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Influences of *Enterobacter Cloacae* Strain Fg 5-2 and Its Vermicompost and Nitrogen Fertilizer Usage Efficiency on Groundnut Yield

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Abstract

Reducing production costs by replacing chemical nitrogen fertilizer (CNF) with natural nitrogen fertilizers from endophytic nitrogen-fixing bacteria is an optimally alternative solution to utilize natural nitrogen sources from biological nitrogen fixation to improve crop yields. Three levels of nitrogen (N) and vermicompost (VMC) application combined with *Enterobacter cloacae* strain Fg 5-2 (EC strain Fg 5-2) inoculation, which were carried out in 2 experiments, aimed to evaluate its impacts on the groundnut output. The 2 experiments consisted of 0, 20, 40 kg N ha^{-1} and 0, 5.0, 10 t VMC ha^{-1} application associated with EC strain Fg 5-2 inoculation and 4 replications. Results observed that 50 % CNF reduction, which increased up to 16.5 % of the groundnut yield compared to no CNF application, was no yield difference of the 100 % CNF application. Application of 10 t VMC ha^{-1} associated with EC strain Fg 5-2 inoculation increased up to 38.4 % compared to no VMC amendment. The groundnut yield of experiment 1 and 2 remarkably increased up to 19.6 and 12.9 %, respectively, with the EC strain Fg 5-2 inoculation compared to the non-EC strain Fg 5-2 inoculation. Application of VMC was the high efficiency on the nodulous number and weight of groundnut. Application of 20 kg N or 10 t VMC ha^{-1} combined with EC strain Fg 5-2 inoculation adequately reached highest groundnut yield. These selected results are the best associated rate to reduce CNF by applying EC strain Fg 5-2 inoculation to improve farmland nutrients and increase the groundnut yield.

Keywords: *Enterobacter cloacae* strain Fg 5-2, Groundnut, Productivity, Vermicompost, CNF, VMC

Introduction

Peanut (*Arachis hypogeae* L.) has been the agriculture's mainstay, which is used to product cooking oil from its seed in tropical and subtropical regions. Peanuts are the 3rd most important grain crop, the 4th most main oilseed crop and the 3rd main foodstuff source for humans globally [1]. Peanut cultivation that is thought to firstly plant South America areas, have originated in this area [2]. Peanuts play a various position in various biochemical and physiological processes of crop growth, including new cell formation [3]. The influences of N fertilizer on crops have sufficiently shown that foliar or farmland fertilization of N fertilizer has been shown to have a significant impact on the yield and quality promotion of many plant kinds [4]. Peas were grown on N deficiency soils, which leaded to low seed protein [5]. Chemical nitrogen fertilizer has exceedingly applied to the soil, with rate higher than the needs of legume plants, which could inhibit N fixation process by rhizobia through the development of inefficient nodules. As a whole, N fertilizer application of higher levels significantly reduced number of green been nodules [6].

In the recent years, the world has been using VMC in agricultural cultivation [7]. There are nearly

3,000 species of worms, which have used to produce VMC and their feed from livestock waste, which is an important material in VMC production [8]. Many N-fixing bacterial strains have fully presented in VMC and farmland such as *Rhizobium* sp., *Bacillus* sp. and *Azotobacter* sp., which are often found in the earthworm's gut. Moreover, *mycorrhizal* fungi are microorganisms that are capably fixed by N_2 from the atmosphere. Therefore, VMC provides huge number of beneficial microorganisms to farmlands. Furthermore, these bacterial strains also use nutrients from VMC [9]. Positive microorganisms, which are abundant in the soil, decompose organic matter from VMC and soil and make it available to plants in a more easily digestible form [10]. Deficiency of SOM content has often happened in the farmlands and the animal manures is totally recycled and reproduced to the soil of An Phu district, where VMC can contribute to waste treatment and are an important part in environmental protection [11].

