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Efficiency of *Enterobacter asburiae* and Vermicompost on the Peanut Growth and Yield

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The combined use of endophytic nitrogen-fixing bacteria (ENFB) and organic manures is a trend in sustainable agricultural production. To investigate the effect of *Enterobacter asburiae* (*E asburiae*) and vermicompost (VCT) on farmland nutrients, yield, quality, and reduce arsenic uptake of peanuts. Three factors, which were: (i) *E asburiae* (inoculum and no inoculum); (ii) irrigation water (River and underground water); (iii) VCT (0.00 and 10.0 t ha^{-1}), were designed by eight treatments (Trt) and four replications. *E asburiae* inoculation combined with 10 t VCT ha^{-1} application and river water (RW) irrigation that both raised soil nutrient properties and traits of peanut agronomy and yield traits, raised significantly fresh seed yield and reduced arsenic (As) absorption of peanut stems and seeds. fresh seed yield of peanut in treatments of *E asburiae* inoculation, RW irrigation and 10.0 t VCT ha^{-1}, which were higher than non *E asburiae* inoculation, deep well water (DWW) irrigation and no VCT application, were 24.3, 26.9 and 15.4%, respectively. The peanut As contents in Trt of E *asburiae* inoculation, RWI and 10.0 t VCT ha^{-1} were lower than 7.78, 45.3 and 1.92% in stems and 13.2, 78.3 and 9.82% in seeds, respectively, compared to non-*E asburiae* inoculation, DWW irrigation and no VCT application. *E asburiae* was found out one potential species to promote soil nutrients, the peanut growth, yield, quality and As resistance, which will be used like biological fertilizers in the future.

Keywords: Arsenic, confined field, deep well water, *Enterobacter asburiae,* river water.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) that is a popular crop that provides oil, foodstuff, and high income for global farmers, have popularly planting around the word (Daudi *et al*., 2021; Hamidou *et al.,* 2013). This is one of the crops that contains high levels of protein, oil, fatty acids, carbohydrates, vitamins, and minerals. Peanut is a crop that provides oil, foodstuff, and high income for global farmers. It is grown in most tropical and subtropical countries such as India, China, the United States, West Africa, and Southeast Asia (Ganesh and Ali *et al.,* 2007). Use of ENFB associated with organic manures has effectively developed on new sandy soil in an Giang province, Vietnam, where most nutrients are lacking or inaccessible because it is not suitable for development on sandy soil. Sandy soil that exploited for recent cultivation in Vietnam, introduced to its low soil nutrients and emissions, decreased to keep farmland water and nutrients. However, the management of organic manures and ENFB in the soil has remarkably improved the soil fertility of these high sandy areas. The high sand composition of the farmland makes it difficult to retain soil nutrients due to leaching and percolation (Nguyen, 2024). Agricultural production is facing environmental changes, which consist of climate change, prolonged drought, intensive farming, crop increase, causes farmland to become increasingly degraded. In addition, urbanization has reduced agricultural land leading to a decrease in global food production. Therefore, the government of each country has developed an intensive farming system to overcome the food shortage, which has led to a decline in the quality of agricultural land (Tully *et al.,* 2015). Sandy soils have good drainage and aeration due to the high sand proportion in the soil structure, but low organic matter content due to easy leaching and high mineralization (Erenstein *et al.,* 2015). Local farmers are increasingly using chemical fertilizers, pesticides and irrigate. As pollution water, which mainly causes environmental pollution due to the accumulation of nutrients from fertilizers and pesticides. Soil and groundwater will be gradually polluted with heavy metals, leading to a further decrease in crop yield and quality.

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Inoculation of native nitrogen-fixing bacteria can replace inorganic N fertilizer and can improve soil fertility and reduce the uptake of toxins such as As and Cd (Chuong and Hung, 2021; Tuan and Chuong, 2022; Roychowdhury *et al.,* 2005). Freshwater shortage for crop irrigation has been a big problem from 2021 to 2024, which is a cause of prolonged drought and dikes surrounding fields for multi-cropping. Therefore, people had to use a deep well water, which contained a high-arsenic concentration to irrigate crops, leaded to serious pollution of agricultural land and agricultural products (Chuong, 2024;Chuong and Hung, 2021). An important role of N is for effective peanut growth, and adequate nitrogen supply is essential for growth and yield due to intensive cultivation. Therefore, farmers have used a large amount of chemical fertilizers, which is the cause of soil degradation. The regular use of biofertilizers is encouraged to obtain high quality and clean agricultural products (Hung and Chuong, 2022). A recent challenge facing advanced agriculture is to achieve higher yields in an environmentally friendly way (Chuong, 2021). Therefore, it is necessary to find environmentally friendly solutions immediately. Among the many species used as biocontrol agents (Chuong and Tri, 2021). Inoculation of rhizobia helps to supplement nitrogen for peanuts because native rhizobia cannot provide enough nitrogen for it (Cuong and Chuong, 2022; Tuan and Chuong, 2022). Peanut growth and yield were significantly reduced by As-contaminated irrigation water and farmland. In addition, farmers continue to use As-contaminated irrigation water to water crops for a long time, which could cause As accumulation in soil to be higher and higher (Clough and Mueller, 2014; Creamer *et al.,* 2014). Endophotic nitrogen fixing bacteria (ENFB) can provide up to 74% of the N required by groundnut plants. Environmental conditions greatly influence the activity and nitrogen fixation ability of bacteria. Applying too much N can inhibit the nitrogen fixation process of bacteria. Soil carbon concentration has a positive effect on the formation and nitrogen fixation of nodules. Especially soils that are adequately fertilized with organic manures (Panke-Buisse *et al.,* 2014). The soil As accumulation and its uptake by both rhizosphere bacteria and plant growth-promoting bacteria is an emerging environmental and farmland. Soil microorganisms can biodegrade As into products that are less toxic to plants, less mobile, and less bioavailable, but are very difficult to remove from contaminated soil environments (Gao *et al.,* 2015; Ouyang, *et al.,* 2018). A wide range of root-zone microorganisms, including both symbiotic and non-symbiotic nitrogen-fixing bacteria, can adapt and reduce the heavy metal toxicity to plants, and even thrives in high metal concentrations. This is the intrinsic result or induced adaptation of ENFB to these metals. Heavy metals are not biodegradable and are wholly indestructible, though their properties and bioavailability can change depending on changes in environmental factors (Scarlett *et al.,* 2021). Intrinsic traits of ENFB demonstrate metal tolerance capabilities.

