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Nitrogen fertilizer use reduction by two endophytic diazotrophic bacteria for soil nutrients and corn yield

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Abstract

Today, the utilization of endophytic diazotrophic bacteria (EDB) purposely to minimize the application of urea fertilizer (UF), and enhance soil fertility, crop quality and corn yield in sustainable agricultural practices is an inevitable trend. The experiment was arranged outside the AGU green house of Agriculture Research Center, An Giang university, Vietnam, which consisted of two factors: (i) two EDB species [*Bacillus* sp. NTLG2-20 (*Bacillus* A) and *Bacillus arybhattai* strain CM44 (*Bacillus* B)] and (ii) three UF ratios (0.0, 100 and 200 kg N ha^{-1}) and four replications. Research data presented that the fresh cob yield of *Bacillus* A was found 4.0% higher than that of *Bacillus* B and 12.7% than that of non EDB inoculation. Furthermore, the inoculation of Bacillus B had 9.02% higher fresh cob yield compared to non-inoculation. The 50% reduction of UF application combined EDB addition obtained the fresh cob with no significant difference, compared to 100% recommended urea application. The interaction between the effectiveness of EDB inoculation and UF reduction was clearly different in favor of corn grown on sandy loam soils with low nutrient contents. Both *Bacillus* A and *Bacillus* B had the abilities of high yield and good nitrogen fixation with the potential to simultaneously improve soil fertility and corn yield. The results of this study demonstrated that two strains of *Bacillus* A and *Bacillus* B have the potential to promote the growth and increase the yield of peanuts, and it should be suggested for future biological fertilizer production.

Keywords: Bacillus; corn; EDB; UF reduction; urea

1. Introduction

Consumed all over the world, corn (*Zea mays* L.) is one of important cereals and a widely cultivated crop providing high profit for farmers. In fact, though the demand for corn consumption has remarkably increasing in Asian countries, corn yields have been decreasing in the recent years in relation to the change in farming practices, chemical fertilizer types, and cultivation methods [1-3]. The high sandy content of land has led farmland to nutrient loss due to both poor soil retention and low soil fertility. Since soil nutrients are under the required levels for plant growth, farmers have to use inorganic fertilizers to compensate for the lack of nutrient supply of these soils [4,5]. In tropical areas with high rainfall, soils are easily affected by nutrient loss leading to soil degradation [4,6]. Moreover, long-term and high-dose use of inorganic fertilizers can lead to the formation of a hardpan layer, later on reducing permeability, water infiltration, retention capacity, as well as corn yield [7]. The use of intensive chemical fertilizer is potential to reduce the rate of organic matter mineralization in the soil as well as the ability of nitrogen fixation by symbiotic microorganisms [8,9]. Nitrogen element is a macronutrient that provides corn plants, combined with Mg to form the crop chlorophyll for raising photosynthesis. In corn production, nitrogen deficiency symptoms can be one of the most common nutritional problems. Chlorophyll content in corn leaves is positively correlated with both N content for photosynthesis and increasing corn yield [10]. Endophytic diazotrophic bacteria in Low-N soil play an important role in the supply and management of the N cycle in soil. The N conversion from air to ammonia is performed by two groups of EDB [11]. The inoculation of *Klebsiella quasipneumoniae* species, which is EDB species and isolated from corn roots, is reduced by the application of nitrogen fertilizer (50% of 300 kg N ha^{-1}). The potential of EDB inoculation remarkably reduces inorganic nitrogen fertilizer to raise the natural N concentration in soil and corn yield and quality [12].

Four EDA species are isolated from maize roots that are capable of growing on N-free medium, consistently reducing acetylene, and being detected positive for the presence of the nifH gene. In addition to the ability to provide positive results for N-fixing activity, two of these four species (identified as *Bacillus* sp.) also exhibit other abilities, such as IAA production, and resistance of root rot fungi. When all of these

