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Effective Appraisement of Enterobacter asburiae and Irrigation Water on Arsenic uptake, Yield of Peanuts

Phan Tran Hai Dang[1] and Nguyen Van Chuong[2*]

[1]Master student of An Giang University, VNU HCM, Vietnam.
[2] *faculty of Agriculture of An Giang University, VNU HCM, Vietnam.

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CORRESPONDING AUTHOR:

Nguyen Van Chuong

nvchuong@agu.edu.vn

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Abstract

The field study was carried out in An Phu town, An Giang province, Vietnam during Summer-Autumn crop of 2023. The field experiment was carried out by eight treatments and four replications. Eight treatments consisted of two factors: (i) *Enterobacter asburiae* (EA) (inoculum and no inoculum); (ii) irrigation water (River and deep well water). The results of the study showed that EA inoculum and river water irrigation remarkably increased agronomic and yield traits such as height, branches, biomass number and weight of nodules and fill pods of peanut. Furthermore, EA inoculum combined with irrigating river water significantly increased peanut yield and reduced arsenic (As) uptake of peanut stems and seeds. *Enterobacter asburiae* inoculation was higher than no EA inoculation by 19.7%. River irrigation had the higher fresh peanut yield (6.17 t/ ha) compared to irrigation of deep well water (3.10 t/ ha). The interaction between bacteria inoculation and irrigated water types was not significant differences at 5% level. Furthermore, The As accumulation of peanut stems and seeds of EA inoculation was lower than 12.3% in stems and 4.06% in seeds. Irrigating river water for peanuts, which absorbed lower As toxicology of stems and seeds than irrigating deep well water from 16.5% (stems) and 39.7% (seeds). *Enterobacter asburiae* may be a potential genus to product biological fertilizers in the future.

INTRODUCTION

Peanut *(Arachis hypogaea* L.), which is one of the world's most popular crops cultivated in tropical and sub-tropical regions, has the high economic value. It contents high protein, oil, fatty acid, carbohydrates, vitamins and mineral. Peanut contains 45-55% oil, 20-25% protein, 16-18% carbohydrates and 5% minerals (Gulluoglu 2011; Gulluoglu et al., 2016). The urbanization has narrowed the agricultural soil, reducing food production. The government developed an intensive system to overcome the food shortage that has resulted in the deterioration of the quality of agricultural soil. Therefore, the agricultural production may be faced with environmental changes that make the soil increasingly apparent depression (Barrow, 2012). The grey degraded soils have good drainage and aeration due to the high rate of sand in the soil structure, but low organic matter content due to easy leaching (Yunilasari et al, 2020). Increasing crop yields, farmers use more and more chemical fertilizers and pesticides. This is the cause of environmental pollution due to the accumulation of nutrients derived from fertilizers and pesticides. Land will be damaged slowly and the productivity of crops will continue to decrease. The entophytic nitrogen fixing bacterium inoculation that replaces for inorganic N fertilizers, can improve the soil fertility (Krishnan et al., 2018).

Peanuts, which like many other plants, the same peanut family, are the symbiosis of *Rhizobium* roots and *Rhizobium*. *Rhizobium* is a type of bacteria, the ability to fix nitrogen. *Rhizobium* are responsible for fixing free nitrogen from the air for plants (Mbah & Dakora, 2018). The amount of nitrogen that was fixed by *Rhizobium* was achieved an average of 94 kg N/ ha / crop (about 200 kg ure/ha/crop) (Awadalla & Mohammed, 2017). In the good condition, it can reach 168 kg N/ ha / crop (Doloum et al., 2017). The amount of nitrogen that fixed by *Rhizobium,* can support to 74% of N the peanut trees. Environmental conditions have a great influence on the activity and ability of nitrogen fixation of *Rhizobium*. If application of N is too high, it can inhibit the nitrogen fixation of bacteria. Phosphorus has a positive effect on the formation and nitrogen fixation of nodules. Especially, the soil is sufficiently applied the calcium or lime (Korir, 2017). Nitrogen is an important element for effective production of peanut, adequate supply of nitrogen fertilizer is essential for growth and yield due to the intensive farming. Therefore, farmers have used a large amount of chemical fertilizer, which has been the cause of soil degradation. The application of bio-fertilizers frequently recommended to get high and clean agricultural product (Doloum et al., 2017). The recent challenge faced by advanced farming is to achieve higher yields in an environment-friendly manner. Thus, there is an immediate need to find eco-friendly solutions. Among various types of species being used as biocontrol agents (Mbah & Dakora, 2018). Inoculation of rhizobia helps to add nitrogen to peanuts because native rhizobia species cannot provide enough nitrogen for it (Anteneh, 2018). Rice growth and yield were significantly reduced by arsenic-contaminated irrigation water. If As concentrations in irrigation water is below 0.5