Farmland microorganisms are dramatically diversified with population of up to 1,011 CFU gr^{-1} [12]. Soil bacteria are related in nutrient cycling and OM decomposition and contribute plants perform many effective actions such as nutrition absorption, stress adaption and disease resistibility [13]. The rhizosphere contains a dynamic community of microorganisms that participate in bacterial-bacterial and bacterial-plant communication mediated by plant exudates [14]. However, the N-fixing microbiota is very dynamical and changed reaction in response to complex environmental factors [15]. *Enterobacter cloacae* strain Fg 5-2 is an endophytic nitrogen-fixing bacteria (ENFB) and isolated from peanut nodules, which has a high capacity to fix nitrogen from the atmosphere into ammonium (NH_4^+) and provides hugely natural N source and is easily uptake for crops. Addition of 10 tons of VMC per hectare in combination with E. *asburiae* genus increased the highest productivity and yield characteristics of peanuts and the lowest values were in the treatments without fertilizer and without E. cloacae strain. The combination of VMC with rhizosphere nitrogen-fixing bacteria remarkably increased agricultural indicators, yield components and peanut yield compared to only applying inorganic fertilizer [16]. Moreover, VMC addition combined with E. *asburiae* increased the number and weight of peanut nodules compared to only applying inorganic fertilizer. Fertilization of organic fertilizer in fertilizer management and the use of rhizosphere microorganisms can maximize soil fertility and productivity while lessening the usage of chemical fertilizer and improving farmland health towards sustainable agricultural production [17]. In addition, the addition of VMC can enrich the soil microbial community such as N-fixing and P-solubilizing microorganisms [18]. Derivatives present in VMC are important growth promoters in plant growth. Waste water of VMC is a type of liquid organic fertilizer, which collected after water passes through the digestive tract of worms [19]. Nitrogen-fixing microorganisms help to reduce the use of inorganic N fertilizer due to inorganic nitrogen is part replaced by the natural nitrogen. The natural nitrogen produced by nitrogen-fixing microorganisms that are symbiotic with legumes. The studied goal could select the best ratio of VMC and N fertilizer when combined with *Enterobacter cloacae* strain Fg 5-2 species in order to raise the growth and yield of groundnuts.

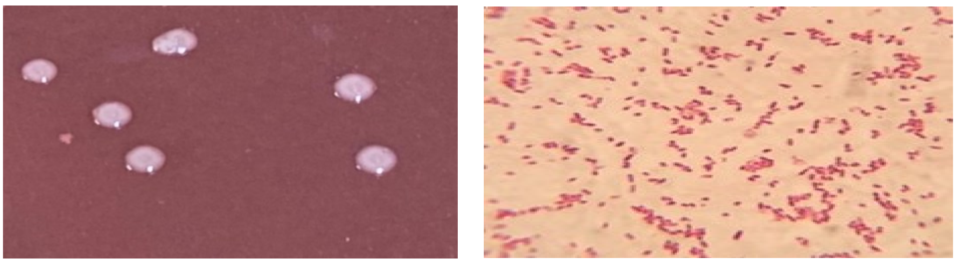
Materials and methods

Enterobacter cloacae strain Fg 5-2 was isolated from groundnuts nodules that collected from Phuoc Hung commune, An Phu district, An Giang province, Vietnam. Pure cultures were isolated and biochemically tested to preliminarily identify as nitrogen-fixing bacteria. And then, pure EC strain Fg 5-2 colonies were molecularly identified and sequenced by amplifying a partial area of the 16S rRNA gene and BLAST against the NCBI gene bank. *Enterobacter cloacae* strain Fg 5-2 species that had a 100 % similarity to the 16S rRNA sequence of the target bacteria, was used in this study.

Two experiments that were conducted outside the net house of An Giang University, consisted of (i) experiment 1: Evaluating the effect of EC strain Fg 5-2 inoculum associated with 3 CNF rates, (ii) Experiment 2: Evaluating the effect of EC inoculum associated with 3 VMC ratios on the peanut growth and yield and 4 replications in a completely randomized block design. The groundnut seeds were incubated with EC strain Fg 5-2 1 day before sowing. Three CNF and VMC ratios and *Enterobacter cloacae* strain Fg 5-2 species inoculum per the experiment were shown in **Table 2**. All treatments of 2 experiments were 12 treatments. Chemical fertilizers, which were used by urea, super phosphate and KCl, was converted to the NPK weight. The experiment area was 480 m^2 (1 m in width×10 m in length×6 treatments×02 experiments×4 replicates). Chemical properties of the experimental farmland samples were collected in a 0 - 20 cm of soil depth, which were tested according to and the data are presented in **Table 1**. The analysis of the experimental soil samples classified the tested soil as sandy loam [20].

