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The Effectiveness of Chemical Fertilizer Combined with Lime, Cow Manure and Indigenous Nitrogen-Fixing Bacteria İnoculation on Soil Fertility and White Bean Yield

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ABSTRACT

In response to the increasing costs, decreasing efficiency of nitrogen fertilizer use, and arsenic-contaminated soil leading to reduced crop yield and quality. The agricultural community has actively looking for sustainable alternatives to exploit the natural nitrogen source from biological N fixation, lime and cow manure supplementation to improve crop yield and quality. This study investigated the combined effects of Indigenously Endogenous Nitrogen Bacteria (IENB) and its combination with cow manure (CM) and lime on soil fertility, available nutrients as well as yield and quality parameters of white bean. The study included the application of lime rates (0 and 3.0 $CaCO_3$/ha); CM (0 and 5.0 t/ha) combined with Bacterium strain BS1897 species on "BI" variety of white bean, using a complete factorial design with 4 treatment levels and 4 replications. The results showed that the application of lime, CM, and Bacterium strain NTLG2-5 (B. NTLG2-5) to the soil had a positive effect on white bean plants. The fresh seed yield significantly raised to compare to the control treatment, which ranged from 17.0 to 33.0%. The As accumulation of white bean stems decreased significantly from 21.3 to 65.8% compared to the control treatment. In particular, there was no As accumulation of white bean seeds in all treatments. The addition of lime and CM, either separately or in combination, fully improved plant yield and quality. Therefore, it is necessary to apply lime, fertilizer, and indigenous IENB inoculation in combination to increase crop yield and improve crop quality on the As contamination soil and irrigation water.

Keywords: Arsenic, *Bacterium* strain NTLG2-5, cow manure, lime, white bean

INTRODUCTION

White bean (*Phaseolus vulgaris* L.) is one of the important export crops in Asian countries in general and Vietnam in particular. It is being grown and used as a popular food all over the world and is an importantly nutrient source for humans (Khan *et al.,* 2018). White bean is a bean type, which contains high levels of protein, lipids, minerals, and antioxidants (Virginia 2014). Climate change has been affecting on agricultural production, such as sea water and temperature increase leading to salinity, drought, and acidity, as well as As contamination from irrigation water and farmland, reducing the yield and quality of crops (Zandalinas *et al,.* 2021; Van Dijk *et al.,* 2021). In the coming years, over 80% of the world's population will be affected by population growth (over 0.4 billion people) in Asian and African countries (Chojnacka *et al.,* 2020). The world's food that needs to meet this population increase, are expected to be very high. Therefore, effective methods to improve crop yields need to be implemented immediately (Van Dijk *et al.,* 2021). The optimal trend that can be achieved is the efficient use of local organic manure sources and the application of biological techniques in agriculture such as inoculation of endogenous nitrogen-fixing microorganisms to reduce chemical fertilizer application for plant, use uncontaminated water as well as pesticides in agricultural production in order to increase crop yields and quality (Young *et al.,* 2021, Chuong *et al.,* 2024).

Good agricultural production is essential to guarantee quality food and environmental security (Menila *et al*., 2022). Traditional farming practices have used excessive amounts of chemical fertilizers to increase food production, which has mainly led to reduced farmland fertility and crop yield. The potential of the N-fixing microbium strategy to enhance the farmland and crop quality has to be better utilised (Pylak *et al*., 2019, Nguyen *et al.,* 2024). Soil microorganisms have the ability to fix nitrogen from the atmosphere (Miao *et al*., 2020) and produce organic acids, enzymes, and hormones, thereby regulating the soil environment (Olanrewaju *et al*., 2017), promoting bacterial growth and a community of agricultural soils (Bhardwaj *et al*., 2014; Rahman and Zhang, 2018). These microorganisms can establish symbiotic relationships with the plant root system and increase the nutrient uptake ability of crops (Armanhi *et al*., 2017). Endophytic N-fixing bacteria have the ability to fix atmospheric nitrogen (Pitiwittayakul *et al*., 2021; Bisht *et al*., 2020; Bononi *et al*., 2020).