Resistance is the ability that allows bacteria to survive at high toxic metal concentrations through detoxification mechanisms, which are activated to directly respond to the heavy metal exist. Therefore, it is necessary to completely remove, transform, or immobilize toxic heavy metals from contaminated soil to create less toxic or non-toxic forms. However, to treat and remove in As contaminated soils, tolerance must be existed in ENFB associated with hosts. Existing in high As pollution environments, Endophotic nitrogen fixing bacteria have developed mechanisms that immobilize, sequester, or transform metals into inactive forms to tolerate the uptake of heavy metal ions. Endophotic nitrogen fixing bacteria can be contributed to the farmland to enhance the soil nutrient values for the plant growth (Mooshammer *et al.,* 2014). Endophotic nitrogen fixing bacteria are a main key in promoting soil fertility and decreasing the inorganic fertilizer use because they can develop rapidly lead to the beneficial bacteria increase that provide soil nutrients for plant growth, help easily nutrient uptake, and crop resistance increase to pathogens, thereby decreasing both diseases and pests (Berendsen *et al.,* 2012). However, the use and promotion of traditional bacterial strains are restricted due to low environmental adaptation and inoculated efficiency and unstable inoculation process. Endophotic nitrogen fixing bacteria, in recent years, was used to replace bio-fertilizers containing many ENFB strains and different functions (Smith *et al.,* 2014; Chu *et al.,* 2007). The main reason for the interaction between ENFB and crops is nutrition shifts, as crops supply the different carbon resources for ENFB growth and life, conversely, ENFB supply fixed N sources to promote the crop growth (Philippot *et al.,* 2023). The benefit interaction between peanut and ENFB has strongly raised crop yields under different farmland types (USDA, 2019). Denitrification, IAA biosynthesis, glutamine oxidation, and oxoglutarate metabolism by aminotransferase enzymes have been used to assess the impact of ENFB on N uptake of crops from soil, its metabolism, and its use efficiency. Endophotic nitrogen fixing bacteria both promote N uptake from farmland to plants and have the impacts of enhancing photosynthesis and carbohydrate metabolism (Guo *et al.,* 2023; Lòpez-Fernàndez *et al.,* 2017). Therefore, the organic manure application supplied huge carbon sources for ENFM growth (Peix *et al.,* 2015). The organic manure application that has been a right alternative to replace chemical fertilizers, lessened the inorganic N use in agricultural crop production (Lòpez-Fernàndez *et al.,* 2017). The main objective of this study was to evaluate the effectiveness of E asburiae inoculation combined with VCT application and two different irrigation water types on peanut yield, quality, and As accumulation

MATERIALS AND METHODS

Isolation and molecular identification of E asburiae species from peanut root nodules: Small nodules of peanuts (<1.5 mm were collected and isolated *E asburiae* species. When peanut plants were planted in the field in An Phu district at 65 days after sowing (DAS), which were taken nodules for isolating ENFB (Hossain *et al.*, 2023). Twenty selected nodules from 4 plants (each plant with five nodules) were cleaned with water to eliminate soil particles on the peanut nodules. The next step, nodulous surface's recleaning was used by 70% alcohol for 60 seconds, and cleaned out with sterile distilled water five times, and finally soaked in 5% NaClO for 120 seconds. The washed nodules were crushed in 1 mL of sterilized phosphate-buffered saline, and the resulting suspension was streaked on YMA agar plates (https://www.himedialabs.com/us/). The plates with multiple dilution rates and the streaked YMA agar plates that were incubated at 25-28ºC for 4 days, and the selected pure colonies were cultured in liquid YMA.

Molecular Characterization Identification of E asburiae species: DNA of *E asburiae* species was extracted from all selected isolates after preliminary biochemical testing using the Promega Genome Isolation Kits (Phu Sa company, Vietnam). The 16S rRNA gene was amplified from these isolates using primer of from *E asburiae* isolates by polymerase chain reaction (PCR) in thermal cycler (M.J. Research PTC-100) using the primer pair PA (5′ AGAGTTTGATCCTGGCTCAG 3′) and PH (5′ AAGGAGGTGATCCAGCCGCA 3′) in three cycles of PCR analysis. The isolated and identified species were characterized at the genus level. To better understand and determine the sequence similarity of these isolates to the previously published peanut nodule microbiota sequences (Cardoso *et al.*, 2018), NCBI BLAST was used to interpret the Sanger sequencing results, and the selected isolate branch had a high similarity of up to 99,74% compared with *E asburiae* species (Figure 2)**.** *Enterobacter asburiae* species were completely increased up to 10^8CFU/mL population of *E asburiae* species, which was well mixed with peanut seeds and soaked in liquid YMA medium for 8 hours before sowing (Hassan Etesami, 2022). *Enterobacter asburiae* species high similarity and were more closely related to other bacterial species, which were deposited in the NCBI database use of the BlastN programmer. Furthermore, a phylogenetic tree was constructed to determine the position of the two isolated species along with sequences from selected reference strains (Figure 1 and 2). *Enterobacter asburiae* species was completely increased in their population up to 10^8CFU/ mL and well inoculated for peanut seeds for applying directly to the soil.

