

EFFICIENT EVALUATION OF *E. ASBURIAE*, *K. QUASIPNEUMONIAE* AND *E. CLOACAE* COMBINATION WITH VERMICOMPOST ON GROWTH AND YIELD OF GROUNDNUT

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Abstract

The influence of three Vermicompost (VC) rates and three N₂-fixing bacteria on the growth, yield attributes and yield of peanut on the low nutrient soil. Three VC rates consisted of 0, 5.0 and 10 tons/ha, and three bacteria were inoculated in the experiment: *E. asburiae*, *K. quasipneumoniae* and *E. cloacae*. The impact of VC rates and three bacterial species was on the growth, yield attributes and yield of peanut. The studied results proved that application of different VC rates was a little impact on the nitrogen fixing ability of three bacteria, but high efficiency on the nodulous number and weight of peanut. Rate of 10 tons VC/ha combined with *E. asburiae* inoculum significantly obtained the highest yield attributes and yield of peanut, while the lowest yield component and yield of peanut observed without VC application and *E. cloacae* inoculation. The differences between rhizosphere bacterial species and different VC rates was better than that caused by the inorganic fertilizer. The main aim of this study is to optimize organic manures management and apply nitrogen fixing bacteria to maximize soil fertility and yields while lessening the usage of inorganic fertilizer and maintaining good soil.

Keywords: *E. asburiae*, *K. quasipneumoniae*, *E. cloacae*, Vermicompost, Peanut, Yield

INTRODUCTION

With human interest to the unpolluted environment, in agricultural cultivation, organic agriculture needs to be gradually superseded, and organic agriculture is expanded. Positive rhizosphere bacteria have outstandingly to sustainably agricultural cultivation, mainly used to crop pest control and bio fertilizer (Ahmad Abdel Gayed et al., 2018). The discovery and usage of plant growth promoting rhizobacteria (PGPR) have used by famers in over the word. Soil contains a huge microbial source base for the discovery of PGPR, the main strains consist of *Bacillus* spp. and *Pseudomonas* spp. Further, *Flavobacteria*, *Azotobacter*, *Enterobacter*, *Arthrobacter* and *Bradyrhizobium* have also found (Bardgett et al., 2014). On other hand, PGPR could promote crop productivity by promoting crop development, seed sprout and yield components. A recent study showed that PGPR could increase interactions between root and soil, and the co-application of rhizosphere N₂ fixing bacteria improved crop growth and soil fertility (Deakin et al., 2018). Groundnut (*Arachis Hypogaea* L.) seeds were pretreated with *Bacillus* sp., which development through different PGPR properties like producing many IAA, increasing N₂ fixing and phosphate solubilization significantly raised peanut yield (Wen-yue Xu et al., 2021). According to prior studies of Lugtenberg and Kamilova (2009); Karlidag et al., (2009) presented that the soil types, which contain low nutrition and pollution, are improved by PGPR. The high benefit efficiency in growth increase has studied during stress

conditions kinds of plant. Lee et al. (2012) proved that some bacterial strains could be zinc solubility, increased the soybean productivity by promoting the mobilization and bio-fortification of zinc. Some PGPR strains have also been found for other efficiency, such as the phosphatase solution (Arafa et al., 2010). Rhizosphere N_2 -fixing bacteria take nitrogen of atmospheric by means of biological N_2 fixation mechanism to alter N_2 from the air into inorganic nitrogen of soil compounds, such as NH_3 , and promote the growth and yield of crops (Montañez et al., 2009). Vermicompost, which is an organic manure with high nutrient concentration, highly airy and porous level, and good water holding capacity, supported by earthworms and microbes. In addition to organic waste management, VC is discovered as a plant growth promoter (Coria-Cayupaán et al., 2009). Nitrogen (N), phosphorus (P), Potassium (K), and other micronutrients have more available abilities thank to microbial activities in VC (Goutam et al., 2009). Nutrients of VC are higher levels of N, K, and Ca than the topsoil at a depth of 15 cm from 5, 7 and 1,5 times, respectively, in which plants grow (Ruz-Jerez et al., 2011; Parkin et al., 1994). The average nutritional concentrations in VC consist of N (2%), P (2.2%), K (1.5%), and organic carbon (18%), along with other micronutrients required for plant growth such as zinc (Zn), magnesium (Mg), and iron (Fe) (Adhikary et al., 2012). Vermicompost is applied by a long term of these macro and micronutrients in a readily available nutrition to crops (Atiyeh et al., 2000). Further, VC application could be enriched with soil microorganism such as N_2 -fixing and P-solubilizing bacteria (Yatoo et al., 2020). Derivativeness of VC, such as leachates and humus, are the important stimulators of crop growth promotion. Waste water of VC is an organic manure to be liquid nutrients, which collected after the water passes through a heap of VC (Ayyobi et al., 2014). The objective of this research could select the best train from three RNFB trains were chosen and the best VC rate to promote on the growth and yield of peanut in order to carry out for the next research.