White bean is a legume, which has the ability to symbiotically fix nitrogen in the farmland and increase soil nitrogen through biological nitrogen fixation between IENB, (Pastor-Bueis *et al.,* 2021), thereby, lead to the need reduction for chemical N fertilizer weight to apply for crops. The biological nitrogen fixation ability of this legume depends on the IENB species, soil nutrition, environmental conditions, and the amount of nitrogen fertilizer applied (Barros *et al.,* 2018; Massa *et al.,* 2020). Reducing As uptake, increasing the quality and yield of legume seeds to the stem, leaves, and seeds by applying organic fertilizer, lime, and IENB genera (Chuong *et al*., 2019). Application of lime combined with organic fertilizer and IENB inoculation can also raise natural N fertilizer and plant yields. In general, lime, organic manure application combined with IENB inoculation has significantly increased the yield of legumes and reduced plant As uptake in farmland and underground water, which was high As contamination (Tuan and Chuong, 2022; Chuong and Bush, 2021). Continuous cultivation without allowing the soil to rest and not using organic fertilizer, especially in small fields, is one of the reasons for the decline in soil fertility and crop yields. (Ghimire *et al.,* 2017).

Local farmers are not familiar with IENB inoculation on bean seeds before planting (De Ron 2015). According to prior study of Chuong (2024) proved that agricultural soil and irrigation water have highly polluted in An Phu town, An Giang. The stem and seed As content of legumes and cereals both exceed WHO standards. Their As uptake reduce is to raise soil pH by applying lime (Chuong and Tri, 2024). In addition, organic manure application also reduces the As uptake of legumes compared to only apply the inorganic fertilizer (Hai Dang and Chuong, 2023). The research objective is to investigate on IENB abilities of nitrogen -fixing, As resistance, improve white bean quality and yield by using lime, CM, and *Bacterium* strain NTLG2-5 on As-contaminated soil and irrigation water.

MATERIAL AND METHODS

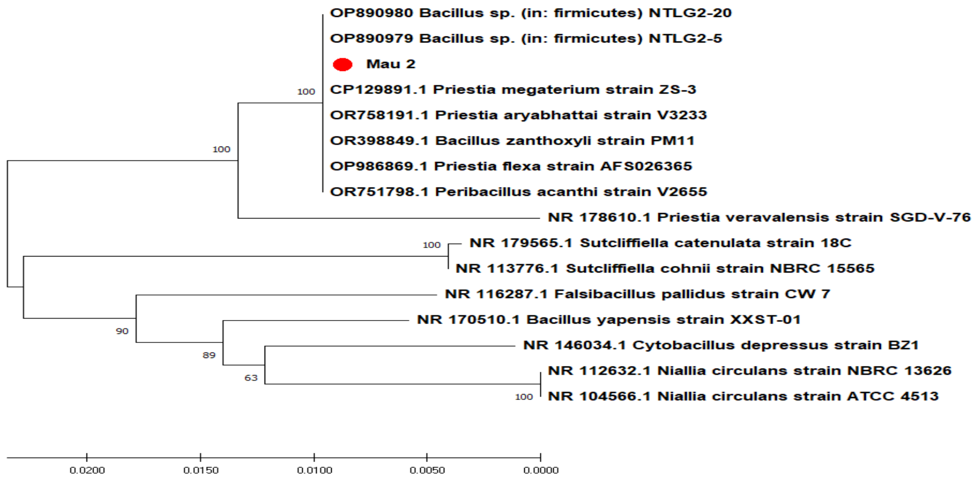
Isolation and identification of IENB

Bacterium strain NTLG2-5 genus was isolated from while bean roots using YMA (Yeast Mannitol Agar) culture and subsequently determined through the 16S rRNA sequencing technology. The homology result is a 99.74% similarity to the known organisms. The isolated colonies that were determined on the AGU campus of VNU HCMC, were initially identified depended on morphological and biochemical traits. Further, molecular identification was achieved through 16S rRNA gene sequencing on the NCBI database, confirming 100% similarity to our target bacteria. The relationship between IENB species and sequencing analysis results are presented in **Figure 1** and **2**, respectively.