mg/L, it can stimulate rice plants grow well, and increase rice yields. However, if As concentrations in irrigation water is higher than 0.5 mg/L, it can significantly reduce rice growth and yield. In addition, if tillers continue to use the As pollution irrigation water to irrigate crops for a long time. it will cause to raise the soil As accumulation (Azad et al., 2012).

Accumulation of As in the soil and their uptake by both plant growth promoting *Rhizobacteria* and crops is a matter of growing environmental concern. Unlike many other pollutants, which can undergo biodegradation and produce less toxic, less mobile and/ or less bio-available products, heavy metals are difficult to be removed from contaminated environment (Stan et al., 2011; Glick, 1995). These metals cannot be degraded biologically, and are ultimately indestructible, though the speciation and bioavailability of metals may change with variation in the environmental factors. The ability to grow even at high metal concentration is found in many rhizosphere microorganisms including symbiotic N2 fixing bacteria and may be the result of intrinsic or induced mechanism (Giller et al., 1998). Tolerance may be defined as the ability to cope with metal toxicity by means of intrinsic properties of the microorganisms, while resistance is the ability of microbes to survive in higher concentrations of toxic metals by detoxification mechanisms, activated in direct response to the presence of heavy metals. Toxic heavy metals therefore, need to be either completely removed from the contaminated soil, transformed or to be immobilized, producing much less or non-toxic species. However, in order to survive and proliferate in metal contaminated soils, tolerance has to be present both in microbes and their associative hosts. For survival under metal-stressed environment, plant growth promoting Rhizobacteria have evolved several mechanisms by which they can immobilize, mobilize or transform metals rendering them inactive to tolerate the uptake of heavy metal ions (Nies, 1999). The nitrogen of *Rhizobium*-legume symbiosis is one of the nutrients that increase the soil fertility. This nutrition makes it help the farmers reduce application of NF. Farmers can increase their productivity and income by maximizing bio-nitrogen fixation through the *Rhizobium* strain. Helping peanuts grow and raise the population of *Rhizobium* strain to improve nitrogen supply, so yield of peanuts will be increased (Trang & Chuong, 2023). The aim of this study find out effects of Rhizosphere N2-fixing bacteria combined with irrigation water types on yield and As uptake of the Peanut in Tri Ton town.

Materials and Methods

Microorganisms

Enterobacter asburiae was isolated and identified from nodules of peanuts on fields of farmers in An Phu district, An Giang province, Vietnam. *Enterobacter asburiae* was mixed well with seeds of peanut. Seeds of peanut were soaked in liquid inoculated after diluted 1:1 with well water for

30 min. before sowing.

Treatments and crop management

The field experiment included eight treatments and four replications. Each treatment has an area of 80 m^2 (1.0 m x 20 m x 4 replications) and the total area was 640 $m^{2.}$ All treatments were designed in Table 1:

Table 1: Nitrogen – fixing bacterium and Irrigation water types in the field study

Treatment	EA (108CFU/ml)	Irrigation water	Inorganic fertilizer (kg/ha)
NT1 (Control)	No	Deep well	40 urea: 60 P_2O_5: 60 KCl
NT2		River	
NT3		DW	
NT4		River	
NT5	Yes	DW	
NT6		River	
NT7		DW	
NT8		River	

Data recorded

Four samples, which were taken at 20, 40 and 65 days after sowing and ten plants were taken from each plot randomly, estimated the average height and number of shoots per plant. At harvest. Random samples of ten plants were taken from each plot to determine number of biomass, number of nodule per plant, dry weight of nodule**,** fresh and dry weight of filled and unfilled pods per plant (g). Plants on the middle two rows in each plot were harvested separately and dried in order to estimate As content in seed.