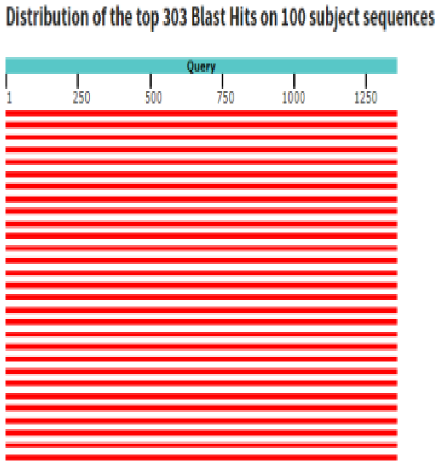
The experimental site was divided into 48 units for 2 experiments. Each unit that was 10 m^2 (10 m in length×1 m in width, consisted of 2 rows in 50 cm distance. Groundnut seeds L14 were collected from the Vietnam Plant Variety Center. Groundnut seeds were collected from the Vietnam Plant Breeding Center, which were sown 2 seeds/hole with a distance of 30 cm between plants. Ground plants had from 3 leaves that will keep a healthy plant in each hole for research until harvest. Immediately before planting 24 h, the seeds were inoculated with EC strain Fg 5-2 in dark and at room temperature with up to 70 % humidity. The 2 experiments were arranged on April 30th, 2023 and harvested manually on August 30th, 2023 in both experiments.

At harvest, 10 groundnut plants were randomly collected from each experimental unit to record agronomic indicators, yield components and fresh yield. Fresh seed samples were taken from each unit to test nutrient compositions. Yield components were calculated according to the groundnut growth time, including height and number (No.) of branches and pods per plant, biomass, nodulous No. and weight (Wt.) per plant, fresh Wt. of firm and unfilled pods per plant. Fresh productivity was counted in t ha^{-1} for fresh pods. farmland parameters such as pH (6.80), CEC (5.07 $cmol^+ kg^{-1}$), SOM (1.17 %), total N (0.15 %) and available P (29.5 mg 100 gr^{-1}). Effective P, which is an essential element for N-fixing microorganisms and peanut plants, is essential for plant growth and the life of N-fixing microorganisms. In particular, exchangeable K was not detected in all soil samples. In general, the experimental soil had poor nutrient status and was deficient in potassium (**Table 1**). Peanut seeds L14 have good disease resistance and high output.

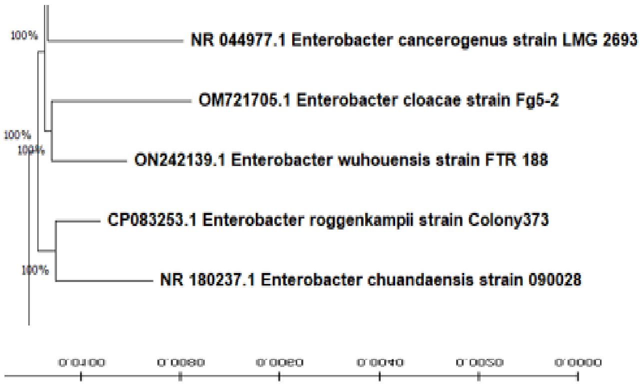


(a) (b)

(c)



(d)



(e)

Figure 1 Isolation and identification of EC Strain Fg 5-2. (a) Globular, slimy, shiny, milky colonies on YEMA medium; (b) Morphology (100× objective) and Gram-negative, short rod-shaped; (c) 1350 bp long, 5’- 3’; (d) Blast Nucleotide on NCBI and (e) Phylogenetic tree.

Table 1 Attributes of the farmland (0 - 20 cm) before planting.

Traits	**Units**	**Results**
	Soil Texture	
Sand		80.4
Silt	%	18.6
Clay		1.00
Soil texture	-	Sandy loam
	Farmland properties	
Soil pH*	-	6.8
Soil organic matter (SOM)	%	1.17
CEC (cmol$^+$ kg^{-1})**	cmol$^+$ kg^{-1}	5.07

Traits	Units	Results
Soil available nutrition		
Total N	%	0.15
Availlable P	mg 100 gr^{-1}	29.5
Exchangeable K	$cmol^{+}$ kg^{-1}	undetected (LOD = 0.30)

*; ** Soil pH and CEC: Ratio of soil/H_2O (1: 2.5, w/v); Average data were 5 soil samples.

The variance (ANOVA) determine technique for research design by using the statgraphics version XVIII software. The comparison of mean values between variables was performed by using the Duncan test at a significance level of 5 % ($p \leq 0.05$). For each experiment, the processed data were calculated according to 02 factors to find the interaction between treatments and factors with each other.

