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Original Article

Effects of *Bacillus songklensis* and *Bacillus siamensis* WD-32 combined with vermicompost on soil fertility, growth, yield and arsenic accumulation in peanut

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Abstract

Arsenic (As) contamination poses a serious threat to crop productivity and food safety in the Mekong Delta. This study evaluated the synergistic effects of *Bacillus songklensis* (BS), *Bacillus siamensis* WD-32 (WD-32), and vermicompost (VA) on soil fertility, peanut growth, yield, and arsenic accumulation. A field experiment was conducted in An Phu commune, An Giang province, Vietnam, using two factors with (factor 1) three vermicompost (VA) rates (0, 5, and 10 t ha^{-1}) and (factor 2) three microbial inoculation treatments: BS, WD-32, and their combination. The studied results revealed that the combined application of 10 t ha^{-1} VA with both bacterial strains significantly improved soil chemical properties, including pH (6.10), CEC (7.98 $cmol^{+}$ kg^{-1}), SOM (2.21%), TN (0.20%), AP (352 mg kg^{-1}), and EK (160 mg kg^{-1}). This integrated treatment also promoted plant development, pod formation, and achieved the highest fresh pod yield (7.34 t ha^{-1}), representing an 11% increase compared to the control (Without BS, WD-32 and VA). Notably, this treatment reduced As accumulation in stems and seeds by 25% and 30%, respectively, relative to the control. The synergistic effects were clearly demonstrated through key interaction parameters, confirming that the co-application of BS, WD-32 and VA is more effective than single applications. These findings highlight the potential of combining VA application with seed inoculation using BS and WD-32 as a sustainable strategy to enhance peanut yield and reduce arsenic uptake under field conditions, particularly in arsenic-contaminated areas.

Keywords: Animal manures, Arsenic, Beneficial bacteria, Groundnut, Organic amendment

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Introduction

Peanut (*Arachis hypogaea* L.) is a widely cultivated legume known for its nutritional and economic value, especially in tropical and subtropical regions. It serves as a key source of plant-based protein, edible oil, and micronutrients, supporting food and nutrition security (Liu et al., 2022; Kumar et al., 2021). In Vietnam, peanuts remain a major cash crop for smallholder farmers, particularly in rural areas of the Mekong Delta, where they contribute to local livelihoods and economic resilience (Hang et al., 2024). However, peanut cultivation in the Mekong Delta faces increasing environmental stress, particularly from soil contamination. Recent studies report that over 41% of agricultural soils in the region exceed the WHO (1998) threshold for arsenic (12 mg kg^{-1}), largely due to long-term use of arsenic-contaminated irrigation water from shallow aquifers (Chuong et al., 2025; Sabbagh, 2023). This contamination poses a direct risk to crop productivity and food safety. In addition, excessive use of chemical fertilizers, particularly NPK, contributes to soil acidification and nutrient imbalance, further exacerbating As bioavailability (Alengebawy et al., 2021). Climate change induced events such as erratic rainfall and heatwaves also disrupt peanut growth and reduce yield stability (Sabbagh, 2023), especially during critical reproductive stages (Pokhrel et al., 2025). To compensate, many farmers adopt high-intensity cultivation practices such as monoculture and multiple cropping cycles (Al-Shammary et al., 2024). Although these methods may offer short-term yield benefits, they have caused long-term soil degradation, notably through reduced organic matter and disrupted microbial communities essential for nutrient cycling and soil structure (Hatano et al., 2024). Excessive use of NPK fertilizers further contributes to soil acidification and nutrient imbalance, and in some cases, increases the risk of contamination with toxic elements like As, particularly in regions dependent on groundwater or surface water irrigation (Alengebawy et al., 2021).

Arsenic contamination has emerged as a serious issue in peanut-growing areas, particularly where irrigation water is tainted with As, leading to its accumulation in edible plant parts (Muehe et al., 2019; Van, 2024). This poses significant public health and trade risks. Moreover, conventional fertilizer-dependent systems often overlook the importance of soil biological health, which plays a key role in long-term productivity and resilience (Chen et al., 2024). In response, researchers and farmers alike are turning toward integrated biological strategies to manage soil fertility and promote crop sustainability. Among these, the use of endophytic N-fixing bacteria and VA has gained attention for their ability to enhance soil function, improve plant tolerance to abiotic stresses, and reduce dependence on chemical inputs (Van Chuong and Le Kim Tri, 2024; Matisic et al., 2024; Figiel et al., 2025). Endophytic bacteria such as *B. songklensis* and *B. siamensis* have demonstrated strong potential in peanut production, due to their multifunctionality in N fixation, P solubilization, indole-3-acetic acid (IAA) production, and suppression of soil-borne pathogens (Wagi and Ahmed, 2019; Chang et al., 2025; Van Chuong and Le Kim Tri, 2024). These strains are particularly effective in improving root development, nodulation, and N uptake, even in soils with low fertility or metal contamination, and may reduce As accumulation in plant tissues (Fonseca de Souza et al., 2025; Awan et al., 2020; Nguyen, 2025).

