14 was used that was collected from Vietnam Institute of Agricultural Sciences. The physical and chemical traits of farmland at the experimental initiation are presented in Table 1.

### Endophytic N-fixing Bacteria

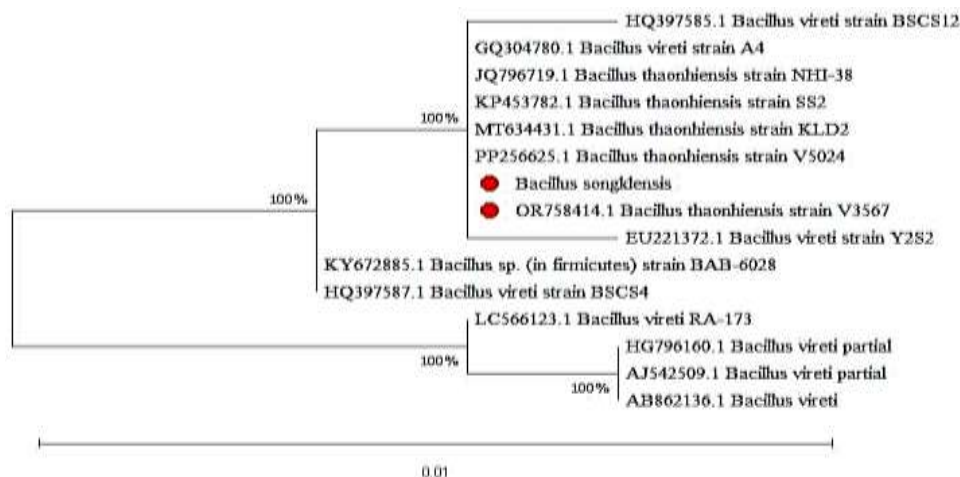
The Symbiotic rhizosphere bacteria were isolated from peanut nodules in the local farmer's fields in An Phu at 65 days after sowing (DAS) (Tian-Yi et al., 2019). *B. sonklengsis* in the YMA growth medium was cultured until it reached a concentration of  $10^8$  CFU/mL before being used for inoculation. The strain, isolated and molecularly identified at Phu Sa Company in Can Tho, Vietnam, was also used to construct a phylogenetic tree to identify similar strains (Fig. 1). The biochemical tests showed a good N-fixing ability in liquid YMA medium after 6 days incubating.

### Experimental Design and Location

Long Xuyen city has a total area of 114,96km<sup>2</sup> and up to 272,365 people. The wet season is covered with clouds, the dry season has hot and stuffy weather all year round. Average temperatures usually vary from 22 to 35°C and rarely fall below 20°C or above 37°C. The rainy period of the year that lasts for 9.0 months, from 23<sup>th</sup> March to 22<sup>th</sup> December, has 31 days of rain falling at least 13mL. The rainiest month is September and an average rainfall of 170ml. Nine different treatments were included in the experiment, which was conducted outside the AGU net house. The experiment followed a completely randomized block design, and each treatment was repeated four times.

**Table 1:** Soil physical and chemical properties before the experiment

Sand (%)	Silt (%)	Clay (%)	Soil pH	SOM (%)	CEC (cmol <sup>+</sup> /kg)
80.0	18.5	1.50	6.70	1.82	1.21
Tissue	Total nitrogen (%)		Available P (mg/kg)		Exchangeable K (cmol <sup>+</sup> /kg)
Sandy loam	0.124		280		Undetected

**Fig. 1:** A phylogenetic tree of *Bacillus sonklengsis*, based on 16S rRNA gene sequences from nodule isolates of peanut and known neighbor sequences from selected reference strains, was constructed using MEGA 11 (Van & Tran, 2024).