Results and discussion

There was a statistically adequately divergence at $p \leq 0.05$ and $p \leq 0.01$ for the groundnut plant height among treatments in the 2 experiments (**Table 2**). Groundnut plant height increased throughout the growth period from 20, 45 and 65 days after sowing (DAS). However, the maximum plant height increased in the treatments of 40 kg N fertilizer application per ha in experiment 1 and 10 t VMC per ha in Exp. 2. On contrary, the minimum height of groundnut reached in application of non CNF or 20 kg N_2 ha^{-1} in Exp. 1, as well as without VMC or 5.0 t VMC ha^{-1} amendment per ha in Exp. 2.

The height of groundnut plants among the CNF and VMC rates at 20 DAS showed that the average height of peanut plants ranged from 11.1 to 12.7 cm in 3 N rates (0, 20 and 40.0 kg ha^{-1}) in Exp. 1 and 13.67 to 15.68 cm in 3 VMC rates (0, 5.0 and 10.0 t ha^{-1}) in Exp. 2. The highest plant height was 12.7 cm when applying 40 N fertilizer per ha and 10 t VMC per ha (15.68 cm) were applied, while the other treatments in Exp. 1 and 2 had the lowest plant height of 11.10 cm and 13.67, respectively. Similar to the 20 DAS stage, the highest plant heights of 45 and 65 DAS were 22.8 and 53.2 cm, respectively, at the fertilizer application of 40 kg N ha^{-1} and 25.77 and 56.52 cm, respectively, at the fertilizer application rates of 10 t VMC ha^{-1}. Further, the minimum results observed at 45 and 65 DAS in the application of no CNF or 20 kg ha^{-1} and non-VMC or 5.0 t VMC ha^{-1} (**Table 2**). There is a sufficient difference between the inoculated and non-EC strain Fg 5-2 inoculation at $p \leq 0.01$. The had higher plant height across the 20, 45 and 65 DAS stages (13.0, 23.1 and 53.7 cm, respectively) compared to the non-inoculated treatments (10.6, 19.6 and 48.4 cm, respectively). However, there was no interaction between CNF rate with EC strain Fg 5-2 inoculation and VMC with EC strain Fg 5-2 inoculation from 20, 45 and 65 DAS (Except 65 DAS at 3 N rates). Research results of Chuong [17] and Motoso *et al.* [21], proved that N-fixing bacterium had a related impact on groundnut height at 38 DAS, while the animal manures treatment had insignificant impact on peanut height. When applying nitrogen fertilizer in moderation, legumes can form more effective nodules on their roots to fix nitrogen from the air, which can further stimulate plant growth [22]. Decomposed organic manures has the ability to increase the height of groundnuts due to the full supply of nutrients for plant growth. According to Mondal *et al.* [23]. Nitrogen is essential for plants to form amino acid and protein many kinds, more importance in the mature period to promote peanut growth processes such as cell creation and cell divergence to increase crop yield. Endophytic nitrogen-fixing bacteria (ENFB), especially those residing in plant roots, are considered to be excellent sources of biofertilizers and biocontrol agents in agriculture. They are not only involved in promoting plant growth and health but can also transmit these benefits for the next crops [24]. Several endophytic nitrogen-fixing bacteria, including *Enterobacter* sp., have been shown to promote groundnut growth. Moreover, several previous studies have shown that ENFB have reduced the amount of nitrogen fertilizer and significantly interacted with the amount of inorganic fertilizer applied [25].

Table 2 Effects of N fertilizer, VMC and EC inoculum on crop height in 2 experiments.

Factor	Experiment 1 (DAS) Crop height (cm)			Factor	Experiment 2 (DAS) Crop height (cm)		
	20	45	65		20	45	65
N fertilizer (A)				VMC (A)			
0.00 kg ha^{-1}	11.1 ± 0.081^{b}	20.4 ± 0.326^{b}	49.3 ± 0.245^{c}	0.00 t ha^{-1}	13.67 ± 0.016^{b}	20.88 ± 0.001^{b}	50.42 ± 0.016^{c}
20.0 kg ha^{-1}	11.7 ± 0.163^{b}	20.9 ± 0.081^{b}	50.6 ± 0.245^{b}	5.00 t ha^{-1}	13.62 ± 0.016^{b}	22.47 ± 0.001^{ab}	53.81 ± 0.008^{b}
40.0 kg ha^{-1}	12.7 ± 0.163^{b}	22.8 ± 0.163^{a}	53.2 ± 0.163^{a}	10.0 t ha^{-1}	15.68 ± 0.016^{a}	25.77 ± 0.001^{a}	56.62 ± 0.016^{a}
EC strain Fg 5-2 (10^8 CFU mL^{-1}) (B)				EC strain Fg 5-2 (10^8 CFU mL^{-1}) (B)			
Yes	13.0 ± 0.081^{a}	23.1 ± 0.081^{a}	53.7 ± 0.163^{a}	Yes	13.04 ± 0.032^{b}	21.27 ± 0.057^{b}	51.97 ± 0.008^{b}
No	10.6 ± 0.245^{b}	19.6 ± 0.245^{b}	48.4 ± 0.326^{b}	No	14.28 ± 0.032^{a}	23.48 ± 0.016^{a}	54.93 ± 0.024^{a}
F (A)	**	**	**	F (A)	*	**	**
F (B)	**	**	**	F (B)	*	**	*
F (A x B)	ns	ns	**	F (A x B)	ns	ns	ns
CV (%)	6.70	4.70	12.2	CV (%)	4.6	5.5	13.5

