Study on *Enterobacter Asburiae* association with organic manures on peanut growth and productivity in sandy soil

Chuong Nguyen Van*

Faculty of agriculture, An Giang University- VNU HCM City, Vietnam

Abstract

Optimal cultivation techniques that are mainly aimed by promoting the farmland fertility, increasing peanut yield and quality, reduce the use of chemical fertilizers, and increase local tillers' profits. *Enterobacter asburiae* was completely isolated on YEMA media and obtained maximum nitrogen-fixing ability. Molecular identification results showed that *Enterobacter asburiae* is an endophytic nitrogen-fixing bacterium based on 16S rRNA BLAST technique. A field experiment was conducted with two factors: (i) *Enterobacter asburiae* inoculation and non-inoculation; (ii) three rates of vermicompost (0.0, 5.0, and 10.0 t ha^{-1}). The molecular identification result of *Enterobacter asburiae*, which was used by16S rRNA BLAST technique, was identified that *Enterobacter asburiae* belongs to the rhizobium strain. The research results observed that the combination of *Enterobacter asburiae* inoculation and vermicompost addition increased the ability to accumulate nitrogen (N_2) in the soil and in the peanut seeds, and promoted the growth of peanut plants. This is not only the result of biological nitrogen fixation, but also related to the growth-promoting effect on the yield and quality of peanuts. The interaction between vermicompost (VT) and *Enterobacter asburiae* increased peanut yield compared to the control. Application of 10 t vermicompost ha^{-1} increased yield by 11% compared to 5 vermicompost ha^{-1} and 38.4% compared to non-VT amendment. *Enterobacter asburiae* inoculation raised yield by up to 12.9% compared to non- *Enterobacter asburiae* inoculation. All traits of yield, nodous number and fresh weight, as well as the peanut quality were significantly increased in co-application of VT and *Enterobacter asburiae* inoculation. The new discovery of this study proved that *Enterobacter asburiae* is quite potential to become a good candidate species to manufactory biofertilizers to promote crop yields.

Keywords: *Enterobacter asburiae*, *groundnut*,16S rRNA BLAST, *VT, YEMA*, isolation

I. INTRODUCTION

Peanuts (*Arachis hypogaea* L.), which has popularly crop grown in many countries around the world, are used to make cooking oil, food for humans and animals. Peanuts that are a versatile crop that can be grown in many different climates, are used to make cooking oil, food for humans and livestock, and even as a source of protein for animal feed. Peanuts contain a high composition of oil (48–50%), protein (25–28%), vitamins, minerals, antioxidants, polyphenols, and flavonoids (Janila et al., 2013; Hamza et al., 2021) and belongs to the Fabaceae family, order Fabales, is mainly utilized for human foodstuff, either by direct consumption or oil production. The grains and bagasse in the form of cake generated in oil extraction are also used in animal feed (Camargo et al., 2017). Peanuts are a major economic crop for local farmers in An Giang, Vietnam and the Mekong Delta. However, there are many factors that can reduce profitability to lead to decrease peanut productivity and quality such as Arsenic (As) polluted irrigation water and poor nutrient soils, insect pests or diseases caused by pathogenic microorganisms. Arsenic pollution in soil and irrigation water is a serious problem when it comes to improving crop yields and quality and its As accumulation. Previous research conducted in a field experiment in Phuoc Hung commune, An Phu district, An Giang province, which found out that high As pollution of crop soil and irrigation water reduced the yield and As accumulation in the stems of white beans (Nguyen et al., 2023).

Enterobacter asburiae inoculation has been considered the main method for controlling *ectomycorrhizae* strains in peanut cultivation. *Enterobacter asburiae* has the ability to completely inhibit the growth of *Phytophthora* root rot and improve soil nutrients by fixing N_2 from the air. Many biological control agents can disrupt the growth and infection

of plant pathogens that live in the root (Kim et al., 2020*). Enterobacter asburiae* was identified based on the analysis of 16S ribosomal RNA gene sequences. *Enterobacter asburiae* cells significantly suppressed spore production and zoospore germination in *Phytophthora* root rot by 87.4% and 66.7%, respectively. When E. asburiae is inoculated into the soil, it effectively prevents Phytophthora root rot by up to 81.1%. Therefore, E. asburiae can be used as a potential agent for the biological control of *Phytophthora* root rot infecting plants (Bakker et al., 2003). Previous studies have clearly demonstrated that the application of endogenous N_2 –fixing bacteria and organic manures to peanuts plays an important role in promoting peanut yield and soil quality. The excessive use of inorganic fertilizers and the lack of organic manure application can significantly reduce the population of beneficial microorganisms in crop soils. Similarly, farmers face multiple pressures and trade-offs with the replacement of chemical nitrogen fertilizer with natural nitrogen sources from biological nitrogen-fixing bacteria and locally available organic manures (Erenstein et al., 2015; Tittonell et al., 2015; Valbuena et al., 2015). Therefore, the application of endogenous N_2 –fixing bacteria combined with the cultivation of legumes are widely recommended as an application method of organic N_2 sources to agricultural ecosystems. They can provide the N_2 necessary for both the healthy nutritional function of the soil and increased crop yields. They also have the potential to replenish organic matter in degraded soils and increase the population of beneficial microorganisms in agricultural soils (Snapp et al., 2018). More importantly, legumes are also important for human health, nutrition, and dietary diversity as a key source of protein, diverse amino acids, micronutrients, dietary fiber, and phytochemicals (Foyer et al., 2016). Due to their many potential benefits, legume cultivation and N_2- fixing bacterium inoculation are recommended as part of ecological nutrient management and conservation agriculture schemes with the ultimate goal of improving soil health, and thus the sustainability and resiliency of low-input agricultural ecosystems (Thierfelder et al., 2013; Drinkwater et al., 2017). Application of VT is one of the positive techniques of organic farming that is considered to be cheap for farmers because of its low cost and safety, hygiene, and solid waste treatment efficiency. The invasion of earthworms to decompose organic waste is considered to be effective because the loss of nitrogen from organic waste is reduced when using earthworms as a source of organic fertilizer (Abbasi et al., 2002). Vermicompost is important for improving soil properties and contains many easily absorbable nutrients, many nutrients essential for plant growth (Abdel-Mouty et al., 2011). It is a stable organic matter and it loosens the soil, enhances aeration when added to the soil. It is considered to be a high-porosity material that can promote plant growth and productivity. The most important characteristic of peanuts is their unique ability to fix nitrogen in a symbiotic relationship and serve as a source of nitrogen in nature as well as in agriculture (Aktar et al., 2019). With the help of plant growth-promoting bacteria, which have a special symbiotic characteristic in nitrogen fixation, peanuts eventually increase the amount of nitrogen in the soil by converting atmospheric nitrogen into ammonia, ultimately enhancing soil fertility (Ali, et al., 2018). The above results, *Enterobacter asburiae*, which is a potential biological control agent for *Phytophthora* root rot infecting peanut crops, is rhizosphere N2 fixing bacteria. The *Enterobacter asburiae* inoculation into the soil can help to improve soil nutrients and effectively prevent *Phytophthora* root rot (An-Dong Gong et al., 2019).