The nine experimental plots included: NT1-control (without NFA, CMA and *B. sonklengsis*); NT2 (*B. sonklengsis* inoculation+no NFA+5.0tCMA/ha); NT3 (*B. sonklengsis* inoculation+no NFA+10.0tCMA/ha); NT4 (no *Bacillus sonklengsis* inoculation+20kgNFA/ha+0.0tCMA/ha); NT5 (*B. sonklengsis* inoculation+20kgNFA/ha+5.0tCMA/ha); NT6 (*B. sonklengsis* inoculation+20kgNFA/ha+10.0tCMA/ha); NT7 [*B. sonklengsis* inoculation+40kgNFA/ha (100% of NFA)+0.0tCMA/ha; NT8 (*B. sonklengsis* inoculation+40kgNFA/ha+ 5.0tCMA/ha) and NT9 (*B. sonklengsis* inoculation+40kgNFA/ha+10.0tCMA/ha). Use of the inorganic fertilizers was a single fertilizer, which included urea, superphosphate, and potassium chloride. All fertilizer weight was converted to N, P and K weight per ha, were applied by 60P and 60K kg/ha for all experimental treatments. The chicken manure was taken by Songhong Company, Vietnam, which contained total N (1.7%), available P (0.6%), Exchangeable K (0.85%), total Ca (2.4%) and total Mg (1.27%). The whole area of the experiment was 360m<sup>2</sup> (1.0m x 10m x 9 treatments x 4 repeats). The distance between two repeats was 0.5m. Peanut plants were planted in single rows with a spacing of 25cm (sowing 2 seeds per hole). All two healthy plants were kept for each hole in order to monitor throughout the experiment.

### Sample Collection and Data Analysis

The agronomy components (plant height, total chlorophyll content, available shoots, and leaf number) were averagely measured per plant during 20, 45 and 65 DAS. Ten healthy plants were selected to observe the growth and yield traits per replication, which consisted of the nodulous number and weight, plant biomass, number of fill and empty pods, 1,000 seed weight and fresh pod yield. Seed nutrition attributes were analyzed for humidity, lipid and protein. Collection of soil samples in the initial and end experiment were under depth of 0 to 20cm. Seed samples were separately taken and dried to analyze nutrient traits. All traits of soil physical chemical were analyzed by Arnold (1986) and nutrition composition (Delahaut & Marega, 2022). Mean data were calculated by

using Statgraphite XVI software for the variance analysis. Research data were analyzed statistically, which were compared between variables and Duncan's test at  $P \leq 0.05$ .

## RESULTS & DISCUSSION

### The Effects of *Bacillus sonklengsis*, CMA and NFA Rates on the Peanut Growth

The results of the study on the growth of peanut plants at 20, 45, and 65 days after sowing (DAS), as shown in Fig. 1, were different significantly from 5.0 to 1.0%. This showed that the average height in the treatments of NFA, CMA, and *B. sonklengsis* was significantly higher than that of the treatment without NFA, CMA, and *B. sonklengsis*. The application of 20 kg NFA/ha + 10 t CMA/ha and *B. sonklengsis* (NT6) had the highest plant height at all three time periods (20, 45, and 65 DAS), in contrast to the one without the application of NFA, CMA and *B. sonklengsis* (NT1).

Similar to plant height, the results of the study on the peanut available branches at stages 20, 45 and 65 DAS showed the significant difference among treatments at levels of 5.0 and 1.0%. (Fig. 3). The number of available branches in the treatments of NFA combined with CMA and *B. sonklengsis* inoculation had a higher average available branches and was significantly different compared to the control treatments (NT1, NT4 and NT7). The application rate of 20kgNFA/ha + 10tCMA/ha and *B. sonklengsis* inoculation (NT6) had the highest branches compared to other treatments, whereas, the lowest height was found in the treatments of without NFA, CMA, and *B. sonklengsis* (NT1) at 20, 45 and 65 DAS (Fig. 3). The available branches well grow by supplying the nutrients from farmland to form pods, as a result, if the peanut plant has available branches, it can produce more pods and fruit (Fan et al., 2019). The application of animal manures significantly enhanced plant height, effective shoot number, stem length, internode length and leaf number after 3, 4, and 5 weeks of cattle manure application (Rekha et al., 2018). The *B. sonklengsis*

inoculation not only promoted the secretion of auxin (IAA) and phosphorus solubilization but also enhanced the biological N-fixation process in plants. Moreover, the highest nitrogenase enzyme activity was observed in the treatments of ENFB amendment, leading to a more pronounced N-fixation process compared to non-bacteria inoculation. In other words, the ENFB inoculation is capable of promoting the highest plant height and shoot number (Zhang et al., 2022).

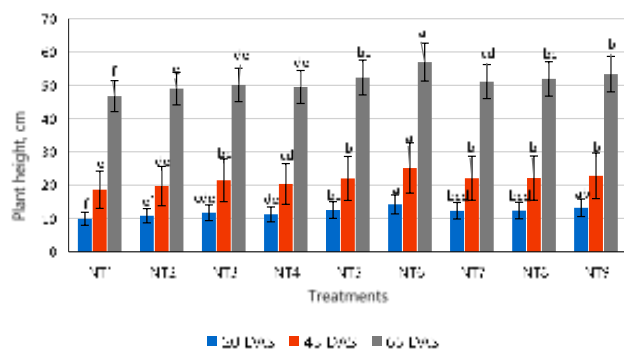


Fig. 2: Plant height during period of 20, 45 and 65 DAS.