MATERIALS AND METHODS

Bacteria

The bacteria isolated from the rhizosphere soils, roots and nodules of groundnuts, which were taken from peanut fields of An Phu district. Three bacteria (*E. asburiae*, *K. quasipneumoniae* and *E. cloacae*), which were isolated and identified by genotypes through sequencing technology. The similarity of *E. asburiae*, *K. quasipneumoniae* and *E. cloacae* were 99.65, 99.74 and 99.93%, respectively, compared to the 16S rRNA sequence of target bacteria (chuong et al., 2023). Theses bacteria were used to evaluate their efficiency on peanut growth and yield.

The population of main microorganism in the rhizosphere of groundnut

Rhizosphere N_2 fixing microorganisms are the result of crop selection from rhizosphere soil, which has plentiful bacterial sources. Plants and combined microbiota form a 'holobiont' that can maintain host functionality by the co-evolution between them (Xiaolin et al., 2020). Our research aimed to explore the bacterial community ability of the nodulous nitrogen fixing bacteria on promoting the growth and yield of peanut.

Design and location of experiment

The outside of the greenhouse of An Giang university was experimentally designed by evaluating and choosing the best bacterium of *E. asburiae*, *K. quasipneumoniae* and *E. cloacae* on groundnut growth and yield. The soil nutrients were quite poor (Table 1). Seeds were incubated by these bacteria one day before sowing. Three vermicompost ratios and three microorganisms were presented in Table 2. There were nine treatments and two factors (three microorganisms and three VC ratios), and four repeats (Table 2). NPK fertilizer, which were used by $\text{CH}_4\text{N}_2\text{O}$, DAP and KCl, was presented in Table 2. The total area of field study was 72 m^2 (1 m x 2 m x 04 repeats x 09 treatments). Soil samples were taken before and after experiment in 0-20 cm of the soil depth to determine the soil properties. Soil texture, total N, available P, exchangeable K, CEC and pH were used by methods of Carter & Gregoric, (2007). Yield components, which were counted by growth time of groundnut had height and shoot number of each plant, No. of pods per plant, number of biomass, number of nodule per plant, weight of fresh nodule, fresh weight of fill and empty pods per plant (g). The fresh yield was recorded by tons/ha for fresh pods. The soil texture was the silt sandy, soil pH (6.30), CEC ($1.19 \text{ Cmol}^+/\text{kg}$), total N (0.08%), the Available phosphorus was quite poor ($2.74 \text{ mg}/100\text{g}$). The available P, which is very essential element of Rhizosphere N_2 fixing microorganisms and peanuts, needs for the plant development and Rhizosphere N_2 fixing microorganisms life. Especially, exchangeable K was undetected at all soil samples. In generally, experimental soil had quite poor nutrients and lacked potassium (Table 1). The L14 seeds were bought from Peanut Research and Development Center, Hanoi-Vietnam, had well diseased resistance and high yield.

