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Synergistic Effects of Bacterium Strain ZM12 and Vermicompost on Yield, Quality, and Nutrient Uptake in Groundnut (*Arachis hypogaea* L.)

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ABSTRACT: Peanut cultivation in nutrient-poor soils often relies heavily on chemical fertilizers, leading to soil degradation, reduced productivity, and lower profitability for farmers. To address this issue, a sustainable alternative combining organic amendments and beneficial microbes was evaluated. The research objective is to assess the influences of vermicompost addition and inoculant with *Bacterium* strain ZM12 on the growth, yield, and quality of peanuts. *Bacterium strain* ZM12 was isolated on YMA medium and identified using 16S rRNA gene sequencing. Field research was conducted using a factorial design with two factors: (i) inoculation vs. non-inoculation with strain ZM12 and (ii) three rates of vermicompost (0, 5, and 10 t ha^{-1}), resulting in six treatments with four replications. The combined application of 10 t ha^{-1} vermicompost and ZM12 inoculation significantly enhanced peanut protein content and growth performance. Peanut yield increased by 38.4% with 10 t ha^{-1} vermicompost compared to the control (0 t ha^{-1}), and ZM12 inoculation alone improved yield by 12.9% compared to non-inoculated plots. These improvements were attributed not only to biological nitrogen fixation but also to the plant growth-promoting effects of strain ZM12. The study demonstrates an effective strategy for improving peanut productivity and soil fertility while reducing dependence on chemical fertilizers. However, future work should include the isolation and testing of additional beneficial microbial strains to further enhance the sustainability of this approach.

KEYWORDS: Composition, endophytic bacteria, peanut, pure colony, rhizosphere

1. Introduction

Groundnut (*Arachis hypogae*a L.) is a globally important legume crop known for its adaptability to a wide range of soil types and climatic conditions. It serves as a vital source of edible oil and protein for both human consumption and animal feed (Janila et al., 2013; Camargo et al., 2017; Hamza et al., 2021). In regions such as An Phu district, groundnut represents a high-value cash crop that supports local livelihoods.

Despite its significance, groundnut cultivation is increasingly challenged by multiple agronomic and environmental constraints. Soil and irrigation water contamination with arsenic (As), excessive application of chemical fertilizers, and the prevalence of root rot diseases caused by pathogenic microorganisms have led to reduced yield, quality, and farmer profitability. Arsenic contamination is a serious concern for crop safety and productivity (Tuan et al., 2021; Bush et al., 2023). Moreover, long-term use of chemical fertilizers has resulted in soil degradation and a decline in beneficial microbial communities.

Biological approaches offer a sustainable alternative to address these challenges. The nitrogen-fixing bacterium, identified as an endophyte in groundnut roots, has shown promising potential in promoting plant growth and controlling Phytophthora-induced root rot. Kim et al. (2020) demonstrated that inoculation with *E. asburiae* significantly reduced disease incidence and enhanced soil nitrogen levels by fixing atmospheric nitrogen. Other studies have confirmed the role of beneficial microbes in suppressing soil-borne pathogens and improving soil health (Bakker et al., 2003). However, the wider application of such inoculants in field conditions is still limited.

In addition to microbial inoculants, organic amendments like vermicompost are known to improve soil fertility and crop productivity. Vermicompost enhances soil aeration, structure, and nutrient availability while supporting microbial activity (Abdel-Mouty et al., 2011; Aktar et al., 2019). Its combination with beneficial microbes may offer synergistic effects in improving plant health and yield.

Given the pressing need for sustainable soil fertility management and biological disease control in groundnut cultivation, this study was conducted to assess the integrated effects of nitrogen-fixing Bacterium strain ZM12 and vermicompost on the growth, yield, nutrient uptake, and quality of groundnut. The study aims to isolate and identify strain ZM12 using biochemical and molecular techniques, evaluate its role in enhancing plant growth and suppressing root rot, and determine the effectiveness of combining microbial inoculation with vermicompost in improving soil fertility and crop performance under field conditions.

2. Materials and methods

2.1 Purification and DNA-based identification

Surface sterilization of groundnut nodules and roots was achieved by immersion in 96% ethanol for 15 seconds, followed by treatment with a 4% sodium hypochlorite (NaClO) solution for 5 minutes. Subsequently, samples were thoroughly rinsed multiple times with sterile distilled water to ensure complete removal of disinfectants (Upasana et al., 2020). The sterilized tissues were transferred aseptically to sterile Petri dishes and macerated using a flame-sterilized glass rod within a drop of 0.9% saline solution (Michel et al., 2000). A 0.1 mL aliquot of the homogenate was plated onto yeast mannitol agar (YMA) medium and incubated at ambient temperature for four days to facilitate colony formation (Vincent, 1970). Molecular identification of the isolate, designated as Bacterium strain ZM12, was conducted via partial sequencing of the 16S rRNA gene. Comparative sequence analysis revealed a 99.93% similarity to reference strains in established databases, confirming its taxonomic classification.