Complementing microbial inoculants, vermicompost-produced through the decomposition of organic waste also plays a pivotal role in restoring degraded soils. Rich in nutrients, humic substances, enzymes, and beneficial microbes, VA enhances soil structure, moisture retention, and microbial activity in the rhizosphere (Mulatu and Bayata, 2025; Nguyen, 2025). Importantly, VA can reduce the bioavailability and movement of heavy metals and toxic elements like As in the soil, thereby lowering food safety risks (Alengebawy et al., 2021). When used together, *B. songklensis* or *B. siamensis* and VA form a synergistic, eco-friendly system that supports sustainable peanut cultivation by improving soil fertility, plant growth, and crop yield while mitigating the harmful effects of chemical overuse and environmental contamination (Rani et al., 2019; Rajput et al., 2024). Based on these findings, this study aims to evaluate the individual and combined effects of *B. songklensis*, *B. siamensis*, and VA on soil quality, peanut yield, and arsenic accumulation in both soil and plant tissues. Conducted in As-contaminated agricultural regions, this research seeks to develop practical, bio-based fertility management strategies that can be adopted by local farmers to enhance food safety, soil health, and sustainable peanut production.

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Material and Methods

Isolation of BS and WD-32

BS and WD-32 were isolated from peanut (*Arachis hypogaea* L.) root nodules collected at 65 days after sowing (Hossain et al., 2023). Fifty nodules were harvested from five representative plants (ten nodules per plant) and initially rinsed with sterile water to eliminate surface contaminants. To ensure effective surface sterilization, nodules were immersed in 70% ethanol, thoroughly washed with sterile distilled water, and finally treated with 5% sodium chloride solution for one minute. Following sterilization, the nodules were macerated in 1 mL of phosphate buffer. The resulting suspension underwent serial dilution and was plated on yeast mannitol agar (YMA). Plates were incubated at room temperature for approximately four days. Well-isolated colonies were selected and subcultured for purification, and distinct colonies were subsequently preserved for molecular characterization (Figure 1).

Molecular identification of BS and WD-32

Genomic DNA was extracted from purified colonies of BS and WD-32 to perform 16S rRNA gene analysis, aiming to compare the obtained sequences with those of reference bacterial strains (Figure 1). DNA extraction was conducted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific™), following the manufacturer's instructions. Amplification of the 16S rRNA gene was performed via PCR using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). Each 25 µL PCR reaction mixture contained 12.5 µL of PCR Master Mix, 1 µL of each primer (10 µM), 1 µL of genomic DNA, and 9.5 µL of nuclease-free water, as outlined by Cardoso et al. (2018). The PCR cycling program included an initial denaturation at 95 °C for 5 minutes; 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and an extension at 72 °C for 90 seconds; followed by a final extension at 72 °C for 10 minutes. PCR amplicons were resolved on 1.5% agarose gels stained with ethidium bromide. Bands of the expected size were purified and submitted for Sanger sequencing. The resulting sequences were analyzed using the BLAST algorithm in the NCBI GenBank database to determine taxonomic identity. Sequence analysis revealed that the isolates exhibited up to 99% identity with BS species and WD-32 strain, as further supported by phylogenetic tree construction (Figure 2). The BS and WD-32 population was subsequently propagated to a density of 10^8 CFU mL^{-1} five days before sowing. Peanut seeds were then inoculated by soaking in the bacterial suspension prepared in liquid YMA medium for 8–12 hours prior to planting (Etesami, 2022).