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CCCCAGTCATAGAATCACAAAGTAGGTAAGCGCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTC
CCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTAGCATTCTGATCTACGATTACTAGC
GATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTC
GCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACTTGA
CGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCTAACCGCTGGCAACAAAGG
ATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTG
TCTCAGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCTGGATGTCAAGAGTAGGTAAGGTTCTTCGC
GTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGG
CCGTACTCCCCAGGCGGTCGACTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGA
CATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCT
TTGTCCAGGGG
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Figure 1. DNA Identification of E asburiae belong to 1350 bp long, 5'- 3'

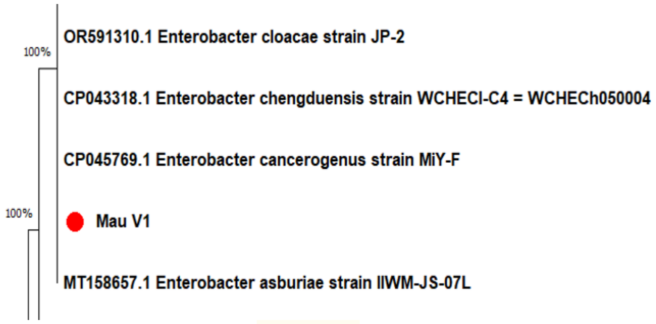

Figure 2. Phylogenetic tree of *E asburiae* based on 16S rRNA sequences of 4 isolates with the closest type species

***Experimental location and materials*:** An experiments were designed in local farmer's field inside the dyke of Phuoc Hung commune, An Phu district, which included three factors (i) the first factor was *E asburiae;* (ii) the second factor was irrigation water types; (iii) the third factor was VCT and 4 replications in a completely randomized block design. The peanut seeds (L14 variety, Vietnam) were incubated with *E asburiae* eight hours before planting. User of Inorganic fertilizers and VCT ratios and *E asburiae* species inoculants were presented in Table 1. Inorganic fertilizers that were used by $CO(NH_4)_2$, P_2O_5 and K_2O, converted to the NPK weight. The total experimental area was 640 m^2 (1 m in width x 20 m in length x 8 treatments x 4 replicates). The distance between two replications was 0.5 m and planted in single rows with a plant distance of 25 cm (sowing 2 seeds $hole^{-1}$) Peanuts have up to three leaves, each hole was chosen a healthy plant for research until harvest. Immediately before planting 24 hours, the seeds were inoculated with EC strain Fg 5-2 in dark and at room temperature with up to 70% humidity. The two experiments were arranged on April 30th, 2023 and harvested manually on August 30th, 2023 in both experiments. The experimental soil texture was silt sandy loam that were evaluated according to USDA (2019), which is suitable for peanut cultivation because of its high sand content (Rajendran *et al.*, 2012).

There were ten healthy peanut plants, which were randomly selected from each replication, observed all traits of agronomy, yield and fresh productivity. Peanut seeds were collected from each replication to analyzed nutrient traits. Peanut yield properties were determined during the peanut growth time, which included crop height, available branches number (No.) and pods per plant, biomass, nodulous No. and weight (Wt.) per plant, fresh Wt. of filled and unfilled pods plant^{-1}, fresh pod yield was counted by tons per hectare (t ha^{-1}). Initial soil properties were pH_{H2O} (4.97), CEC (2.627 cmol^{+} kg^{-1}), SOM (1.24%), total N (0.125%), available P (25.4 mg kg^{-1}) and exchangeable K (75.1 mg kg^{-1}). Arsenic concentration, which was 697 µg L^{-1} (DWW) and no detection (RW), was 84.55 mg kg^{-1} (soil inside the dyke). Available phosphorus is a macronutrient for ENFB and peanuts. In general, the research area had low soil nutrient compositions. Peanut seeds of L14 variety were a high ability of disease resistance and high yield. The field experiment included eight treatments and four replications.

Table 1. *E. asburiae*, VCT and irrigation water types in experimental Treatment (Trt).

Treatments (Trt)	*E asburiae* (10^8CFU mL^{-1})	Irrigation	VCT (t ha^{-1})	NPK fertilizers (kg ha^{-1})
P1	No	DWW	0	20N:60P:60K
P2		RW		
P3		DWW	10.0	
P4		RW		
P5	Yes	DWW	0	
P6		RW		
P7		DWW	10.0	
P8		RW		

Research data: Ten plants were randomly selected from each replicate. The height, total chlorophyll content, available branches, and leaf number were averagely measured during 20, 45 and 65 DAS, and the value per plant was counted. Ten plant samples were taken from each plot to determine growth parameters and yield components, which consisted of the nodule number and fresh nodule weight, fresh biomass weight, No. of filled and unfilled seeds, 100-grain weight, 1,000-grain weight, fresh fruit yield. Soil, stem, leaf and grain samples were collected per replication at harvest and dried separately for As analysis. Dry grain samples were further analyzed for moisture, lipid, protein, total N, P, and K.