Table 1: soil properties before the experimental (n = 5)

Soil attributes						Soil texture (%)		
pH	OM (%)	CEC (Cmol^+/kg)	Total N (%)	Available P ($\text{mg}/100\text{g}$)	Exchangeable K (Cmol^+/kg)	Sand	silt	clay
6.30	1.79	1.19	0.08	2.74	undetected	80.0	18.7	1.3

Table 2: experimental treatments

Treatment	Three rhizosphere N_2 - Fixing Bacteria ($10^8\text{CFU}/\text{ml}$)			Vermicompost (t/ha)	N, P, K fertilizers (kg/ha)
	<i>E. asburiae</i>	<i>K. quasipneumoniae</i>	<i>E. cloacae</i>		
G1 (control)	0	0	0	0.00	40N-60P-60K
G2	inoculated	0	0	5.00	
G3	inoculated	0	0	10.0	
G4 (control)	0	0	0	0.00	
G5	0	inoculated	0	5.00	
G6	0	inoculated	0	10.0	
G7(control)	0	0	0	0.00	
G8	0	0	inoculated	5.00	
G9	0	0	inoculated	10.0	

Statistical analysis

The studied data were counted by software of statgraphics version XVIII. The one-way and ANOVA analysis of variance was used by comparison with the significance and LSD (standard deviation). $P_{\text{value}} < 0.05$ was counted by significant differences.

RESULTS AND DISCUSSION

Plant height

The results of Table 3 showed that there was a significant difference of 5% in the peanut plant height between the treatments at the 20 DAS. The average height of peanut plant ranged from 12.67 to 15.58 cm in three VC rates (0, 5.0 and 10.0 t/ha). The highest plant height (15.58 cm) observed at application of 10 tons VC /ha, while in the control treatment without applying VC and no *E. asburiae* inoculum gave the lowest plant height (12.67 cm). Similarly, three bacteria, which were inoculated, were significantly different at P value ≤ 0.05 , and the highest plant height (14.50 cm) observed at treatment of *E. asburiae* inoculation, while inoculating *E. cloacae* had the lowest plant height (13.04 cm) in 20 DAS. The developmental stages of 45 and 65 DAS were significantly different at P value ≤ 0.01 in both factors (VC and bacteria). In general, the maximum plant heights of 45 and 65 DAS in two factors were acknowledged in the applied rate of 10 tons VC/ha and *E. cloacae* inoculation, while the minimum plant heights observed without VC application and *E. cloacae* inoculation. However, there was not interaction between VC rates and bacteria (Table 3). According to prior study of Manisha Basu et al., (2008), presented that co-application of 20N:40P:30K kg/ ha and 2.5 t farmyard manure / ha improved the growth, nitrogen fixation, yield and kernel quality such as concentration of oil, protein, mineral n and hydration coefficient of peanut crop.

Table 3: average height of maize plant during the developmental period

Factor	Height of maize plant (cm)		
	20 DAS	45 DAS	65 DAS
Vermicompost (A)			
- 0.0 (t/ha)	12.67 ^b	20.88 ^b	50.42 ^c
- 5.0 t/ha	13.62 ^b	22.47 ^{ab}	53.81 ^b
- 10.0 t/ha	15.68 ^a	25.77 ^a	56.62 ^a
Bacteria (B)			
<i>E. asburiae</i>	14.50 ^a	24.48 ^a	55.93 ^a
<i>K. quasipneumoniae</i>	14.28 ^b	23.48 ^b	54.93 ^b
<i>E. cloacae</i>	13.04 ^c	21.27 ^c	51.97 ^c
F (A)	*	**	**
F (B)	*	**	*
F (A x B)	ns	ns	ns
CV (%)	14.6	15.5	13.5

DAS: days after sowing; ns: insignificant difference at P value ≤ 0.05 ; *, **: significant difference at P value ≤ 0.05 and 0.01%

