



Effects of bacteria and nitrogen fertilizer on promoting maize (*Zea mays* L.) growth, yield and chemical constituents

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Abstract: A field experiment was carried out during two successive summer seasons (2016/ 2017-2017/2018), at the College of Agricultural Studies, Sudan University of Sciences and Technology, to assess the effect of bacterial strains, nitrogen and their combinations on the performances of two maize genotypes Hudibi2 (C1) and ZML309 (C2). The form of two types of bacterial strains *Bacillus megatherium* var. *phosphorous* + *Azotobacter* spp + *Azospirillum* spp (B1) and *Bacillus megatherium* var. *phosphorous* + *Azotobacter* spp + *Flavobacterium* spp (B2), nitrogen applied at rates of 80kg/f (N) plus control. The treatments were arranged in a Randomized Complete Block Design (RCBD) in four replicates. The results showed that the combinations of B1 and B2 with N increased plant growth compared to B1, B2 and N each alone at both seasons. The general trend was the combinations (B2+N, B1+N) had significant effect on all growth parameters in both seasons. Yield components increased with combination (B2+N) followed by (B1+N), the maximum seed yield (5.0 and 2.8t/ha) was achieved by the interaction of (C2*B2+N) in the first season and (C2*B1+N) in the second season, respectively. Generally, in among all treatments the combination of B2+N increased nitrogen content, crude protein and fiber irrespective to maize genotypes. Moreover, C2 showed the highest values as compared to C1. In conclusion, improvement in maize plant growth and yield are more prominent and significant when genotype ZML309 inoculated with *Bacillus megatherium* var. *phosphorous* + *Azotobacter* spp + *Flavobacterium* spp and supplemented with 80kg/f nitrogen.

Keywords: *Azotobacter*; *Azospirillum*; *Flavobacterium*; Maize; Nitrogen%; Protein; Fiber.

Introduction

Maize (*Zea mays*) is cultivated throughout the world and greater amount of maize are produced each year than any other grain (El Toum, 2016). It is cultivated globally, being one of the most important cereal crops worldwide, superior position of maize is due to it is very wide and variety utilization. Maize provides food for human, feed for animals and poultry, and fodder for livestock it is a rich source of raw materials for the industry (ABPSD, 2008).

Maize's central role as a staple food in Africa and Central America is comparable to that of rice or wheat in Asia, with consumption rates being the highest in eastern and southern Africa (CGIAR, 2016). Maize ranks the fourth important cereal crop in Sudan after sorghum, wheat and pearl millet (Tagne *et al.*, 2008). In developing countries including Sudan maize is a major source of income to many farmers. Moreover, the possibility of blending maize with wheat for

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bread making has also increased the demand of maize in Sudan.

Nitrogen deficiency is a key factor for limiting maize yields (Alvarez and Grigera, 2005). The using of bio-fertilizers increases the biological nitrogen fixation, growth hormone, plant antibiotics and improve the root systems of corn (Garg et al., 2005). Application of bio-fertilizers highly considered to limit the use of mineral fertilizers and decreasing agricultural costs, maximizing crop yield by providing the available nutritive elements and growth promoting substances (Metin et al., 2010). One of the environmentally sound approaches for nutrient management and ecosystem function is the use of soil microorganisms which can either fix atmospheric nitrogen, solubilize phosphate, synthesizing growth promoting substance or by enhancing the decomposition of plant residues to release vital nutrients and increase humid content of soil (Wu et al., 2005). In this field, many experiments were conducted in Sudan to study the effect of soil borne microorganisms alone or in combination with other chemical fertilizers on different crops (Rugheim et al., 2017; Mohammed et al., 2012; Rugheim and Abdelgani, 2012; Abdalla et al., 2011; Osman et al., 2011). The aim of the present study was to evaluate the effect of seed inoculation with bacterial strains, nitrogen fertilization and their combinations on growth and yield of maize plants.

Materials and Methods

A field experiment was conducted during summer seasons (2016/2017-2017/2018) at the demonstration farm of the College of Agricultural Studies Sudan University of Sciences and Technology, Shambat, Khartoum North, Sudan. Shambat climate is tropical, usually hot and humid in summer, cold and dry in winter. The temperature reached a maximum value (45.9°C) in June and a minimum value (22°C) in January

(El Toum, 2016). Two open pollinated maize genotypes seeds Hudibi2 (C1) and ZML309 (C2) were obtained from Wad Madani Research Station, El-Gazzira State.