Analysis method: Soil, stem, leaf, and seed samples from each treatment were harvested separately and dried to determine As and grain nutrient compositions. Arsenic in soil, stem, and grain was determined by using the method described by AOAC. At the end of the experiment, soil samples were taken from all plots to determine the effects induced by the implemented treatments on some soil properties. Soil pH was measured using a pH meter with a soil-to- water ratio of 1:2.5. Mineral nitrogen content was analyzed using the Kjeldahl method, while available phosphorus was determined employing the alkaline hydrolysis method, as outlined by All parameters were analyzed by using the USDA (2019) method.

***Statistical analysis*:** 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 significant difference at $P\ value \leq 0.05$. For each experiment, the processed data were calculated according to 02 factors to find the interaction between treatments and factors with each other.

RESULTS

***Soil properties at harvest season*:** The analysis results presented in Table 2 showed the significant diversity between the *E asburiae* inoculation and non- *E asburiae* inoculation between the two factors (B) and (C) at level 1% at harvest season (expect soil pH of VCT). The interaction among factors (AxBxC) were statistically insignificant differences (except for the 5% diversity in total N and CEC content). However, the Table 2's results proved that interaction between factors, which were both soil total N [expect for F(AxB)] and CEC [expect for F(BxC)], had a statistically significant difference at 5% level. In general, all chemical traits consisted of farmland pH, available P, SOM and exchangeable K were not interaction among three factors (AxBxC) (expect for N and CEC). and CEC (expect soil pH and exchangeable K). The farmland property values that were pH, total N, pH, available P, SOM and exchangeable K and CEC at treatments of *E asburiae* inoculation, RW irrigation and the 10 t ha^{-1}application of VCT (except for pH in the VCT factor) at harvest season, had higher than those of the non- *E asburiae* inoculation, DWW irrigation and no VCT application (Table 2).

Plant height and available branches: Results in Table 3, showed that the average height of peanut plants in the treatments with E *asburiae* inoculation combined with VCT and RWI were remarkably higher than the plots of non *E asburiae* inoculation, non VCT application, and DWW irrigation during the growth period of 20, 45, and 65 DAS, (except for 20 DAS). The interactions between factors A, B, and C were only significant differences for AxC and BxC at 1% and 5%, respectively. The number of available branches per plant did not differ significantly between factors A and B (except for RWI, which had a higher number of branches than DWW irrigation at 45 DAS and a 5% difference) at 20 and 40 Days after sowing (DAS). However, the number of branches was significantly different (1%) between three factors (*E asburiae*, irrigation water types, and VCT at 65 DAS). The number of branches at 20, 45, and 65 DAS in the plots with the *E asburiae* inoculants, RWI, and VCT application were all higher than the other experiments (Table 3). The

Table 2. effect of *E asburiae, irrigation water and* VCT on soil chemical attributes at harvest .

Factor	pH	Total N (g kg^{-1})	Available P (mg kg^{-1})	SOM (g kg^{-1})	Exchangeable K (mg kg^{-1})	CEC (Cmol^{+} kg^{-1})
E *asburiae* (A)						
No	5.02^{b**}	1.64^{b**}	24.2^{b**}	23.1^{b**}	118^{b**}	3.30^{b**}
Yes	5.41^{a**}	2.10^{a**}	26.6^{a**}	25.4^{a**}	145^{a**}	5.23^{a**}
Irrigation (B)						
DWW	4.76^{b**}	1.78^{b**}	22.9^{b**}	23.4^{b**}	121^{b**}	3.29^{b**}
RW	5.67^{a**}	1.96^{a**}	27.9^{a**}	25.1^{a**}	141^{a**}	5.24^{a**}
Vermicompost (C)						
0.00 t ha^{-1}	5.14^{ns}	1.69^{b**}	$24{,}7^{b*}$	22.4^{b**}	125^{b**}	4.10^{b**}
10.0 t ha^{-1}	5.29^{ns}	2.05^{a**}	26.1^{a*}	26.0^{a**}	137^{a**}	4.43^{a**}
F (AxB)	*	ns	ns	ns	*	**
F (AxC)	ns	**	ns	ns	ns	*
F (BxC)	ns	*	ns	ns	ns	ns
F (AxBxC)	ns	*	ns	ns	ns	*

Means with different letters, in the same column differ significantly *, ** = significant at Pvalue ≤0.05 and ≤0.01, respectively; ns = insignificant difference

Table 3. Peanut plant height and available branches during the growth.

Factor	Plant height (cm)			Available branches (branches plant^{-1})		
	Days after sowing (DAS)					
	20	45	65	20	45	65
E *asburiae* (10^{8}CFU mL^{-1}) (A)						
No	11.8^{b**}	21.3^{b**}	50.5^{b**}	5.38^{ns}	11.2^{ns}	17.3^{b**}
Yes	12.8^{a**}	22.6^{a**}	53.0^{a**}	5.94^{ns}	12.1^{ns}	19.7^{a**}
Irrigation (B)						
DWW	12.1^{ns}	21.5^{b*}	51.2^{b**}	5.63^{ns}	10.9^{b*}	17.3^{b**}
RW	12.5^{ns}	22.4^{a*}	52.4^{a**}	5.69^{ns}	12.4^{a*}	19.8^{a**}
Vermicompost (C)						
0.00 t ha^{-1}	12.0^{b*}	21.5^{b*}	50.7^{b**}	5.63^{ns}	11.3^{ns}	17.6^{b}
10.0 t ha^{-1}	12.6^{a*}	22.4^{a*}	52.9^{a**}	5.69^{ns}	11.9^{ns}	19.4^{a}
F (AxB)	ns	ns	ns	ns	ns	ns
F (AxC)	**	**	**	ns	*	**
F (BxC)	*	**	*	ns	ns	ns
F (AxBxC)	ns	ns	**	ns	ns	ns

Means with different letters, in the same column differ significantly *, ** = significant at Pvalue ≤0.05 and ≤0.01, respectively; ns = insignificant difference

interaction between the factors was not significant at 5% (except for the interaction between the *E asburiae* and VCT factors at 45 and 65 DAS).