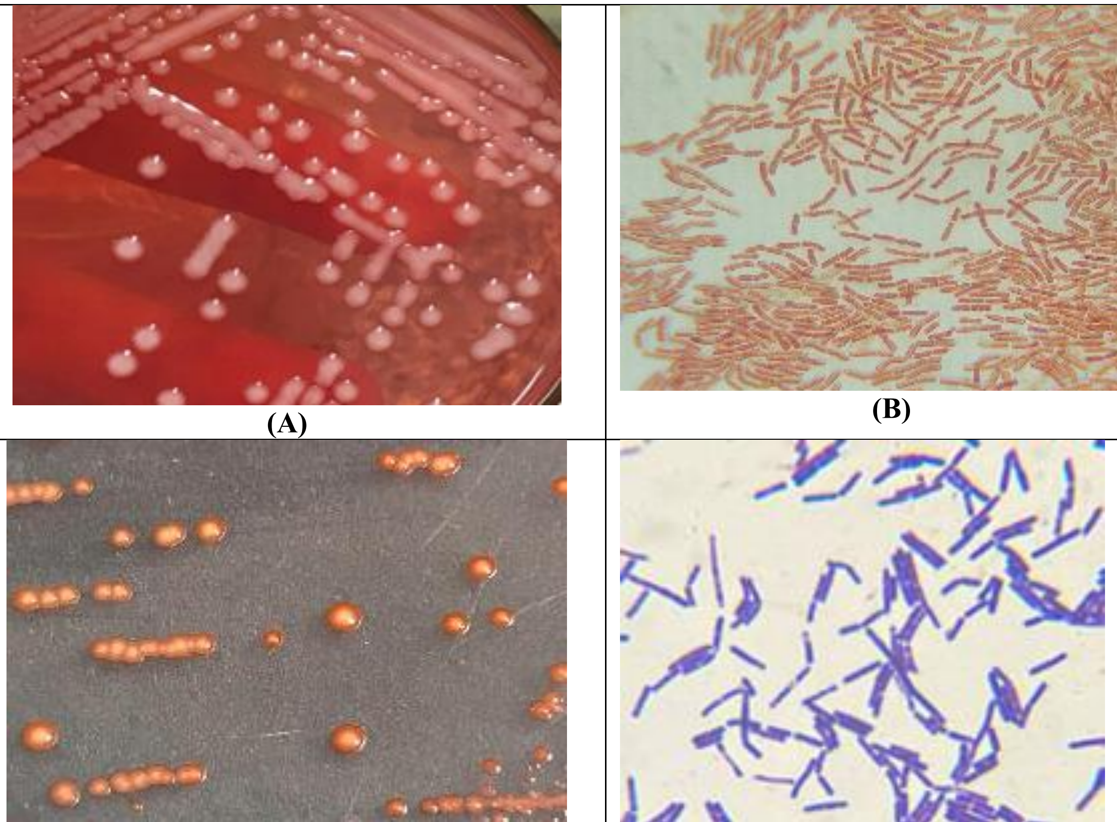

Figure-1. (A) and (C) represent pure colonies, while (B) and (D) show 100X microscopic images of BS and WD-32, respectively.

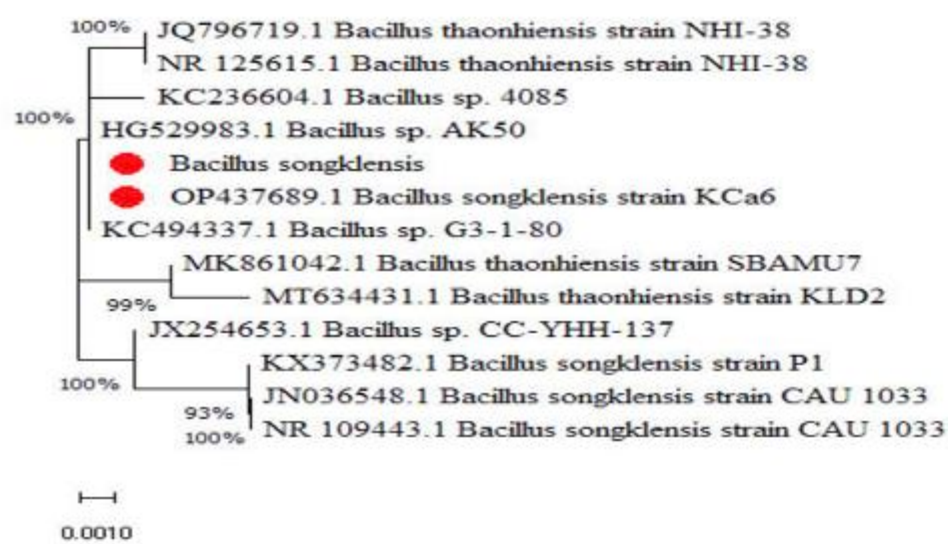

Figure-2. Phylogenetic tree of BS based on 16S rRNA gene sequences from selected reference strains, constructed using MEGA 11. The scale bar represents 0.0010 and 0.00050, respectively, substitutions per nucleotide position. Bootstrap values based on 1,000 replicates are shown at the nodes

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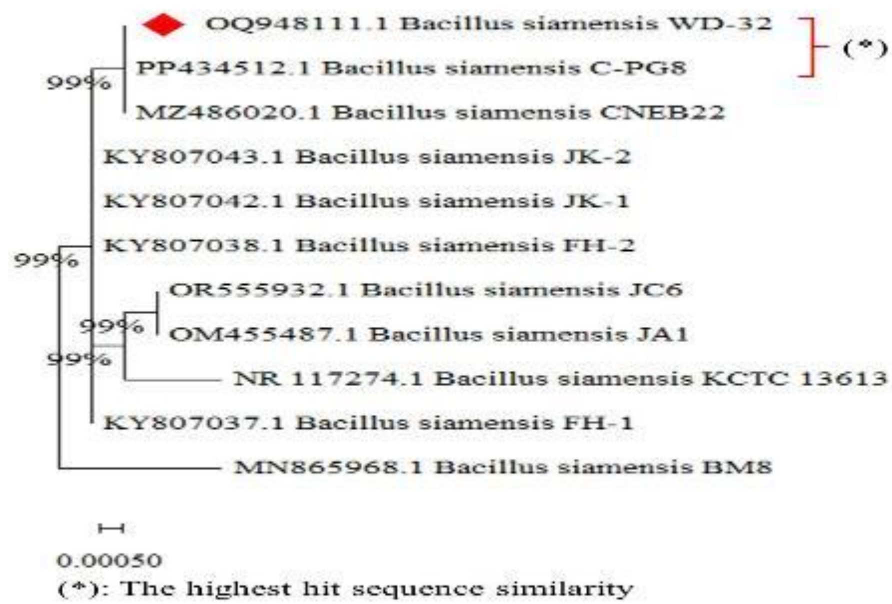