II. MATERIALS AND METHODS

Isolation of *Enterobacter asburiae* from peanut nodules

Roots and nodules of peanuts, which were collected from local fields, surface-disinfected with 95% ethanol for 10 seconds and transferred to NaClO 3% (v/v) solution for 3-4 minutes. The surface-disinfected nodules were, then, rinsed with 5 changes of sterile distilled water to completely wash away the disinfectant chemicals (Upasana et al., 2020). The nodule was then transferred to a sterile Petri dish and crushed with a sterile glass rod that has been flamed in a drop of normal saline solution (0.85% NaCl) inside a laminar flow cabinet (Michel et al., 2000). After that 0.1ml (loop) of suspension was streaked on a plate containing Yeast Extract Mannitol Agar (YEMA) and incubated at 28 ± 2^0C for 3-5 days. YEMA composition (Vincent, 1970). *Enterobacter asburiae* colonies that were purely isolated from the root and noodle samples of groundnuts, were studied during experiment, was determined by sequencing technology of 16S rRNA.

Pure test and protection of *Enterobacter asburiae* colonies

Enterobacter asburiae colonies that were significantly grown on YEMA plates after 3-5 days, were chosen from single dome-shaped at 28 ± 2^0C. The pure isolates were carefully tested by biochemical before determined by sequencing technology of 16S rRNA. Each colony was examined for purity using peptone, gram (+/-) staining and development reaction to YEMA-CR culture (Jordan, 1984).

Inoculation for peanut seeds before sowing

Enterobacter asburiae were identified and cultured in 250 ml flasks containing 200 ml dilute YEMA medium on an orbital shaker at 100 rpm for 12 hours at 20 ± 2°C. Cells of *Enterobacter asburiae* were taken from centrifugation at 14000 rpm for 1 min at 2°C, and each pellet was washed twice with distilled water. E. *asburiae* pellets were added in 0.6 ml distilled water, and used for sowing seeds. All peanut seeds were surface sterilized in 4% NaCl for 1 min and washed many times in distilled water. Taking to dry seeds, which were immersed in each *Enterobacter asburiae* suspension, stirred for 5 min. The treated peanut seeds were spread at room temperature. The population of *Enterobacter asburiae* cells per seed determined dilutions and was approximately 10^8 CFU/seed.

Experimental design

A field experiment was conducted by using a 2-factor factorial design: (i) 3 levels of VT (0, 5, and 10 t/ha) and (ii) *Enterobacter asburiae* (inoculation and non-inoculation). There were 6 treatments with 4 replications (6 treatments x 4 replications = 24 experimental units). The total area of the experiment was 480 m^2 (2 m in width x 10 m in length x 6 treatments x 4 replications). The experiment was laid out in a completely randomized complete block design. Peanut seeds were planted in single rows with a planting distance of 30 cm (2 seeds/hole). 15 days after sowing, one healthy plant was chosen for each hole. The planting density was 30 cm x 30 cm.

The experimental treatments of this field experiment were conducted in the Phuoc Hung commune, An Phu, An Giang province, Vietnam, in the Summer-Autumn season of 2023. Six treatments were included: PN1 (application of NPK fertilizer at the rate of 40N-60P-60K kg/ha); PN2 (NPK + *Enterobacter asburiae* inoculation); PN3 (NPK + 5 t 5.0 t VCP /ha); PN4 (NPK + *Enterobacter asburiae* inoculation + 5.0 t VT /ha); PN5 (NPK + 10.0 t VT/ha); and PN6 (NPK + *Enterobacter asburiae* inoculation + 10.0 t VT/ha) (Table 1). The soil characteristics in the experiment were determined according to the method of Brady, N.C. (1988). Total As concentration of soil, water, stem and seed of peanut was determined by using atomic absorption spectrophotometry. All the chemical-physic properties of soil and As results of irrigation water and soil before the experimental design were shown in Table 2. The yield components and yield of peanut, which were observed from the growth stages to harvest of the plants, were biomass, number of pods and seeds/plant, 1,000-seed weight. Fresh seed yield was determined by t/ha for each treatment.