***Leaf number and total chlorophyll*:** There was insignificant differences in the leaf number per peanut plant at 20 DAS between factors B and C, on contrary, factor A has a higher number of leaves than the control. There was no interaction between three factors (Table 4). However, the leaf number per plant in plots with *E asburiae* inoculation combined with VCT application and RWI were significantly higher and different in the leaf number compared to the treatments without *E asburiae* inoculation, without VCT application and DWW irrigation at 45 and 65 DAS, (except for the treatments with and without VCT application at 65 DAS). There were interactions between factors (FAxB) and (FAxBxC) at 45 DAS and (FAxC) and (FAxBxC) at 65 DAS. Total chlorophyll contents at 20 and 40 DAS was higher in the treatments of *E asburiae* inoculation, VCT amendment and RW irrigation compared to the control treatments, and statistically significant differences at 5% and 1% levels (except for the factor C at 65 DAS). However, there was insignificant interaction between factors A, B, and C [except for (AxB) and (AxC) at 20 and 40 DAS].

***Peanut yield components*:** Table 5's results presented that *E asburiae* inoculation, irrigation water types and VCT application were significant effects on the nodulous number, nodulous weight, fresh biomass, number of filled and unfilled pods per plant at level 1% (P≤0.01). Treatments of *E asburiae*

Table 4. Peanut leaf number and Total chlorophyll during the growth.

Factor	Leaf number (leaves plant^{-1})			Total chlorophyll (mg per m^2)		
	Days after sowing (DAS)					
	20	**45**	**65**	**20**	**45**	**65**
***E asburiae* (10^{8}CFU mL^{-1}) (A)**						
No	16.4^{b*}	50.0^{b**}	102^{b**}	379^{b**}	423^{b*}	412^{b**}
Yes	17.9^{a*}	57.4^{a**}	113^{a**}	405^{a**}	437^{a*}	472^{a**}
Irrigation (B)						
DWW	16.8^{ns}	52.4^{b*}	105^{b*}	375^{b**}	421^{b*}	417^{b**}
RW	17.4^{ns}	55.9^{a*}	110^{a*}	410^{a**}	439^{a*}	467^{a**}
Vermicompost (C)						
0.00 t ha^{-1}	16.8^{ns}	52.2^{b**}	105^{ns}	386^{b*}	426^{ns}	438^{ns}
10.0 t ha^{-1}	17.4^{ns}	56.1^{a**}	110^{ns}	398^{a*}	434^{ns}	445^{ns}
F (AxB)	ns	**	ns	**	**	ns
F (AxC)	ns	ns	**	*	*	ns
F (BxC)	ns	ns	ns	ns	ns	ns
F (AxBxC)	ns	**	**	ns	ns	ns

Means with different letters, in the same column differ significantly *, ** = significant at Pvalue ≤0.05 and ≤0.01, respectively; ns = non-significant difference

Table 5. Yield components of Nodulous Number (No.) and weight, biomass, No. of filled and unfilled pods per peanut plant.

Factors	No. of nodules (nodules plant^{-1})	Wt. of nodules (gr. plant^{-1})	Wt. of biomass (gr. plant^{-1})	No. of filled pods (pods plant^{-1})	No. of unfilled pods (pods plant^{-1})
E *asburiae* (10^{8}CFU mL^{-1}) (A)					
No	241^{b**}	1.28^{b**}	257^{b**}	66.5^{b**}	8.38^{a**}
Yes	277^{a**}	1.52^{a**}	288^{a**}	73.2^{a**}	5.63^{b**}
Irrigation (B)					
DWW	246^{b*}	1.32^{b**}	257^{b**}	65.4^{b**}	8.63^{a**}
RW	272^{a*}	1.49^{a**}	288^{a**}	74.3^{a**}	5.38^{b**}
Vermicompost (C)					
0.00 t ha^{-1}	227^{b**}	1.32^{b**}	265^{ns}	67.3^{b**}	7.88^{a**}
10.0 t ha^{-1}	291^{a**}	1.48^{a**}	280^{ns}	72.4^{a**}	6.13^{b**}
F (AxB)	ns	ns	ns	**	**
F (AxC)	ns	ns	ns	ns	ns
F (BxC)	**	**	ns	ns	ns
F (AxBxC)	ns	ns	ns	ns	*

Means with different letters, in the same column differ significantly *, ** = significant at Pvalue ≤0.05 and ≤0.01, respectively; ns = non-significant difference

inoculation, DWW irrigation and VCT application (10.0t ha^{-1}), which had the nodulous number, nodulous weight, fresh biomass, number of filled and unfilled pods per plant, were higher than those of non-*E asburiae* inoculation, DWW irrigation and no VCT application (0.00t ha^{-1}). However, there were not their interaction between F(AxB), F(AxC), F(BxC) and F (AxBxC) about the nodulous number, nodulous weight, fresh biomass, number of filled and unfilled pods per plant [Except for nodulous number and weight of F(BxC); number of filled and unfilled pods of F(AxB) and unfilled pod number of F(AxBxC)].