2.2 Biochemical assays and application of Bacterium strain ZM12

Colonies of Bacterium strain ZM12 were isolated and cultured on yeast mannitol agar (YMA) medium under room temperature conditions for four days to obtain pure cultures. Biochemical characterization of the isolates was conducted prior to molecular identification using 16S rRNA gene sequencing, following the method described by Jordan (1984). For inoculum preparation, *Bacterium* strain ZM12 was cultured in 200 mL of diluted YMA medium in a 250 mL Erlenmeyer flask and incubated on a rotary shaker at 100 rpm for 12 hours at 20°C. Bacterial cells were harvested by centrifugation at 13,000 rpm for one minute at 2°C. The resulting pellets were washed twice with sterile distilled water to remove any residual medium. The bacterial suspension was adjusted to a final volume of 0.6 mL per Eppendorf tube and used for seed inoculation. Groundnut seeds were air-dried, then soaked in the prepared Bacterium strain ZM12 suspension (10^8 CFU mL^{-1}) and gently shaken for five minutes to ensure uniform coating. After soaking, the seeds were incubated in the dark at room temperature for 12 hours. The final concentration of Bacterium strain ZM12 on each seed was estimated to be approximately 10^8 colony-forming units (CFU) per seed.

2.3 Experimental arrangement

A field experiment was conducted to evaluate the effects of vermicompost-derived plant treatment (VPT) and *Bacterium* strain ZM12 inoculation on groundnut (*Arachis hypogaea* L.) growth and yield. A field experiment was conducted to evaluate the effects of vermicompost-derived plant treatment (VPT) and *Bacterium* strain ZM12 inoculation on groundnut (Arachis hypogaea L.) growth and yield. The study employed a factorial design with two main factors: VPT application at three levels (0, 5.0, and 10.0 t ha^{-1}) and *Bacterium* strain ZM12 inoculation (with and without), resulting in six treatment combinations. Each treatment was replicated four times, and the trial was arranged in a completely randomized block design. The total experimental area covered 240 m², with plot dimensions of 1 m × 10 m per treatment replication. Groundnut variety L18, obtained from the Vietnam Seed Company, was seeded at a spacing of 30 cm between plants, with two seeds per hole. Fifteen days after sowing, only one healthy seedling was retained per planting hole.

Soil characteristics were assessed using the methods described by Brady (1988), and total arsenic content in the soil, water, stems, and seeds was measured using atomic absorption spectrophotometry. Initial soil and water quality assessments conducted fifteen days before sowing revealed an acidic soil pH of 4.8 and a water pH of 0.30. The arsenic (As) concentration in the soil was 90.0 mg kg^{-1}. Soil nutrient analysis showed a total nitrogen (N) content of 110%, available phosphorus (P) at 2.60%, and exchangeable potassium (K) at 130%. The soil texture was predominantly sandy, comprising 70.0% sand, 25.0% silt, and 5.0% clay. Groundnut yield and related agronomic traits were recorded from maturity through harvest, with fresh seed yield expressed in tons per hectare for each treatment. A study employed a factorial design with two main factors: VPT application at three levels (0, 5.0, and 10.0 t ha^{-1}) and *Bacterium* strain ZM12 inoculation (with and without), resulting in six treatment combinations.

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Fifteen days before sowing, the soil exhibited acidic conditions with a pH of 4.8, while the irrigation water had a near-neutral pH of 6.3. The arsenic (As) concentration in the soil was measured at 0.30 mg kg^{-1}. Regarding soil nutrients, the total nitrogen content was 2.60%, available phosphorus was 110 mg kg^{-1}, and exchangeable potassium reached 130 mg kg^{-1}. The soil texture analysis revealed a sandy loam composition, including 70.12% sand, 5.80% clay, and 24.08% silt.

2.4 Statistical analysis

Statistical analysis was conducted using Statgraphics XV and Microsoft Excel 2010. The data presented represents the outcomes of four independent experimental replicates that yield consistent results. Treatment effects were evaluated through analysis of variance (ANOVA), and mean comparisons were performed using the Least Significant Difference (LSD) test at a significance level of $p \leq 0.05$.