Table 1. NPK fertilizer, VCP and *Enterobater asburiae* in each treatment

Treatments	***Enterobacter asburiae*** **(10^8 CFU/ml)**	Vermicompost **(t/ha)**	**N-P-K fertilizer (kg/ha)**
PN1 (control)	Non inoculation	0	40-60-60
PN2	Inoculation		
PN3	Non inoculation	5	
PN4	Inoculation		
PN5	Non inoculation	10	
PN6	Inoculation		

Table 2. Physical and chemical properties of soil before the experiment (n=5)

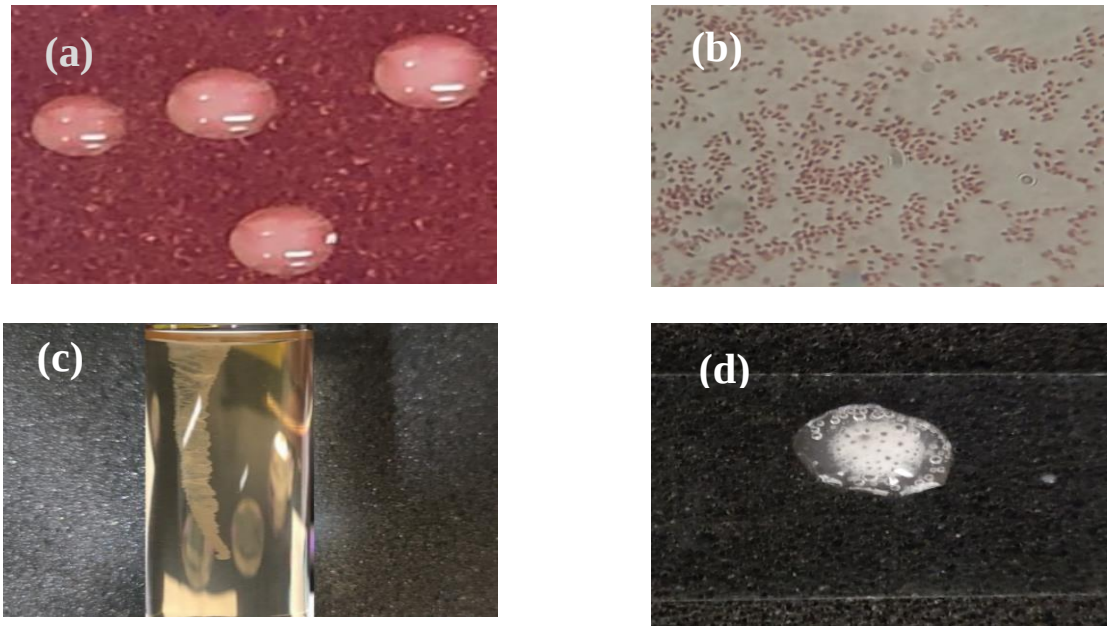
pH	As contents		Soil nutrition			Soil texture (%)		
Soil	Water (μg/L)	Soil (mg/kg)	Total N (mg/kg)	Available P (mg/kg)	Exchangeable K (ppm)	Sand	Clay	Silt
4.8	370	120	110	2.59	137	65.9	4.0	30.1

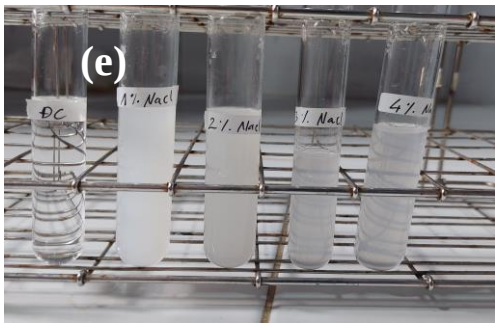
Statistical analysis

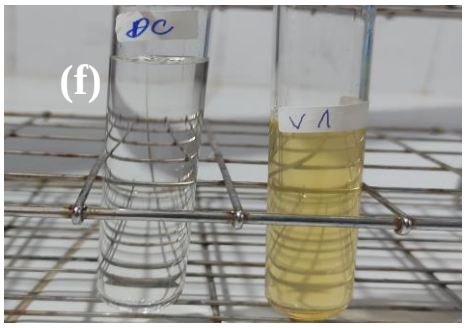
Statistical analyses were performed using Statgraphic XVIII and Microsoft Office Excel 2010. A completely randomized design was used for all experiments, with 4 replicates for each treatment. The data were presented from representative experiments that were repeated 4 times with similar results. Treatments were compared via ANOVA using the least significant differences test (LSD) at 5% ($P \leq 0.05$) significance level.

III. RESULTS AND DISCUSSION

Isolation and identification of *Enterobacter asburiae*







CCCCAGTCATAGAATCACAAAGTAGGTAAGCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCA

ACCCACTCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTCTGAT

CTACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCAC

TTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGTGTGTAGC

CCTACTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTC

CTTTGAGTTCCCGGCCTAACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCC

AACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAGAGTTCCCGAAGGCACCAA

TCCATCTCTGGAAAGTTCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAAC

CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCC

CAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGA

CATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCG

TCAGTCT TTGTCCAGGGG

(g)

Figure 1: (a) E. asburiae colony was purely isolated on YEMA medium; (b) Gram staining: Gram negative, short rod shaped bacteria; (c) Reaction with Nessler reagent; (d) Catalase examination: gas bubble appearance showed the existence of catalase in E. asburiae colony. (e) salt test: four salt contents for 12 h, transparent solution turned to muddy solution, which showed a positive result in 1, 2, 3 and 4% of salt solution. (f) Nessler test: creation of a pink solution indicated a positive result; (g): Sequencing data.