***Weight of 100 pods, 1,000 seeds and productivity*:** Table 6's result presentation showed that yield components and peanut yield at harvest were statistically significant at 1%. The yield components of peanut plants corresponding to the 100-pod weight, 1000-seed weight, and fresh fruit yield indicators in the experimental trials with *E. asburiae* inoculum, RW irrigation, and VCT application, which were always higher than those of without *E. asburiae* inoculum, DWW irrigation, and no VCT application (Table 6). In particular, the fresh fruit yield of the experimental plots with *E asburiae* inoculation, RW irrigation, and VCT application was always higher than that of without *E asburiae* inoculation, DWW irrigation, and no VCT application by 24.4%, 26.9%, and 15.4%, respectively. Furthermore, As-contaminated irrigation water had a significant impact on *E asburiae* species. When As-contaminated water irrigation water was used to irrigate peanut plants, which reduced the number and weight of all yield components and peanut yield. The interaction between factors (AxB) was 1% different for all indicators. However, there was no interaction between (AxC), (BxC), and (AxBxC). Except for the 100-pod weight, there was a 5% interaction between irrigation water types and VCT (BxC) for total empty pods, 100-pod weight, and fresh fruit yield (Table 6).

Arsenic contents**:** Peanut As contents of stems, leaves and seeds at harvest showed that the plots with *E asburiae* inoculum and river water irrigation had lower As concentrations than those of without *E asburiae* inoculum and deep well water irrigation by 7.76%, 13.2%, and 45.3%, 78.3%, respectively, and were statistically significant at 1% (Table 6). However, VCT application with 10 t ha^{-1} weight did not affect As accumulation in stems, but affected As accumulation of peanut seeds. As content of peanut seeds at harvest showed that treatments with 10 t VCT ha^{-1} application were 9.82% lower than those of without VCT application and statistically significant at 5%. There was no interaction between factors such as *E asburiae* species (A), irrigation water types (B), and VCT application (C) on As in stems, but there was an interaction between factors of (AxC) at 5% ($P \leq 0.05$), (AxB), (AxBxC) at 1% ($P \leq 0.01$) in seeds at harvest season. The results of the study showed that *E asburiae* bacteria, RW irrigation I, and VCT application significantly reduced As accumulation in stems, leaves, and especially in peanut seeds (Table 6). Furthermore, peanut yield increased significantly compared to no *E asburiae* inoculation, DWW irrigation, and no VCT application by 24.4, 26.9, and 15.4%, respectively (Table 6).

Table 6. Yield components, yield and As content of peanut at harvest season.

Factor	Weight of 100 pods (gr)	Weight of 1,000 seeds (gr)	Pod yield (t ha^{-1})	Arsenic concentration (µg kg^{-1}) Stems and leaves	Arsenic concentration (µg kg^{-1}) Seeds
E asburiae **(10^8CFU mL^{-1}) (A)**					
No	104^{b**}	312^{b**}	4.38^{b**}	$1{,}070^{a**}$	114^{a**}
Yes	107^{a**}	345^{a**}	5.79^{a**}	987^{b**}	99.0^{b**}
Irrigation (B)					
DWW	103^{b**}	312^{b**}	4.29^{b**}	$1{,}330^{a**}$	175^{a**}
RW	108^{a**}	345^{a**}	5.87^{a**}	728^{b**}	38.0^{b**}
Vermicompost (C)					
0.00 t ha^{-1}	104^{b**}	313^{b**}	4.66^{b**}	1.04^{ns}	112^{a*}
10.0 t ha^{-1}	107^{a**}	344^{a**}	5.51^{a**}	1.02^{ns}	101^{b*}
F (AxB)	**	*	**	ns	**
F (AxC)	ns	ns	ns	ns	*
F (BxC)	*	ns	ns	ns	ns
F (AxBxC)	*	ns	**	ns	**

Means with different letters, in the same column differ significantly *, ** = significant at Pvalue ≤0.05 and ≤0.01, respectively; ns = non-significant

Table 7. Nutrition traits of peanut seeds at harvest season.

Factor	Water	Lipid	Protein	Nitrogen	Phosphorus	Potassium
	(gr 100gr^{-1})					
E. ***asburiae*** **(10^8CFU mL^{-1}) (A)**						
No	28.3^{ns}	25.2^{b**}	17.3^{b**}	2.77^{b**}	0.287^{ns}	0.398^{b**}
Yes	28.8^{ns}	26.4^{a**}	18.1^{a**}	2.90^{a**}	0.292^{ns}	0.424^{a**}
Irrigation (B)						
DWW	27.9^{b**}	25.2^{b**}	17.0^{b**}	2.72^{b**}	0.289^{ns}	0.404^{b**}
RW	29.1^{a**}	26.4^{a**}	18.4^{a**}	2.95^{a**}	0.291^{ns}	0.418^{a**}
Vermicompost (C)						
0.00 t ha^{-1}	27.0^{b**}	25.6^{ns}	17.5^{b**}	2.80^{b**}	0.286^{b*}	0.401^{b**}
10.0 t ha^{-1}	30.0^{a**}	26.0^{ns}	17.9^{a**}	2.87^{a**}	0.294^{a*}	0.421^{a**}
F (AxB)	**	**	*	**	**	**
F (AxC)	ns	**	ns	ns	**	**
F (BxC)	ns	**	*	*	ns	**
F (AxBxC)	ns	**	ns	ns	**	**

Means with different letters, in the same column differ significantly *, ** = significant at Pvalue ≤0.05 and ≤0.01, respectively; ns = non-significant