Four bacterial strains *Bacillus megatherium var phosphors*, *Azotobacter* spp, *Azospirillum* spp, and *Flavobacterium* spp, were kindly supplied from the Environment, Natural Resources and Desertification Research Institute, National Centre for Research, Sudan. A broth medium of meat peptone was prepared by adding the following constituents (gm) 7.5 peptone, 5 meat extract and 5 NaCl to one liter of distilled water. Then the medium was sterilized by autoclaving at 121°C for 15 minutes. Then the broth was inoculated by the bacterial strains each alone. The inoculated broth was incubated in incubator shaker for 48 hours (Mohammed et al., 2012). Briefly, one kilogram of sterilized charcoal powder (sterilized by autoclaving at 121°C for 30 minutes) was mixed with 500 ml of the bacterial broth culture. Maize seeds were mixed carefully with sugar solution (10%) and charcoal until completely coated then the seeds were left to dry in the shade for minutes before sowing (Osman et al., 2013).

The field was disc ploughed and harrowed. Each experimental plot has divided in to four rows of 5.6 m long spaced 40 cm a part with a plot area of 1.2 m (1mx1.2m) 1 m distance between replications and 0.5 m between plots. A spacing of 40cmx10cm was used for planting the seed (Ukonze et al., 2016).

The treatments used in this experiment were two maize genotypes, 2 combinations of bacterial strains and nitrogen fertilizer {80kg/f (N)} each alone or in combinations. Un-inoculated unfertilized treatment was used as a control. Sowing was done manually on the last week of July in the two seasons (first season 2016/17 and second season 2017/18). Seeds (5-7) coated by bacterial strains were sown. Nitrogen ferti-

lizer was applied two times, one half at two weeks after sowing (WAS) and the other at 4 WAS. Irrigation immediately applied after sowing, then each ten days intervals the field was irrigated. Hand weeding was done each two weeks. All necessary agronomic practiced was done uniformly ((Ukonze *et al.*, 2016).

Data collection:

Plant growth: After 30, 45 and 60 days after sowing (DAS), data were collected from a sample of five plants at random from each plot to measure the following growth parameters:

Plant height (cm): The height of the main stem from ground level to the tip of the plant, using a tape meter.

Stem thickness (cm): Measured at 10 cm above the ground level, using tape meter.

Number of leaves/plants: By counting the number of leaves per plant.

Chlorophyll content: Using chlorophyll meter

Leaf area (cm²): The leaf area was calculated as follows:

$$\text{Leaf area (LA)} = \text{maximum length (cm)} \times \text{maximum width (cm)} \times 0.75.$$

The data on each parameter was collected from the central rows in each plot.

Weight of root (gm)/m²: The average roots weight was determined from one-meter square harvested plant from each plot, dried roots samples at sun until a constant weight was reached, and then weighed in grams.

Cobs Length (cm): The mean cobs length was determined from five cobs selected randomly.

Number cobs/plant: The mean number of cobs per plant was counted from the randomly selected sample of five plants per plot.

Number of rows /cobs: The mean number of rows per cob was counted from randomly selected sample of five cobs of the five plants of the sample.

Number of grains /rows: The mean number of grains per row was counted from randomly

selected sample of five cobs of the five plants of the sample.

Hundred grains weight (gm): Hundred grains in grams were obtained after threshing 100-grain taken randomly from each plot and then weighed.

Harvest index %: Was calculated as follows:

$$\text{Harvest index} = (\text{Economic yield} / \text{Biological yield}) \times 100$$

Yield (t/ha): Was calculated as follows:

$$\text{Total grain yield (t/ha)} = \text{Grain weigh (kg/m}^2) / 100$$

Grain quality analysis:

Seed crude protein, organic nitrogen and crude fiber contents were determined following the standard methods of the Association of Official American Analytical Chemists (AOAC, 1990). The organic nitrogen content was determined using the micro Kjeldahl method, while the crude protein content was estimated by multiplying the organic nitrogen content by a factor of 6.25 (Sosulski and Imafidon, 1990).

Statistical analysis: The data collected were subjected to analysis using statistic8 computer program. Mean separations were made by Least Significance Difference test (LSD) at P≤5%.

Results and Discussion

Plant height

First season: At 30 days after sowing (DAS), results showed that un-inoculated unfertilized genotypes (C1 and C2) gave 21 and 23 cm height, respectively (Table 1). All treatments increased plant growth as compared to the corresponding control. Among all treatments the combinations of B2+N and B1+N irrespective to maize genotypes sustained the highest significant ($p \leq 0.05$) growth as compared to the control. At 45 and 60 DAS, C1 inoculation with B2+N, irrespective to genotypes, sustained the highest significant ($p \leq 0.05$) growth as compared to other treatments and control. Over all

means results showed that the combination of B2+N gains the highest growth irrespective to maize genotypes as compared to the control. **Second season:** All treatments achieved significant ($p \leq 0.05$) increment of plant growth as compared to the corresponding controls. Among all treatments the combination of B2+N followed by B1+N irrespective to maize genotypes sustained the highest significant ($p \leq 0.05$) growth as compared to the other treatments.