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EDB species are inoculated with other endophytic strains belonging to the *Enterobacter* genus, it can significantly enhance seed germination by 47% and root volume by 44% [13]. Increasing crop yields without negatively impacting the environment has been becoming a major challenge for the global agriculture. The use of EDB species, which is a sustainable solution for agriculture, can provide soil nutrients as well as protect plant health. The interaction's research among plant-bacteria-environment can support the development of more effective bio-products in the future, which can contribute to more sustainable agricultural practices [14]. Endophytic diazotrophic bacteria do not form nodules in a large number of agriculturally important plants, but produce the large amount of nitrogen taken from the air and directly into the soil. This highlights the importance of research with an aim to produce non-nodulating diazotrophic bacteria as probiotics [15] . *Bacillus* strain inoculation, in addition to its role in nitrogen fixation and growth promotion for some agricultural crops, is an attractive agricultural practice that is deemed more efficient with insignificant impact on products and environment in comparison to chemical pesticides and fertilizers. This then has made the use of endophytic nitrogen-fixing microorganisms as a more sustainable solution from the perspective of profit and environment [16]. Today, some EDB species have been increasingly being studied and applied to improve soil fertility and increase crop yields [17]. Endophytic diazotrophic bacteria have an ability to decompose organic matters, fix natural nitrogen, and resist root rot diseases and the toxicity of heavy metals in the soil, which helps healthy roots to absorb more available nutrients for promoting plants grow better [18]. The strain number of EDB in the soil is closely related to the physical and chemical properties of the farmland [19,20]. Thus, it reflects that the ability to increase soil fertility highly depends on the microbial species and soil texture [21,22]. For this reason, study on these two EDB species and their use reduction efficiency of chemical nitrogen fertilizer to improve to soil fertility, and corn yield and increase profits for corn farmers needs to be carried out.

2. Materials and Methods

2.1 Location description

An experiment on corns was conducted at agriculture Research Center of AGU, Vietnam, located in a tropical climate zone and on both banks of the Mekong River. The northernmost point is located at the latitude of 10°57'N, the southernmost point is at the latitude of 10°10'60"N, the westernmost point is at 104°46'E, and the easternmost point is on longitude 105°35'E. The largest distance north - south direction is 86 km and east - west direction is 87.2 km with the latitude of 29°N and 79.3°E. Rainy season has the maximum temperature up to 38°C (March to May), while the minimum temperature is at 22°C (November to December). The relative humidity was high during the experiment period and the heaviest rainfall was recorded from July to October.

2.2 Two EDB species and growth conditions

Throughout the experiment, river water was used for irrigation. Soil temperature was in the range of 25 to 32°C, and soil pH was between 6 and 7. These conditions were highly suitable for the growth and activity of the two bacterial