Available branches

Table 4: average height of maize plant during the developmental period

Factor	Branches/ plant		
	20 DAS	45 DAS	65 DAS
Vermicompost (A)			
- 0.0 t/ha	2.4	4.0 ^a	5.1 ^b
- 5.0 t/ha	3.5	5.1 ^{ab}	7.2 ^{ab}
- 10.0 t/ha	3.7	6.2 ^a	8.4 ^a
Bacteria (B)			
<i>E. asburiae</i>	3.6	5.5 ^a	7.1 ^a
<i>K. quasipneumoniae</i>	3.5	4.4 ^b	6.5 ^b
<i>E. cloacae</i>	3.6	4.2 ^a	6.1 ^c
F (A)	ns	**	**
F (B)	ns	**	*
F (A x B)	ns	*	*
CV (%)	12.2	9.7	5.8

ns: insignificant difference at P value ≤ 0.05 ; *, **: significant difference at P value ≤ 0.05 and 0.01%

The results in Table 4 showed that there was insignificant difference of 5% between the different doses of VC and three bacteria in the number of branches per the peanut plant at the stage of 20 DAS. However, the growth period of 45 and 65 DAS was significant difference at level 5 and 1% between VC rates and three bacteria. The maximum branch values observed at dose of 10 tons VC/ha and *E. asburiae* inoculum, and the minimum branch number of 0. Ton VC/ ha and *E. cloacae* inoculation in 45 and 65 DAS. From these results, it was shown that application of 10 tons VC/ha combined with *E. asburiae* inoculum helped peanuts raise more the number of branches compared with other VC rates and other bacteria. Purbajanti et al., (2019) showed that the application of vermicomposting helped plants increase in height, number of leaves, number of branches, and width of burial compared to treatments without tons VC/ha application.

Number and weight of peanut nodules

Table 5: Number and weight of peanut nodules

Factors	75 DAS	
	No. of nodules (plant)	Wt. of nodules (gr/plant)
Vermicompost (A)		
- 0.0 t/ha	317 ^a	1.80 ^a
- 5.0 t/ha	305 ^b	1.57 ^b
- 10.0 t/ha	281 ^c	1.06 ^c
Bacteria (B)		
<i>E. asburiae</i>	259 ^a	1.41 ^a
<i>K. quasipneumoniae</i>	250 ^b	1.29 ^b
<i>E. cloacae</i>	229 ^c	1.15 ^c
F (A)	**	**
F (B)	*	*
F (AxB)	**	**
CV (%)	12.6	12.8

*, **: significant difference at P value ≤ 0.05 and 0.01%

The results (Table 5) presented that *E. asburiae* inoculation was the highest nodulous number (259) and nodulous weight (1.41gr/plant), while the lowest value of nodulous number (229) and nodulous weight (1.15 gr/plant) observed at treatment of *E. cloacae* inoculant and significant difference at $LSD \leq 0.05$. Without VC application also had the maximum nodulous number (317 nodules/plant) and the maximum nodulous weight (1.08 gr/plant) and a significant difference at level 1%. In contrast, the application of 10.0 tons VC /ha achieved the lowest nodulous number and nodulous weight of peanut (281 and 1.06 gr per plant, respectively). The positive relation between the N_2 -fixing bacteria and nodule and rhizosphere of legume has been produced by a major nitrogen fixing source (Zhou et al., 2007). According to recent study of Yan et al. (2022), discovered that The effects of organic manure application on the different soil structures and forms of peanut rhizosphere microorganism during different developmental period of plant was found out in the paddy soil. Application of VC improved the soil biochemical attributes under various years of continuous growth, thereby raising growth, yield quality of tomato compared with urea application. Further, considering the higher EC and lower soil pH achieved by applying VC may be a better recommendation for soils. The study showed the efficiency of VC on soil microbial population and nutrients on different soil types, to increase tomato yields under sustainable production systems (Fu et al., 2017)