Figure-3. Phylogenetic tree of WD-32 based on 16S rRNA gene sequences from selected reference strains, constructed using MEGA 11. The scale bar represents 0.0010 and 0.00050, respectively, substitutions per nucleotide position. Bootstrap values based on 1,000 replicates are shown at the nodes

Experimental design and location

An Phu commune, located on an island bordering Cambodia, spans approximately 225.3 km² with a population of around 200,000. The region receives 500–650 mm of annual rainfall, mostly from June to October, and experiences temperatures ranging from 27–38 °C in summer and 20–32 °C in winter. Its alluvial sandy loam soil, formed by seasonal Mekong River flooding, is low in organic matter and prone to nutrient depletion due to intensive rice farming. Local agriculture is dominated by smallholders practicing rice-based systems, increasingly supplemented by integrated nutrient management and microbial inoculants to enhance soil fertility and productivity.

The field experiment was conducted within a dike-protected area in An Phu commune, An Giang province, Vietnam, using a randomized complete block design (RCBD) with two experimental factors: NFB inoculation and VA rates. Nine treatment combinations were coded from 1 to 9 and arranged in a factorial layout, with each plot replicated four times (Table 1).

Table-1. VA rates and NFB inoculation by plots.

Plots	NFB inoculation (10^8CFU mL^{-1})	VA rates (t ha^{-1})	Chemical fertilizers (kg ha^{-1})
1	BS	0.0	40N:60P: 60K
2	WD-32	0.0	
3	BS + WD-32	0.0	
4	BS	5.0	
5	WD-32	5.0	
6	BS + WD-32	5.0	
7	BS	10.0	
8	WD-32	10.0	
9	BS + WD-32	10.0	

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The field experiment was conducted on a total area of 720 m² (20 m in length × 1 m in width × 9 treatments × 4 replicates). Each replicate measured 20 m², with four replicates per treatment. Adjacent plots were spaced 0.5 m apart. Peanuts were sown in single rows with a row spacing 25 cm, and two seeds were placed per hill. At the three-leaf stage, the healthier seedling was retained. For each treatment, 10 healthy plants were randomly selected per replicate and monitored throughout the experimental period.

Chemical fertilizers were applied individually, consisting of urea, superphosphate, and potassium chloride. These fertilizers were standardized and applied at rates equivalent to 40 kg N, 60 kg P, and 60 kg K per hectare across all nine experimental treatments (Table 1). The VA used in this study was sourced from the from Nongnghieppho Company (Vietnam), and its chemical composition is presented in Table 2.

At the beginning of the experiment, soil samples were collected from a depth of 0–20 cm to determine soil properties such as pH, cation exchange capacity (CEC), soil organic matter (SOM), total nitrogen (TN), available phosphorus (AP), exchangeable potassium (EK), and As. Plant height and the number of branches were measured at 20, 45, and 65 DAS. Biomass, yield components, and peanut yield were recorded at harvest. Seed nutritional components, including humility, lipid, and protein content, were analyzed according to Aulia et al. (2023). Soil pH was measured using distilled water at a 1:2.5 soil-to-water ratio. Soil organic matter content was determined using the Walkley-Black method (Walkley and Black, 1934). Total nitrogen was analyzed by the Kjeldahl method. Available phosphorus was determined using the Olsen-P method (Olsen et al., 1982). Exchangeable potassium was analyzed by flame photometry, and total arsenic content in soil, shoots, and seeds was determined using the hydride generation technique with an atomic absorption spectrophotometer (Shraim et al., 1999).

Table 2 presents the results of soil analysis conducted prior to the experiment, including pH, CEC, SOM, total N, available P, exchangeable K, and As concentration. The soil texture was classified as sandy clay loam based on USDA classification guidelines (USDA, 2019), which are considered suitable for peanut cultivation (Rajendran et al., 2012).

Data collection

Growth parameters (plant height, chlorophyll, branch and leaf number) were recorded at 20, 45, and 65 days after planting (DAP). Ten plants per treatment were assessed for nodules, biomass, pod characteristics, and seed yield. The seed samples were analyzed for moisture, lipid, and protein content. At harvest, trunk, leaf, and seed samples were collected and dried to determine As accumulation.

Table-2. Physicochemical properties of farmland prior to the experiment.

Property	Result	Property	Result
Soil depth (0-20 cm) (n=36)			
Sand (%)	58.5	Total N (%)	0.125
Silt (%)	35.1	Available P (mg kg^{-1})	254
Clay (%)	6.40	Exchangeable K (mg kg^{-1})	117
pH soil	4.97	SOM (%)	1.24
As in soil (mg kg^{-1})	84.6	CEC (cmol kg^{-1})	2.627
VA composition (n = 2)			
Property	**Result**	**Property**	**Result**
Total N (%)	0.98	Ca (%)	1.18
Total P (%)	1.05	Mg (%)	0.27
Total K (%)	0.29	As (μg kg^{-1})	Undetected

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Analysis methods
Soil, trunk, leaf, and seed samples were separately collected and dried for analysis of As, EK, and grain nutrients. As concentrations were determined following Korkmaz et al. (2017). Soil pH (1:2.5), total nitrogen (Kjeldahl method), and available phosphorus (alkaline hydrolysis) were analyzed. All parameters followed USDA standard procedures (USDA, 2019).