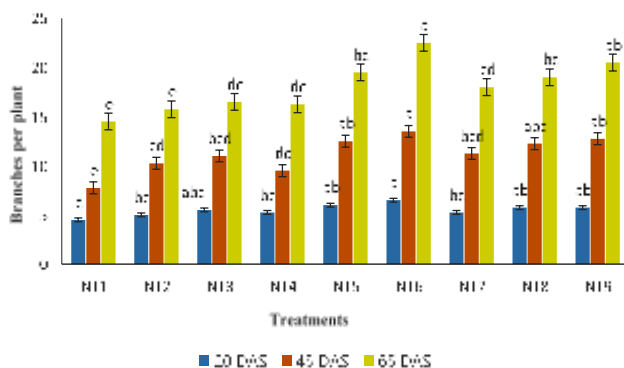


Fig. 3: Branch number during period of 20, 45 and 65 DAS.

The leaf number per peanut plant at the stages of 20, 45 and 65 DAS showed that the average leaf number in the treatments of the ENFB inoculation combined with NFA and CMA statistically was different and significantly increased the growth period (Table 2). For the fertilization of 20kgNFA/ha + 10 t CMA/ha associated with similar to plant height, the results of the study on the peanut available branches at stages 20, 45, and 65 DAS showed significant differences among treatments at level 5.0 and 1.0%. (Fig. 3). The number of available branches in the treatments of NFA combined with CMA and *B. sonklengsis* inoculation had a higher average available branches and was significantly different from the control treatments (NT1, NT4 and NT7). The application rate of 20 kgNFA/ ha + 10tCMA/ha and *B. sonklengsis* inoculation (NT6) had the highest branches compared to other treatments, while the lowest height was found in the treatments without NFA, CMA and *B. sonklengsis* (NT1) at 20, 45, and 65 DAS (Fig. 3). The application of vermicompost significantly enhanced plant height, effective shoot number, stem length, internode length, and leaf number after 3, 4 and 5 weeks of CMA (Rekha et al., 2018; Fan et al., 2019; Nguyen & Tran, 2024). The *B.*

*sonklengsis* inoculation not only promoted the secretion of auxin (IAA) and phosphorus solubilization but also enhanced the biological N-fixation process in plants. Moreover, the highest nitrogenase enzyme activity was observed in the treatments of ENFB inoculation, leading to a more pronounced N-fixation process compared to non ENFB inoculation. In short, the ENFB inoculation is capable of promoting the highest plant height and shoot number (Zhang et al., 2022; Chuong, 2024b).

Table 2: Number of leaves and chlorophyll concentration per peanut plants

Treatments	Leaves/plant			Total chlorophyll (mg/m <sup>2</sup> )		
	Days after sowing (DAS)					
	20	45	65	20	45	65
NT1	14.5e	41.3e	92.5d	339d	403c	417
NT2	15.5de	43.5e	95.3cd	345cd	410c	429
NT3	16.5bcd	52.3cd	109ab	383b	416bc	439
NT4	15.8cde	50.3d	99.3bcd	360c	414bc	430
NT5	17.8ab	57.8abc	107abc	411a	436ab	440
NT6	19.0a	61.5a	115a	411a	447a	449
NT7	16.8bcd	54.8bcd	102bcd	406a	427abc	439
NT8	17.5abc	55.3bcd	106abc	407a	444a	446
NT9	18.3ab	58.5ab	110ab	415a	449a	462
F	**	**	*	**	**	ns
CV (%)	10.5	7.38	9.80	8.16	5.33	5.02

The average value followed by the same letter in the same column is insignificantly different (ns); \*, \*\*: significantly different at level 5% and 1%, respectively.

