Beneficial Effects of Nitrogen-Fixing Bacteria on the Growth and the Yield of Corn Cultivated at an Giang Province

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Abstract**—** *A net house and a field experiment were conducted in An Phu district, An Giang Province to assess the effectiveness of bacterial strains applied to the NK7328 corn. Bacterial cultures of Bacillus aryabhattai ADR3 and Klebsiella pneumoniae DNR5, which were isolated from rehabilitated corn soils in the Mekong delta Vietnam, were used in this study.The experimented soil type was silty clay loam, pH5.64, and total N (0.063%). The recommended fertilizer dose was NPK 180 kg N+135 kg* P_2O_5*+90 kg* K_2O*/ha and corresponding to 100% N+100%* P_2O_5*+100%* K_2O*. In the treatments, the percentage of nitrogen (% N) increased gradually from 0% N, 25% N, 75% N, 100% N. There were 15 treatments that were growing in pots in the greenhouse, and 20 treatments that were in the randomize complete block designed in the fields experiment. Each treatment had 4 replications.The results of the experiments showed that corns, which inoculated with nitrogen-fixing bacteria, and applied 75% N, helped increasing plant height, stem diameter, leaf color, number of leaves, ear corn height, fresh shoot weight, fresh root weight, dry weight of the plant, amount of N, 1000-seed weight, and the higher yield compared to the corns which applied 100% N and without inoculation. Inoculated corn with Klebsiella pneumoniae DNR5 strains gave a higher yield than inoculated corn with Bacillus aryabhattai ADR3. Thus, inoculating corn with bacterial strains, 25% N could be saved.*

Keywords**— *Bacillus aryabhattai ADR3, fertilizer, hybrid corn NK7328, Klebsiella pneumoniae DNR5, Klebsiella pneumoniae HN1, nitrogen-fixing bacteria.***

I. INTRODUCTION

Nitrogen is a very important nutrient source for plants. Supplying nitrogen to plants is very necessary to meet the growth and the development needs of the plants, and partially compensate for the amount of nitrogen that the plants have been absorbed. To increase productivity, farmers often used a lot of chemical nitrogen fertilizers in their fields. That causes many harmful effects on the soil, changing the physicochemical properties of the soil, reducing soil fertility, losing ecological balance, causing pollution due to nitrate loss, and adverse effects on the ecosystem. Application microbial fertilizers in crops can help improving the microenvironment of rhizosphere soil and alleviating the damage of saline stress.

That free nitrogen-fixing bacteria convert nitrogen into NH_3 under the normal physiological conditions due to the activity of the enzyme nitrogenase [1]. Plants absorb nitrogen to synthesize plant protein. Over the past 10 years, several papers mentioned the effects of plant growth promotion of plant-associated bacteria on corn were published. Test pot and field experiments have been conducted in many countries. According to the study of Piromyou et al. (2011) of *Pseudomonas* sp. SUT19 and *Brevibacillus* sp. SUT 47 promoted the germination and the growth of corn plants grown in Leonard jars. In the field experiment, strains Pseudomonas sp. SUT 19 and Brevibacillus sp. SUT 47 mixed with compost could promote the best growth of corn among all treatments and could be applied as PGPR inoculum for forage corn [2]. Seven trains, which belonged to genera *Klebsiella*, *Enterobacter*, and *Pantoea* isolated from sugarcane showed abilities of nitrogen fixation and the production of IAA. These bacterial strains were tested for their abilities to promote plant growth on potted corn plants at 40 days old [3].

The study of Puneet (1998) showed that the *Azotobacter* sp. strain stimulated seed germinating, rooting and the yield of wheat and corn and increased by 10-15% compared to the control [4]. When applying *Azospirillum lipoferum* strain to corn, the results showed that it reduced 50% of the amount of N used for plants, even the corn yield was still guaranteed [5]. Bacterial *Klebsiella pneumoniae* bacteria fix atmospheric nitrogen to supply wheat [6].

Corn (*Zea may* L.) is an important food crop in the global economy. Corn needs a huge amount of fertilizer for its growth, but this cost is high. In addition, the excessive use of inorganic fertilizer will cause pollution, affect the health of humans and animal [7]. A recent trend in agricultural production is how to improve soil fertility, reduce the number of chemical fertilizers, increase bio-fertilizers and to reduce production costs, reduce environmental pollution, contribute to creating safe products, and developed sustainable ecological agriculture. In previous studies, a collection of bacteria-associated with corn, which had the abilities of nitrogen fixation and IAA production, were selected and stored in the microbiology laboratory of Can Tho University, Vietnam. In this study, two strains of well-characterized bacteria, which were selected from the collection, were continued to be evaluated for their abilities of plant growth-promoting on the host plant as corn. Bio-fertilizer, which was composed of bacterial strains and peat, was applied to the experimented corn at the greenhouse and in the field.

II. MATERIALS AND METHODS

2.1 Description of Study Area

A field experiment was conducted at Vinh Truong village, An Phu District, An Giang Province in Vietnam during spring-summer cropping season 2018 and 2019 (September to January). The study area was 10^o78'72''East and 105^o11'74'' North with a mild climate, and an average annual temperature of 27.93°C, sunshine duration of 213.90 hours, rainfall of 142.7 mm and humidity of 82.25% per month occurring from September to January, Statistical Yearbook of An Giang [8]

2.2 Soil characteristics

Alluvial soil used in the greenhouse experiment was collected at the depth from 0-20 cm, before sowing seeds maize field of Mr. Loi's family, An Phu district, An Giang province. Eighty soil samples were taken in the field (zigzag style).Each soil collection site corresponds to 1 point in the experimental plot, 10 kg were collected and the total weight of 800 kg of soil was collected for the experiment in the greenhouse. The soil was transported to the lab and air-dried, sheltered, naturally in a dry place. Then the soil was mixed evenly into a uniform mass, minced and screened through a mesh (2x2 mm size).500 g sample of mixed soil were put it in a glass jar. Three replication were used. These samples jars were kept at 18-25°C in the plastics box and transferred to the laboratory and were used to do soil physicochemical analysis immediately in Can Tho University.