DAS: Days after sowing; ns: No significant different at 5 % ($p > 0.05$); * $p \leq 0.05$, ** $p \leq 0.01$

Similarly, the number of effective branches in the 2 experiments increased throughout the growth period from 20, 45 and 65 DAS and was statistically adequate differences at $p \leq 0.01$. However, the highest branches increased in the treatments of 40 kg N fertilizer application per ha in experiment 1 and 10 tons VMC per ha in experiment 2 during the growth period (20, 45 and 65 ADS). Otherwise, the lowest branches of peanut plants increased without CNF or 20 kg CNF application per ha in experiment 1, as well as without VMC or 5 tons VMC addition per ha in experiment 2. The combined application of chicken manure and Rhizobium sp. inoculation increased the height and branch number of groundnut plants compared to the control treatments (no chicken manure and no *Rhizobium* sp. inoculation). The research also showed that the nitrogen fixation ability of *Rhizobium* sp. on sandy soil was significantly enhanced in treatments of *Rhizobium* sp. strain inoculation combined with chicken manure [26].

Table 3 Effects of N fertilizer, VMC and EC inoculum on available branches in 2 experiments.

Factor	Experiment 1 (DAS) Available branches per plant			Factor	Experiment 2 (DAS) Available branches per plant		
	20	45	65		20	45	65
N fertilizer (A)				VMC (A)			
0.00 kg ha^{-1}	3.75 ± 0.040	4.50 ± 0.408^{c}	5.12 ± 0.408^{b}	0.00 t ha^{-1}	2.4 ± 0.326^{b}	4.0 ± 0.081^{b}	5.1 ± 0.081^{b}
20.0 kg ha^{-1}	3.50 ± 0.041	5.13 ± 0.025^{b}	6.18 ± 0.025^{a}	5.00 t ha^{-1}	3.5 ± 0.408^{a}	5.1 ± 0.081^{ab}	7.2 ± 0.163^{ab}
40.0 kg ha^{-1}	3.86 ± 0.049	5.75 ± 0.041^{a}	6.25 ± 0.041^{a}	10.0 t ha^{-1}	3.7 ± 0.163^{a}	6.2 ± 0.163^{a}	8.4 ± 0.326^{a}
EC strain Fg 5-2 (10^8 CFU mL^{-1}) (B)				EC strain Fg 5-2 (10^8 CFU mL^{-1}) (B)			
Yes	3.67 ± 0.057	5.50 ± 0.408^{a}	6.50 ± 0.481^{a}	Yes	3.5 ± 0.408	4.4 ± 0.327^{b}	6.5 ± 0.082^{b}
No	3.50 ± 0.408	4.75 ± 0.408^{b}	5.50 ± 0.408^{b}	No	3.6 ± 0.500	5.5 ± 0.408^{a}	7.1 ± 0.481^{a}
F (A)	ns	**	**	F (A)	**	**	**
F (B)	ns	**	**	F (B)	ns	**	*
F (A x B)	ns	ns	ns	F (A x B)	ns	*	*
CV (%)	6.70	4.70	12.2	CV (%)	12.2	9.7	5.8

ns: No statistically significant at 5 % ($p > 0.05$); * $p \leq 0.05$, ** $p \leq 0.01$