Plant growth-promoting bacteria (PGPB) promote plant growth by secretion of hormones {indole-3-acetic acid (IAA), enzymes, or by facilitating the nutrient uptake such as (Ahemad &

Kibret, 2014). Previous studies have shown positive growth responses in maize, when inoculated with PGPB (Widawati and Suliasih, 2018). Molina *et al.*, (2017) reported that 22% improvement of maize plant height was obtained when inoculated with bacteria. Similarly, Arruda *et al.*, (2013) revealed that the maize inoculation with different bacteria strains significantly promoted root (50-68 %) and shoot (25-54 %) growth. In general, the performance of ZML309 (C2) was better than Hudibi2 (C1) at both seasons. Differences in plant height observed among maize varieties could be attributed to genetic variability.

Table (1). Effects of bacterial strains and nitrogen fertilizer on two maize genotypes plant height

Genotypes	Treatments	1 st Season 2016/2017				2 nd Season 2017/2018			
		DAS			Overall mean	DAS			Overall mean
		30	45	60		30	45	60	
C1	Control	21.1 ^d	65.8 ^h	108.72 ^{de}	65.23	10.8 ⁱ	60.8 ^f	88.3 ^h	52.63
	B1	22.9 ^{cd}	71.2 ^{fg}	110.5 ^{cde}	68.20	12.6 ^g	71.1 ^d	99.6 ^{fg}	61.13
	B2	23.5 ^{cd}	79.0 ^{bcd}	119.4 ^{bc}	73.83	14.9 ^{ef}	74.0 ^{bc}	106.8 ^d	65.23
	N	22.4 ^{cd}	77.0 ^{cde}	114.8 ^{bcd}	71.40	14.5 ^f	73.0 ^{bcd}	103.7 ^e	63.73
	B1+N	28.5 ^{ab}	82.0 ^{bcd}	118.9 ^{bc}	76.47	17.0 ^c	74.8 ^b	112.6 ^c	68.13
	B2+N	29.8 ^a	90.3 ^a	123.1 ^{ab}	81.07	19.5 ^b	81.1 ^a	120.9 ^a	73.83
C2	Control	23.0 ^{cd}	72.0 ^{efg}	97.2 ^f	64.07	11.9 ^h	59.1 ^f	84.0 ⁱ	51.40
	B1	24.2 ^{bcd}	76.0 ^{def}	106.0 ^{ef}	68.70	14.5 ^f	69.5 ^e	97.0 ^g	60.33
	B2	24.5 ^{bcd}	86.0 ^{ab}	112.7 ^{cde}	72.60	16.1 ^d	72.6 ^{cd}	103.7 ^e	64.13
	N	25.7 ^{abc}	82.2 ^{bcd}	110.7 ^{cde}	72.87	15.2 ^e	71.7 ^d	100.6 ^f	62.50
	B1+N	29.3 ^a	83.4 ^{abc}	117.7 ^{bcd}	76.80	17.6 ^c	73.1 ^{bcd}	111.5 ^c	67.40
	B2+N	29.7 ^a	89.4 ^a	130.5 ^a	83.20	21.1 ^a	79.8 ^a	116.0 ^b	72.30
LSD		4.47	7.08	9.80		0.71	2.08	2.87	

Mean values bearing different superscripts within columns are significantly different ($P < 0.05$). Key: DAS; (Days After Sowing) C1; (Hudibi2) C2; (ZML309.) Control; (un-inoculated, unfertilized) B1; (*Bacillus megatherium var phosphorous*+*Azotobacter spp* +*Azospirillum spp*) B2; (*Bacillus megatherium var phosphorous*+*Azotobacter spp* + *Flavobacterium spp*) N; (Nitrogen 80kg/F).

Stem thickness

First season: At all sampling times results revealed that maize genotypes C1 and C2 inoculated with B2+N followed by B1+N and B2 achieved the highest significant ($p \leq 0.05$) stem thickness as compared to other treatments and control (Table 2). From overall means results showed that the combination of B2+N gains the maximum stem thickness irrespective to maize genotypes as compared to the control.