Seed nutrition traits**:** Results from Table 7 showed that the peanut seed's moisture ranged from 27.0 to 30.0 gr 100gr^{-1} and was statistically significant at the 1% level (except for factor *E asburiae*). Seed's moisture that was found in plots of the RW irrigation (29. gr 100gr^{-1}) and 10 t VCT ha^{-1} application (30.0%), obtained higher than that non-VCT application (27.0 gr 100gr^{-1}). Furthermore, the peanut seeds's lipid content (26.4 gr 100gr^{-1}) was achieved in the *E asburiae* inoculation and RW irrigation plots, and conversely, the lipid content (25.2 gr 100gr^{-1}) was found in the non- *E asburiae* inoculation and DWW irrigation. However, VCT application did not affect the moisture content of the peanut seeds and was not statistically significant. Protein reached (18.1 gr 100gr^{-1}) in plots of the *E asburiae* inoculation, 18.5 gr 100gr^{-1} in the RW irrigation, and 17.9 gr 100gr^{-1} in the 10 t VCT ha^{-1} application. All traits of which were higher than those in the non *E asburiae* inoculation, DWW irrigation, and non- VCT application experiments. Similarly, nitrogen and potassium in the seeds were higher in plots of the *E asburiae* inoculation, RW irrigation, and 10 t VCT ha^{-1} application than that of the non- *E asburiae* inoculation, DWW irrigation, and non- VCT application. Except for the seed P content, where *E asburiae* inoculation and the two irrigation water types did not affect the seed P content, but 10 t VCT ha^{-1} application resulted in higher seed P than that of no application and was statistically significant at 1% (Table 7). Lipid and potassium concentration in the seeds had interactions between the three factors (AxB), (AxC), BxC), and (AxBxC) at the 1% significance level. Meanwhile, moisture ($P \leq 0.01$) only had (AxB), protein ($P \leq 0.05$) had factors (AxB) and (BxC), nitrogen had factors (AxB) ($P \leq 0.01$) and (BxC) ($P \leq 0.05$), and phosphorus had (AxB), (AxC), and (AxBxC) at the 1% significance level.

DISCUSSION

Previous research proved that ENFB species play a crucial role in combining with the nodules formation on peanut roots and converting atmospheric nitrogen into a usable form for the peanut. These combinations enhanced plant growth, yield and the importance of nodulation and nitrogen fixation in peanut plants, facilitated by the symbiotic relationship with ENFB (Li *et al.*, 2019). The nodulous formation of peanut roots is due to the exchange of biochemical functions through a symbiotic relationship between peanuts and ENFB, which relies on specialized structures formed on peanut roots. The main function of these nodules is to facilitate the conversion of atmospheric nitrogen into nodule nitrogen by ENFB, providing a readily available natural nitrogen source for peanuts as NH_4^+ or NO_3^- through a process called biological nitrogen fixation (Li *et al.*, 2019). Recent studies have shown that ENFB do not coexist with rhizobium strains. They can enter the host plant by invading the internal cells/tissues of the roots or exploit discontinuities on the root surface to gain entry. Once inside, they establish a symbiotic relationship with the host, receiving a nutrient-rich environment in exchange for enhancing and protecting the health of the host plant they colonize (Sgroy *et al.*, 2009; Oremland, and Stolz, 2005). The results of this study are agreed with the prior discovery of Satapute (2019) showed that ENFM have the potential to increase the amount of nitrogen at harvest seasons due to their nitrogen fixation ability. Moreover, they had the ability to produce IAA and P-solubilization, which decomposed from insoluble phosphorus to soluble phosphorus provided to peanuts (Román-Ponce *et al.*, 2018; Mesa *et al.*, 2017). Animal manures have a significant impact on reducing environmental pollution and improving soil physical and chemical indicators due to affect the physical and natural properties of the soil type. Organic fertilization to increase soil fertility and the nitrogen fixation capacity of ENFB affected the number of nodules and peanut yield on degraded sandy loam soil (Dang and Chuong, 2023). Increasing the use of inorganic nitrogen fertilizers can be detrimental to the nodulation process, which can affect the natural nitrogen source. Applying high amounts of inorganic nitrogen in peanut production can be harmful to the soil and plants. Instead, the addition of animal manure to reduce the amount of inorganic fertilizer needed should be applied not only to increase soil fertility and yield, but also to enhance the nodulation process in peanuts for sustainable peanut cultivation (Mesa *et al.*, 2017; Mekhemar *et al.*, 2005). In this study, it was observed that the application of E *asburiae* species combined with VCT and with RW irrigation (non As pollution), increased all soil chemical and nutrient triats such as pH, SOC, CEC, total nitrogen, available P, and exchangeable K (Table 2). This is similar to previous findings of Chand, (2023); Borase *et al.* (2020), organic manures were mainly attributed to the superior organic acid release capacity when combining chemical fertilizers and ENFB, leading to increased microbial activity and microbial abundance. Consequently, nutrients become more readily available for uptake and utilization by plants. The combined approach proved more effective in consistently meeting the nutrient requirements of peanut plants, facilitating yield increases, while also supporting microbial organic matter decomposition and nitrogen release, thereby enhancing soil fertility.