Analysis methods

Plants on the middle two rows in each plot were harvested separately and dried in order to estimate weight of pods yield per ha and weight of seeds per ha. Seed samples were grinded into fine powder and stored in brown glass bottles for chemical analysis. All data were analyzed by the generalized linear model analysis of variance using Genstat v10 (VSN International Ltd, UK, 2007). Arsenic of soil, stems and seeds were determined according to methods described by AOAC. *Enterobacter Asburiae* was isolated and identified in laboratory of An Giang University. Chemical fertilizers were used urea, KCl and P_2O_5 fertilizers. Peanut seeds L14 were collected from local famers.

Statistical analysis

For statistical analysis, F-test was use by using LSD test [11]. Data was analyzed by using STATISTIX software and the means were separated by LSD test at P≤ 0.05 probability level.

Results and Discussion

Plant height

Results in Table 2 showed that plant height between treatments in the EA inoculation and irrigation water types was a significant difference at P ≤ 0.05 and 0.01 (Except 20DAS in bacteria and 40 DAS water types) during the peanut growth time. Peanut height in treatments of EA inoculation was always higher than without inoculation (Table 3). Similarly, the higher height peanut plant was watered by river water than plant height of watering deep well water t *Rhizobium* inoculation treatments and unpolluted water irrigation had different results to the un-inoculated and polluted water irrigation in plant height; stem dry weight, number of nodules per plant and nodule dry weight (Sajid et al., 2011; Ahmad et al., 2019).

Table 2: Peanut plant height during the growth

Factor	**Plant height (cm)**		
	20 DAS	40 DAS	65 DAS
Enterobacter Asburiae **(10^8CFU) (A)**			
No	6.33	24.3^b	43.5^b
Yes	6.56	29.1^a	49.9^a
Irrigation water (B)			
Deep well	5.49^b	25.5	42.1^b
River	7.40^a	28.0	51.3^a

F(A)	ns	*	*
F (B)	**	ns	**
F_{A*B} (%)	ns	ns	ns
CV_{A*B} (%)	15.1	21.2	17.3

*DAS: Days after seeding; Means with different letters, in the same column differ significantly *, ** = significant at 0.05 and 0.01 level, respectively; ns = non-significant. Mean in the same column with*
different letters are significantly different at p ≤ 0.05, as determined by LSD

Branch number per plant

The number of branches per plant was insignificantly affected by various treatments in the growing stages from 20 to 65 DAS between bacterial inoculation and irrigation water types (Table 3). The results of statistical analysis in Table 3 observed that the peanut plant branch was found in all three stages of 20 – 45 – 65 DAS when EA inoculation with values of 6.51 – 15.7 – 15.9 branches, respectively. In addition, river water irrigation also promoted the branch number per plant of 6.55 – 15.4 – 15.7 branches at the 20 – 45 – 65 DAS stages, respectively. However, its interaction was insignificantly statistical differences between bacteria and water types (Except 40 DAS). In particular, the treatment of EA inoculum combined with irrigating river water had higher branch number than the treatments of non-bacterium inoculation and deep well water irrigation. The unpolluted water use to water crops and combination of rhizosphere nitrogen-fixing microorganisms helped to increase the height, number of leaves, and total chlorophyll content of baby corn plants grown on poor nutrient soil compared to no nitrogen application and *Priestia aryabhattai* inoculation (Le et al., 2023). Recent study showed that the use of nitrogen has significantly improved the photosynthetic efficiency of peanuts, especially the photosynthetic rate and respiration rate of peanut populations in the flowering and pod filling stages (Yang et al., 2014).