3. 3. Results and discussion

3.1 Biochemical test of Bacterium strain ZM12

Endophytic nitrogen-fixing bacteria were isolated from peanut roots and nodules using a YMA medium, followed by biochemical and molecular identification. Preliminary assessments included Gram staining to determine cell morphology, alongside biochemical tests to characterize the strains according to the research objectives. Among the isolates, Bacterium strain ZM12 was identified, showing a negative reaction on LAM medium after flooding, and demonstrating a strong ability to grow on alkaline media, an important trait used for its classification (Deshwal & Chaubey, 2014; Mir et al., 2020). All isolates formed pale pink to pink colonies on the YMA medium, a typical feature of Bacterium strain ZM12 (Somasegaran & Hoben, 1985). The nitrogen-fixing capacity of the isolates was evaluated using Burk's nitrogen-free medium, on which Bacterium strain ZM12 exhibited robust growth, confirming its potential as a nitrogen-fixing bacterium (Park et al., 2005; Singh et al., 2008; Muñoz et al., 2011). Additionally, this strain tested positive for both oxidase and catalase activities, supporting its identification as an endophytic nitrogen-fixing bacterium. These findings are consistent with the work of Tyagi et al. (2017) and Hossain et al. (2019), who reported that some endophytic nitrogen-fixing bacteria also show positive responses in oxidase and catalase assays (Figure 1). The results highlight the metabolic diversity of rhizospheric bacteria, which can vary significantly even among strains colonizing the same plant root type, depending heavily on environmental factors (Thin et al., 2021).

The identification of Bacterium strain ZM12 was carried out through molecular techniques and biochemical assays, both of which confirmed its classification within the predicted genus. The 16S rRNA gene sequencing showed a 99.74% similarity to reference sequences available in the NCBI database (https://www.ncbi.com/), supporting its identity as a root-nodule nitrogen-fixing—