The research results of El-sherbeny *et al.*, (2023); Alves *et al.* (2018) showed that inoculating the soil with two strains of ENFB in combination with biofertilizers helped to save nitrogen fertilizer and increase the height of peanut plants. Legumes have a close relationship with N-fixing bacteria that affect plant characteristics related to nutrient supply, plant growth, defense ability, and abiotic stress (Wang *et al.*, 2018). It is argued that the full exploitation of the potential of ENFB supplied and enhanced soil nutrients. The present study evaluated the efficacy of ENFB inoculation enhanced soil fertility and crop peanut for next germination under greenhouse conditions. Data showed that co-inoculation of *Bradyrhizobium* and *Trichoderma* raised the growth, development, and yield of groundnut (Neelipally *et al.,* 2020). Previous researches have proved that ENFB can be included by species such as E asburiae (Nguyen and Chuong, 2024). ENFB species are present in the peanut nodules, which can change and combine together, can enhance nodulation number and nitrogen fixation ability of peanuts under harsh environment conditions such as heavy metal polluted soil. The high increase of the peanut growth and yield in plots inoculated with ENFB compared to apply chemical fertilizers (Dhawi *et al.,* 2016). An increase of the nodulous number and dry weight of peanuts inoculated with ENFB (Mehmood *et al.,* 2021). The *E asburiae* species, which has high nitrogen fixing ability, can use a biofertilizer and other plant nutrient requirements besides nitrogen are readily available in the soil (Conlon *et al.,* 2022). Inoculation of legumes with ENFB improved nodulation and dry biomass yield and enhanced nitrogen uptake (Lindström, and Mousavi, 2020). Therefore, the objective of this experiment was to determine the potential of *E asburiae* species. ENFB living in symbiosis with peanut plants increased the number of nodules, nodule mass, increased the number of pods and decreased the mass of empty pods compared to the experiments without inoculation of ENFB, additional application of composted VCT combined with NPK helps to increase the yield and yield components of peanut (Orji *et al.,* 2022).

The ENFB effectiveness of peanuts leads to increase the N uptake in plant tissues, which in turn reflects chlorophyll synthesis. The leaf number and chlorophyll index were higher in the treatments with nitrogen-fixing bacteria strains than in other treatments, which could be due to the basic function of ENFB, which is to fix biological nitrogen (Pandey *et al.,* 2023). Inoculation with nitrogen-fixing bacteria increased the number of leaves, promoted chlorophyll synthesis and plant height growth, leading to a significant increase in dry weight and yield (Garg *et al.,* 2024) . According to a study by Mu *et al.* (2010), the influence of organic matter on the number of leaves and chlorophyll content of plants showed that the chlorophyll parameter values under different shades in the studied conditions had a close relationship between photosynthetic characteristics and dry matter accumulation and crop yield. This is expected to provide some theoretical basis for high-yielding dryland crop cultivation and long-term nitrogen management under shade and in fruit-peanut intercropping (Hassen *et al.,* 2023). When unpolluted water was used for irrigation and rhizosphere ENFB were applied, the height, leaves and branch number, and total chlorophyll content of crops grown in nutrient-poor soil increased significantly compared to plants that did not receive nitrogen or inoculation with ENFB (Hossain *et al.,* 2023, Peraltab *et al.,* 2020).

Recent research by Kitwetch *et al.* (2023) showed that ENFB inoculation in agricultural soils can promote the growth of productive components and crop yields, improve fruit quality, and soil nutrition. Based on these results, the simultaneous application of VCT, NPK fertilizer, and ENFM can increase the quality components and yield of peanuts. According to Nguyen and Chuong (2024) discovered that the biochar application from rice husk and sawdust combined with the bacterial strain *B aryabhattai* M2C reduced As soybean's uptake from the As pollution of farmland and irrigation water in Phuoc Hung commune, An Phu district. This further effects that reduced the As damage to the root system, caused by arsenic toxicity in the cultivated soil. The yield and quality of soybeans also raised higher than those of without the *B aryabhattai* M2C inoculation and biochar application from rice husk and sawdust. This result is consistent with the research results of Chuong (2024), which showed that the application of rice husk biochar, sawdust combined with *Priestia aryabhattai* M2C species, an ENFB isolated from the peanut growing area with As-contaminated soil in Phuoc Hung commune, An Phu district, has shown the ability to resist As in soil, inhibit As uptake by legumes from cultivated soil, and reduce root damage caused by As toxicity in cultivated soil. The yield and quality of peanuts in inoculation of *E Asburiae* Strain Cjy141 were higher than those without inoculation (Chuong, 2023).

***Conclusion*:** Vermicompost fertilization associated with *E asburiae* (10^8CFU mL^{-1}**)** inoculation and RWI on farmland significantly increased available soil nutrients, agronomical traits, the nodulous number and weight, and the peanut yield traits and productivity. Furthermore, *E asburiae species*, which remarkably decreased the As accumulation in peanut stems and seeds, raised nutritional compositions of peanut seeds. This research deduced that *E asburiae* species was isolated from small nodules of peanut plants, which was planted on As pollution soil and irrigation water. *Enterobacter asburiae*, which was isolated and identified to be the aboriginally ENFB and had the ability to decrease As uptake from soil to peanut stems and seeds, protected plant roots, thereby increasing pod yield and seed quality. *Enterobacter asburiae* is a potentially bacterial species to product biofertilizer for using in peanut cultivation. Vermicompost co-ordinated with *E asburiae* species inoculum and As-uncontaminated irrigation for peanut plants significantly increased both all soil nutrient components, yield, grain nutrient composition, reduced As uptake and accumulated in peanut trunks, leaves, and grains compared to the control. The continuous research of *E asburiae* will contribute to positively bacterial source for producing bio-fertilizers in peanut cultivation.

***Ethical approval*:** This paper did not contain any researches regarding human or animal**.**

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***SDG's Addressed*:** Zero Hunger.

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