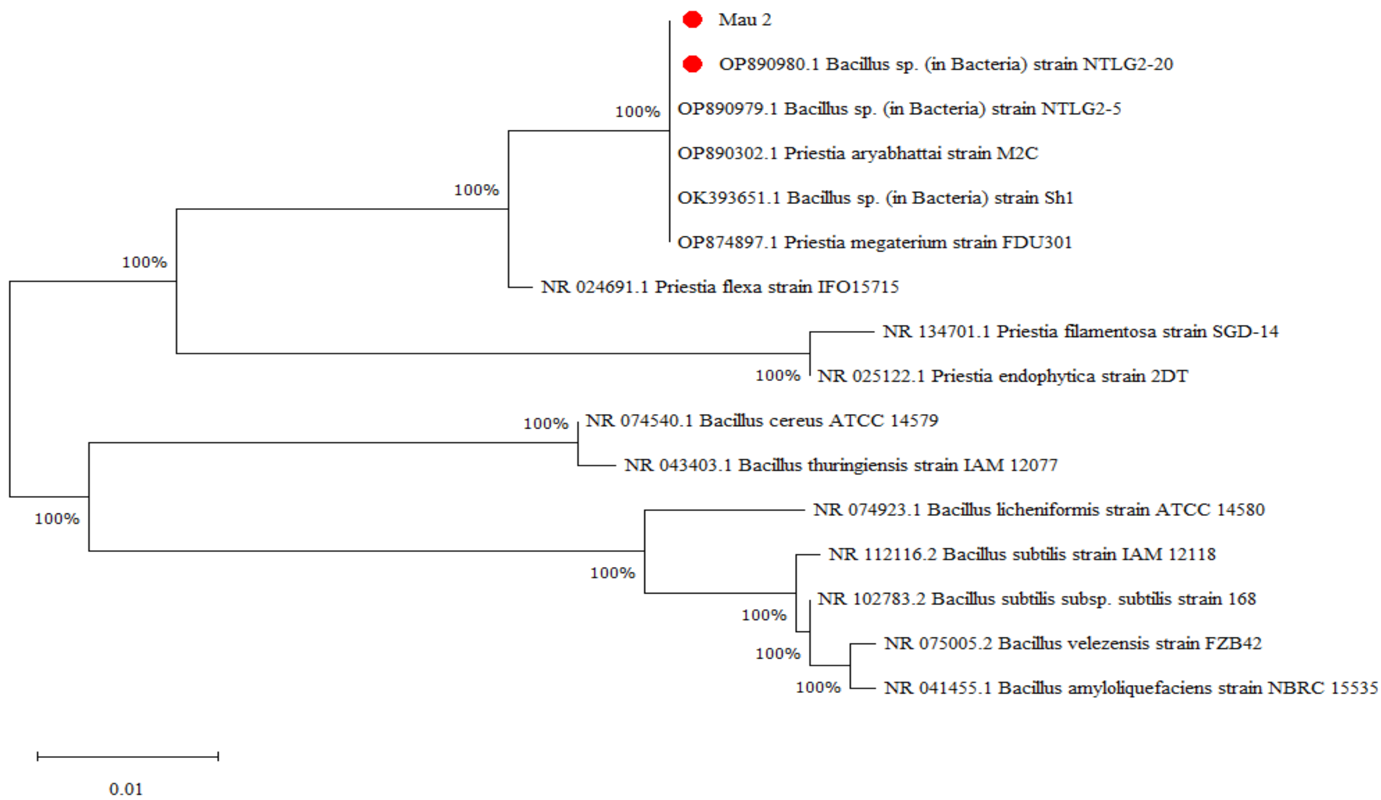

Fig. 1. *Bacillus* sp. NTLG2-20 (Mau 2), 16S ribosomal RNA gene, partial sequence; chloroplast; and large subunit ribosomal RNA gene, partial sequence, results describe organisms with highest percent identity with sample sequence (100%)

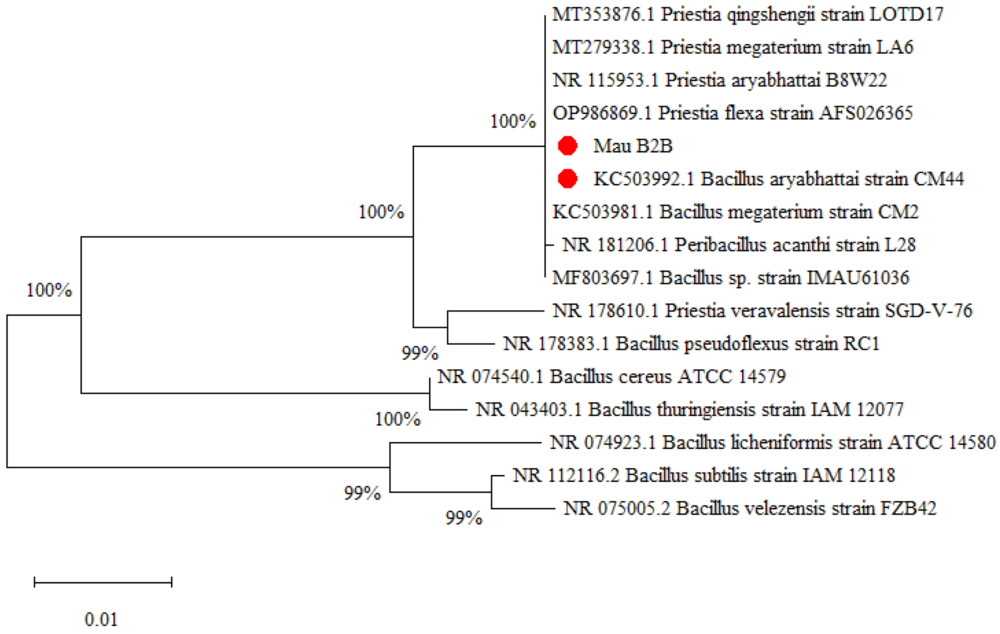

Fig. 2. *Bacillus arybhattai* strain CM44 (Mau B2B), ribosomal RNA gene, partial sequence; chloroplast; and widely molecular ribosomal RNA gene, partial sequence. Results describe organisms with highest percent identity with sample sequence (100%)

strains. Two EDB species were isolated and identified from corn roots taken from the corn farms in Cho Moi district, An Giang, Vietnam. Pure colonies were selected and sequenced by the 16S rRNA gene of all chosen isolates. Subsequently, the obtained sequences of the two species (*Bacillus* sp. NTLG2-20 and *Bacillus arybhattai* strain CM44) proclaimed 100% of similarity and were more closely related to other bacterial species, deposited in the NCBI database use of the BlastN programme. Furthermore, a phylogenetic tree was constructed to determine the position of the two isolated species along with sequences from selected reference strains (Fig. 1 and 2). Two selected EDB species that were completely increased in their population up to 108CFU mL^{-1}, were well inoculated for corn seeds for applying directly to the soil [23].