Isolation and genomic identification of N_2-fixing bacteria from groundnut roots and nodules by YEMA medium for colony identification and Gram staining for determining the shape, cell wall properties of strains. The identified strains showed that E. *asburiae* colonies was negative on LAM medium after flooding. Furthermore, the results on Hofer's alkaline medium were used as the main factor to classify E. *asburiae* colony due to their ability to grow in high pH conditions (Deshwal & Chaubey, 2014; Mir et al., 2020). All the obtained colonies produced pink colonies on YEMA. This is a main characteristic of E. *asburiae* colony, which was already mentioned by Somasegaran & Hoben (1985). Instead of a nodulation check, all E. *asburiae* colonies were checked the ability to nitrogen-fixing by Burk's N-free medium according to their colony size. This result represented that the selected strains grew well on N-free medium, so they should be a top priority when working with these selected bacteria (Muñoz et al., 2011; Park et al., 2005, Singh et al., 2008). All E. *asburiae* isolates were positive for oxidase and catalase tests. These results are consistent with previous studies of Hossain et al., (2019); Tyagi et al., (2017), which showed that rhizosphere N2 fixing bacterial

strains usually give positive results for the oxidase and catalase reaction. When biochemical tests such as urea hydrolysis, nitrate reduction, and citrate utilization were performed, there were different results depending on the E. *asburiae* species (Figure 1). However, this result demonstrated that diverse features of rhizosphere bacteria metabolism, even on the same rhizobial species, are highly influenced by their living location (Thin et al., 2021).

Genomic identification of pure E. *asburiae* isolates showed results consistent with the predicted genus of E. *asburiae* species, which was used by simple selective media, would help distinguish E. *asburiae* quickly. The results showed that the 16S rRNA nucleotide sequences with a similarity of 99.74% to the reference bacteria, retrieved from the NCBI database (https://www.ncbi.com/), were used for identification. The 16S rRNA sequences for selected E. *asburiae* derived from rhizosphere N2-fixing bacteria. New E. *asburiae* species have been contributed as a result of rapid and modern identification methods based on new molecular techniques, which may change their taxonomic identities, a useful understanding of important distinguishing features of each typical rhizobial species. It would be good to reduce unnecessary confusion when comparing with long-standing molecular techniques (Chibeba et al., 2017; Hameed et al., 2004). The results in Figure 1 showed that E. *asburiae* adapted to a temperature of 25 to 40°C as the optimum temperature for growth. However, the most optimum temperature for growth is 37 - 40°C. When soil temperature for cultivation is too hot or too cold, it can negatively affect their growth and nodulation activity, thus making low and ineffective N_2 fixation ability (Rao, 2014). Figure 1 showed the salt tolerance ability of E. *asburiae*, with better NaCl tolerance in the medium is greater than from 0.5 to 1%. According to previous studies have proved that their select rhizobia can be highly salt tolerant up to 3 - 4% (Baber et al., 2015) or even to 5% (w/v) NaCl (Küçük et al., 2006). However, NaCl concentration below 1% (w/v) is adapted for most Rhizobium Sp. E. *asburiae* was adapted in YEMA medium with pH from 5 and 8 (Yanni, et al., 2005; Yarza, et al., 2014, Zahran et al., 1999).

Testing efficiency of** E. asburiae **on peanut growth and productivity

The experimental results in Table 5 showed that the plant biomass of peanuts at different rates of VCP were statistically significant differences ($p<0.01$). The application of 10 t VCP/ha had the highest plant biomass and the lowest being the treatment of no VCP application. There was significantly statistical difference ($p<0.01$) at number and weight of filled pods in various rates of VCP application (0, 5.0 and 10.0 t/ha). The highest values of number and weight of filled pods, which obtained in the treatments of 10.0 t VCP/ ha application were 82.5 fill pods/plant and 175g/plant, respectively, followed by the treatments of 5.0 t VCP/ha application (76.8 fill pods/plant and 160g/plant, respectively,) and the lowest in the treatment of no VCP application (62.8 fill pods/plant and 105g/plant, respectively). Contrariwise, the highest values of number and weight of unfilled pods, which observed in the treatments of non VCP application were 7.25 unfilled pods/plant and 6.0g/plant, respectively, followed by the treatments of 5.0 tons VCP / ha application (4.5 unfill pods/plant and 5.2g/plant, respectively) and the lowest in the treatment of no VCP application (4.37 unfilled pods/plant and 2.8g/plant, respectively).

In factor B of E. *asburiae* inoculation ($p<0.01$), which had the highest number and weight of filled pods obtained in the treatments of E. *asburiae* inoculation were 80.3 fill pods/plant and 162g/plant, respectively, and the lowest in the treatment of no E. *asburiae* inoculation (69.3 fill pods/plant and 132g/plant, respectively). Contrariwise, the highest values of number and weight of unfilled pods, which observed in the treatments of no E. *asburiae* inoculation were 6.20 unfilled pods/plant and 5.6g/plant, respectively, and the lowest in the treatment of E. *asburiae* inoculation (5.0 unfilled pods/plant and 3.75g/plant, respectively). However, there was not their interaction between VCP rates and *Enterobacter asbu*riae inoculation.

Table 5. The yield attributes and yield of peanuts at harvest

Factor	biomass (g/plant)	Yield components							Fresh yield t/ha
		Number of pod/plant		pod weight (g/plant)		No. of nudole	Wt. of nudole (g/plant)	Wt. of 100 seeds (g)	
		filled	unfilled	filled	unfilled				
Vermicompost (VT: A)									
0.0 t/ha	237[b]	62.8[b]	7.25[a]	105[b]	6.00[a]	223[b]	1.00[b]	176[b]	4.50[b]

5.0 t/ha	278^{ab}	76.8^{ab}	4.5^{b}	160^{ab}	5.20^{a}	257^{ab}	1.30^{ab}	225^{ab}	6.50^{ab}
10.0t/ha	304^{a}	82.5^{a}	4.37^{b}	175^{a}	2.80^{b}	274^{a}	1.40^{a}	240^{a}	7.30^{a}
***Enterobater asburiae* (B)**									
Non inoculation	254^{b}	69.3^{b}	6.20^{a}	132^{b}	5.60^{a}	211^{b}	1.00^{b}	193^{b}	5.40^{b}
Inoculation	291^{a}	80.3^{a}	5.00^{b}	162^{a}	3.75^{b}	284^{a}	1.60^{a}	234^{a}	6.20^{a}
F (A)	**	**	*	**	*	**	**	**	**
F (B)	**	**	*	**	*	**	**	**	**
F (A x B)	ns	ns	ns	ns	ns	*	*	ns	*
CV (%)	11,.2	8.4	20.6	15.0	24.6	4.70	7.60	6.00	7.80

ns: no significant statistical difference *, **: significant statistical difference at $p < 0.05$ and 0.01, respectively.