Experimental design and location

The farm experiment was performed in Phuoc Hung, An Phu town, An Giang, Vietnam from August to October of 2023. To characterize the soil traits of the experimental site, soil samples were taken from the 0- 20 cm depth prior to the experiment, and certain physicochemical properties of the collected soil sample were analyzed according to the methodology reported by Carter and Gregoric (2007). The study comprised four treatments labeled WB0 (NPK:40

Urea-60 P_2O_5-60 K_2O kg/ ha), WB1 (NPK+3.00 t$CaCO_3$ + B. NTLG 2-5 inoculation), WB2 (NPK+5.0 tCM/ ha + B. NTLG2-5 inoculation) and WB3 (NPK+3.00 t$CaCO_3$ +5.0 tCM/ ha + B. NTLG2-5 inoculation), each treatment replicated four times (**Table 1**). In WB0, received 0 tons of the required lime + CM and non- IENB inoculation for the plant was supplied, WB1 amended the required 3.0 tons of $CaCO_3$ per ha and B. NTLG2-5 inoculation, WB 2 amended 5.0 tons of the required CM per ha and B. NTLG2-5 inoculation, WB3 got significantly 3.0 tons of lime + 5.0 tons of CM per ha and B. NTLG2-5 inoculation, all applied through inorganic fertilizer. In the experiment, alongside seed sowing, nitrogen (40 kg urea/ ha), phosphorus (60kg P_2O_5/ ha) and potassium fertilization (60 kg K_2O/ ha) was fertilized to all treatments. The planting layout included all hole distances of 30 cm x 25 cm, with two seeds sown per hole. The "BI" while bean variety has a growth period of approximately 90 days, ensuring rapid growth, health, and high yield. The whole experiment area covered 320 m^2 (2 m x 10 m x 4 replicates x 4 plots).



(a)

GCTTCTATGACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGA
CTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGA
GATGATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTA
GTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATC
GGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAAT
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GGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAG
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GTGCAGAAGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGA
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CCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTA
ATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGA

(b)

Figure 1(a):The relationship between different species of IENB isolated from while bean roots on a phylogenetic tree; (b):Sequencing results (5'-3' direction) of B. NTLG2-5 length 1369 bp (Van and Tri, 2024).

TABLE 1
Dosage of different composition in all treatments

Treatment	B. NTLG2-5* (10^8CFU/g)	$CaCO_3$ (t/ha)	CM (t/ha)	Chemical fertilizers (kg/ha)
WB0	No	0.00	0.00	
WB1	Yes	3.00	0.00	40 Urea-60P_2O_5-60K_2O
WB2	Yes	0.00	5.00	
WB3	Yes	3.00	5.00	

*: Bacterium (in: firmicutes) strain NTLG 2-5 genus

Seed preparation and inoculation

Bacterium strain NTLG 2-5 genus species was increased to a quantity of 10^8 colony-forming units per milliliter (10^8 CFU/ mL) on diluted YMA medium. The "BI" variety of while bean seeds, collected from a breeding Mekong delta company in Vietnam, was used for this study. Twenty-four hours before sowing in darkness, the while bean seeds were evenly sprayed and subsequently inoculated with *Bacterium* strain BS1897. The inoculation of B.NTLG 2-5 species was applied to while bean seeds intended for sowing in all plots, except for treatment WB0.

Measurement of agronomic and yield traits

Agronomic, yield, and while bean productivity traits were systematically observed from 20, 45 and 60 DAS. The properties were followed such as plant height, available branches, pod number, seed number per pod, 1,000 seed weight and fresh pod yield per ha. Furthermore, for determining the As uptake of while bean sterms and seeds.