Yield composition and yield of peanuts

Table 6: Effect of VC and bacteria on yield composition and yield of peanuts

Factors	Biomass (g/plant)	No. of fill pods (pods/plant)	Wt. of fill pods (gr/plant)	Wt. of 100 seeds (gr)	Fresh yield (t/ha)
Vermicompost (A)					
- 0.0 t/ha	244 ^c	54.0 ^b	135 ^b	86.8 ^c	6.31 ^c
- 5.0 t/ha	260 ^b	69.4 ^a	159 ^{ab}	92.7 ^b	7.29 ^b
- 10.0 t/ha	282 ^a	69.1 ^a	179 ^a	106 ^a	7.91 ^a
Bacteria (B)					
<i>E. asburiae</i>	299 ^a	73.9 ^a	172 ^a	115 ^a	8.17 ^a
<i>K. quasipneumoniae</i>	224 ^b	55.9 ^b	132 ^b	85.9 ^b	7.15 ^b
<i>E. cloacae</i>	201 ^c	45.5 ^c	112 ^c	85.1 ^b	6.50 ^c
F (A)	*	*	*	**	**
F (B)	**	**	**	**	**
F (A x B)	ns	ns	ns	ns	**
CV (%)	12.5	20.6	21.9	13.3	12.1

ns: insignificant difference at P value ≤ 0.05 ; *, ** significant difference at P value ≤ 0.05 and 0.01%

The biomass and fill pods No. of peanut were remarkably affected by applying VC rates, and insignificantly different at level 5%. However, three bacteria were significantly different at level 1% (Table 6). Three inoculated bacteria consisted of *E. asburiae*, *K. quasipneumoniae* and *E. cloacae*, *E. asburiae* was the maximum biomass and fill pods number per plant (299 gr/plant and 73.9 pods/plant, respectively). While, the minimum values of the biomass and fill pods number per plant observed at *E. cloacae* inoculum (201 gr/plant and 45.5 pods/plant, respectively). The values of biomass and fill pods number per plant of peanut ranged from 201 to 299 gr/plant and 45.5 to 73.9 pods/plant, respectively. Furthermore, weight of fill pods and 100 seeds were significant differences at level 5 and 1% in three different VC rates (0, 5 and 10 tons/ha). The highest values of biomass, No. of fill pods, Wt., of fill pods, Wt. of 100 seeds

and fresh yield obtained in application of 10 tons VC /ha. Contrariwise, the lowest values observed at treatments without VC application (Table 6). In general, all yield components and productivities of groundnut obtained the highest values in application of 10 tons VC/ha and *E. asburiae* inoculation. Contrariwise, the lowest values of all yield components and yields had without VC application and *E. cloacae* inoculation. There were an interaction of yield components and not significantly different between VC rates and bacteria (Expect fresh yield). The recent study discovered that VC application was affected on population of N₂-fixing bacteria and soil nutrition. In particular, the long-term application of organic fertilizer can markedly improve soil quality (Jindo et al., 2016). Thus, VC application improved crop yield and quality in study. Application of VC more effectively improved plant growth, such as agronomy, yield components, yield and fruit quality in each type of soil (Wang et al., 2017). Inoculation of the different N₂-fixing bacteria significantly raised on soil properties and/or Groundnut discharges, which has not been a detection. Furthermore, the fixing bacteria should be applied as a potential bacterium to increase development and yield of crops (Yang, et al., 2021).

CONCLUSION

In conclusion, studied results offer that VC combination with rhizosphere N₂ fixing bacteria could promote the agronomy, yield components and yield peanut, therefore raising peanut growth and yield compared with inorganic fertilizers. Moreover, considering application of VC combination with *E. asburiae* inoculation, which achieved the highest yield attributes and yield of peanut, could be planted on low nutrient soils. Especially, VC combination with *E. asburiae* can produce better improvements in nodulous number and nodulous weight compared with inorganic fertilizers. These research should be carried out to clear the effects of VC on rhizosphere microbial processes and nutrient cycling on different soil kinds, to increase yields of different plants under sustainable cultivation systems. The final aim is to optimize organic manures management and use N₂ – fixing bacteria to maximize soil fertility and yields while lessening the usage of inorganic fertilizer and maintaining good soil.

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