Comparing the six experimental treatments, the highest leaf number was observed in the treatment of NT6, while the lowest one was in NT1 (Control). Similar to plant height, the number of effective peanut branches at 20, 45, and 65 DAS was found significantly different ( $P < 0.05$  and  $P < 0.01$ ) among the various treatments. (Fig. 3). The number of available branches in the treatments of NFA combined with CMA and *B. sonklengsis* inoculation had on average a higher available branches and was significantly different from the control treatment (NT1, NT4 and NT7). The application rate of 20 kgNFA/ ha + 10tCMA/ha and *B. sonklengsis* inoculation (NT6) had the highest branches compared to other treatments, while the lowest height was found in the treatments without NFA, CMA, and *B. sonklengsis* (NT1) at 20, 45, and 65 DAS (Fig. 3). The available branches well grow by supplying the nutrients from farmland to form pods; thus the peanut plant has available branches, it will produce more pods and fruits (Fan et al., 2019; Nguyen, 2024). The application of vermicompost significantly enhanced plant height, effective shoot number, stem length, internode length and leaf number after 3, 4 and 5 weeks of CMA (Rekha et al., 2018; Mustafa, 2023). The *B. sonklengsis* inoculation not only promoted the secretion of auxin (IAA) and phosphorus solubilization but also enhanced the biological N-fixation process in plants. Moreover, the highest nitrogenase enzyme activity was observed in the treatments of ENFB inoculation, leading to a more pronounced N-fixation process compared to non-ENFB inoculation. In other words, the *B. sonklengsis* inoculation promoted the maximum plant height and shoot number (Zhang et al., 2022, Chuong, 2024a).

Table 2 showed that the total chlorophyll content at three growth stages of 20, 45 and 65 DAS between the NFA rates of 0, 20 and 40 kg/ha and the CMA rates of 0, 5

and 10t/ha was significantly different at 1% (except for 65 DAS). Here, the chlorophyll content in peanut leaves with the rate of 20kgNFA/ha + 10tCMA/ha + *B. sonklensis* (NT6) had the highest value (411 and 447 mg/m<sup>2</sup>) at 15 and 45 DAS, in contrast to the one, without NFA, CMA and *B. sonklensis*. Plant height, shoot number, and root length gradually increased from the seedling stage to harvest in treated seed plots with endophytic N-fixing bacteria inoculation compared to untreated seeds. Chlorophyll content and leaf number of peanuts are the indirect indicators of health and determine peanut yield. The effectiveness of organic manure application combined with *B. sonklensis* reduced 25 to 50% of NFA without reducing chlorophyll content and leaf number per plant (Maroniche et al., 2024).

The data as presented in Table 3 indicated that at the end of the growing season, the average biomass varied from 208g (NT1) to 304g (NT9). A 1% significance level was observed between these treatments. The highest average biomass achieved in the highest CMA (10 t/ha) and NFA in combination with *B. sonklensis*. Peanut seeds treated with *B. sonklensis* had good green leaves, while the control plots without *B. sonklensis* inoculation had slightly yellow leaves. At 65 DAS, there were nodules on peanut roots in the inoculated plants of *B. sonklensis*, which had many large nodules to concentrate on the root crown and dark red color. These results indicated that non-*B. sonklensis* inoculation was ineffective (low effectiveness). Meanwhile, the application of 40kg NFA + 5.0tCMA ha combined with *B. sonklensis* inoculation (NT8) had the highest number and fresh nodule weight. The number of nodules ranged from 180 to 330 nodules/plant and it was significantly different at the level 1%. In particular, the treatments of NT2, NT3, NT5, NT6, NT8, and NT9 had significantly different numbers of nodules (Table 3). The nodule weight ranged from 0.935 to 1.82g/plant and it was significantly different at the level 1%. The number and weight of peanut nodules in the ENFB inoculation treatments were higher than that of the non-amended treatments of *B. sonklensis* species. Peanuts are a major host plant for symbiotic *B. sonklensis* in the legume family. The *B. sonklensis* inoculation help the host plant resist pathogens to reduce cellulose biosynthesis processes, which consists of lignin biosynthesis, nodulous formation, and biomass increase (Caño-Delgado et al., 2003; De Andrade et al., 2023).

**Table 3:** Biomass, nodulous number and weight per peanut plants

Treatments	Biomass (gr/plant)	Nodulous number (nodules/plant)	Nodulous weight (gr/plant)
NT1	208d	180e	0.993ef
NT2	226cd	211de	1.17de
NT3	256bc	293ab	1.62ab
NT4	251bcd	184e	0.935f
NT5	294ab	284b	1.58bc
NT6	313a	258bc	1.37cd
NT7	271abc	273bc	1.50bc
NT8	280ab	330a	1.82a
NT9	304a	238cd	1.25d
F	**	**	**
CV (%)	16.5	21.9	23.2

The average value followed by the same letter in the same column is insignificantly different (ns); \*\*, significantly different at level 1% ( $P \leq 0.01$ ).