Soil analysis

Farmland samples of the initial and end the experiment, which were collected from all repeated sites to analyzed the impacts induced by the performed treatments on some soil traits. Farmland pH value was determined by a pH meter with a soil-to- water ratio of 1:2.5. Mineral nitrogen content was analyzed using the Kjeldahl method, while available phosphorus was determined employing the alkaline hydrolysis method, as outlined by Carter and Gregoric (2007). The As content of farmland before and after the experiment were analyzed following the methodology outlined by Atomic Absorption Spectroscopy (AAS).

Statistical data

The effects of treatments were counted through ANOVA using Statgraphics software version XVIII. Datum Means were analyzed and compared with stastically significant differences $P \leq 0.05$.

RESULTS AND DISCUSSION

Farmland chemical-physic compositions at the initial and end experiment

The soil samples'results collected from the experimental sites prior to the planting commencement of the study, it was shown that the initial soil properties of the study area consisted of sandy loam texture (60.5% sand, 35.0% silt, and 4.5% clay), exhibited a low soil and irrigated water pH of 5.35 and 4.99, respectively, and possessed medium levels of soil organic matter (SOM:2.60%), total nitrogen (0.122%), medium available phosphorus (153 mg/kg) and low exchangeable potassium (124 mg/kg). Especially, As contents of experimental soil (86.1 mg/kg) and irrigated water (356 µg/L) were quite high (**Table 2**).

Soil pH, SOM, total N, available P and exchangeable K contents resulting from the amendments were observed through soil analysis results, as presented in **Table 3**. According to the attained results, it was proved that the B. NTLG2-5 species, applied in combination with lime, CM application significantly affected the examined farmland attributes. Despite the first soil pH of 5.35, which valued from 4.92 to 6.17 in all treatments, the highest pH was attained in the WB3 treatment, where B. NTLG2-5 inoculation was associated with 3.0 t$CaCO_3$/ha + 5.0 t CM/ha and all required chemical fertilizers for the plant was supplied. On contrary, the minimum pH was shown in the control (WB0) treatment. Similarly, all soil nutrient traits of SOM, the total N, available P and exchangeable K contents, which obtained the maximum in the WB3 treatment and the lowest values was found in the WB0 treatment (**Table 3**). Studies showed that the B. NTLG2-5 inoculation of the seeds, combined with chemical fertilizers at different doses increases all traits of pH, SOM, total N, P and K concentration in the farmland at harvest season (**Table 3**). The prior research of Etesami, (2022) proclaimed that IENB had the positive ability to contribute the huge amount of natural N sources for the crops from their N-fixing process. Furthermore, IENB can convert from an insoluble P to a soluble P and produce IAA in order to provide available nutrition for plants (Rajendran *et al.,* 2012). Soil organic matters increased and had more available nutrients of farmland, which supplied a remarked carbon content for IENB, helped to raise the N-fixing fixation capacity and peanut productivity (Wang *et al.,* 2021, Nie *et al.,* 2019).

TABLE 2

Physic and chemical of soil samples took from plots at the initial experiment

Farmland properties		Values	Farmland properties		Values
Mechanics	Sand (%)	60.5	Soil nutrients	Total N (%)	0.112
	Clay (%)	4.50		Available P (mg/kg)	153
	Silt (%)	35.0		Exchangeable K (mg/kg)	124
	Texture	Sandy loam		Soil organic matter (%)	2.60
Chemical	pH_{soil}	5.35	As contents	Irrigation water (µg/L)	356
	$pH_{\text{deep well water}}$	4.99		Experimental Soil (mg/kg)	86.1

TABLE 3

Changes in soil chemical traits of soil samples collected from plots at harvest season

Treatments	pH	SOM (%)	Total N (%)	Avail. P (mg/kg)	Exch. K (mg/kg)
WB0	4.92 ± 0.016d	2.31 ± 0,008d	0.180 ± 0.008c	113 ± 3.86c	122 ± 1.63c
WB1	5.45 ± 0.040c	2.51 ± 0.011c	0.177± 0.012d	200 ± 1.00a	150 ± 2.59ab
WB2	5.90 ± 0.080b	2.61 ± 0.052b	0.210± 0.026b	155 ± 3.09b	132 ± 3.15b
WB3	6.17 ± 0.025a	2.87 ± 0.057a	0.258 ±0.026a	208 ± 4.52a	167 ± 1.60a
F_{test}	**	**	**	**	*
CV (%)	9.50	8.50	17.6	21.7	18.2

*: $p \leq 0.05$; **: $p \leq 0.01$; CV (%) expresses the coefficient of variation; Sign (±) indicates the mean standard deviation of four repeats; The same letter denotes inadequately different means in a column for each trait.