The pH and EC of the soil were determined in distilled water using a soil/liquid ratio of 1:2.5. After stirring for 30 minutes the pH value was read using a glass electrode pH meter. Total Nitrogen was determined by the Kjeldahl method [9]. Useful nitrogen NH_4^+ and NO_3^- in soil has determined by colorimetric method at 650 nm for NH_4^+ and 540 nm for NO_3^-. Total phosphorus was measured by extraction method HCl: HNO_3 with the ratio 3:2. Phosphorus dissolved available in the soil: measured by method of Olsen. Mechanical analysis soil textured sand, silt and clay by method hydrometer of Gavlak. Soil sample was analysed by Department of Soil Sciences, College of Agriculture, Can Tho University. Some physical and mechanical properties were showed in Table 1.

TABLE 1
PHYSICAL AND CHEMICAL PROPERTIES OF SOILS OR THE EXPERIMENTAL SITE

Soil depth (cm)	pH	EC (mS/cm)	N			P		Soil texture		
			Total N (%)	NH_4^+ mg/kg	NO_3^- mg/kg	Total P_2O_5 (%)	dissolved P mg/kg	Sand (%)	Heavy (%)	Clay (%)
0-20	5.64	5.64	0.063	2.74	36.20	0.16	89.50	18.86	62.68	18.46

Origin: Soil analysis Lab. Department of Soil Sciences, college of Agriculture, Can Tho University

2.3 Bacterial strains, mixed microbial fertilizers, experimental corn

Bacterial cultures of *Bacillus aryabhattai* ADR3 and *Klebsiella pneumoniae* DNR5, which were isolated from rehabilitated roots of corn in the Mekong delta Vietnam, were used in this study. All the isolates were previously identified based on 16S rRNA gene sequence similarities and were also characterized based on morphological and biochemical properties. *Klebsiella pneumonia* HN1) from Biotechnology R&D Institute, Can Tho University. Bacterial cultures were produced in Nfb broth for 4 days, reaching to $>10^9$ CFU/mL. Carrier material peat was dried, ground by pestle and porcelain mortar, sieved through rails with the size of 2x2 mm, and then disinfected at 121°C for 20 minutes. A mixture of microbial fertilizers including a carrier with bacteria culture. VK1 biofertilizer was a peat carrier mixture with *Bacillus aryabhattai* ADR3. VK2 fertilizer was a peat carrier mixture with *Klebsiella pneumoniae* DNR5. VKDC fertilizer was a peat carrier mixture with *Klebsiella pneumonia* HN1. Hybrid corn NK7328 seed was used in all treatments of the experiment.

2.4 Treatments and Experimental Design

2.4.1 Experimental of corn grown in a greenhouse

Greenhouses plot size was 9 m x 9 m = 81 m^2 covered by insect nets. The experiment contained 60 pots. The cross-sectional area on the top is 30 cm, height 25 cm, bottom 24 cm of each pot. Each pot was cleaned and disinfected with 75° alcohol, containing 12 kg of dry ground soil. The evaluation of nitrogen fixation ability of bacterial strains was done in a completely randomized design, with 4 replications at 15 treatments. Row to row distance was 75 cm. Corn kernels were disinfected with 75° alcohol, soaked for 2 minutes, removed alcohol, soaked in H_2O_2 (3%) for 3 minutes, then washed 4 times with sterilized distilled water. Continue soaring in distilled water for 12 hours, incubating for 3 hours. Mix 10 g of microbial fertilizer with corn kernels, incubated for 1 hour. Then sow 3 seeds into each pot according to the respective treatments, 7 days after sowing (DAS), kept 1 plant/pot. The temperature difference between the experiment in the greenhouse and the experiment in the field is about 1-2°C.

There were 15 treatments for growing pots in greenhouses as follows 0% N (control without nitrogen, biofertilizer) [Treatment 1], 0% N+VK1 (VK1) [Treatment 2], 0% N+VK2 (VK2) [Treatment 3], 25% N (45 kgN) [Treatment 4], 25% N+VK1(45 kgN+VK1) [Treatment 5], 25% N+VK2 (45 kgN+VK2) [Treatment 6], 50% N (90 kgN) [Treatment 7], 50% N+VK1 (90 kgN+VK1) [Treatment 8], 50% N+VK2 (90 kgN+VK2) [Treatment 9], 75% N (135 kgN) [Treatment 10], 75% N+VK1 (135 kgN+VK1) [Treatment 11], 75% N+VK2 (135 kgN+VK2) [Treatment 12], 100% N (180 kgN) [Treatment 13], 100% N+VK1 (180 kgN+VK1) [Treatment 14], 100% N+VK2 (180 kgN+VK2) [Treatment 15]. Treatments 1, 2 and 3 were without NPK, Other all treatments were supplied (from 4 to 15) 135 kgP_2O_5/ha and 90 kgK_2O/ha.

The recommended fertilizers were NPK: 180 kg N+135 kg P_2O_5+90 kg K_2O/ha (equivalent to 100% N+ 100% P_2O_5+100% K_2O). Treatments were fertilized with the percentage of nitrogen (% N) increasing gradually from 0% N, 25% N, 75% N, 100% N. The fertilizer schedule for each treatment was different. There were 4 times of fertilization including the first time the first time [10 days after sowing (DAS) with 30% N+30% P_2O+20% K_2O], the second time 20 DAS with 30% N+20% P_2O+30% K_2O, 3rd time (40 DAS with 40% N+40% P_2O+30% K_2O), 4th time (60 DAS with 10% P_2O+20% K_2O). All treatments were sprayed with the same amount of water at the spray location. Pest protection followed the guidelines of the Department of Plant Protection, An Giang province. Use according to Syngenta's NK7328 corn pest control solution. Depending on the growth stage of corn, all treatments were similar in caring, spraying insecticides, and controlling corn diseases.

2.4.2 Experimental of corn grown in a field

The experiment was arranged with a randomized complete block design with four replications. The experimental area had a total area of 0.35 ha. There were 80 plots and each plot was 21 m^2 (7 m x3 m) square meters, rows to rows of 70 cm and in rows of 25 cm. The number of plants was about 57,000 plants/ha. The ground was plowed to a depth of about 40 cm. The horizontal surface was 3 m and 30 cm high (measured from the surface to the bottom of the ditch). The experiment was four blocks which a horizontal dike 60 cm and a 70 cm ditch surrounding the treatments. The experimental area was protected by two rows of planted corn, with a 1.2 m width row. Mixing 120 g of microbial fertilizer with 1.5 kg of germinated corn kernels (disinfected surface as described in the greenhouse experiment) for each type of fertilizer, incubating for 1 hour, then sowing every 3 seeds in 1 hole, at 7 DAS only 1 plant was kept.