Statistical analysis
Statgraphite XV software was used for variance analysis (ANOVA). The mean comparison between variables was performed by Duncan's test with a significant difference at *P value* ≤0.05. Each treatment data was calculated according to 3 factors to find the interaction between the experiments and the factors.

Results

Soil chemical properties at harvest
Table 3 indicates that the application of 10 VA t per ha significantly enhanced all measured soil chemical parameters, including pH, CEC, SOM, TN, AP, and EK ($p \leq 0.01$). Compared to the control, these values were markedly higher, confirming the positive impact of organic matter on soil fertility. Among plots of microbial inoculants, the combined application of BS and WD-32 led to the greatest improvements in SOM (2.21%), TN (0.20%), and CEC (7.98 $cmol^+ kg^{-1}$), significantly outperforming individual inoculations. While BS alone achieved higher AP and EK values than WD-32, the combined treatment produced superior overall soil enrichment. Factorial analysis revealed that vermicompost (Factor A) significantly affected all six parameters, while microbial inoculation (Factor B) had a significant effect on all except soil pH. Importantly, the interaction between vermicompost rates and microbial inoculants (A×B) was significant for SOM, TN, AP, and EK, indicating a synergistic effect in enhancing nutrient availability. Although WD-32 alone had a weaker effect on TN, combining it with BS and vermicompost yielded stronger results. Soil pH was mainly improved by vermicompost application, rising from 5.14 to 6.10. These findings support the integrated use of organic and microbial amendments to restore soil quality in degraded systems.

Table-3. Effects of BS and WD-32 with VC on soil chemical traits.

Factors	**pH**	**CEC ($cmol^+ kg^{-1}$)**	**SOM (%)**	**TN (%)**	**AP ($mg kg^{-1}$)**	**EK ($mg kg^{-1}$)**
VA rates (A)						
0.0 t ha^{-1}	5.14±0.03c	6.09±0.04c	1.18±0.02c	0.09 ± 0.01b	208 ±0.08 b	118 ± 0.04 b
5.0 t ha^{-1}	5.80±0.08b	6.61±0.01b	2.00±0.08b	0.14 ± 0.03ab	329 ± 0.08a	145±0.08a
10.0 t ha^{-1}	6.10±0.08a	7.98±0.01a	2.21±0.08a	0.18 ± 0.07a	352 ± 0.16a	160±0.12a
NFB inoculation (10^8 CFU mL^{-1}) (B)						
BS	6.00±0.41	7.56±0.04a	1.59±0.01c	0.18 ± 0.01a	296 ± 0.16a	141 ± 0.08b
WD-32	6.04±0.03	5.05±0.04b	1.80±0.08b	0.10 ± 0.40b	259 ± 0.08b	121 ± 0.08c
BS + WD-32	6.00±0.08	7.98±0.01a	2.21±0.08a	0.20 ± 0.10a	273 ± 0.08ab	137 ± 0.08a
F (A)	**	**	**	**	**	**
F (B)	ns	**	**	**	**	**
F (AxB)	ns	ns	**	**	**	**

F(A) and F(B) represent the main effects of three VA rates and inoculation of BS and WD-32, respectively; F(A×B) denotes the interaction between these two factors. ns: differences were not significant unless indicated: ** denote significance at LSD ≤ 0.01

Peanut agronomy components

As shown in Table 4, the main effects of VA [F(A)] and microbial inoculation [F(B)] showed varying influence on plant height and available branch number at different growth stages. VA significantly increased both strains at 45 and 65 DAP, where 10.0 t ha^{-1} resulted in the tallest plants (22.8 and 53.2 cm, respectively) and the highest number of branches (5.75 and 6.25 branches per plant). However, plant height was not significantly affected by VA rates at 20 DAP. Regarding microbial treatments, the combination of BS and WD-32 showed the best performance, significantly enhancing plant height at all stages and available branches at 45 and 65 DAP. For instance, the dual inoculation produced the tallest plants at 20 DAP (13.4 cm) and the most branches at 45 DAP (6.03 branches). Notably, the interaction between vermicompost and microbial inoculation [F(A×B)] had no significant effect on plant height but significantly influenced branch number at all three stages ($P \leq 0.01$). This interaction suggests a synergistic effect in enhancing shoot development. Overall, the combined application of vermicompost and dual microbial inoculation was most effective in promoting peanut growth.

Table-4. Effects of BS and WD-32 with VA rates on plant height and available branches.