2.3. Experimental location and materials

An experiment, conducted outside the greenhouse at the Agricultural Research Center of An Giang University (Table 1), included two factors: (i) without EDB (*Bacillus* sp. NTLG2-20 and *Bacillus arybhattai* strain CM44) and (ii) three UF ratios (0.0, 100 and 200 kg UF ha^{-1}) and four replications. The experiment was conducted by means of a randomized, completely randomized block experiment design with two factors. The first factor referred to the application of EDB (no inoculum and inoculum of *Bacillus* sp. NTLG2-20 and *Bacillus arybhattai* strain CM44); The second factor was evaluated by reducing UF rates (50% and 100%) and by using the urea resource as the N source before being converted to the N weight. In addition, there were three controls treatments, consisting of no N fertilization (0%) and no inoculum of the two EDB and having a total of 9 treatments (Table 1). Corn seeds were taken from Northern Seed Research Center, Vietnam. All experimental plots were repeated and were designed in a completely randomized block design. Each replicate measured 1m wide and 2m long, which are spaced 0.5m apart. The total experimental area was 288 m^2 (1m x 4m x 4 replicates x 9 treatments). Each replicate consisted of 16 holes (two seeds per hole) and the distance of two holes were 30 cm. The plants had 3 leaves that only kept one heathy plant per hole for the experimental duration. Soil chemical properties before the experiment included pH (7.2), soil organic matter (SOM:1.54%), total N (TN: 681 mg kg-1), available P (AP:485 mg kg^{-1}), and total K (undetected). Meanwhile, fertilization was based upon the physicochemical properties of the soil and the fertilization rates used in the field (200 UF kg ha^{-1}), diammonium phosphate (P_2O_5) (80 kg ha^{-1}) and KCl (100 kg ha^{-1})]. Here, P2O5 was applied one day before sowing; while urea and KCl were applied after seed germination. Bacterial suspension with a density of 108 CFU mL^{-1} was directly applied to the soil around the plants (10 mL^{-1} flask). Thinning of weak plants aimed to leave only one strong plant when the plants have three leaves. The soil

Table 1. Used methods of two EDB species and UF rates per experimental treatment

Treatments	EDB species (10^{8}CFU mL^{-1})	UF ratios (kg ha^{-1})	Inorganic fertilizers (kg ha^{-1})
Corn 1	No inoculation (No)	0	80 P_2O_5-100 KCl
Corn 2		100	
Corn 3		200	
Corn 4	*Bacillus* sp. NTLG2-20 (*Bacillus* A)	0	
Corn 5		100	
Corn 6		200	
Corn 7	*Bacillus arybhattai* strain CM44 (*Bacillus* B)	0	
Corn 8		100	
Corn 9		200	

texture of experiment was sandy loam, containing traits of sand (80.2%), silt (18.6%) and clay (1.2%) of the 0–20 cm soil layer. Humidity, lipid, protein and dry matter contents of corn seeds were determined by Silva et al. (2016) [24]. The average values of all 10 corn plants were randomly selected per replication, observed and counted all traits of agronomy, yield and fresh pod productivity. Corn earns were carefully selected to analyze the seed's nutrition. Agronomic, yield components and yield were observed during the growth period of corn plants, including plant height, leaf number (No.), total chlorophyll, cob length, cob diameter, number of row per cob, number of seeds per row, weight (Wt.) of biomass, Wt. of 1,000 seeds and fresh cob yield.

2.4. Research data

Each replication that was randomly chosen eight plants, was averagely measured the developmental period of 15, 30 and 60 DAS such as the plant height, total chlorophyll content and No. of leaves per plant. Yield traits and yield of corn consisted of Cob length (cm), No. of row per cob (rows) and seeds per row (seeds), plant biomass (t ha^{-1}), Wt. of 1,000 seeds (gr.) and fresh cob yield (t ha^{-1}). Nutrition composition included percentage of humidity, lipid, protein and dry matter content

2.5. Statistical data

To analyze the variance (ANOVA) for all data studied, the stat graphics version XVI software was used. The mean values between variables were compared by means of the Duncan test at statistically significant differences of *P value ≤ 0.05, **P value ≤ 0.01 and ^{ns}P value > 0.05. The interaction between two factors was calculated based upon research data to find their interaction.