There were 20 treatments for growing in the field as follows 0% N (control without nitrogen, biofertilizer) [Treatment 1], 0% N+VK1 (VK1) [treatment 2], 0% N+VK2 (VK2) [Treatment 3], 0% N+VSDC (VSDC) [Treatment 4], 25% N (45 kgN) [Treatment 5], 25% N+VK1 (45 kgN+VK1) [Treatment 6], 25% N+VK2 (45 kgN+VK2) [Treatment 7], 25% N+VKDC (45 kgN+VKDC) [Treatment 8], 50% N (90 kgN) [Treatment 9], 50% N+VK1 (90 kgN+VK1) [Treatment 10], 50% N+VK2 (90 kgN+VK2) [Treatment 11], 50% N+VKDC (90 kgN+VKDC) [Treatment 12], 75% N (135 kgN) [Treatment 13], 75% N+VK1 (135 kgN+VK1) [Treatment 14], 75% N+VK2 (135 kgN+VK2) [Treatment 15], 75% N+VKDC (135 kgN+VKDC) [Treatment 16], 100% N (180 kgN) [Treatment 17], 100% N+VK1 (180 kgN+VK1) [Treatment 18], 100% N+VK2 (180 kgN+VK2) [Treatment 19], 100% N+VKDC (180 kgN+VKDC) [Treatment 20]. Treatments 1, 2, 3 and 4 were without NPK.

Other treatments were supplied (from 5 to 20) 135 kgP_2O_5/ha and 90 kg K_2O/ha. Weeds were removedat 9, 19, 39, and 59 DAS. The experiment was watered by rainy or flooded fertilizer application as mentioned in section 2.4.1

2.5 Yield component and grain yield

Each treatment was four rows with 54 corn plants included rows 1, 4 (protection rows), and 2, 3 (lines for monitoring indicators). Twelve corn plants were marked with a zigzag line in 2 rows inside the tracking plot. Ripe corn harvested at 105 DAS. The monitored indicators of growth and yield included plant height, stem diameter, leaf color, number of leaves, ear corn height, dry biomass, total N, length of corn ear, a diameter of corn ear, number of row/ corn, number of seed/ row, the weight of one thousand grains, actual yield [10]. Corns were collected at 60 DAS and 105 DAS. Leaf color (Spad index): Chlorophyll meter Minolta Spad 502 Plus were used. Spad readings were taken on three young fully expanded leaves (above silking) and index leaf (first leaf below the ear) according to the recommendation of Costa et al. [11]. Four Spad measurements were taken per leaf, on either side of the midvein, at mid-silking near the midpoint of the leaf blade about 20 cm from the stalk and an average of absolute Spad values were recorded. Twenty plants were measured for each plot. Sample plants were dried at 55°C, approximately 5 days until weight was constant. Dry matters were weighed to record the biomass of each part as leaf, stem, root, grains, biological yield, and total nitrogen analysis. Samples were analysed at the Genetics laboratory of Dinh Thanh agricultural research center.

Actual yield: At maturity 42 corn plants per plot were collected. The grain yields were dried to 14% moisture content. Grains per plot were weighed and then converted to its hectare equivalent.

$$Actual\ yield\left(\frac{t}{ha}\right) = \frac{P_1}{S_0} \times \frac{P_2(100 - A_0)}{P_3(100 - 14)} \times \frac{1000\ m^2}{1000}$$

Calculation of yield is based on the formula (t/ha)

P_1: Fresh fruit weight of 42 plants in a plot

A_0: Grain moisture when weighing sample grain.

S_0: The planted area of 42 plants, at a density of 57,000 trees/ha (7.37 m^2).

P_2: Grain weight of 10 corn samples (weighed when measuring grain moisture"A_0").

P_3: Fresh corn weight of 10 corn samples.

$$\frac{(100 - A_0}{(100 - 14)} = \text{Conversion factor of NTT at 14\% humidity}$$

1000: is the number to convert from kg to tons. 10,000 is the number to convert from m^2 to hectare (ha).

2.6 Data analyses

The data was saved and calculated with Microsoft Office Excel 2013 and statistically tested by one-factor analysis of variance. All analyses conducted using the Statgraphics centurion xv software. The experimental results were from plant height, stems diameter, index of leaf chlorophyll, number of leaves, dry matter weight, 1000 seeds weight and yield corn considered significantly different at $P<0.05$, Total N considered significantly different at $P<0.01$.

III. RESULTS AND DISCUSSION

3.1 Evaluation of the effect of nitrogen fixing bacteria on the growth of corn under greenhouse conditions

3.1.1 The Growth of corn in the greenhouse at 60 DAS

The results showed that the average height of corn plants in the VK1 or VK2 treatments was different from the average height of corn in the non-VK1, VK2 treatments at the same level of nitrogen (Table 2). At 50% N, 75% N with the VK1 and VK2 treatments, the height of corns was as similar as the height plants in the treatment that applied only 100% N. Similarly, the treatments at level 75% N+VK1 had a significantly greater difference in root internode diameter, chlorophyll index, and number of leaves than ones only applied nitrogen fertilizer at the same level. The treatments at 75% N+VK1 were not significantly different from the treatment with only 100% NPK strain without bacterial (Figure 1).