The results from Table 5 showed that the number and weight of nodules at the 75-day days after sowing (DAS) in the factor A (VCP) were significantly statistic differences at p<0.01, with the treatment of 10 t/ha of VCP giving the highest number of nodules (274 nodules/plant), nodule weight (1.40 g/plant) compared to the treatments of 5 t VCP t/ha and non VCP application. The factor B (*Enterobacter asburiae)* also recorded significant differences in the number and weight of nodules at p<0.01, with the treatment of *Enterobacter asburiae* inoculation giving more nodules and nodule weight than the treatment without Enterobacter asburiae inoculation. In addition, the VCP factor had an interaction with the *Enterobacter asburiae* factor in the number and weight of peanut nodules, which was recorded to be statistically significant at p<0.05, with the treatment of 10 t VCP /ha giving the number of peanut nodules (310 nodules/plant), nodule weight (7.1 g/plant) more than the treatment of no fertilizer + no bacteria. This showed that the application of VCP + *Enterobacter asburiae* inoculant helped the plant to form more nodules than no application + no *Enterobacter asburiae* inoculation, and VCP contained many nutrients and beneficial microorganisms that help the plant to form more nodules. However, there was their interaction between VCP rates and *Enterobacter asburiae* inoculation in number and weight of peanut nodules (p<0.05) (Table 5). Weight of 100 seeds and yield of fresh pods were significant difference (p<0.01). The 10 t/ha application of VCP gave the highest weight of 100 seeds (240g) and yield of fresh pods (7.3 t/ha) compared to the treatments of 5 t VCP /ha [weight of 100 seeds (225g) and yield of fresh pods (6.5 t/ha)] and non VCP application [weight of 100 seeds (176g) and yield of fresh pods (4.5 t/ha)]. The factor B (*Enterobacter asburiae)* also recorded significant differences in weight of 100 seeds and yield of fresh pods at p<0.01. *Enterobacter asburiae* inoculation had more weight of 100 seeds (234g) and yield of fresh pods (6.2 t/ha) than the treatment without Enterobacter asburiae inoculation [(weight of 100 seeds (1930g) and yield of fresh pods (5.4 t/ha)]. In addition, the VCP factor had an interaction with the *Enterobacter asburiae* factor in the yield of peanut at p<0.05, (expect weight of 100 seeds) (Table 5).

The research results of Argaw (2017) showed that the application of organic fertilizer and nitrogen-fixing bacterial strains significantly increased the number and weight of nodules on peanut plants. Organic fertilizers are fertilizers derived from the remains of plants and animals, such as animal manure, compost, green manure, straw, and other materials that can play an important role in improving the physical, chemical, and biological properties of the soil. The most widely used organic fertilizer by farmers has been using by many animal manures. Organic manure has been shown to increase plant growth (Rahimabadi et al., 2017). This is due to the presence of nutrients in organic manures that are essential for plant growth. Additionally, organic manures can improve soil quality, which can also lead to increased plant growth (Amos et al., 2015).

Testing efficiency of *Enterobacter asburiae* on peanut quality

The results in Table 6 presented that oil and protein contents of peanut seeds in the treatments of VCP application were significantly statistic differences at p<0.01 (Table 6). Treatments of 10 tVCP/ha gave the highest oil (27.5%) and protein (18.3%) content of peanut seeds, followed by the treatments of 5.0 t VCP/ha application (25.2 and 17.3%, respectively) and the lowest in the treatment of no VCP application (23.6 and 15.2%, respectively). Furthermore,

treatments of *Enterobacter* asburiae inoculation ($p<0.01$), which had the higher oil and protein content of peanut seeds obtained than the treatments of without *Enterobacter*. asburiae inoculation, were 25.5 and 16.4%, respectively. The treatment of non *Enterobacter*. asburiae inoculation had oil (23.4%) and protein (15.5%) content of peanut seeds. There was their interaction (F_{AxB}) between VT rates and *Enterobacter*. asburiae inoculation at $p<0.05$.

The contents of nitrogen, phosphorus, and potassium in peanut seeds in the VCP factor (A) all showed a statistically significant difference at $p<0.05$. The highest contents of nitrogen (2.92%), phosphorus (0.43%), and potassium (0.51%) in peanut seeds obtained in the treatments of 10 tVT/ha application, and the lowest values were nitrogen (2.43%), phosphorus (0.24%), and potassium (0.22%) in the treatments of no VCP application. Similarly, the treatments of *Enterobacter asburiae* inoculation also showed a statistically significant difference at $p<0.05$ in the contents of nitrogen, phosphorus, and potassium in peanut fruit, in which the treatment of *Enterobacter asburiae* inoculation had a higher content of nitrogen (2.63%), phosphorus (0.33%), and potassium (0.35%) than the treatment of no *Enterobacter asburiae* inoculant (2.50, 0.25 and 0.24%, respectively). There was no interaction [F(AxB)] between VCP rates and *Enterobacter asburiae* inoculation. According to previous research by Singh et al., 2021, it has been shown that *Enterobacter. asburiae in* agricultural soil can promote the growth of crop yield components and productivity, improve fruit quality and nutrient content in fruit. There was a significant interaction between Rhizobium and liquid organic fertilizer on the yield and quality of peanuts per crop. This interaction had positive effects on yield components, yield, and seed quality (Alfandi et al., 2019).