### Impacts of *Bacillus sonklensis*, NFA and CMA on the Peanut Yield Traits

As depicted in Table 4, the peanut yield components corresponding to the total number of full pods, empty pods, weight of filled pods and weight of unfilled pods were significantly different at level 5% ( $P \leq 0.05$ ) (Except unfilled pod weight). The weight of unfilled pods per plant statistically was not different in all experiments. The results showed that the weight and total number of unfilled pods per plant were not affected by CMA, NFA and *B. sonklensis*. Other traits, however, were significantly affected at the significance level of 5%. The *B. sonklensis* inoculation of peanut seeds grown on low nutrient soil and under water stress conditions improved shoot length, root length, total chlorophyll concentration, nodulous number, lipid concentration and seed output (Van Chuong, 2021; Chuong, 2025). Moreover, endophytic bacteria inoculation stimulated drought tolerance mechanisms and led to higher nitrogen, phosphorus, potassium, and electrolyte uptake efficiency. The amendment of *B. sonklensis* controlled peanut's water uptake capacity, enhanced photosynthesis and stress adaptability, and reduced water lack's damages. Therefore, ENFB species have positive abilities for application in improving crop productivity (Kumar et al., 2022).

### Impacts of *Bacillus sonklensis*, N Fertilizer and CMA on Peanut Yield and Quality

The weight of 100 pods, 1,000 seeds and fresh yield of peanuts grown on infertile soil in the outdoor area of the experimental field of AGU showed a number of significant differences ( $P \leq 0.01$ ) in all experimental treatments (Table 5). The largest weight of 100 pods, 1,000 seeds and fresh yield were 120g, 383g and 6.54 t/ha, respectively, for the treatment of 20kgN/ha + 10tCMA/ha combined with *B. sonklensis* (NT6). Whereas, the lowest values of 100-pod weight, 1,000-seed weight and fresh yield were 76.7g, 250g and 3.82t/ha, respectively, for the NT1 treatment (The yield in NT6 was 41.6% higher than that in NT1). This result proved that the N fixing level fixed from root-zone microorganisms can help to increase the availability of nutrients for the peanut plant, enabling the roots to take up nutrients from the soil more effectively compared to the one without co-fertilization of these two types, which in turn helps to increase the peanut yield (Nagaraju et al., 2021).

Table 6 shows that the moisture content of peanut seeds ranged from 25.2 to 33.8% and was significantly different at the 1% level ( $P \leq 0.01$ ). The highest moisture content was observed in NT1 and NT7 (33.8%), while the lowest one was found in NT8 (25.2%). However, the highest lipid content in the seeds (28.8%) was achieved in the treatment of *B. sonklensis* inoculation and 10 CMA/ha + no NFA (NT3). Conversely, the lowest lipid content (23.7%) was observed in the control treatments (NT1 and NT4). Protein was found the highest (19.5%) in the treatment of 5 t CMA/ha (no application of NFA and *B. sonklensis*) (NT2) and the lowest one was in NT1 (15.9%) (Application of P and K fertilizers).

**Table 4:** Number and weight of filled and unfilled pods

Treatments	No. of filled pods (pod/ plant)	Filled pod weight(g/plant)	No. of unfilled pods (pod/ plant)	Unfilled pod weight (g/plant)
NT1	41.9b	94.7b	9.25ab	9.42
NT2	68.4a	161a	11.0a	10.8
NT3	74.5a	169a	4.75c	4.17
NT4	66.8a	173a	6.75bc	4.83
NT5	72.1a	175a	6.25bc	4.92
NT6	79.5a	200a	6.50bc	5.75
NT7	74.5a	177a	4.75c	4.92
NT8	76.6a	183a	7.50abc	7.50
NT9	77.7a	187a	8.50ab	8.83
F	*	*	*	ns
CV (%)	22.2	26.1	41.2	55.4

The average value followed by the same letter in the same column is insignificantly different (ns); \*: significantly different at level 5% ( $P \leq 0.05$ ).