Table 3: Peanut branch number during the growth

Factor	**Number of branches (branch)**		
	20 DAS	40 DAS	65 DAS
Enterobacter Asburiae (10^8CFU) **(A)**			
No	4.95^b	12.5^b	12.1^b
Yes	6.51^a	15.7^a	15.9^a
Irrigation water (B)			
Deep well	4.91^b	11.7^b	12.5^b
River	6.55^a	15.4^a	15.7^a

F(A)	*	**	**
F (B)	*	**	**
F_{A*B} (%)	ns	**	ns
CV_{A*B} (%)	11,7	13.5	12.1

*DAS: Days after seeding; Means with different letters, in the same column differ significantly *, ** = significant at 0.05 and 0.01 level, respectively; ns = non-significant. Mean in the same column with different letters are significantly different at $p \leq 0.05$, as determined by LSD*

Peanut yield traits and yields

Table 4's results showed that EA inoculation were significant effects on the number and fresh weight of peanut nodules. EA inoculation increased remarkably the number and fresh weight of root nodules in the 75 DAS. The number of root nodules ($p<0.01$) and fresh weight of root nodules ($P\leq 0.01$) in peanut roots at 75 DAS decreased by 69.7% and 69.1%, respectively, compared to no EA inoculation. Similarly, the number of root nodules with river irrigation increased by 20.8% and nodulous weight was not difference compared to irrigate deep well water. The results in Table 4 presented that. EA inoculation and water irrigated types was impacted on biomass of peanut plants ($P\leq 0.01$ and $P\leq 0.05$, respectively). Further, peanut biomass of EA inoculant and river irrigation were higher than treatments of non-EA inoculant and deep well water irrigation. However, there were no interaction between the Bacteria inoculation and irrigation types.

Table 4: Number and weight of nodules and biomass per plant at harvest season

Factor	**Number of nodules (nodule)**	**Weight of fresh nodules (gr/plant)**	**Plant Biomass (gr/plant)**
Enterobacter Asburiae (10^8CFU) **(A)**			
No	80^b	0.63^b	201^b
Yes	264^a	2.04^a	292^a
Irrigation water (B)			
Deep well	152^b	1.35	226^b
River	192^a	1.32	267^a
F(A)	**	**	**
F (B)	**	ns	*
F_{A*B} (%)	ns	ns	ns
CV_{A*B} (%)	19.5	17.3	29.1

*Means with different letters, in the same column differ significantly *, ** = significant at 0.05 and 0.01 level, respectively; ns = non-significant. Mean in the same column with different letters are* *significantly different at p ≤ 0.05, as determined by LSD*

Symbiotic nitrogen-fixing bacteria strains can convert atmospheric nitrogen into nitrate nitrogen, which can be absorbed by roots due to its unique nitrogen-fixing function (Huang et al., 2017). Therefore, the full enhancement of rhizobia's nitrogen-fixing ability plays an important role in reducing nitrogen fertilizer use. According to the research results of Walters et al., (2018), the N source for peanut plants throughout the entire growth stage mainly comes from root nodules, soil, and inorganic N fertilizer. The N supply ratio from root nodules, soil, and N fertilizer is 5:3:2. Etesami, (2018) also found similarly that the increased nitrogen-fixing ability of rhizobia directly contributes to promoting the growth and increasing the yield of peanut plants.

Table 5: Number and weight of fresh fill pods per plant and weight of 1,000 seeds

Factor	**Number of fill pods (pod/plant)**	**Fresh weight of fill pods (gr/plant)**	**Weight of 1,000 seeds (gr)**	**Fresh yield (t/ha)**
Enterobacter Asburiae (10^8CFU) **(A)**				
No	51.9^b	127^b	260^b	4.13^b
Yes	74.9^a	178^a	316^a	5.14^a
Irrigation water (B)				
Deep well	55.6^b	130^b	272^b	3.10^b
River	71.3^a	176^a	305^a	6.17^a
F(A)	**	**	**	**
F (B)	*	**	**	**
F_{A*B} (%)	ns	ns	ns	ns
CV_{A*B} (%)	16.9	16.7	17.2	13.6

*Means with different letters, in the same column differ significantly *, ** = significant at 0.05 and 0.01 level, respectively; ns = non-significant. Mean in the same column with different letters are significantly different at p ≤ 0.05, as determined by LSD*

The higher number and weight of filled pods (P≤0.01) was 74.9 pods/plant and 178 gr/plant, respectively, per plant in EA inoculation than those of without EA inoculation (51.9 pod/ plant and 130 gr/plant). Similarly, the number and weight of filled pods was 71.3 pods/plant and 176 gr/plant, per plant in river irrigation higher than those of deep well water irrigation, which were 55.6 pod/ plant (number of fill pods) and 127 gr/plant (weight of fill pods). Weight of 1,000 seeds was significantly different (P≤0.01) in both EA inoculant and irrigated water types. *Enterobacter Asburiae* inoculation gave the 1,000 seeds' weight was (316 gr) higher than N_2 non-EA inoculation