Factors	**Average height per plant (cm)**			**Available branch number (branches per plant)**		
	Days after planting (DAP)					
	20	45	65	20	45	65
VA rates (A)						
0.0 t ha^{-1}	11.1±0.01b	20.4±0.33b	49.3±0.25b	3.75±0.04b	4.50±0.41b	5.12±0.02b
5.0 t ha^{-1}	12.4±0.33a	22.1±0.08a	51.9±0.08a	3.80±0.08a	5.13±0.02	5.88±0.07a
10.0 t ha^{-1}	12.7±0.16a	22.8±0.16a	53.2±0.16a	3.86±0.16a	5.75±0.04a	6.25±0.04a
NFB inoculation (10^8 CFU mL^{-1}) (B)						
BS	11.0±0.81b	23.1±0.08a	53.7±0.16a	3.67±0.02a	5.50±0.41b	5.50±0.41ab
WD-32	10.6±0.16c	19.6±0.16b	48.4±0.33b	3.50±0.41b	4.75±0.04c	5.10±0.41b
BS+ s WD-32	13.4±0.33a	24.1±0.08a	51.9±0.08a	3.80±0.08a	6.03±0.02a	5.88±0.07a
F (A)	ns	ns	ns	**	**	**
F (B)	ns	**	**	ns	ns	ns
F (AxB)	ns	ns	ns	**	**	**

F(A) and F(B) represent the main effects of three VA rates and inoculation of BS and WD-32, respectively; F(A×B) denotes the interaction between these two factors. ns: differences were not significant unless indicated: ** denote significance at LSD ≤ 0.01

Peanut yield traits

Table-5. Effects of BS and WD-32 with VA rates on the peanut yield traits at harvest.

Factors	**Biomass (g $plant^{-1}$)**	**No. of full pods (pod $plant^{-1}$)**	**Full pod Wt. (g $plant^{-1}$)**	**Nodule No. (nodules $plant^{-1}$)**	**Nodule Wt. (g plant-^{1})**	**Wt. of 1,000 seeds (g)**
VA rates (A)						
0.0 t ha^{-1}	244±2.00c	63.8±0.65b	158±1.60b	275±4.08a	0.96±0.02b	860±8.16c
5.0 t ha^{-1}	260±4.08b	76.5±0.408a	171±0.82a	307±1.63a	1.70±0.08a	925±2.88b
10.0 t ha^{-1}	282±1.63a	78.3±0.25a	176±0.96a	175±4.08b	1.77±0.02a	1,050±4.08a
NFB inoculation (10^8 CFU mL^{-1}) (B)						
BS	260±4.08 b	76.5±0.408b	171±0.82a	307**±1.63a	1.70±0.08a	925**±2.88b
WD-32	228±1.63b	64.9±0.08c	135±0.82b	155±4.08c	1.22±0.02b	840±8.16c
BS+ WD-32	296±1.63a	80.8±0.65a	175±0.82a	257±1.63b	1.79±0.01a	1,050±4.08a
F (A)	**	**	**	**	**	**
F (B)	**	**	**	ns	ns	**
F (AxB)	**	**	**	**	**	**

F(A) and F(B) represent the main effects of three VA rates and inoculation of BS and WD-32, respectively; F(A×B) denotes the interaction between these two factors. ns: differences were not significant unless indicated: ** denote significance at LSD ≤ 0.01

Based on Table 5, both VA rates [F(A)] and NFB inoculation [F(B)] had a significant ($P \leq 0.01$) positive effect on peanut biomass and yield traits, with strong interaction effects [F(A×B)] observed across all parameters. The highest biomass (296 g plant^{-1}) was recorded in the combined treatment of BS and WD-32, along with 10.0 t VA ha^{-1}. This combination also resulted in the greatest number (80.8 pods plant^{-1}) and weight (175 g plant^{-1}) of full pods. In contrast, the lowest biomass (244 g plant^{-1}) was observed in the control treatment (0 t VA ha with no NFB inoculation).

The significant F(A×B) interactions indicate a strong synergistic effect between organic amendment and microbial inoculation in enhancing nutrient availability and pod formation. For example, pod and nodule production was significantly greater in treatments combining 10 t VA per ha and dual inoculants, likely due to improved root-soil-microbe dynamics. The weight of 1,000 seeds also increased notably, reaching 1,050 gr, further confirming the beneficial interaction of these two factors. These findings highlight the potential of integrated organic and microbial management to optimize peanut yield and physiological development.

Nutrition composition and As content of peanut seeds

According to Table 6, VA rates [F(A)] and NFM inoculation [F(B)] had significant individual effects on several peanut yield and quality traits, with limited interactive effects [F(A×B)]. Seed humidity was significantly improved by BS inoculation (32.8%), while VA alone showed no significant influence on this trait. Conversely, lipid content was significantly enhanced by 10 t VA per ha (25.9%) but was not affected by NFB inoculation, indicating that organic amendments played a dominant role in oil accumulation. Fresh pod yield increased significantly with both vermicompost and microbial treatments. The highest yield (7.34 t ha^{-1}) was recorded at 10 t VA ha, and BS+WD-32 inoculation also showed notable improvement. The interaction effect F(A×B) was significant for both protein content and fresh yield, indicating that specific combinations of VA and NFB inoculation can synergistically enhance yield quality traits. These results suggest that while individual applications of vermicompost or microbial inoculants enhance specific traits, their interaction has greater influence on traits like protein accumulation and final pod yield. This highlights the potential of integrated biological and organic management for improving peanut productivity and seed nutritional quality under sustainable cultivation practices.

Table-6. Effects of BS and WD-32 with VA rates on fresh peanut yield and quality.