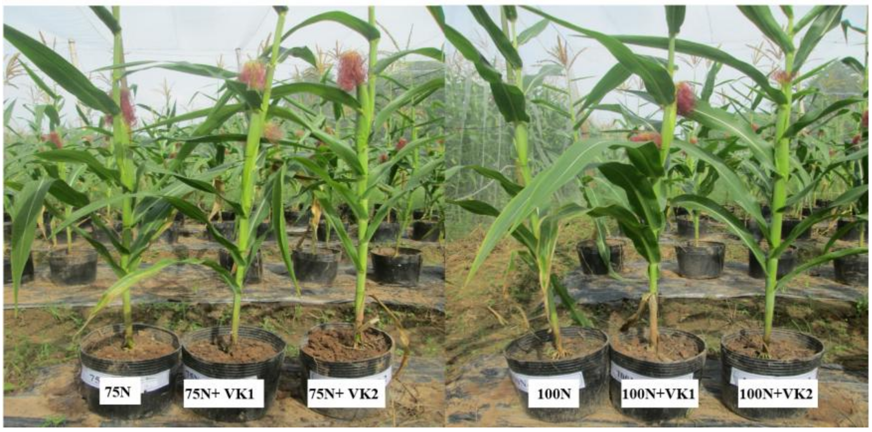

FIGURE 1: Treatments of 75% N and 100% N were grown pots in a greenhouse at 60 DAS

TABLE 2
SOME AGRICULTURAL PARAMETERS OF CORN AT 60 DAS AFTER SOWING AT GREENHOUSE

Treatment	Plant height (cm)	Stem Diameter (cm)	Leaf color (Spad index)	Number of leaves
0% N	105.50 g	1.68 g	22.78 h	17.50 f
0% N+VK1	116.45 f	2.04 f	26.23 g	18.25 e
0% N+VK2	114.10 fg	1.92 f	25.38 g	18.00 ef
25% N	148.18 e	2.17 e	28.98 f	18.50 de
25% N+VK1	160.96 d	2.59 c	31.10 ef	19.00 cd
25% N+VK2	161.40 d	2.42 cd	30.25 ef	19.25 bc
50% N	166.85 cd	2.66 bc	31.80 e	19.00 cd
50% N+VK1	176.55 ab	2.76 b	40.25 bc	19.25 bc
50% N+VK2	175.98 abc	2.78 b	39.85 c	19.25 bc
75% N	171.13 bc	2.71 b	35.73 d	19.25 bc
75% N+VK1	182.48 a	3.16 a	42.43 ab	20.00 a
75% N+VK2	180.28 ab	3.14 a	42.10 abc	19.75 ab
100% N	183.64 a	3.17 a	43.08 a	19.75 ab
100% N+VK1	182.55 a	3.15 a	42.93 a	19.75 ab
100% N+VK2	184.70 a	3.12 a	43.45 a	19.75 ab
F	**71.09***	**134.77***	**79.63***	**10.96***
C/V (%)	**4.02**	**3.24**	**4.71**	**2.31**

Means within a column followed by the same letter/s are not significantly different at $p < 0.05$

According to research by Adoko, bacterial fertilizer contained *Pseudomonas putida* showed significant improvements in height (49.49%), stem diameter (32.70%), leaf area (66.10%) on ferrallitic soil with the application of biostimulant compared to the control [12]. *Bacillus aryabhattai* S10 reported increase in water contents (19% in mungbean and 32% in corn), chlorophyll “a” and chlorophyll “b” contents in mungbean and corn crops as compared to uninoculated control [13].

3.1.2 Agronomic index and biological yield of corn at harvest stage

At the treatment fertilized 75% N with VK1 or VK2, the plant height was not significant difference as compared to the 100% N treatment without any bacterial strains. Similarly, first ear corn height and fresh stem leaf weight of corn, only fertilized with nitrogen at 0% N, 25% N, 50% N, 75% N, had lower values and statistically significant differences compared to corn fertilized with VK1 or VK2 strains and inorganic nitrogen 0%-75%. Corn with VK1 or VK2 strains fertilized with 75% N had the same first ear corn height, fresh stem weight, and root weight as the positive control treatment with only 100% NPK and no bacterial strains. The fresh root weight of corn plants, which had significantly different at 25% N, 50% N, 75% N was lower than ones of with nitrogen fertilizer combined with bacterial strain VK1 or VK2 (Table 3).

The biomass of corn’s dry matter in the treatments with the VK1 or VK2 strain was significantly different from ones in the treatments that applied only inorganic fertilizer at the same amount of nitrogen fertilizer without the bacterial strain. This result was similar to the result in the experiment of Montañez and Sicardi (2013), which had the ability to fix nitrogen of 4 strains of bacteria on the potted corn [14]. The results showed that the dry matter weight of the root increased to 42-53% and the dry matter weight of the leaves increased to 22%-50% compared to the control at the 90 DAS-old corn stage. When adding the endogenous bacteria *Lactococcus lactis* and *Klebsiella* sp. to corn plants 25% of the amount of NPK fertilizer could be saved and help to increase the dry biomass of rhizomes as equivalent to the corn which fertilized 100% NPK without bacteria strains [15].

TABLE 3
EFFECTS OF VK1 AND VK2 FERTILIZERS ON PLANT HEIGHT, EAR CORN HEIGHT, FRESH STEM AND LEAF, ROOT WEIGHT AND DRY BIOMASS CORN AT THE STAGE OF HARVEST

Treatment	Plant height (cm)	Ear corn height (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry Biomass (g)
0% N	106.50 h	33.28 g	99.63 g	67.10 j	45.82 j
0% N+VK1	116.45 g	40.28 f	108.18 f	71.25 ij	49.31 ij
0% N+VK2	114.10 gh	39.13 f	113.17 f	74.61 i	55.77 i
25% N	148.18 f	61.35 e	173.43 e	108.58 h	148.15 h
25% N+VK1	160.96 e	72.18 d	199.53 d	131.13 f	171.81 f
25% N+VK2	161.40 e	71.63 d	204.23 d	119.18 g	163.63 g
50% N	166.85 de	72.58 d	203.35 d	128.25 f	172.72 f
50% N+VK1	176.55 bc	77.55 c	261.00 b	179.46 cd	260.93 d
50% N+VK2	175.98 bc	79.08 bc	250.25 c	176.25 d	248.18 e
75% N	171.13 cd	75.85 cd	245.45 c	169.56 e	247.44 e
75% N+VK1	182.48 ab	82.65 ab	269.55 a	181.31 bc	272.43 c
75% N+VK2	180.28 ab	82.98 ab	268.88 ab	185.71 ab	277.36 bc
100% N	183.64 ab	86.10 a	275.43 a	183.76 abc	286.28 a
100% N+VK1	182.55 ab	83.08 ab	273.25 a	181.16 bc	283.89 ab
100% N+VK2	184.70 a	87.38 a	271.85 a	187.75 a	285.02 ab
F	**97.98***	**108.63***	**534.01***	**795.15***	**1051.73***
C/V (%)	**3.41**	**4.94**	**2.59**	**2.27**	**2.81**

Means within a column followed by the same letter/s are not significantly different at $p < 0.05$

3.1.3 Components of yield and theoretical yield of corn kernels

Corn, fertilized with 50% N, 75% N without bacterial strain, had its length shorter than the one which fertilized the same amount of nitrogen and with the VK1 or VK2 (Figure 2). The length and the stalk diameter of the corn with 75% N fertilization and with VK1 or VK2 strains were not statistically different from those of 100% NPK (Table 4).