The available peanut branches of the Exp. 1 (N fertilizer rates) and Exp. 2 (VMC rates) at 20 DAS observed that the average branches per peanut plant valued from 3.50 to 3.86 branches in 3 N rates (0, 20 in experiment 1 and 2.40 to 3.70 branches in experiment 2, however, which were not significantly statistical differences ($p > 0.05$). The highest plant branches of 45 and 65 DAS were 5.75 and 6.25 branches, respectively, at the fertilizer application of 40 kg N ha^{-1} and 6.20 and 8.40 branches, respectively, at the VMC rate of 10 tons per ha. Conversely, the minimum number of available branches were observed at 45 and 65 DAS without CNF or 20 kg N ha^{-1} fertilization and non VMC or 5 tons VMC per ha (**Table 3**). Similarly, to plant height, there are a significant difference among the EC strain Fg 5-2 no or inoculation from 45 and 65 DAS (except 20 DAS) in both experiments at 1 % level. The EC strain Fg 5-2 inoculation had available branches raised during 20, 45 and 65 DAS stages. The EC strain Fg 5-2 inoculation raised a higher branch number than that of without EC inoculation at 45 and 65 DAS (except for 20 DAS), which was not significantly different in both experiments. However, there was no interaction between N fertilizer rates with EC strain Fg 5-2 inoculation from 20, 45 and 65 DAS, but, had interaction between VMC and EC inoculation from 45 and 65 DAS (except for 20 DAS) at $p \leq 0.05$. Organic manure amendment raised No. of leaves per plant, the agronomy traits, achieved the maximum results when applying a combination of N and organic fertilizers. Nitrogen concentration in inorganic fertilizers could be easily exchanged and contributed directly to the farmland. Effective nutrition in bio-fertilizers need to be changed by microorganism processes before they can be taken by plants and this alterableness longer than the inorganic fertilizer. Therefore, the yield traits of peanuts lessened under rapid denitrification, while in the mature and harvest, the yield components and yield of peanuts reached to treat with additional organic fertilizers [27].

Table 4 Nodulous number and weight of groundnut in 2 experiments.

Factors	Experiment 1		Factors	Experiment 2	
	75 DAS			75 DAS	
	Nodulous number per plant	Nodulous weight (gr $plant^{-1}$)		Nodulous number per plant	Nodulous weight (gr $plant^{-1}$)
	N_2 fertilizer (A)			VMC (A)	
0.00 kg ha^{-1}	181 ± 0.817^{c}	0.96 ± 0.008^{c}	0.00 t ha^{-1}	223 ± 2.45^{b}	1.00 ± 0.081^{b}
20.0 kg ha^{-1}	235 ± 0.817^{b}	1.27 ± 0.016^{b}	5.00 t ha^{-1}	256 ± 0.816^{ab}	1.30 ± 0.081^{ab}
40.0 kg ha^{-1}	307 ± 1.63^{a}	1.70 ± 0.008^{a}	10.0 t ha^{-1}	274 ± 3.26^{a}	1.40 ± 0.081^{a}
EC strain Fg 5-2 (10^8CFU mL^{-1}) (B)			EC strain Fg 5-2 (10^8CFU mL^{-1}) (B)		
Yes	257 ± 1.63^{a}	1.39 ± 0.008^{a}	Yes	283 ± 2.44^{a}	1.60 ± 0.081^{a}
No	245 ± 4.08^{b}	1.22 ± 0.163^{b}	No	211 ± 0.816^{b}	1.00 ± 0.081^{b}
F (A)	**	**	F (A)	**	**
F (B)	*	*	F (B)	**	**
F (A x B)	ns	ns	F (A x B)	*	*
CV (%)	11.6	11.8	CV (%)	4.70	7.60

ns: no statistically significant at 5 % ($p > 0.05$); * $p \leq 0.05$, ** $p \leq 0.01$