(260g). Watering N deep well water reduced 33gr/1,000 seeds compared to irrigate river water. The fresh yield was insignificantly statistical different (P≤0.01) in both EA inoculant and irrigation types. Yield of EA inoculation was higher than no EA inoculation by 19.7%. River irrigation had the higher fresh peanut yield (6.17 t/ ha) compared to irrigation of deep well water (3.10 t/ ha). The interaction between bacteria inoculation and irrigated water types was not significant differences at 5% level (Table 5). The number and weight of fresh fill pods per plant were affected by co-application of differently irrigated water types associated with *Rhizobium* inoculation on peanut yield traits and yield (Anteneh A. 2017). Application of organic and inorganic fertilizer could significantly increase growth and yield of peanut in the next crop (after 4 months) (Nguyen & Chinh, 2018).

Arsenic contents

The As accumulation of soil that valued 82.35 mg/kg in no EA inoculation and 67.65 mg/kg EA inoculation, was 81.45 mg/kg in deep well water irrigation and 68.55 mg/kg in river water irrigation and sufficiently different at 1% level. The As content of peanut stems (0.268 mg/kg and peanut seeds (0.16 mg/kg) in RV3. Further, there were the lowest As content of peanut stem (0.70 mg/kg) and seed (0.69 mg/kg)) in no EA inoculation (Table 6). Furthermore, The As accumulation of peanut stems and seeds of EA inoculation was lower than 12.3% in stems and 4.06% in seeds. Irrigating river water for peanuts, which absorbed lower As toxicology of stems and seeds than irrigating deep well water from 16.5% (stems) and 39.7% (seeds). In general, the As uptake of peanut stems and seeds in both EA inoculation and river water irrigation was lower than non EA inoculation and deep well water irrigation. The addition of entophytic microorganisms showed positive effects on plant growth, reduced As uptake into plants, and increased population of P. *vittata* bacterium. The results of this study provided important clues to elucidate the mechanism of As remediation enhanced by microorganisms in plants, thereby promoting related studies on plant-associated microbial remediation in As-contaminated soils (Li et al., 2023).

Table 6: Arsenic concentration of soil, peanut stems and seeds at harvest season

Factor	**Arsenic concentration (mg/kg)**		
	Soil	**Stem**	**seed**
Enterobacter Asburiae (10^8CFU) **(A)**			
No	82.35^a	0.268^a	0.069^a
Yes	67.65^b	0.235^b	0.041^b
Irrigation water (B)			
Deep well	81.45^a	0.285^a	0.063^a
River	68.55^b	0.238^b	0.038^b

F(A)	**	**	**
F (B)	**	**	**
F_{A*B} (%)	ns	ns	ns
CV_{A*B} (%)	10.2	11.1	19.2

*Means with different letters, in the same column differ significantly *, ** = significant at 0.05 and 0.01 level, respectively; ns = non-significant. Mean in the same column with different letters are significantly different at $p \leq 0.05$, as determined by LSD*

Conclusion

Inorganic fertilizer application combined with EA inoculation and river water irrigation on alluvial soil remarkably increased agronomic traits, the number and weight of nodules, and the peanut yield components and yield. In particular, EA bacterium significantly reduced the Arsenic absorption of peanut stems and seeds. This study concluded that EA genus was isolated from peanut nodules, which was grown on As-contaminated soil. *Enterobacter asburiae* that was identified to be the entophytic nitrogen-fixing genus and had the ability to reduce As absorption into the peanut stems and seeds, could help the plant to develop well, thereby increasing yield and seed quality. This is a potential bacterial species for the production of organic bio-fertilizer for peanut cultivation.

COMPETING INTERESTS

The authors have no competing interests to declare.

Author's Affiliation

Phan Tran Hai Dang[1] and Nguyen Van Chuong[2*]

[1]Master student of An Giang University, VNU HCM, Vietnam.

[2] *faculty of Agriculture of An Giang University, VNU HCM, Vietnam.

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