The number of rows of seeds/corns and the number of seeds/rows of corn fertilized with 50% N with VK1 strain and 50% N with VK2 strain were not statistically different from those of corn fertilized with only 100% NPK and without bacterial strain.

FIGURE 2: Effects of VK1 and VK2 fertilizers on the length of corn ear in greenhouse

TABLE 4
EFFECTS OF VK1 AND VK2 FERTILIZERS ON LENGTH OF CORN EAR, DIAMETER OF CORN EAR, NUMBER OF ROW SEAD, WEIGHT OF ONE 100 GRAINS, GRAIN YIELD AT HARVEST

Treatments	Length of corn ear (cm)	Diameter of corn ear (cm)	Number of row/ corn	Number of seed/ row	Weight of 100 grains (g)	Grain yield/ pot (g)
0% N	11.43 g	2.24 f	6.00 d	9.50 f	17.67 h	10.37 h
0% N+VK1	12.48 f	2.78 e	9.50 c	12.00 e	19.50 g	23.33 g
0% N+VK2	13.28e	2.82e	9.00 c	11.50 e	19.57 g	22.29 g
25% N	13.43 e	3.14 d	12.50 ab	20.25 d	21.24 f	53.48 f
25% N+VK1	15.23 d	3.29 c	13.00 b	24.25 c	23.91 de	72.29 e
25% N+VK2	15.40 d	3.25 cd	13.00 b	23.75 c	23.76 de	73.67 e
50% N	14.90 d	3.17 d	13.00 b	23.25 c	23.21 e	70.26 e
50% N+VK1	17.95 b	3.67 b	13.50 ab	28.75 ab	26.31 abc	106.17 bc
50% N+VK2	17.80 b	3.58 b	13.50 ab	28.50 ab	25.55 bc	100.06 cd
75% N	16.95 c	3.64 b	13.50 ab	27.00 b	25.17 cd	91.68 d
75% N+VK1	19.05 a	3.87 a	15.00 a	29.50 a	27.14 a	121.18 a
75% N+VK2	19.23 a	3.92 a	15.00 a	28.75 ab	27.15 a	113.36 ab
100% N	18.95 a	3.91 a	15.00 a	29.25 a	26.86 ab	116.86 ab
100% N+VK1	19.08 a	3.90 a	15.00 a	28.75 ab	27.55 a	120.54 a
100% N+VK2	19.13 a	3.92 a	15.00 a	29.27 a	27.32 a	116.56 a
F	**126.56***	**160.12***	**24.56***	**118.16***	**42.69***	**95.21***
C/V (%)	**2.97**	**2.37**	**8.34**	**5.53**	**4.13**	**9.74**

Means within a column followed by the same letter/s are not significantly different at $p < 0.05$

The weight of 100 seeds had an average value ranging from 17.67 g to 27.55 g. In the control treatment 0% N (Control), the weight of 100 seeds was lowest and significantly different from other treatments. In the treatment with 75% N+VK1 or 75% N+VK2 fertilizer, the weight of 100 seeds was significantly different and respectively higher at 1.08 times than corn which only fertilized 75% N and was not significantly different from the treatment with 100% N. The mean corn kernel yield had an average value ranging from 10.37 g (0% N) to 121.18 g (100% N+VK1). Corn yield in control treatments without fertilizer and without strain (0% N) was completely lower than those of the treatments with fertilizer and lower than the treatment with bacterial strains. At the same level of nitrogen fertilization of 0% N, 25% N, 50% N, 75% N with VK1 or VK2 of bacterial strain had a higher grain yield than the treatment with only nitrogen fertilizer. The treatment 75% N fertilization with bacterial strains VK1 or VK2 had a high yield, respectively 1.32 or 1.24 times higher than the treatment with only 75% N nitrogen fertilizer. Treatments 75% N+VK1 or 75% N+VK2 fertilizer were not significantly different and were equivalent in yield of the treatment with 100% NPK and without bacteria. This result proved that when applying 75% N+VK1 or 75% N+VK2 helped the plant to grow better and the grain yield/pot was similar to ones with only 100% NPK nitrogen fertilizer without bacterial strain.

This result of plant height, the length, the diameter of corn, the number of seeds/corn, and 1000-grain weight similarly increased in the treatments with the nitrogen-fixing bacteria *Gluconacetobacter diazotrophicus* with 75% N compared to when the treatment was fertilized only 100% N and without bacterial strain [16]. Inoculation with rhizobacteria could be efficiently improved the growth and grain yield of corn, reduced fertilizer costs and reduced the emission of the greenhouse gas, N_2O as well as reduced leaching of NO_3^-, N to groundwater even [17].

Six potential corn endophytic bacterial isolates were positive influenced on corn plants in a pot-house experiment under glasshouse conditions. Endophytic bacterial as *Pantoea dispersa*, *Pantoea* sp., *Klebsiella variicola*, *Lactococcus lactis*, *Bacillus cereus*, and *Staphylococcus hominis* inoculated corn, made increased the growth and yield of corn and save about 25% NPK of chemical fertilizers. *Lactococcus lactis* inoculation influenced maximum, followed by *Klebsiella variicola* isolates [18]. The study results of Ahmad revealed that inoculation of *Bacillus aryabhattai* S10 made increasing of nodules number, and nodules fresh and dry weight of mungbean compared to the control. This inoculation of bacterial strain also increased nitrogen (N) concentration up to 142%, phosphorus (P) concentration up to 90%, and potassium (K) concentration up to 71% in shoots of mungbean and corn crops. In conclusion, the tested strain *Bacillus aryabhattai* S10 had the potential to use as such promising bio-inoculants that maximize plant growth and nutritional status of the crop in sustainable crop production [13].