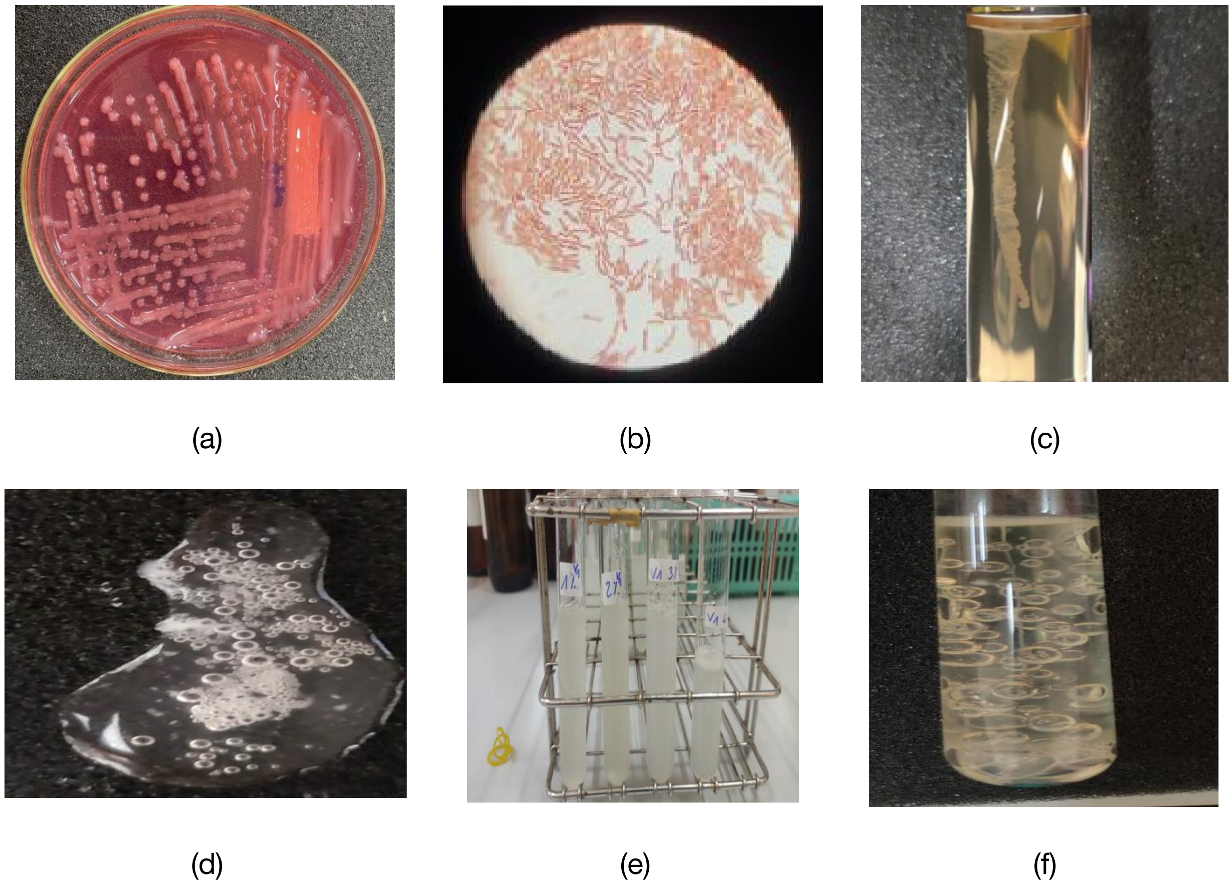

Figure 1. (a) Isolated pure colony grown on YMA medium; (b) Gram-negative, short rod-shaped bacterial cells; (c) Result of the Nessler's reagent test; (d) Formation of bubbles indicating catalase activity in Bacterium strain ZM12 colonies; (e) Growth tolerance at salt concentrations of 1%, 2%, 3%, and 4%; (f) Motility capability.

bacterium (Hameed et al., 2004; Chibeba et al., 2017). Bacterium strain ZM12 demonstrates strong adaptability to temperatures ranging from 25°C to 40°C, aligning with the optimal conditions for endophytic bacteria, although the most favorable temperature for plant development is around 37°C. Rapid fluctuations in soil temperature can negatively impact bacterial development and nodule formation, ultimately reducing the efficiency of nitrogen fixation (Chuong & Tri, 2024; Rao, 2014). As shown in figure 1, Bacterium strain ZM12 also exhibits tolerance to salinity, maintaining growth in media with NaCl concentrations ranging from 0.5% to 1%. Previous studies have shown that some rhizobia strains can endure salinity levels of 3–4% (Baber et al., 2015) or even up to 5% NaCl (w/v) (Küçük et al., 2006; Chuong & Tri, 2024). However, most rhizobia adapt best in environments with NaCl concentrations below 1% (w/v) and in YMA media with pH values between 5 and 8 (Zahran et al., 1999; Yanni et al., 2005; Yarza et al., 2014).

3.2 Bacterium ZM12 boosts groundnut productivity

The biomass of peanut plants showed significant variation across different levels of vermicompost-derived plant treatment (VPT), with a statistical significance of $p < 0.01$. The greatest biomass was observed in plants treated with 10 t ha^{-1} of VPT, whereas the treated control group exhibited the lowest biomass. Likewise, the number and weight of filled pods differed significantly (*p ≤ 0.01*) among the VPT application rates of 0, 5.0, and 10.0 t ha^{-1}. Specifically, the 10.0 t ha^{-1} VPT treatment produced the highest pod count and weight, averaging 82.8 pods and 178 g per plant, respectively. This was followed by the 5.0 t ha^{-1} treatment, which resulted in 77.1 pods and 163 g per plant, while the control group had the lowest values, with 63.1 pods and 108 g per plant. Conversely, the highest number and weight of unfilled pods were found in the control treatment (7.28 pods per plant and 6.03 g per plant), decreasing with increased VPT application. The lowest values were observed in the 10t ha^{-1} treatment, with 4.40 unfilled pods per plant and 2.83 g per plant. These findings align with previous research indicating that organic amendments like vermicompost enhance pod filling and overall biomass through improved soil structure and nutrient availability (Arancon et al., 2004; Mahesh et al., 2022; Choudhary et al., 2022).

Inoculation with Bacterium strain ZM12 significantly enhanced peanut pod production, with the highest recorded values being 80.6 pods per plant and 165 g per plant (LSD ≤ 0.01). In contrast, plants that did not receive inoculation exhibited lower pod numbers and weights, averaging 69.6 pods per plant and 135 g per plant, respectively.

Table 1. Groundnut productivity traits and productivity.