Agronomic compositions at 20, 45 and 65 days after sowing (DAS)

The effect of lime, CM, B. NTLG2-5 inoculation and As-contaminated irrigation water were sufficient influences on the height and effective branch number of white bean plants throughout the observation period of 20, 45, and 65 DAS (**Figures 2** and **3**), and were statistically significant differences at the 5% level among the experimental treatments. White bean height and available branch number gradually increased in the growth time period. The highest height and effective branch number of of white bean plants obtained in control treatment at harvest. On contrary, The lowest height of white bean plants observed in treatments WB3 , and the lowest effective branch number in the WB1 and WB2 treatment at harvest. The height and effective branch numbe of white bean was remarkably affected by soil nutrients and environmental factors, which consisted of irrigation water, cultivation techniques, and climate (Lozano et al., 2017). Aipa and Michael, (2018) demonstrated that the beneficial effects of CM, lime, and *Rhizobium* sp. helped to increase the yield components of peanut plants such as height and effective branches.

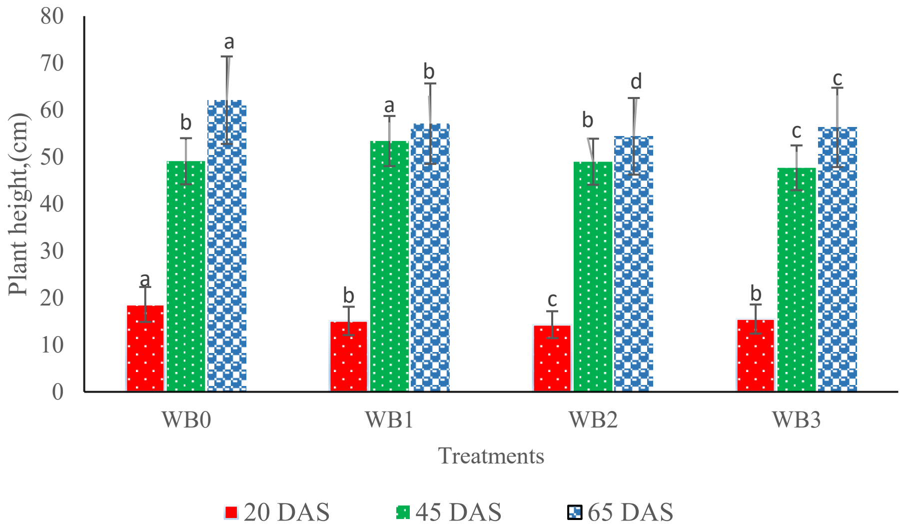

Figure 2. Changes in plant height of while bean during growth period from plots of the experiment