Table 6. Peanut quality at harvest

Factor	Peanut seed quality				
	Oil (%)	**Protein (%)**	**N (%)**	**P (%)**	**K (%)**
Vermicompost (A)					
0.0 t/ha	23.6^{b}	15.2^{b}	2.43^{b}	0.24^{b}	0.22^{b}
5.0 t/ha	25.2^{ab}	17.3^{ab}	2.74^{ab}	0.35^{ab}	0.38^{ab}
10.0t/ha	27.5^{a}	18.3^{a}	2.92^{a}	0.43^{a}	0.51^{a}
***Enterobater asburiae* (B)**					
Non inoculation	23.4^{b}	15.5^{b}	2.50^{b}	0.25^{b}	0.24^{b}
Inoculation	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

ns: no significant statistical difference *, **: significant statistical difference at $p < 0.05$ and 0.01, respectively.

IV. CONCLUSION

Enterobater asburiae which was isolated and identified at the molecular level, is a multifunctional plant growth-promoting bacterium strain, it showed high nitrogen-fixing ability when combined with VT amendment as well as N fixation and increased peanut yield and quality. *Enterobater asburiae* inoculation combined with VT application increased the symbiotic ability and increased peanut yield and quality. The high yield and quality of peanut was achieved when applying 10 t VT/ha and E. *asburiae* inoculation. Application of 10 t VT per ha, the yield increased by 11% compared to applying 5 t VT/ha and *Enterobater asburiae* increased by 38.4% compared to no VT application. *Enterobater asburiae* inoculant increased yield up to 12.9% compared to no inoculation. The yield components and number and fresh weight of nodules increased significantly in the VT application and *Enterobater asburiae* inoculant. *Enterobater asburiae* is a potential candidate for commercial formulations used as biofertilizers in the future.

Enterobacter asburiae has been isolated, biochemically characterized, and molecularly identified as an endophytic nitrogen-fixing bacterium associated with legumes. It has shown high nitrogen-fixing ability when combined with vermicompost and increased peanut yield and quality. The combination of *Enterobacter asburiae* and vermicompost application has enhanced the symbiotic ability, leading to increased peanut yield and quality. High peanut yield and quality were achieved when applying a formulation of 10 tons of vermicompost/ha and *Enterobacter asburiae* inoculation. Application of 10 t vermicompost per ha increased yield by 11% higher than 5.0 t vermicompost per and Enterobacter asburiae increased by 38.4% compared to non-VT application. *Enterobacter asburiae* increased yield by up to 12.9% compared to no *Enterobacter asburiae* inoculation. The agronomic and yield components such as number and fresh weight of nodules increased significantly when applying vermicompost and *Enterobacter asburiae* inoculation were applied. *Enterobacter asburiae* is a potential candidate for commercial formulations used as biofertilizers in the future.

REFERENCES

[1] Abbasi, P. A., Al-Dahmani, J., Sahin, F., Hoitink,H. A. J. and Miller, S. A. 2002. Effect of compost amendments on disease severity and yield of tomato in conventional and organic production systems. *Plant disease*. 86(2), 156-161. DOI: https:// doi: 10.1094/PDIS.2002.86.2.156

[2] Abdel-Mouty, M.M., Mahmoud, A.R., EL-Desuki, M. and Rizk, F.A. 2011 Yield and fruit quality of Egg plant as Affected by organic and Mineral Fertilizers Application. *Research Journal of Agriculture and Biological Sciences*. 7, 196-202.

[3] Aktar, S., Quddus, M. A., Hossain, M. A., Parvin, S. and Sultana, M. N. (2019). Effect of integrated nutrient management on the yield, yield attributes and protein content of lentil. *Bangladesh Journal of Agricultural Research*. 44(3), 525-536. DOI: https:// doi:10.3329/bjar.v44i3.43483

[3] Albuena, D., Tui, S. H. K., Erenstein, O., Teufel, N., Duncan, A., Abdoulaye, T., et al. (2015). Identifying determinants, pressures and trade-offs of crop residue use in mixed smallholder farms in Sub-Saharan Africa and South Asia. *Agric. Syst.* 134, 107–118. DOI: https://doi: 10.1016/j.agsy.2014.05.013.

[4] Alfandi, S. and Wahyuni, Nisa, W.W. 2019. Effect of Rhizobium Inoculum and Liquid Organic Fertilizer on Growth and Yield of Peanut (*Arachis Hypogaea* L.) CV. Takar-2. *IOP Conf. Ser.: Earth Environ. Sci.* 391 012004. DOI: https:// doi.org.10.1088/1755-1315/391/1/012004.

[5] Ali, O. A., El-Tahlawy, Y.A. and Abdel-Gwad, S.A. 2018. Impact of Compost Tea Types Application on Germination, Nodulation, Morphological Characters and Yield of Two Lentil Cultivars. *Egyptian Journal of Agronomy* 1-19. DOI: https:// doi:10.21608/AGRO.2018.5678.1126.

[6] Amos, H., Voncir, N., Fagam, A.S.and Garba, A. 2015. Effect of cattle manure on the growth and yield performance of vegetable maize (*Zea mays Saccharata*) varieties under irrigation. *Sch J Agric Vet Sci*. 2(4), 319-323. DOI:https://doi.org/10.22146/ipas.72990

[7] An-Dong Gong, Fei-Yan Dong, Meng-Jun Hu, Xian-Wei Kong, Fen-Fen Wei, Shuang-Jun Gong, Yi-Mei Zhang, Jing-Bo Zhang, Ai-Bo W.U. and Yu-Cai Lia. 2019. Antifungal activity of volatile emitted from *Enterobacter asburiae* Vt-7 against Aspergillus flavus and aflatoxins in peanuts during storage. *Food Control*.106, 106718. DOI: https://doi.org/10.1016/j.foodcont.2019.106718

[8] Argaw, A. 2017. Organic and inorganic fertilizer application enhances the effect of *Bradyrhizobium* on nodulation and yield of peanut (*Arachis hypogea* L.) in nutrient depleted and sandy soils of Ethiopia. *Int J Recycl Org Waste Agricult*. 6, 219–231. DOI: https:// doi: 10.1007/s40093-017-0169-3.