3. Results and Discussion

3.1. Farmland traits in initial and end experimental

As shown in Table 2, it was observed that there were no sufficient differences between inoculation methods of the *Bacillus* A and *Bacillus* B species and three NF rates for traits of soil pH, SOM, N and available P at the experimental initiation. However, as shown in Table 3, it could be observed the sufficient differences between plots of factor (A) and factor (B) at * Pvalue ≤0.05 and **Pvalue ≤0.01, respectively in the end of experiment. Soil properties consisted of pH, SOM and AP (Except TN for factor B). Nonetheless, still in Table 3 it was observed that an interaction between two factor (FAxB) were sufficiently different at **Pvalue ≤0.01 between the traits of SOM, TN and AP, except for pH. The inoculation of *Bacillus* A and B depicted the highest pH and SOM values compared to the non-two species. Other traits, consisting of TN and AP, had the highest values with the plots of *Bacillus* A inoculation, followed by *Bacillus* A inoculation, and the lowest one was found in the one without *Bacillus* A and *Bacillus* B inoculum. Similarly, the soil properties, those are pH, SOM, TN, and AP at the treatments without urea application obtained the lowest values, followed by 100 kg UF ha^{-1}, and the highest in application of 200 kg UF ha^{-1}.

Table 2. Farmland chemical traits at the initial experiment

Factors	pH	SOM (%)	TN (mg kg^{-1})	AP (mg kg^{-1})
EDB species (10^{8}CFU mL^{-1}) (A)				
No	7.01±0.075	1.52±0.017	676±11.2	480±14.6
Bacillus A	7.05±0.074	1.54±0.017	692±9.40	500±5.33
Bacillus B	7.02±0.074	1.53±0.017	683±8.75	492±7.74
Urea ratios (kg UF ha^{-1}) (B)				
0.00	7.01±0.075	1.52±0.017	692±4.46	507±9.11
100	7.04±0.074	1.53±0.018	675±13.9	491±7.97
200	7.04±0.075	1.54±0.017	683±8.75	474±11.1
F(A)	ns	ns	ns	ns
F(B)	ns	ns	ns	ns
F (AxB)	ns	ns	*	ns

*: *significant differences at P ≤0.05 level; ns: no statistically significant differences.*

Table 3. Farmland chemical traits in the experimental end

Factors	pH	SOM (%)	TN (mg kg^{-1})	AP (mg kg^{-1})
EDB species (10^{8}CFU mL^{-1}) (A)				
No	6.75±0.092b	1.45±0.018b	617±50.0c	333±7.98c
Bacillus A	7.29±0.086a	1.75±0.072a	750±54.0a	583±9.42a
Bacillus B	7.32±0.087a	1.73±0.070a	700±44.8b	400±4.26b
Urea ratios (kg UF ha^{-1})				
0.00	6.93±0.119b	1.42±0.016b	467±14.5c	433±37.9
100	7.17±0.101ab	1.75±0.066a	783±29.1b	433±26.1
200	7.26±0.110a	1.77±0.063a	817±19.9a	450±32.9
F (A)	**	**	**	**
F (B)	*	**	**	ns
F (AxB)	ns	**	**	**

*,**: *significant differences at Pvalue ≤0.05 and 0.01 level, respectively; ns: no statistically significant differences.*

The synergistic effects of EDB inoculation and their nitrogen fertilizer use efficiency on soil health improvement were on plant growth and yield. Enhanced soil fertility and microbial activity contributed to better nutrient availability, INF use reduction and ultimately, higher peanut yields and profits [25]. Endophytic diazotrophic bacteria have been being used as biofertilizers to minimize the chemical fertilizers use, thus improving nutrient use efficiency, increasing crop productivity, and reducing environmental pollution. The root nodule creation of plants is the metabolism functions through a symbiotic relationship between crops and EDB. The nodule function that contains the N conversion of atmosphere by EDB provides a majorly natural nitrogen weight for plants and this process is realized by N-fixing bacteria, which is called as biology N fixation [26]. Penetrating the host plant is through the inner cells/tissues of the roots or the junctions between the intercellular junctions on the root surface. When ENFB enters inside the plant host's root, it can establish a symbiotic relationship with the host plant and quickly adapt in the nutrient-rich environment in exchange to protect the health of their host plants. [27-29]. EDB has a beneficial ability to supply the large amount of natural nitrogen of the next crops from their N-fixing process. Furthermore, it is able to convert from an insoluble P to a soluble P and produce IAA to provide available nutrition for plants [30]. Organic manure fertilization increases the available nutrients of farmland, which supplies a carbon element for EDB, and helps to raise the N-fixing fixation capacity and peanut productivity [31].