Factors	Humidity	lipid	Protein	Fresh yield	As contents (µg kg^{-1})	
	(%)	(%)	(%)	(t ha^{-1})	Stems	Seeds
VA rates (A)						
0.0 t ha^{-1}	30.5±0.8	25.3±0.25a	17.3±0.245a	6.53±0.03b	1,264±3.27a	126±0.82a
5.0 t ha^{-1}	30.1±0.82	23.5±0.41b	15.2±0.16b	6.03±0.02b	1,060±4.98b	118±1.63ab
10.0 t ha^{-1}	29.9±0.8	25.9±0.08a	17.4±0.25a	7.34±0.03a	958±1.63c	101±0.82b
NFB inoculation (10^8 CFU mL^{-1}) (B)						
BS	32.8±0.16a	25.8±0.16	17.3±0.24	6.87±0.02bc	1,077±1.63a	99.5±0.41b
WD-32	29.2±0.16b	25.4±0.33	17.4±0.33	6.40±0.08b	944±3.27b	88.2±1.63a
BS+ WD-32	30.0±0.82b	25.5±0.41	17.2±0.16	7.03±0.02a	940±4.98b	88.1±1.63a
F (A)	Ns	**	**	**	ns	**
F (B)	*	ns	ns	**	ns	**
F (AxB)	Ns	ns	**	**	ns	**

F(A) and F(B) represent the main effects of three VA rates and inoculation of BS and WD-32, respectively; F(A×B) denotes the interaction between these two factors. Ns: differences were not significant unless indicated: * and ** denote significance at LSD ≤ 0.05 and 0.01, respectively.

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According to Table 6, both VA rates [F(A)] and NFB inoculation [F(B)] significantly reduced As accumulation in peanut stems and seeds. The application of 10 t VA ha^{-1} resulted in the lowest As concentrations in stems (958 µg kg^{-1}) and seeds (101 µg kg^{-1}), compared to 1,264 µg kg^{-1}and 126 µg kg^{-1}in the control (0 t VA ha^{-1}), respectively. This highlights the potential of VA in immobilizing As and improving soil buffering capacity against heavy metal uptake. Among microbial plots, the dual inoculation of BS and WD-32 showed the greatest reduction in seed As content (88.1 µg kg^{-1}), compared to 126 µg kg^{-1} in the control, indicating the role of NFB in mitigating As toxicity. Although the interaction [F(A×B)] was not statistically significant, both factors independently contributed to lowering As levels in plant tissues.

Discussion

The results of this study support previous findings that organic amendments improve soil chemical properties. Application of 10 t VA ha^{-1} significantly increased soil pH, CEC, SOM, TN, AP, and EK compared to the control (Table 3), consistent with studies reporting that organic manures raise soil pH and nutrient content, especially in acidified or contaminated soils (Chuong, 2021). The improved pH, from 5.14 to 6.10, demonstrates vermicompost's buffering role against H^+ release from As-contaminated irrigation water (Kumar et al., 2013; Van Nguyen, 2024). Combined inoculation of BS species and WD-32 trains also significantly improved SOM, TN, and nutrient availability. Notably, their synergy with vermicompost resulted in higher AP and EK levels than when applied separately. These effects align with reports that NFB inoculation and organic manures enhance phosphorus uptake, reduce soil acidity, and promote nutrient cycling (Banik et al., 2006, Nguyen Chuong, 2024). This statement emphasizes the chemical buffering effect of organic amendments specifically VA raising soil pH by neutralizing excess hydrogen ions (H^+) released from acidified conditions or As-contaminated irrigation water. The increase in pH from 5.14 to 6.10 clearly reflects this direct chemical improvement. In contrast, the subsequent sentence referring to Banik et al. (2006) and Nguyen Chuong (2024) highlights a broader biological impact, including enhanced phosphorus uptake and nutrient cycling. These effects are mediated by improved microbial activity and organic acid interactions that help mobilize nutrients and reduce long-term acidity. Thus, while both statements support the role of organic inputs, the first focuses on direct chemical buffering of soil pH, whereas the second describes integrated biological and nutrient cycling processes that contribute to sustainable soil fertility.

Significant interactions (A×B) for SOM, TN, AP, and EK indicate the importance of integrating microbial inoculants with vermicompost to restore soil health. This confirms earlier conclusions that precise organic and microbial input combinations are essential for sustainable crop production in As-contaminated areas (He et al., 2022; Gao et al., 2023).

The results in Table 3 demonstrate that applying 10 t VA ha^{-1} combined with dual inoculation of BS and WD-32 significantly enhanced peanut plant growth, particularly in branch development (Table 4). These improvements reflect the synergistic benefits of organic amendments and microbial inoculants in promoting nutrient availability and hormonal stimulation (Mahmud et al., 2021). Although plant height was not influenced by interaction effects, the increased branch number suggests improved shoot architecture, which is vital for pod formation and yield (Zhao et al., 2021). Such integrated practices offer sustainable alternatives for boosting crop productivity under low-input systems.