[9] Asnake, B. and Fassil, A. 2019. Isolation,Identification and Characterization Effective Rhizobium Species Nodulating Mung Bean (Vigna radiata) from some places of North Shewa. *Int J Environ Sci Nat Res.* 20 (1), 5556026. DOI: https://doi: 10.19080/IJESNR.2019.20.556026

[10] Baber, M., Fatima, M., Rameesha, A., Muther, M.Q., Sidra, N., Muhammad, K.H. and Tahir, N. 2018. Weed rhizosphere: a source of novel plant growth promoting rhizobacteria (PGPR). *Int. J. Biosci.*13(1), 223-233. DOI: http://doi.org/10.12692/ijb/13.1.223-233.

[11] Bakker, P.A.H.M., Ran, L.X., Pieterse, C.M.J., van, Loon, L. C. 2003. Understanding the involvement of rhizobacteria-mediated induction of systemic resistance in biocontrol of plant diseases. Can. *J. Plant Pathol.* 25, 5–9. DOI: https:// doi: 10.1080/07060660309507043.

[12] Brady, N.C. 1988. The Nature and Properties of Soils. Macmillan Publishing Co., Inc. Doran, J.W. and Parkin, T.B. 1996. Quantitative Indicators of Soil Quality: A Minimum Data Set. Soil Science Society of America Special Publication No. 49, SSSA, Madison, Wisconsin, USA

[1] Camargo, A.C., Regitano-d'Arce, M.A.B., Rasera, G.B., Canniatti-Brazaca, S.G., Prado-Silva, L., Alvarenga, V.O., Sant'Ana, A.S. and Shahidi, F. 2017. Phenolic acids and flavonoids of peanut by-products: Antioxidant capacity and antimicrobial effects. *Food Chem.* 237,538–544. DOI: https://doi.org/10.1016/j.foodchem.2017.05.046

[14] Chibeba, A. M., Kyei-Boahen, S., de Fátima Guimarães, M., Nogueira, M. A. and Hungria, M. (2017). Isolation, characterization and selection of indigenous Bradyrhizobium strains with outstanding symbiotic performance to increase soybean yields in Mozambique. *Agriculture, Ecosystems & Environment*. 246, 91-305.DOI: https://doi.org/10.1016/j.agee.2017.06.017

[15] Deshwal, V.K. and Chaubey, A. 2014. Isolation and characterization of Rhizobium *leguminosarum* from root nodule of Pisum sativum L. *Journal of Academia and Industrial Research*. 2(8), 464-467. DOI: https://doi.org/10.20546/ijcmas.2019.803.227

[16] Drinkwater, L. E., Schipanski, M., Snapp, S. and Jackson, L. E. (2017). "Ecologically based nutrient management," in Agricultural Systems: Agroecology and Rural Innovation for Development: Second Edition, eds S. Snapp, B. Pound (London: *Elsevier Inc*), 203–257.DOI: https://doi: 10.1016/B978-0-12-802070-8.00007-4.

[17] Erenstein, O., Gérard, B. and Tittonell, P. 2015. Biomass use trade-offs in cereal cropping systems in the developing world: overview. *Agric. Syst*. 134, 1–5. DOI: https://doi: 10.1016/j.agsy.2014.12.001.

[18] F. B. 1997. Natural endophytic association between Rhizobium *leguminosarum* bv. Trifolii and rice roots and assessment of its potential to promote rice growth. *Plant and Soil*. 194, 99-114. DOI: https://doi:10.1023/A:1004269902246.

[19] Foyer, C. H., Lam, H. M., Nguyen, H. T., Siddique, K. H. M., Varshney, R. K., Colmer, T. D., et al.2016. Neglecting legumes has compromised human health and sustainable food production. *Nat. Plants.* 2, 1–10. DOI: https://doi: 10.1038/NPLANTS.2016.112

[20] Hameed, S., Yasmin, S., Malik, K. A., Zafar, Y. and Hafeez, F. Y. 2004. *Rhizobium*, *Bradyrhizobium* and *Agrobacterium* strains isolated from cultivated legumes. *Biology and Fertility of Soils*. 39(3), 179-185. DOI: https:// doi:10.1007/s00374-003-0697-z.

[21] Hamza, M., Abbas, M., Abd Elrahman, A., Helal, M. and Shahba, M. 2021. Conventional versus Nano Calcium Forms on Peanut Production under Sandy Soil Conditions. *Agriculture*, 11, 767. DOI: https:// doi.org/10.3390/agriculture11080767

[22] Hossain, A., Gunri, S. K., Barman, M., Sabagh, A. E. and da Silva, J. A. T. 2019. Isolation, characterization and purification of Rhizobium strain to enrich the productivity of groundnut (*Arachis hypogaea* L.). *Open Agriculture*. 4(1), 400-409. DOI: https:// doi:10.1515/opag-2019-0040.

[24] Janila, P., Nigam, S.N., Pandey, M.K., Nagesh, P. and Varshney, R.K. 2013. Groundnut improvement: Use of genetic and genomic tools. *Front. Plant Sci.* 4, 23. DOI: https://doi.org/10.3389/fpls.2013.00023.

[24] Jordan, D. 1984. *Rhizobaceae.* In: Bergey's Manual of Systematic Bacteriology, 234-256, (D.P. Hendricks *et al.*, eds). *Orient Longman, New York*.