3.2. Plant height, leaves number and total chlorophyll of corns

As shown in Table 4, it was observed that the plant height growth in the period of 15, 30, and 60 DAS was insignificantly different during 15 and 30 DAS [(Excepting for 60 DAS of factor EDB (A)]. In contrast, there was a significant difference at level 1% in three different urea ratios (0.0, 100 and 200 kg urea ha^{-1}). The maximum plant height was obtained at the rate of 200 kg urea ha^{-1}, followed by 100 kg UF ha^{-1} and minimum value was found in the ones without urea application. The next trait, i.e. number of leaves per plant, had no significant difference in the period of 30 and 60 DAS (expect for 20 DAS) in factor EDB species (A). Inversely, leaf number per plant of three urea ratios were significant different among plots at level 1% (Table 4). The highest leaf number was attained at the application of 200 kg urea ha^{-1}, followed by 100 kg urea ha^{-1}, and the lowest value was found in the treatment without urea application. The total chlorophyll was statistically different at 30 and 60 DAS between plots of EDB species and urea rates at level 5% and 1% (expect 20 DAS). Overall, the inoculation of *Bacillus* A and B had the highest values of plant height at 60 DAS and total chlorophyll at 30 and 60 DAS. However, there were remarkable differences in the ratios of urea application, obtaining the maximum values of plant height, leaf number and total chlorophyll in the plots of 200 kg UF per ha, followed by 100 kg urea ha^{-1}, and the lowest values were found in the one without urea application. Their interactions were unremarkably different between the factors (Table 4)

EDB inoculation significantly increased the peanut plant height, leaf number and total chlorophyll and reduced 50% of UF application, which were both 50% reduction of UF and raised peanut output higher than non-EBB inoculum and be applied by 100% of UF [32]. The height, leaf number and total chlorophyll content of corn plants in the inoculated experiments of EDB and urea fertilization were higher than that of the control (no bacteria and no urea fertilization), which contributed to better bioavailability and nutrient uptake in the corn root zone. Plant growth stimulation due to EDB inoculation into the soil was able to improve nutrient acquisition due to increasing root volume and plant photosynthetic function [30]. EDB strains played a main role to raise natural N resources into the agricultural soil, transform insoluble N into soluble N to help crops to absorb more available nitrogen easily. Nitrogen is a macro element coordinated with Mg t to form and synthesize chlorophyll, and increase their photosynthetic capacity. Crops absorb more nitrogen to contribute to develop higher plant height, more shoots, leaves and chlorophyll index compared to treatments without EDB inoculum [31, 33].

3.3. Corn yield traits

Results in Table 5 showed that inoculated and uninoculated plots of *Bacillus* A and *Bacillus* B were insignificantly affected on cob diameter, number of rows per cob and seeds per row (Expect Cob length). Inversely, the plots of three urea ratio fertilization (0.0, 100 and 200 kg ha^{-1}) were insignificantly affected on all yield traits at level 1% (**$P \leq 0.01$). The traits of cob length, cob diameter, number of row per cob and seeds per row attained the maximum values in the plots of 200 kg urea per ha, followed by 100 kg urea ha^{-1}, and lowest values were found in the one without urea application. Their interactions were not different between facto A and B (Table 5).

The inoculation of three EDB species for planting baby corn, which consisted of *Bacillus* sp., *Pseudomonas* DJ06, and

Table 4. The growth traits of corn during the growth period of 15, 30 and 60 DAS

Factors	Plant height (cm)			No. of leaves (leaves $plan^{-1}$) DAS			Total chlorophyll (mg per m^2)		
	15	30	60	15	30	60	15	30	60
EDB species (10^8CFU mL^{-1}) (A)									
No	17.8±0.584	38.1±3.09	121±5.03b	4.44±0.250b	5.52±0.289	7.81±0.497	371±4.24	419±7.34b	470±15.2b
Bacillus A	18.8±0.732	44.8±2.98	131±3.25a	5.08±0.210a	5.83±0.311	8.69±0.508	373±4.32	442±8.52a	510±15.5a
Bacillus B	18.5±0.696	41.3±3.70	127±3.31a	4.81±0.254a	5.71±0.290	8.58±0.404	372±4.40	438±8.05a	506±16.7a
Urea ratios (kg UF ha^{-1})									
0.00	16.8±0.442b	31.1±1.70c	112±2.73c	3.92±0.149c	4.44±0.116c	6.83±0.308c	369±3.87	408±4.43c	447±8.72c
100	18.5±0.675ab	39.9±2.24b	127±2.29b	4.83±0.104b	6.10±0.152b	8.27±0.335b	371±4.16	431±5.80b	493±12.9b
200	19.8±0.590a	53.2±1.88a	140±1.23a	5.58±0.175a	6.52±0.109a	9.98±0.227a	375±4.71	460±6.22a	547±11.7a
F (A)	ns	ns	**	**	ns	ns	ns	**	*
F (B)	**	**	**	**	**	**	ns	**	**
F (AxB)	ns	ns	ns	ns	ns	ns	ns	ns	ns