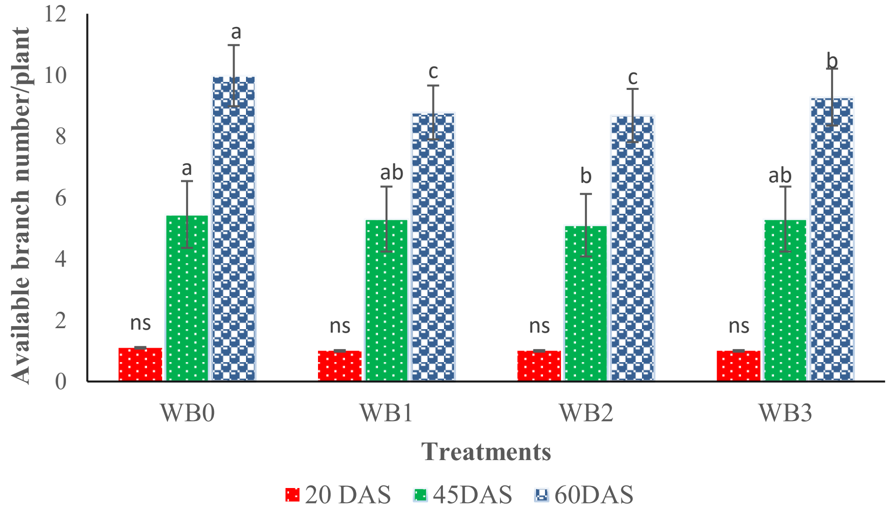

Figure 3. Increases in available branches of while bean during growth period from experimental plots

Yield composition and yield at harvest

The pod and seed number, and the weight of 1,000 seeds showed significant differences among the fertilizer treatments. The highest values of the pod number per plant (39.5 pods), the seed number per pod (14.3 seeds), and the weight of 1,000 seeds (199 g) were obtained in the treatment with NPK + lime + CM and B. NTLG2-5 inoculation compared to the other treatments (**Table 4**). Treatment WB3 (NPK + lime + cow manure and B. NTLG2-5 inoculation) with the application of 40 kg urea + 60 kg P_2O_5 + 60 kg K_2O per ha and the addition of 3.0 t$CaCO_3$/ha + 5.0 t CM/ha with B. NTLG2-5 inoculation gave the highest yield (5.59 t/ha). Meanwhile, the lowest white bean yield (3.70 t/ha) was found in the control treatment (WB0) using only 40 kg urea + 60 kg P_2O_5 + 60 kg K_2O per ha. The following high values for white bean yield were obtained by using 40 kg urea/ha + 60 kg P_2O_5/ha + 60 kg K_2O/ha + 5.0 t CM/ha (4.48 t/ha) and 40 kg urea/ha + 60 kg P_2O_5/ha + 60 kg K_2O/ha + 3.0 t CaCO3/ha (5.13 t/ha), respectively (Table 4). The white bean yield was calculated by the growth process of the plant from the seedling to the end of the experiment in which the nutrients in the soil play an important role. The application of lime, CM, inorganic fertilizer and B. NTLG2-5 inoculation significantly affected the production components and yield of white bean such as the pod number per plant and average seed number per the pod, the weight of 1,000 seeds and fresh yield (**Tables 4**). Recent studies have demonstrated the strong impact of the combined use of inorganic, organic fertilizers, and the nitrogen-fixing bacterium addition on crop growth and yield (Li *et al.,* 2017; Han *et al.,* 2021, Van, 2024). A study conducted by Sihi *et al.* (2020) and Sheoran *et al.* (2019) showed that a formulation containing indigenous nitrogen-fixing bacterial species, when combined with a reduction in the amount of chemical fertilizer to various rates, increased crop yield and improved soil microbial activity. Additionally, Ye *et al.* (2022) and Nguyen (2024a) found that a balanced combination of microbial formulation with fertilizer increased the seed yield in plants under field conditions. However, there is limited research on the impact of the simultaneous use of chemical fertilizer and microbial formulation on soil quality and crop nutrition. Timmusk *et al.* (2017) called this strategy integrated nutrient management, which includes the use of organic manures, adjusted chemical fertilization, and supplemental microbial formulations.

TABLE 4

Effects of treatments on pods, seeds, weight of 1,000 seeds and fresh yield of while bean

Treatment	Pods/plant	Seeds/pod	1,000 seeds weight (g)	Fresh yield (t/ha)
WB0	27.8±0.653d	10.5±0.408b	188±2.44b	3.70±0.163d
WB1	36.3±0.245b	9.50ᶜ±0.408c	200±8.16a	5.13±0.024b
WB2	33.5±0.408c	11.0±0.817b	194±3.27ab	4.48±0.065c
WB3	39.5±0.408a	14.3±0.245a	199±7.37a	5.59±0.073a
Ftest	**	**	*	**
CV(%)	12.9	16.9	3.68	15.6

*: $p \leq 0.05$; **: $p \leq 0.01$; CV (%) expresses the coefficient of variation; Sign (±) indicates the mean standard deviation of four repeats; The same letter denotes inadequately different means in a column for each trait.