Factor	Biomass (g plant^{-1})	Yield components							Fresh yield (t ha^{-1})
		Pod number plant^{-1}		pod weight (g plant^{-1})		Nodous number	Nodous weight (g plant^{-1})	weight of 100 seeds (g)	
		filled	unfilled	filled	unfilled				
Vermicomposting (A)									
0.0 t ha^{-1}	240^{b}	63.1^{b}	7.28^{a}	108^{b}	6.03^{a}	226^{b}	1.03^{b}	179^{b}	4.53^{b}
5.0 t ha^{-1}	281^{ab}	77.1^{ab}	4.53^{b}	163^{ab}	5.23^{a}	260^{ab}	1.33^{ab}	228^{ab}	6.53^{ab}
10.0 t ha^{-1}	307^{a}	82.8^{a}	4.40^{b}	178^{a}	2.83^{b}	277^{a}	1.43^{a}	243^{a}	7.33^{a}
***Bacterium* strain ZM12 (B)**									
No	257^{b}	69.7^{b}	6.23^{a}	135^{b}	5.63^{a}	214^{b}	1.03^{b}	196^{b}	5.43^{b}
Inoculation	294^{a}	80.6^{a}	5.03^{b}	165^{a}	3.78^{b}	287^{a}	1.63^{a}	233^{a}	6.23^{a}
F (A)	**	**	*	**	*	**	**	**	**
F (B)	**	**	*	**	*	**	**	**	**
F (A x B)	ns	ns	ns	ns	ns	*	*	ns	*
CV (%)	11.5	8.7	20.1	21.0	21.6	14.7	17.6	16.0	17.8

Note: The F(A); F(B) are the interaction of the between-VPT rates and Bacterium strain ZM12; F(AxB) are the interaction of the A and B group: insignificantly different; *, **: significantly different at LSD ≤ 0.05 and 0.01, respectively.

Conversely, the greatest number and weight of unfilled pods were observed in the non-inoculated group, with 6.23 pods per plant and 5.63 g per plant, while the inoculated plants showed reduced unfilled pod counts and weights of 5.03 pods per plant and 3.78 g per plant. Notably, no significant interaction was detected between vermicompost-derived plant treatment (VPT) levels and Bacterium strain ZM12 inoculation in these parameters. At 75 DAS, significant differences (LSD ≤ 0.01) were observed in both the number and weight of nodules across varying VPT application rates. The highest nodule count (277 nodules per plant) and nodule weight (1.43 g per plant) were found in plants fertilized with 10 t ha^{-1} of VPT, outperforming those treated with 5.0 t ha^{-1} and those without any VPT application. Similarly, inoculation with Bacterium strain ZM12 led to a significant increase in both nodule number and weight compared to non-inoculated plants (LSD ≤ 0.01). Furthermore, there was a statistically significant interaction between VPT rates and Bacterium strain ZM12 inoculation regarding nodulation (LSD ≤ 0.01). Specifically, inoculated plants receiving VPT exhibited the highest nodulation, with 287 nodules per plant and a nodule weight of 1.63 g per plant, substantially greater than plants without either treatment. These results suggest that combining VPT applications with Bacterium strain ZM12 inoculation synergistically promotes nodule formation more effectively than either treatment alone (Table 1).

Significant differences were also noted in 100-seed weight and fresh pod yield (LSD ≤ 0.01). Fertilization with 10 t ha^{-1} of VPT resulted in the heaviest 100-seed weight (243 g) and the highest fresh pod yield (7.33 t ha^{-1}), surpassing the 5.0 t ha^{-1} treatment, which produced 228 g per 100 seeds and 6.53 t ha^{-1} fresh yield, and the control group without VPT, which had the lowest values of 179 g and 4.53 t ha^{-1}, respectively. Bacterium strain ZM12 inoculation also had a significant positive effect on these yield components. Inoculated plants showed increased 100-seed weight (233 g) and fresh pod yield (6.23 t ha^{-1}) compared to non-inoculated plants, which had 196 g per 100 seeds and 5.43 t ha^{-1} fresh yield. Additionally, there was a significant interaction between inoculation and non-inoculation treatments on peanut yield parameters, except for the 100-seed weight (Table 1).

These findings align with previous research by Argaw (2017) and Chuong et al. (2024), who demonstrated that the combined use of organic manures and nitrogen-fixing bacterial inoculants substantially increases the number and weight of peanut nodules. Organic manures, derived from plant and animal residues, play a crucial role in enhancing soil properties and fertility. The integration of organic manures with nitrogen-fixing bacteria, commonly applied through various animal manures, has been widely adopted due to its effectiveness in boosting soil nutrient content and improving crop productivity (Chuong et al., 2023; Rahimabadi et al., 2017). These manures contain abundant nutrients and beneficial microorganisms essential for plant growth, which contribute to enhanced soil nutrition and ultimately lead to improved crop yield and quality (Tra Amos et al., 2015; Trang and Chuong, 2025).