**Table 5:** Weight of 100 pods, 1000 seeds and fresh pod yield

Treatment	Weight of 100 pods (g)	Weight of 1,000 seeds (g)	Fresh pod yield (t/ha)
NT1	76.7d	250f	3.82c
NT2	103bc	273ef	4.82bc
NT3	105bc	300de	4.81bc
NT4	100c	313cd	5.11b
NT5	114ab	343bc	5.15b
NT6	120a	383a	6.54a
NT7	109abc	333bcd	5.16b
NT8	107bc	323bcd	5.20b
NT9	110abc	360ab	5.35b
F	**	**	**
CV (%)	13.2	14.4	18.2

The average value followed by the same letter in the same column is insignificantly different (ns); \*\*: significantly different at level 1% ( $P \leq 0.01$ ).

**Table 6:** Nutrition composition (%) of peanut seeds

Treatments	Moisture	Lipid	Protein	Nitrogen	Phosphorous	Potassium
NT1	33.8a	23.7e	15.9e	2.54e	0.280c	0.415ab
NT2	30.1bc	25.7cd	19.5a	3.12a	0.291bc	0.421ab
NT3	30.7b	28.8a	17.6bcd	2.81cd	0.289bc	0.433a
NT4	30.6b	23.7e	17.0d	2.72d	0.312a	0.327c
NT5	28.5c	26.8bc	17.3cd	2.77cd	0.314a	0.438a
NT6	28.4c	27.4ab	17.9bcd	2.86bcd	0.296ab	0.427a
NT7	33.8a	24.9de	18.3bc	2.93bc	0.302ab	0.418ab
NT8	25.2d	26.7bc	18.6ab	2.98ab	0.294bc	0.428a
NT9	28.6c	25.4cd	16.9de	2.70de	0.260d	0.395b
F	**	**	**	**	**	**
CV (%)	9.37	7.34	6.83	6.83	6.41	8.60

\*\* : significantly different at level 1% ( $P \leq 0.01$ ).

The results are similar with the findings of Ludwig et al. (2003); Chuong et al. (2024) reporting that the nodules are the habitat of nitrogen-fixing bacteria and that these bacteria produce enzymes synthesizing protein for the plant. The mutually beneficial symbiotic relationship between plants and ENFB in vegetative tissues is controlled by the plant, allowing the peanut plant to take up protein from soil into the plant and pod better than in the treatments without root-zone nitrogen-fixing microbial inoculation. The composition of N, P and K in peanut seeds was found highest in the treatments with NFA, *B. sonklengsis* inoculation and CMA (NT4, NT5, NT6 and NT7). While the lowest results were found in the treatments with only P and K fertilization, without both *B. sonklengsis* inoculation and NFA (NT1) (Table 6). The cattle manure application had a significant effect on most peanut yield and quality parameters, including pod number, pod quality, pod weight, seed weight. The CMA treatments combined with *B. sonklengsis* inoculation increased the peanut yield and quality components compared to the control one. Other studies showed that the NFA combined with organic manures significantly increased the peanut yield and quality. The positive interaction between ENFB inoculation and CMA impacted remarkably on the nitrogen

fertilizer use efficiency and peanut output (Li et al., 2019; El-Sherbeny et al., 2023; Chuong, 2024b).

## Conclusion

The fertilization of 10tCMA/ha and 20kgNFA/ha associated with *Bacillus sonklengsis* inoculation obtained the highly positive increases on peanut traits of agronomy, yield and quality. The chicken manure application in combination with *B. sonklengsis* amendment reduced the amount of inorganic nitrogen fertilizer up to 50%, which was the higher pod yield of the recommended NFA (100%). The application of 20kgNFA/ha + 10tCMA/ha combined with *B. sonklengsis* amendment had the highest yield. In contrast, the lowest yield was achieved in the treatments only using phosphorous and potassium fertilization. The NT6 treatment's yield increased by 41.5% compared to the control treatment and by 18.2% compared to the NT9 treatment (10tCMA/ha+40kgNFA/ha) and *B. sonklengsis* amendment. From this result, it can be concluded that the combined application of CMA and *B. sonklengsis* increased yield, quality and reduced by 50% of NFA.

**Conflict of Interests:** The authors state that there are no conflicts of interest with this study.

**Acknowledgement:** The authors thank the central laboratories and Agricultural faculty of AGU for supporting this research.

**Author's Contribution:** Nguyen, V. Chuong oversaw and coordinated the experiments. Nguyen, N. P. Trang isolated and test the bacteria. Phan, T. H. Dang and Tran, T. Liem analyzed the experimental data statistically. All authors contributed to the critical revision of the manuscript and approved the final version.



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