[25] Kim, Sang Yeob Lee, Seong Ho Ahn, Ji Hee Han and Jin Woo Park. 2020. Biological Control of Gom-chwi (Ligularia fischeri) Phytophthora Root Rot with *Enterobacter asburiae* ObRS-5 to Suppress Zoosporangia Formation and Zoospores Germination. *Plant Pathol J*. 36(3), 244–254. DOI: https:// doi.10.5423/PPJ.OA.11.2019.0283

[26] Michel, D., Claude, B., Antoine, C., Rolland, M., Jean-Pierre, G. and Didier, R. 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J. Clin. Microbiol* 38,3623-3630. DOI: https:// doi: 10.1128/jcm.38.10.3623-3630.2000

[27] Mir, M.I., Nagabhushanam, B., Quadriya, H., Kumar, B. K. and Hameeda, B. 2020. Morphological, biochemical and intrinsic antibiotic resistance of rhizobia isolated from root and stem nodules of various leguminous plants. *Plant Cell Biotechnology and Molecular Biology*. 21(71), 126-138. DOI:https//doi:10.5897/AJB09.1337.

[28] Muñoz, V, Ibañez, F, Tonelli, M.L., Valetti, L., Anzuay ,M.S. and Fabra, A. 2011. Phenotypic and phylogenetic characterization of native peanut Bradyrhizobium isolates obtained from Córdoba, Argentina. *Syst Appl Microbiol*. 34(6):446-52. DOI: https: // doi: 10.1016/j.syapm.2011.04.007

[29] Nguyen, V.C., Le, M.T., Tran, L.K.T., Nguyen, N.P.T. Ho, T.T. and Nguyen, T.T.D. 2023. Efficient evaluation of E. *asburiae*, K. *quasipneumoniae* and E. *cloacae* combination with vermicompost on growth and yield of groundnut, *Seybold report*. 18, 862-1870. DOI: https://doi 10.17605/OSF.IO/P879S

[30] Park, M., Kim, C., Yang, J., Lee, H., Shin, W., Kim, S. and Sa, T. 2005. Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. *Microbiological Research*. 160(2), 127-133. DOI: https://doi.10.1016/j.micres.2004.10.003

[31] Rahimabadi, R.E., Ansari, M.H. and Nematollahi, A.R. 2017. Influence of cow manure and its vermicomposting on the improvement of grain yield and quality of rice (Oryza sativa L.) in Field conditions. *Applied Ecology and Environmental Research*. 6(1), 97-110. DOI: http://dx.doi.org/10.15666/aeer/1601_097110

[32] Rao, D.L.N. (2014). Recent advances in biological nitrogen fixation in agricultural systems. *Proceedings of the Indian National Science Academy*. 80(2), 359-378. DOI: https://doi:10.16943/PTINSA/2014/V80I2/55114.

[33] Singh, B., Kaur, R. and Singh, K. 2008. Characterization of *Rhizobium* strain isolated from the roots of *Trigonella foenumgraecum* (fenugreek). *African Journal of Biotechnology*. *7*(20), 3671-3676.

[34] Singh, P., Singh, R. K., Li, H. B., Guo, D.J., Sharma, A., Lakshmanan, P., Malviya, M. K., Song, X.P., Solanki, M.K., Verma, K.K., Yang, L.T. and Li, Y. R. 2021. Diazotrophic Bacteria *Pantoea dispersa* and *Enterobacter asburiae* Promote Sugarcane Growth by Inducing Nitrogen Uptake and Defense-Related Gene Expression. *Frontiers in microbiology*. 11, 600417. DOI: https://doi.org/10.3389/fmicb.2020.600417.

[35] Snapp, S., Rahmanian, M. and Batello, C. 2018. Pulse Crops for Sustainable Farms in Sub-Saharan Africa. *Rome: FAO*. DOI: https://doi: 10.18356/6795bfaf-en.

[36] Somasegaran, P. and Hoben, H. J. 2012. Handbook for rhizobia: Methods in legume-Rhizobium technology. Berlin, Germany: Springer Science & Business Media.

[37] Thierfelder, C., Chisui, J. L., Gama, M., Cheesman, S., Jere, Z. D., Trent Bunderson, W., et al. 2013. Maize-based conservation agriculture systems in Malawi: Long-term trends in productivity. *F. Crop. Res.* 142, 47–57. DOI: https://doi: 10.1016/j.fcr.2012.11.010.

[38] Thin, K. K., He, S. W., Wang, X., Wang, Y., Rong, M., Han, J. G. and Zhang, X. 2021. Rhizobium rhizolycopersici sp. nov., isolated from the rhizosphere soil of tomato plants in China. *Current Microbiology*. 78(2), 830-836. DOI:https://doi: 10.1007/s00284-020-02323-6

[39] Tittonell, P., Gérard, B., and Erenstein, O. 2015. Tradeoffs around crop residue biomass in smallholder crop-livestock systems – What 's next. *Agric. Syst.* 134, 119–128. DOI: https:// doi: 10.1016/j.agsy.2015.02.003.

[40] Tyagi, A., Kumar, V. and Tomar, A. 2017. Isolation, identification, biochemical and antibiotic sensitivity characterization of Rhizobium strains from *Vigna mungo* (L.) *Hepper, Cicer arietinum* (L.) and *Vigna radiata* (L.) Wilczek in Muzaffarnagar, Uttar Pradesh India. *International Journal of Current Microbiology and Applied Sciences*. 6(12), 2024-2035. DOI: https://doi.org/10.20546/ijcmas.2017.612.233.

[41] Upasana, B., Shipra, M., Sarita, J. and Dalal, A.S. 2020. A study of isolation and identification of bacteria from lake water in and around Udaipur, Rajasthan. *J Family Med Prim Care*. 9(2): 751–754. DOI: https:// doi: 10.4103/jfmpc.jfmpc_1032_19

[42] Vincent, J.M. 1970. A Manual for the Practical Study of Root Nodule Bacteria', Blackwell, *Oxford and Edinburgh*. 164 pp.

[43] Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F.O., Ludwig, W., Schleifer, K.H. and RosselloMóra, R. 2014. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews Microbiology*. 12(9), 635-645. DOI: https://doi:10.1038/nrmicro3330.

[44] Zahran, H.H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Reviews*. 63(4), 968-989. DOI: https://doi:10.1128/MMBR.63.4.968-989.1999.