3.3 Effect of Bacterium strain ZM12 on peanut nutrition

The application of vermicompost-derived plant treatment significantly influenced the lipid and protein content in peanut seeds, with significant differences at LSD ≤ 0.01 (Table 2). The highest lipid (27.8%) and protein (18.6%) concentrations were recorded under the 10t ha^{-1} VPT treatment, followed by 5.0 t ha^{-1} VPT (25.5% and 17.6%, respectively), while the lowest values were observed in the control without VPT application (23.9% lipid and 15.5% protein). In addition, inoculation with Bacterium strain ZM12 also had a significant effect (LSD ≤ 0.01), increasing lipid and protein content to 25.8% and 16.7%, respectively, compared to 23.7% and 15.8% in non-inoculated treatments. A significant interaction between VPT levels and Bacterium strain parameters. Strain ZM12 inoculation was observed for these parameters. Furthermore, the concentrations of nitrogen, phosphorus, and potassium in peanut seeds also showed significant variation across VPT treatments. The 10t ha^{-1} VPT treatment led to the highest levels of nitrogen (2.95%), phosphorus (0.48%), and potassium (0.54%), while the control exhibited the lowest values (2.46%, 0.27%, and 0.25%, respectively). Similarly, the use of Bacterium strain ZM12 significantly enhanced these nutrient concentrations (LSD ≤ 0.05), with increases to 2.66% nitrogen, 0.36% phosphorus, and 0.38% potassium, in contrast to 2.53%, 0.28%, and 0.27% in the uninoculated group. No significant interaction between VPT levels and Bacterium strain ZM12 inoculation was found for these nutrient contents. These results align with findings from other studies that highlight the synergistic effects of organic amendments and microbial inoculants in improving seed nutritional quality (Zhang et al., 2023; Rehman et al., 2023; Mthiyane et al., 2024).

Table 2. Peanut nutrient compositions in peanut seeds.

Factors	Nutrition of peanut seed (%)				
	Lipid	Protein	N	P	K
Vermicomposting (A)					
0.0 t ha^{-1}	23.9^{b}	15.5^{b}	2.46^{b}	0.27^{b}	0.25^{b}
5.0 t ha^{-1}	25.5^{ab}	17.6^{ab}	2.77^{ab}	0.38^{ab}	0.41^{ab}
10.0 t ha^{-1}	27.8^{a}	18.6^{a}	2.95^{a}	0.46^{a}	0.54^{a}
***Bacterium* strain ZM12 (B)**					
No	23.7^{b}	15.8^{b}	2.53^{b}	0.28^{b}	0.27^{b}
Inoculation	24.8^{a}	16.7^{a}	2.66^{a}	0.39^{a}	0.38^{a}
F (A)	**	**	*	*	*
F (B)	**	**	*	*	*
F (A x B)	*	*	ns	ns	ns
CV (%)	12.7	13.9	17.0	18.1	14.9

Note: The F(A); F(B) are the interaction of the between-VPT rates and *Bacterium* strain ZM12; F(AxB) are the interaction of the A and B group: insignificantly different; *, **: significantly different at *LSD* ≤ 0.05 and 0.01, respectively.

Previous research by Chuong et al. (2025) highlighted that inoculating agricultural soils with nitrogen-fixing bacteria in the rhizosphere can enhance crop yield, improve yield components, and increase both seed quality and nutrient content. Additionally, a significant interactive effect between Rhizobium species and liquid organic fertilizer was observed on peanut yield and quality, with the combination showing a positive influence on yield parameters, total yield, and seed nutritional value (Alfandi et al., 2019).

4. Conclusion

The bacterium strain ZM12, isolated and identified from groundnut nodules as an endogenous nitrogen-fixing species, demonstrated strong potential for promoting peanut growth and development. When combined with vermicompost (VPT) fertilization, especially at the rate of 10 t ha^{-1} ZM12 significantly enhanced yield and nutrient composition. Compared to 5 t ha^{-1} and 0 t ha^{-1} VPT treatments, 10 t ha^{-1} VPT increased peanut yield by 11.0% and 38.4%, respectively. Inoculation with ZM12 alone improved yield by 12.9% compared to non-inoculated plants. The combination of ZM12 and VPT also significantly increased nodule number and fresh weight. These results highlight strain ZM12 as a promising candidate for development as a biofertilizer, offering an eco-friendly alternative to enhance peanut productivity and soil health in sustainable agricultural systems.

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