3.2 Evaluating the effectiveness of nitrogen-fixing bacteria on corn grown in the field

3.2.1 Growth of corn in the field at 60 DAS

The study results showed that the plant height, stem internode diameter, and chlorophyll index of corn leaves, which applied with 75%VN+VK1 or 75% N+VK2 fertilize, were significantly different than those of the treatment with only 75% N without strain, and were not significantly different from the 100% NPK fertilization treatment. Similarly, corn in the fertilized nitrogen treatments with VK1 or VK2 fertilizes had the number of leaves as same as those in the 100% NPK treatment without bacteria strain. In the treatment 50% N+VK2, the chlorophyll index and leaf were higher than those of the other treatments (Table 5). Thus, corn at 60 DAS required a large amount of additional nitrogen fertilizer to grow and develop. Corn with VK1 or VK2 strain helped reduce 25% N but also increased the growth of corn as same as the ones fertilized with 100% N.

TABLE 5
SOME AGRICULTURAL PARAMETERS OF CORN AT 60 DAS AFTER SOWING AT FIELD

Treatment	Plant height (cm)	Stem Diameter (cm)	Leaf color (Spad index)	Number of leaves
0% N	96.94 g	1.71 g	21.29 f	17.48 f
0% N+VK1	98.77 g	1.87 f	21.61 f	17.75 ef
0% N+VK2	99.02 g	1.86 f	21.37 f	18.02 ef
0% N+VKDC	98.77 g	1.90 f	22.02 f	18.20 e
25% N	141.25 f	2.11 e	29.53 e	17.18 d
25% N+VK1	144.94 e	2.18 e	29.36 e	19.24 d
25% N+VK2	143.10 ef	2.16 e	29.68 e	19.23 d
25% N+VKDC	144.43 e	2.18 e	29.3 e	19.20 d
50% N	168.99 d	2.57 d	31.70 d	19.16 d
50% N+VK1	179.83 c	2.70 bc	31.80 d	19.35 cd
50% N+VK2	184.34 b	2.76 b	33.74 c	20.18 a
50% N+VKDC	182.23 bc	2.70 b	31.12 d	19.38 bcd
75% N	183.44 b	2.63 cd	37.37 b	19.65 a-d
75% N+VK1	197.24 a	2.92 a	40.02 a	20.08 a
75% N+VK2	195.88 a	2.95 a	40.44 a	20.11 a
75% N+VKDC	196.46 a	2.90 a	40.05 a	20.00 a
100% N	195.39 a	2.94 a	40.84 a	20.10 a
100% N+VK1	195.22 a	2.95 a	41.04 a	19.98 abc
100% N+VK2	196.71 a	2.92 a	41.25 a	20.15 a
100% N+VKDC	197.45 a	2.96 a	41.36 a	20.13 a
F	**1922.45***	**214.66***	**224.22***	**14.34***
C/V(%)	**6.93**	**2.44**	**2.98**	**2.32**

Means within a column followed by the same letter/s are not significantly different at p < 0.05

Plant growth-promoting bacteria (PGPB), which was inhabiting the phyllosphere, improved the growth and yield of plants by producing natural growth regulators. There was a beneficial effect of most bacterial treatments, especially on the chlorophyll content and chlorophyll content index of inoculated plants [19]. Bacterial strain *Azotobacter* significantly increased plant height, a number of leaves corn [20]. *Azospirillum brasilense* supported development of corn, increased plant height and stem diameter [21]. This result was in agreement with the results of Amogou et al. when they applied bacterial strain *Bacillus panthothenicus* and got an increasing of 49.65% in stem diameter at the plants treated, and *Pseudomonas putida*+ 50% NPK was 32.08% was greater than those of the non-inoculated control [22]. Mucilage of Sierra Mixe corn harbors native diazotrophs and inoculated *Bacillus unamae* confirmed a significant enrichment of 15N in chlorophyll (converted to pheophytin for analysis) of these roots compared to the negative controls [23].

3.2.2 Agronomic index and biological yield of corn grown in the field at harvest stage

In our study, the values from 97.41 cm (0% N) to 201.26 cm (100% N+VKDC) were obtained from plant lengths (Table 6). Plant height of corn with 50% N, 75% N, and inoculated nitrogen bacteria (VK1 or VK2 or VKDC) had higher values and statistically significant differences compared to those applied fertilized at the same amount of 50% N and 75% N. In particular, the treatments 50% N and 75% N, which inoculated with this bacterial fertilizer, had no statistically significant difference in height compared to the treatment of 100% N. The Ear corn height had a contribution to the easy transfer of nutrients to the fruit to increase the size of the fruit. Corn plants, which had the first ear corn height higher, produced larger fruits. The treatments 50% N, 75% N with VK1 or VK2 or VKDC gave higher values and statistically significant differences compared to those which was fertilized at the same 50% N and 70% N. In addition, treatments 50% N+VK2 had the ear corn height of corn highly different from the treatment with 50% N+VK1 strain at the same 50% N fertilizer level. The results also showed that corns, which only were fertilized with 75% N with bio- fertilize, had the same height as of 100% NPK.

TABLE 6
EFFECTS OF VK1 AND VK2 FERTILIZERS ON PLANT HEIGHT, EAR CORN HEIGHT, DRY AERIAL BIOMASS AND TOTAL N OF CORN AT THE STAGE OF HARVEST