Based on the results from Table 5, peanut yield traits including biomass, pod and nodule characteristics, and 1,000-seed weight were significantly enhanced ($P \leq 0.01$) by both VA and BS and WD-32 inoculation. The plot with 10 t ha^{-1} vermicompost and dual inoculation (BS + WD-32) produced the highest biomass (296 g plant^{-1}) and seed weight (1,050 g), confirming a strong synergistic interaction [F(A×B)]. These improvements reflect the positive relationship between organic matter input, microbial activity, and soil nutrient dynamics, particularly under As-polluted farmlands (Mathenge et al., 2019). Similar findings by Jan et al. (2021) and Van Chuong and Le Kim Tri (2024) support the idea that organic manure combined with beneficial microbes improves peanut yield components and soil fertility. Notably, plots without VA or with single inoculation (WD-32) showed significantly lower performance, emphasizing the critical role of integrated nutrient and microbial management (Mondal et al., 2020). Additionally, microbial diversity and rhizosphere interactions are essential drivers of nutrient availability and plant productivity, yet remain underexplored in peanut systems (Paudel et al., 2023).

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The findings from Table 5 align with recent studies highlighting the benefits of combining organic inputs and microbial inoculants. Amendment at 10 tVA ha^{-1} significantly increased lipid content (25.9%) and fresh yield (7.34 t ha^{-1}), supporting its role in enhancing soil nutrient availability and plant productivity (Hamad et al., 2022). Meanwhile, BS inoculation markedly improved seed humidity (32.8%), likely due to its ability to enhance water retention and microbial activity in the rhizosphere (Hadian et al., 2025). Although their combined effect [F(A×B)] was limited, significant interaction on protein content and yield indicates potential synergy when both are applied together. These results underscore the importance of integrating biological and organic strategies to sustainably improve peanut yield and quality.

The reduction in As accumulation in peanut stems and seeds with the VA and endophytic bacterial inoculants, as shown in Table 5, is strongly supported by recent findings. VA improves soil organic matter and pH buffering capacity, enhancing As immobilization and reducing its bioavailability to plants (Oyege and Balaji Bhaskar, 2023; Van Chuong and Le Kim Tri, 2024). Similarly, NFB strains such as BS and WD-32 contribute to As detoxification by producing siderophores and organic acids, which bind As in the rhizosphere and restrict its translocation to shoots and seeds (Hnini et al., 2024). In this study, applying 10 tVA ha^{-1} and inoculating with BS + WD-32 significantly reduced seed As from 126 µg kg^{-1} (control) to µg kg^{-1}. These integrated practices present a sustainable approach to reduce heavy metal accumulation in crops cultivated on As-contaminated soils (Bhat et al., 2022).

The observed reduction in As accumulation in peanut tissues can be attributed to several possible mechanisms employed by BA and WD-32. One likely pathway is the production of siderophores, which are high-affinity iron-chelating compounds that can also bind to arsenic in the rhizosphere, thereby limiting its mobility and bioavailability (Das and Barooah, 2018). Additionally, both strains may secrete organic acids such as oxalic or citric acid, which have been reported to alter the rhizosphere pH and enhance the adsorption of As onto soil particles, reducing uptake by plant roots (Amadou et al., 2021; Das and Barooah, 2018). Another contributing mechanism is the modulation of root architecture or root membrane permeability induced by microbial colonization, which may influence the expression of aquaporins or metal transporter genes, thus restricting As translocation from roots to shoots and seeds (Oyedoh et al., 2025). These effects are synergistically enhanced when vermicompost is present, as it improves soil buffering capacity and organic matter content, further promoting As immobilization. Overall, the interaction between endophytic bacteria and organic inputs likely triggers a combination of biochemical and physiological responses in the plant–microbe–soil system, leading to improved arsenic exclusion and safer crop production under As-contaminated field conditions (Wang et al., 2022; Tamma et al., 2025). As shown in Table 5, Arsenic content in peanut stems and seeds was significantly reduced by microbial inoculation and vermicompost application. The lowest As levels were recorded in the combined BS + WD-32 treatment with 10 t ha^{-1} VA (940 µg kg^{-1} in stems and 88.1 µg kg^{-1} in seeds). While stem concentrations remain high, seed As levels fell below the WHO (1998) guideline of 100 µg kg^{-1}, indicating improved food safety under integrated treatments.

Conclusion

This study confirms the synergistic benefits of combining vermicompost and BS and WD-32 for sustainable peanut production on As-contaminated soils. The integrated plots improved soil fertility indicators such as pH, CEC, SOM, TN, AP, and EK. Importantly, it led to a marked increase in peanut yield compared to control and single amendments. Concurrently, this approach significantly reduced As accumulation in both stems and seeds of peanut plants. The findings highlight that using 10 t VA ha^{-1} in conjunction with both bacterial strains achieves superior effects on both productivity and As mitigation than treatment alone. This eco-friendly solution not only promotes healthier crop growth and soil conditions but also offers a practical approach for farmers facing heavy metal contamination, supporting safer, more resilient, and productive agriculture in affected regions like the Mekong Delta

Future Recommendations

This dataset and the bacterial source can be utilized to produce biofertilizers in the future.

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Contribution of Authors

Van Chuong N: Collected samples and contributed all research funds and wrote down the whole manuscript.
Thanh Liem T, Tran Hai Dang P, Le Kim Tri T & Ngoc Phuong Trang N: Carried out the laboratory work including both isolation and molecular identification.
All authors read and approved the final version of this manuscript.

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