**, **: significant differences at Pvalue ≤0.05 and 0.01 level, respectively; ns: no statistically significant differences.*

E asburiae, increased the ear yield of baby corns by 23.1%, 54.1%, and 64.4%, respectively, compared to the un-inoculated plots. *E asburiae* species here achieved the highest ear yield (5.50 t ha^{-1}). In particular, *E asburiae* had the best ability to reduce the use UF amount and increased both soil fertility and the highest baby corn ear yield [34]. The studied results of *E cloacae* ASD-21 and E. cloacae ASD-48 used to product bio-fertilizers showed that they reduced 15% of UF application up to 15% while maintaining yield compared to 100% UF application. Furthermore, they significantly increased the $NH4^{+}$ content in the soil and improved better N uptake compared to no EDB inoculation. The EDB combination showed the higher values in plant height, stem diameter, corn cob length, and cob diameter compared to 100% UF application. Bio-fertilizer containing these two species reduced UF application by 15% while maintaining corn grain yield [35].

Table 5. Yield traits of corn at two different factors of two EDB species and three UF rates

Factors	Cob length (cm)	Cob diameter (cm)	No. of row per cob (row)	No of seeds per row
EDB species (10^8CFU mL^{-1}) (A)				
No	15.1±0.482b	3.98±0.097	11.3±0.446	27.3±1.12
Bacillus A	16.2±0.417a	4.13±0.086	11.7±0.333	29.3±1.02
Bacillus B	15.6±0.523ab	4.08±0.080	11.5±0.289	28.5±0.900
Urea ratios (kg UF ha^{-1})				
0.00	13.8±0.286	3.90±0.064b	10.8±0.270b	25.6±0.645c
100	15.8±0.225	3.98±0.083b	11.3±0.376ab	28.3±1.03b
200	17.3±0.255	4.31±0.068a	12.3±0.305a	31.2±0.638a
F (A)	**	ns	ns	ns
F (B)	**	**	**	**
F (AxB)	ns	ns	ns	ns

***: significant differences at P ≤0.01; ns: no statistically significant difference.*

3.4 Weight of corn Biomass, 1,000 grain and fresh cob

As shown in Table 6 it was observed that plant biomass (t ha-1), Wt. of 1,000 fresh seeds (gr.) and fresh cod yield (t ha-1) were sufficiently different from two factors at *Pvalue ≤0.05 and **P ≤0.01 (Expect for plant biomass at factor A). Wt. of 1,000 fresh seeds (gr.) and fresh cod yield (t ha-1) had the maximum value in the plots of *Bacillus* A, followed by *Bacillus* B and lowest without these two *Bacillus* A and B. Nevertheless, plant biomass, Wt. of 1,000 fresh seeds and fresh cod yield (t ha-1) that were sufficiently different at **P ≤0.01 obtained the highest biomass value in plots of *Bacillus* A inoculation, followed by *Bacillus* B inoculation and lowest in no EDB inoculation. The weights of fresh seeds and fresh cobs attained the highest values in plots *Bacillus* A and B inoculation, and the lowest value was at the plots of without these two *Bacillus* A and B inoculation. The interaction between the two factors A and B was significantly different at 1% level (Expect plant biomass) (Table 6). The fresh cob yield of *Bacillus* A was 4.0% higher than that of *Bacillus* B and 12.7% higher than that of non EDB inoculation. Recent studies have shown the beneficial effects of EDB strains on corn growth and yield in which EDA strains have been reported to have positive effects on soil fertility, corn growth and yield [36]. Corn plants inoculated with EDB species exhibited a maximum plant height and biomass increase of 37.32% and 56%, respectively, while the combination of P fluorescens and P putida resulted in a 59.11% higher stem dry matter compared to the non-EDB inoculated control. Co-inoculation with B megaterium, A chroococcum, and B. mucilaginous has also been reported to significantly improve corn biomass and height with an efficiency equivalent or higher than 50% compared to chemical fertilizer alone [36, 37].