Treatment	Plant height (cm)	Ear corn height (cm)	Dry Aerial Biomass (t/ha)	Total N (%)		
				Leave	Stem	Grain
0% N	97.41 f	48.49 h	15.95 h	0.4 k	0.22 j	0.58 e
0% N+VK1	100.47 f	50.17 g	19.73 g	0.64 i	0.34 hi	0.79 d
0% N+VK2	100.72 f	50.13 g	19.82 g	0.64 i	0.37 gh	0.78 d
0% N+VKDC	100.46 f	50.28 g	19.79 g	0.55 j	0.35 hi	0.81 d
25% N	142.99 e	67.82 f	21.87 f	0.95 h	0.29 i	1.10 c
25% N+VK1	146.93 e	68.48 f	25.21 e	1.47 fg	0.34 hi	1.08 c
25% N+VK2	145.08 e	68.50 f	25.87 de	1.57 e	0.39 fgh	1.09 c
25% N+VKDC	146.45 e	68.58 f	25.25 e	1.44 g	0.42 efg	1.07 c
50% N	170.97 d	77.66 e	24.46 e	1.55 ef	0.46 b-e	1.28 b
50% N+VK1	182.69 c	81.65 d	29.73 c	1.71 bcd	0.47 a-e	1.28 b
50% N+VK2	187.18 bc	84.72 c	30.20 c	1.69 cd	0.45 cde	1.30 b
50% N+VKDC	191.85 b	82.56 d	29.00 c	1.65 d	0.43 def	1.31 b
75% N	186.71bc	85.25 c	27.24 d	1.66 d	0.42 efg	1.47 a
75% N+VK1	200.79 a	96.49 ab	36.17 ab	1.77 abc	0.49 abc	1.49 a
75% N+VK2	199.39 a	97.04 a	37.01 a	1.78 ab	0.52 a	1.48 a
75% N+VKDC	199.96 a	95.63 b	35.78 ab	1.79 ab	0.49 abc	1.46 a
100% N	199.27 a	96.55 ab	34.95 b	1.79 ab	0.51 ab	1.48 a
100% N+VK1	199.09 a	96.46 ab	36.10 ab	1.80 a	0.50 abc	1.49 a
100% N+VK2	200.54 a	97.10 a	36.61 a	1.80 a	0.49 abc	1.47 a
100% N+VKDC	201.26 a	96.19 ab	35.49 ab	1.79 ab	0.49 a-d	1.46 a
F	**341.41***	**1508.53***	**136.06***	**493.03****	**27.40****	**369.73****
C/V (%)	**2.57**	**1.18**	**4.12**	**3.10**	**7.44**	**2.46**

Means within a column followed by the same letter/s are not significantly different at Means within a column followed by the same letter/s are not significantly different at 5% (*) p< 0.05 or 1% (**) p< 0.01

The dry aerial biomass varied from 15.95 t/ha (0% N) to 37.01 t/ha (75% N+VK2). In the treatment 0% N, corn's dry leaf weight was very small and trendily increased when the plant was applied more % N. In the treatments that fertilized only 0% N, corn had the lowest biomass. In the treatments 75% N+VK1 or 75% N+VK2, the corn had the same biomass as those in the treatment with only 100% NPK without bacterial strain. Control corn had lower nitrogen deficiency content in leaves, stems, and seeds than those of other treatments. The treatment 50% N+VK1 in the leaves and stems had a high total nitrogen content and was not statistically different from the treatment 100% NPK. Similarly, the treatment 75% N with bacterial fertilizer had no statistical difference compared to the treatment of 100% NPK.

The experimental results of Lopez-Ortega showed that the endogenous bacteria *Klebsiella variicola* increased the biomass of corn's shoots and roots up to 39% compared to the control without bacterial strain, and at once accumulated 10% more phosphate in the plant [24]. Inoculation with *Azotobacter* significantly increased ear height, number of ears per m^2, ear length of corn [20]. Inoculation with *Azospirillum brasilense* had a positive effect on the characteristics of yield and productivity of corn, independent of growing season and hybrid used. The agronomic characteristics of corn seeds, which inoculated with *Azospirillum brasilense*, were grown on black oat and ryegrass straw and managed under different doses of nitrogen (0, 50 and 100 kg/ha). In two agricultural seasons (2012/2013 and 2013/2014) under drought conditions, *Azospirillum brasilense* to supported the corn development increased plant height and ear height [21]. The foliar spray of corn treatments included *Bacillus subtilis+Stenotrophomonas maltophilia*, *Bacillus megaterium*, and *Enterobacter hormaechei* significantly increased the shoot dry weight by 9.53, 8.73 and 6.00% compared to the control [19]. The same results were found in the experiment of Amogou, bacteria where bacterial strains *Serratia marcescens+50%* NPK increased the best results of height (41.09%), fresh underground biomass (217.5%), dry aboveground biomass (213.34%) and dry underground biomass (93.82%) compared to the control [22]. The co-inoculation with *Bacillus aryabhattai* S10 and *Bacillus subtilis* ZM63 performed that there was the highest increase in N concentration (18%), and protein contents (19%) in the shoot of corn over uninoculated control [13].

3.2.3 Components of yield and the yield of corn kernels in the field experiment

Corns applied 50% N or 75% N and VK1 or VK2, had their stem length equivalent with those which only were fertilized at the same level of nitrogen (50% N or 75% N) (Table 7). In particular, the treatment with the 50% N+VK2 showed that the length of the corn was not significantly different from those which were applied 100% NPK (only 100% NPK and without bacterial strain) (Figure 3)

FIGURE 3: Effect of bacterial fertilizers VK1, VK2, and VKDC on increasing the length of corn in the field

The number of rows varied from 9.55 to 14.55 rows. The number of seeds/ row varied from 13.38 to 33.22 seeds (75% N+VK1). The results in Table 7 showed that when adding the strain to corn VK1 or VK2 and 75% N, the number of seeds/ row had no statistical difference compared to those in which the corn was fertilized 100% NPK.

TABLE 7
EFFECTS OF VK1, VK2 AND VKDC FERTILIZERS ON LENGTH OF CORN EAR, DIAMETER OF CORN EAR, NUMBER OF ROW, NUMBER OF SEADS/ROW, WEIGHT OF ONE THOUSAND GRAINS, GRAIN YIELD AT HARVEST