Arsenic concentrations in soil, stems, leaves and seeds at harvest

The As uptake in stems ranged from 0.410 to 1.20 mg/kg and was significantly different at the 1% level. The greatest As content of 1.18 mg/kg in the white bean stems was observed in the treatment WB0 and the minimum value was 0.410 mg/kg in the treatment with application of chemical fertilizer combined with 5.0 tCM/ha and B. NTLG 2-5 inoculation. Conversely, the As concentration from agricultural soil was not absorbed into the white bean seeds in all treatments (**Table 5**). However, the As concentration of the farmland in the initial and end experiment showed significant differences at the 1% level, which ranged from 85.1 to 86.7 mg/kg and 89.6 to 117 mg/kg, respectively. Overall, the highest and lowest As level of the farmland were 89.6 mg/kg when using inorganic fertilizers, CM, lime, associated with B. NTLG2-5 inoculation and 117 mg/kg when using inorganic fertilizers, lime associated with B. NTLG2-5 inoculation (**Table 5**). Table 5's presentation proved that the combined fertilization of inorganic fertilizers, CM, lime, and B. NTLG 2-5 inoculation remarkably reduced As accumulation and increased the white bean yield, grown on As-contaminated soil and irrigation water in An Phu. There is an inverse relationship between soil pH and As uptake by plants when soil pH is appropriately raised by lime and organic fertilizer (Nguyen, 2024b; Anh *et al*., 2018). Tuan and Chương, (2022) found out that the addition of lime, vermicompost, and *Rhizobium* inoculation reduced the As store in stems, leaves, and seeds and increased peanut yield. Benvindo *et al*. (2013) presented that the application of 10 t CM/ha or 5 t CM/·ha in combination with lime or mineral fertilizer improved soil fertility, increase N uptake and soybean yield. The use of inorganic fertilizers in combination with the organic manure addition has been shown to be a solution to improve N uptake in soybeans.

TABLE 5

Effect of CM, lime, B. NTLG 2-5 on the white bean output and As uptake

Treatment	As concentrations (mg/kg)			
	stems	seeds	experiment Soils	
	harvest		initiation	harvest
WB0	1.20±0.065a	undetected	86.0±0.817	96.7±0.168b
WB1	0.940±0.033b	undetected	85.1±0.49	117±0.816a
WB2	0.410±0.025b	undetected	86.7±0.082	96.6±0.245b
WB3	0.940±0.033c	undetected	85.8±0.082	89.6±0.082c
*F*test	**	-	**	**
CV(%)	32.7	-	0.57	9.13

*: $p \leq 0.05$; **: $p \leq 0.01$; CV (%) expresses the coefficient of variation; Sign (±) indicates the mean standard deviation of four repeats; The same letter denotes inadequately different means in a column for each trait.

CONCLUSION

From the research results, it was shown that the effect of chemical fertilizer application with the addition of lime, CM, and the B. NTLG2-5 inoculation has improved soil fertility in a positive direction, promoted the white bean growth and increased fresh yield compared to the control treatment. Furthermore, As accumilation remarkably reduced on the stems in treatments of lime, CM application combined with B. NTLG 2-5 inoculation and did not accumulate As on the white bean seeds. Application of 40 kg urea/ha + 60 kg P_2O_5/ha + 60 kg K_2O/ha combined with 3.0 t $CaCO_3$/ha + 5.0 t CM/ha and the B. NTLG2-5 inoculation showed over 33.0% increase in fresh pod yield compared to only chemical fertilizer application. Moreover, lime applied alone or mixed with CM reduced As accumulation of white bean in the stems from up to 60% compared to the control treatment. These research results encourage the simultaneous use of lime, CM, endolopic N-fixing bacteria, and chemical fertilizers to have a positive effect on increasing yield and reducing As uptake of white bean, reducing environmental impacts due to As pollution in agricultural soil and irrigation water.

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