Number and weight of groundnut nodules ($p \leq 0.01$), which ranged from 181 to 307 nodules and 0.96 to 1.70 gr $plant^{-1}$, respectively in 3 CNF rates, obtained the highest value to be 307 nodules and 1.70 gr $plant^{-1}$, respectively, at a CNF level of 40 kg ha^{-1}. Conversely, the lowest value for both the number and weight of nodules was 181 nodules and 0.96 gr $plant^{-1}$, respectively, without CNF application (**Table 4**). *Enterobacter cloacae* strain Fg 5-2 inoculation attained the higher nodulous number (257 nodules) and nodulous weight (1.39 gr $plant^{-1}$) compared without EC strain Fg 5-2 inoculation. Number (245 nodules) and nodulous weight (1.22 gr plant^{-1}) of peanuts achieved at treatment of non-EC strain Fg 5-2 inoculation and significant difference $p \leq 0.05$. There was the interaction between 3 N rates and EC strain Fg 5-2 among treatments. Number and weight of groundnut nodules ($p \leq 0.01$), which ranged from 223 to 274 nodules and 1.00 to 1.40 gr plant^{-1}, respectively, in 3 VMC rates, reached the greatest number to be 274 nodules and 1.40 gr plant^{-1}, respectively, at a VMC level of 10 t ha^{-1}. On contrary, the minimum values of the Number and Weight of fresh groundnut nodules were 223 nodules and 1.0 gr plant^{-1}, respectively, without VMC application (**Table 4**). *Enterobacter cloacae* strain Fg 5-2 inoculation had the higher nodulous number (283 nodules) and nodulous weight (1.60 gr plant^{-1}) compared without EC strain Fg 5-2 inoculation. Number (211 nodules) and nodulous weight (1.00 gr plant^{-1}) of groundnuts achieved at treatment of non-EC strain Fg 5-2 inoculant and significant difference $p \leq 0.01$. Previous research of Chuong [26] showed a positive interaction between the application of organic manure, NPK fertilizer and endophytic bacteria, which were isolated from peanut nodules, increased the number and nodulous weight of groundnut in experimental trials. The combination of *Rhizobium* sp. strains with chicken manure increased the dry weight of nodules compared to NPK fertilization alone. There was the interaction between 3 VMC rates and EC among treatments ($p > 0.05$). Positive relationships between N-fixing bacteria, nodules and the roots of legume plants have been established by the main source of nitrogen fixation [28]. Latest study of Mendes *et al.* [29], the impact of organic manure on different soil structures and the forms of peanut root-associated microorganisms in different crop growth stages were found in peanut-growing soils.

Table 5 Yield traits and productivity of groundnut in 2 experiments.

Factor	Experiment 1			Factor	Experiment 2		
	No. of filled pods (pod plant^{-1})	Wt.of 100 fresh pods (gr plant^{-1})	Fresh pod yield (t ha^{-1})		No. of filled pods (pod plant^{-1})	Wt.of 100 fresh pods (gr plant^{-1})	Fresh pod yield (t ha^{-1})
	N fertilizer (A)				VMC (A)		
0.00 kg ha^{-1}	54.9 ± 0.081	175 ± 4.08^{b}	6.38 ± 0.016^{b}	0.00 t ha^{-1}	62.8 ± 0.163^{b}	176 ± 1.63^{b}	4.5 ± 0.408^{b}
20.0 kg ha^{-1}	69.4 ± 0.326	226 ± 0.81^{a}	7.64 ± 0.032^{a}	5.00 t ha^{-1}	76.8 ± 0.163^{ab}	225 ± 4.08^{ab}	6.5 ± 0.408^{ab}
40.0 kg ha^{-1}	69.1 ± 0.081	230 ± 0.81^{a}	7.84 ± 0.032^{a}	10.0 t ha^{-1}	82.5 ± 0.408^{a}	240 ± 4.08^{a}	7.3 ± 0.244^{a}
	EC strain Fg 5-2 (10^{8} CFU mL^{-1}) (B)				EC strain Fg 5-2 (10^{8} CFU mL^{-1}) (B)		
Yes	73.2 ± 0.163^{a}	241 ± 0.816^{a}	8.07 ± 0.016^{a}	Yes	80.25 ± 0.040^{a}	234 ± 3.26^{a}	6.2 ± 0.163^{a}
No	55.8 ± 0.163^{b}	180 ± 4.08^{b}	6.49 ± 0.008^{b}	No	69.25 ± 0.040^{b}	193 ± 2.44^{b}	5.4 ± 0.326^{b}
F (A)	ns	*	**	F (A)	**	**	**
F (B)	**	**	**	F (B)	**	**	**
F (A x B)	ns	ns	ns	F (A x B)	ns	ns	*
CV (%)	20.6	21.3	18.2	CV (%)	8.40	6.00	7.80

ns: No statistically significant at 5 % ($p > 0.05$); * $p < 0.05$, **$p < 0.01$

Observation's results from **Table 5** showed that a 50 % reduction in nitrogen fertilizer did not decrease 100-seed weight or fresh yield compared to 100 % recommended CNF application. The number of pods per plant did not show a statistically adequate differences at the 3 CNF application levels (0, 20 and 40 kg ha^{-1}). The highest 100-seed weight and yield of groundnuts were observed at the 2 application levels of 20 and 40 kg/ha (226 gr plant^{-1}, 7.64 t ha^{-1} and 230 gr plant^{-1}, 7.84 t ha^{-1}, respectively). In contrast, the 100-seed weight and yield values in the unfertilized treatment were the lowest (175 gr plant^{-1}and 6.38 t ha^{-1}, respectively) and had an adequate effect at the 5 and 1 % levels. However, the pods number per plant in the EC strain Fg 5-2 inoculation was statistically significant at the 1 % level. The number of pods, 100-seed weight and yield in the EC strain Fg 5-2 inoculation treatment was always higher than the non- EC strain