Table 6. Biomass, Wt. of 1,000 seeds and fresh cob yield at harvest season

Factors	Biomass (t ha^{-1})	Wt. of 1,000 seeds (gr.)	Fresh cob yield (t ha^{-1})
EDB species (10^8CFU mL^{-1}) (A)			
No	11.0±0.974	415±6.79c	13.1±0.494c
Bacillus A	12.0±0.985	453±12.5a	15.0±0.444a
Bacillus B	11.4±0.940	432±7.51b	14.4±0.286b
Urea ratios (kg UF ha^{-1})			
0.00	7.62±0.152c	395±4.35b	12.3±0.311b
100	12.2±0.575b	447±7.35a	15.0±0.252a
200	14.6±0.453a	458±6.80a	15.2±0.224a
F (A)	ns	**	**
F (B)	**	**	**
F (AxB)	ns	*	**

**, **: significant differences at Pvalue ≤0.05 and 0.01 level, respectively; ns: no statistically significant differences.*

3.5 Fresh cob nutrition

The synergistic effects of EDB inoculation included UF reduction, soil health improvement on corn growth and yield. Enhanced soil fertility and microbial activity contribute to better nutrient availability, nodulation, and ultimately, higher peanut yields and quality. Table 7 presents that plots of EDB species inoculation and ability of UF use reduction had no impact on the percentage of humidity, lipid, protein, dry matter contents of corn seeds (except for protein in factor B). The percentage of seed protein obtained the maximum content (5.03%) in the plots of 200 kg UF ha-1, followed by 100 kg UF ha-1, and lowest in no urea application. There was an interaction between factor A and B at all seed nutrition compositions. The study corroborated previous research indicating that EDB can enhance nitrogen fixation, increase nutrient availability, and improve soil fertility. Previous studies have shown that corn plants inoculated with EDB strains could enhance beneficial nutrients in the soil, promote plant growth and yield. Furthermore, some EDB strains have an ability to resist root-pathogenic microorganisms, and lead to healthier roots and the production of plant growth hormones. However, prior studies indicated that EDB strains had no significant effects on nutrient components of corn seeds (except for seed protein) [38,39].

Table 7. Nutrient composition of corn seed at harvest season

Factors	Humidity	Lipid	Protein	Dry matter content
		(%)		
EDB species (10^8CFU mL^{-1}) (A)				
No	22.1±0.246	2.74±0.030	4.82±0.066	77.9±0.246
Bacillus A	21.9±0.254	2.74±0.030	4.95±0.063	78.1±0.254
Bacillus B	22.0±0.246	2.74±0.030	4.89±0.062	78.0±0.246
Urea ratios (kg UF ha^{-1})				
0.00	22.3±0.234	2.74±0.030	4.74±0.056b	77.7±0.235
100	22.0±0.236	2.73±0.030	4.89±0.052ab	78.0±0.236
200	21.7±0.237	2.74±0.029	5.03±0.057a	78.3±0.237
F (A)	ns	ns	ns	ns
F (B)	ns	ns	**	ns
F (AxB)	ns	ns	ns	ns

***: significant differences at P ≤0.01; ns: no statistically significant difference*

4. Conclusion

The application of EDB species in combination with a 50% reduction of urea fertilizer in maize cultivation has shown equivalence or higher maize nutrient use efficiency, particularly with the use of 50% UF compared to the full application of 100% urea. The inoculation experiments of *Bacillus* A and B improved soil nutrient components such as pH, SOM, TN, and AP, as well as agronomic parameters (plant height, number of leaves, and chlorophyll content). The plots of EDB species showed a 50% lower UF use efficiency compared to the non-inoculated treatment. Furthermore, the inoculated treatments of *Bacillus* A and *Bacillus* B showed higher urea use efficiency than that of without urea application and no *Bacillus* A and *Bacillus* B inoculation. *Bacillus* B inoculation associated with a 50% reduction of UF fertilization showed a more positive effect on corn agronomic and yield parameters compared to chemical fertilizers and no urea application. This research results indicated that two species of *Bacillus* A and *Bacillus* B have the remarkable function of maize growth promotion. The use of EDB species together with chemical fertilizers has proven to reduce 50% of UF application without affecting maize yield sustainability compared to the maximum use of UF fertilization. Both of *Bacillus* A and B strains have good potential in use as bio-fertilizers in the future.

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