Treatments	Length of corn ear (cm)	Diameter of corn ear (cm)	Number of rows/ corn ear	Number of seeds/ row	Weight of one thousand grains (g)	Grain Yield (t/ha)
0% N	12.28 i	3.12 h	9.55 g	13.38 i	209.17 j	2.41 k
0% N+VK1	13.09 h	3.17 gh	10.33 f	14.43 h	226.67 i	2.83 j
0% N+VK2	13.60 g	3.15 h	10.28 f	14.34 h	230.08 hi	3.28 hi
0% N+VKDC	13.48 gh	3.16 h	10.20 f	14.52 h	227.04 hi	3.07 ij
25% N	14.30 f	3.25 fg	11.15 e	21.19 g	232.21 gh	4.88 e
25% N+VK1	14.36 f	3.31 ef	11.60 d	23.20 f	242.51 de	4.58 fg
25% N+VK2	14.39 f	3.42 d	11.93 d	23.87 e	236.34 fg	4.82 ef
25% N+VKDC	14.33 f	3.37 de	11.95 d	24.54 d	236.68 ef	4.44 g
50% N	14.36 f	3.39 de	11.60 d	24.38 de	246.85 d	3.36 h
50% N+VK1	17.91 d	3.92 c	12.85 c	30.40 c	291.94 bc	5.69 c
50% N+VK2	18.05 bcd	3.90 c	13.40 b	30.22 c	293.35 abc	5.94 c
50% N+VKDC	17.19 e	3.90 c	13.35 b	30.12 c	289.34 c	5.35 d
75% N	17.93 cd	3.91 c	12.85 c	31.76 b	289.89 c	5.71 c
75% N+VK1	18.30 a-d	4.14 b	14.25 a	33.22 a	297.20 a	8.06 b
75% N+VK2	18.625 a	4.20 ab	14.55 a	33.12 a	296.88 ab	8.35 a
75% N+VKDC	18.35 ab	4.18 ab	14.35 a	32.93 a	295.99 ab	8.14 ab
100% N	19.38 ab	4.15 ab	14.30 a	33.10 a	296.18 ab	8.11 ab
100% N+VK1	18.39 ab	4.13 b	14.35 a	33.12 a	297.98 a	7.99 b
100% N+VK2	18.31 abc	4.20 ab	14.30 a	32.95 a	296.75 ab	8.21 ab
100% N+VKDC	18.40 ab	4.25 a	14.40 a	33.03 a	295.78 ab	8.07 b
F	**276.15*****	**206.84***	**163.07***	**1089.01***	**311.14***	**464.71***
C/V (%)	**1.71**	**3.9**	**2.09**	**1.84**	**4.51**	**3.43**

Means within a column followed by the same letter/s are not significantly different at $p < 0.05$

The weight of 1000-seed of corn was also affected by nitrogen-fixed bacteria in biofertilizer. The treatments with VK1 or VK2 or VKDC bacterial strains also gave 1000-seed weights heavier than those in the treatment of inorganic fertilizers applied from 25% N to 75% N. In particular, when applied 50% N+VK1 or 50% N+VK2 the weight of 1000-seed was not significantly different from the treatment with only 100% NPK.

The actual productivity of corn had a variable average value from 2.41 t/ha (0% N) to 8.35 t/ha (75% N+VK2). The applied non-nitrogen treatment (Control) gave the lowest productivity and had significant differences compared to the other treatments. At a similar level of 75% N, corn of treatment 75% N+VK2 gave higher productivity than those of 75% N+VK1 (0.29 t/ha difference). The results also showed that VK2 gave an efficiency in increasing productivity of corn better than VK1. Corn, which applied 75% N and with VK2 fertilizer, gave the actual productivity equivalent to the treatment of 100% NPK (without bacterial strain). The treatment applied 100% N+VK1 or 100% N+VK2 or 100% N+VKDC, increased actual productivity but was not significantly different from the treatments that were only fertilized 100% NPK (without bacterial strain).

Similarly, the results of Montañez and Sicardi (2013) showed that when nitrogen-fixing bacteria inoculated to corn NK900 in the field supported the 1000-seed weight and corn productivity significantly higher compared to the control treatments with only chemical fertilization. On the other hand, when calculating profits, the treatments with bacterial strains helped increase 10.5% profit compared to the control treatment at the same level of chemical fertilization [14]. Inoculation with *Azotobacter* significantly increased kernel per row, 1000 grain weight, grain, and stover yield of corn [20]. The productivity of corn plants increased from 20-70% when the bacterial strain was applied to corn grown in Mexico [25]. It found that a positive effect of *Azotobacter* application on corn grain yield increase at organic field condition. Only inoculation of *Azotobacter* increased corn grain yield upto 35% over non inoculated treatment [26]. The benefit of *Azotobacte*r inoculation was higher when not using chemical fertilizer. A positive additive (15% yield increased) effect of 10 t/ ha FYM with *Azotobacter* inoculation was seen [20]. The experimental results of Oliveira et al (2018), showed that *Azospirillum brasiliense* increased corn yield compared to non-N-fertilized plants. The inoculation with *A. brasiliense*+100 kg/ha N at topdressing provided a similar grain yield as 100 kg/ha N at sowing [27]. Bacterial strain *A. brasilense* had a positive effect on corn productivity different from the treatment with *A. brasilense*+0 kgN increases of 38% at the same only N fertilizer level. The treatment with *A. brasilense*+50 kgN increases of 74% at the same only 50% N and *A. brasilense*+100 kgN increases of 41% at the same only 100% N [21]. The research results of Salvo et al (2018) showed that the bio-fertilizer with *Azospirillum Brasilense* and *Pseudomonas Fluorescens*, which were applied to corn plants, made increased productivity grain yield (11.03 t/ha) respectively compared to the treatment 50% N (10.94 t/ha) and but it was significantly different from the treatment with 100% N and without bacterial strain (12.05 t/ha) [28]. Bacterial strains *Serratia marcescens*+50% NPK gave the best results for grain yield (39.05%) respectively compared to the control [22].. The combined use of *Bacillus* aryabhattai and *Bacillus subtilis* had good effect on the biofortification of corn as well as improvement in growth and yield parameters [29].

IV. CONCLUSION

Bacterial fertilizer *Bacillus aryabhattai* ADR3 with peat or *Klebsiella pneumoniae* DNR5 with peat positively affected the growth, and yield of hybrid corn NK7328. The treatments of 75% N with bio-fertilizers (VK1 or VK2) always had higher values than those at the same level of fertilizer with those applied only 75% N chemical fertilizers. The nitrogen-fixing bacteria strain and fertilizing 75% N contribute to the increase of the number of leaves, plant height, stem diameter, the number of leaves, ear corn height, dry weight of the plant, total N, the weight of 1000 seeds, productivity as same as treatments applied only fertilizing 100% N and without inoculation.

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