Fg 5-2 inoculation treatment, respectively, in Exp. 1. **Table 5** presented that 50 and 100 % reduction in VMC amendment decreased dramatically on number of filled pods, 100-seed weight or fresh yield compared to 100 % VMC application and a statistically significant difference ($p \leq 0.01$) at the 3 VMC application levels (0, 5.0 and 10.0 t ha^{-1}). The greatest number of filled pods, 100-seed weight or fresh yield of peanuts were observed at the application levels of 10 t ha^{-1} (82.5 pod $plant^{-1}$, 240 gr $plant^{-1}$ and 7.3 t ha^{-1}, respectively). In contrast, the lowest number of filled pods, 100-seed weight or fresh yield of peanuts had without VMC application (62.8 pod $plant^{-1}$, 176 gr $plant^{-1}$ and 4.5 t ha^{-1}, respectively). the 100-seed weight and yield values in the unfertilized treatment were the lowest (175 gr $plant^{-1}$and 6.38 t ha^{-1}, respectively) and had a significant effect at the 5 and 1 % levels. Furthermore, filled pods per plant in the EC strain Fg 5-2 inoculation was statistically significant at the 1 % level. The filled pod number, 100-seed weight and yield in the EC strain Fg 5-2 inoculation treatment was always higher than the non- EC strain Fg 5-2 inoculation treatment, respectively, in Exp. 2 (**Table 5**). Application of VMC more effectively improved plant growth, such as agronomy, yield components, yield and fruit quality in each type of soil [30-32]. The different N-fixing bacterial inoculation that significantly reduced application of 50 % N level and raised on soil properties, which was not been no N reduction. In general, all groundnut productivity traits and productivity of groundnut obtained the maximum results in addition of 10 t VMC per ha associated with EC strain Fg 5-2 inoculation. Contrariwise, the minimum results of all groundnut productivity traits and productivity of groundnut were without VMC application and non- EC strain Fg 5-2 inoculation. There is not an interaction between VMC rates and EC strain Fg 5-2 inoculation (Expect fresh yield) on yield components and not significantly different between VMC rates and bacteria (Expect fresh yield in Exp. 2). The latest study found out that VMC application was raised on N-fixing microbial community and soil nutrients. The animal manure fertilization in a long period may remarkably enrich farmland quality [33,17].

Recent studies have shown that using ENFB in groundnut cultivation reduced the applied amount of inorganic nitrogen fertilizer up to 50 % while maintaining the same yield as 100 % inorganic nitrogen fertilizer [34]. However, there have insignificantly been many researches on the combination of ENFB inoculation and animal manures application [35]. Nevertheless, soil carbon element plays a role in the development of ENFB community and increases the N-fixing ability increase of ENFB in groundnut cultivation [36,37]. This studied results, which raised the groundnut yield and agronomic and yield components, reduced 50 % of the CNF application. In particular, groundnut yield increased up to 16.5 % compared to the unfertilized treatment and insignificantly different to the 100 % application of CNF. Groundnut yield increased from 12.9 to 19.6 % with the *Enterobacter cloacae* Fg 5-2 strain inoculation compared to the non-*Enterobacter cloacae* Fg 5-2 strain. The results of this study are consistent with the previous research findings of Zhou *et al.* [38], Etesami [39] and Hossain *et al.* [40].

Conclusions

Application of 20 kg N or 10 t VMC ha^{-1} combined with EC strain Fg 5-2 inoculation adequately obtained the highest groundnut yield, which could promote beneficially agronomic properties to plants, thereby improving crop growth and development. Groundnut yield did not differ between 20 and 40 kg N rates per ha, but was much more than non-CNF application. However, amendment of 10 t VMC per ha yielded the highest yield when compared to 0.0 and 5.0 t ha^{-1} of VMC. In particular, EC strain Fg 5-2 inoculation always had higher yield than non-EC strain Fg 5-2 inoculation when combined with CNF and VMC application. In conclusion, fertilization of 20 kg N ha^{-1} or 10 t VMC associated with EC strain Fg 5-2 inoculation is the best choice for practical application, which can reduce the amount of CNF by 50 % while still achieving high yield in sustainable peanut cultivation to increase farmers' profits.

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