Second season: The results of the second season were ongoing with the results of the first season, at all sampling times results showed that maize genotypes C1 and C2 inoculated with B2+N followed by B1+N gained the highest significant ($p \leq 0.05$) stem thickness as compared to other treatments and control. Similar results reported by researchers Lin *et al.*, (2018); Chattha *et al.*, (2017) where Molina *et al.*, (2017) measured 12% thicker stem at PGPR inoculated conditions. Gholami *et al.*, (2012) reported Azo-

tobacter s-5 + Azospirillum s-21 inoculated maize seeds significantly increased the stem height by 17% and stem diameter by 28% under field condition. Moreover, Aquino *et al.*, (2019)

reported that 17 PGPB isolates promoted a higher stem diameter in maize than in uninoculated maize plants supplied with N.

Table (2). Effects of bacterial strains and nitrogen fertilizer on two maize genotypes stem thickness

Genotypes	Treatments	1 st Season 2016/2017			Overall mean	2 nd Season 2017/2018			Overall mean
		DAS				DAS			
		30	45	60		30	45	60	
C1	Control	4.4 ⁱ	5.3 ^g	6.8 ^f	5.5	3.0 ^g	3.5 ^e	5.5 ^h	4.0
	B1	5.1 ^{gh}	5.8 ^{ef}	6.8 ^f	5.9	3.5 ^{def}	3.8 ^d	6.2 ^{ef}	4.5
	B2	5.7 ^{cdf}	6.8 ^{cd}	7.2 ^{df}	6.6	4.5 ^c	4.8 ^c	6.6 ^{cd}	5.3
	N	5.2 ^{fgh}	6.1 ^{de}	7.2 ^{df}	6.2	3.5 ^{ef}	3.8 ^d	6.4 ^{de}	4.6
	B1+N	5.8 ^{cdf}	6.8 ^{bc}	7.7 ^{bc}	6.6	4.8 ^c	5.2 ^b	6.9 ^b	5.5
	B2+N	6.1 ^{bc}	7.2 ^{ab}	7.8 ^b	7.0	5.4 ^b	5.8 ^a	7.3 ^a	6.2
C2	Control	4.8 ^{hi}	5.6 ^{fg}	6.9 ^{ef}	5.8	3.3 ^{fg}	3.8 ^{ef}	5.2 ⁱ	4.1
	B1	5.5 ^{efg}	6.6 ^{cd}	7.2 ^{df}	6.4	3.9 ^d	4.6 ^d	5.9 ^g	4.7
	B2	6.0 ^{bcd}	6.9 ^{bc}	7.4 ^{cd}	6.8	4.8 ^c	5.4 ^c	6.3 ^{ef}	5.4
	N	5.6 ^{def}	6.6 ^c	7.7 ^{bc}	6.6	3.8 ^{ed}	4.7 ^d	6.2 ^f	4.9
	B1+N	6.3 ^{ab}	7.3 ^{ab}	7.9 ^b	7.2	5.2 ^b	6.2 ^b	6.6 ^c	6.0
	B2+N	6.7 ^a	7.7 ^a	8.3 ^a	7.6	5.8 ^a	6.6 ^a	7.1 ^b	6.3
LSD		0.49	0.48	0.32		0.38	0.34	0.22	

Mean values bearing different superscripts within columns are significantly different ($P < 0.05$). Key: DAS; (Days After Sowing) C1; (Hudibi2) C2; (ZML309.) Control; (un-inoculated, unfertilized) B1; (*Bacillus megatherium var phosphorous + Azotobacter spp + Azospirillum spp*) B2; (*Bacillus megatherium var phosphorous + Azotobacter spp + Flavobacterium spp*) N; (Nitrogen 80kg/F).

Table (3). Effects of bacterial strains and nitrogen fertilizer on two maize genotypes leaf area/cm²

Genotypes	Treatments	1 st Season 2016/2017			Overall mean	2 nd Season 2017/2018			Overall mean
		DAS				DAS			
		30	45	60		30	45	60	
C1	Control	137.3 ^g	305.7 ^g	354.7 ^g	265.9	76.9 ⁱ	190.7 ^{jk}	318.7 ⁱ	195.4
	B1	170.8 ^{efg}	327.7 ^{fg}	388.1 ^{efg}	295.5	87.0 ⁱ	205.8 ⁱ	341.6 ^g	211.5
	B2	204.7 ^{cd}	379.4 ^{cd}	422.3 ^{cde}	335.5	126.0 ^e	275.6 ^e	368.0 ^e	256.5
	N	200.3 ^{cde}	357.1 ^{def}	392.2 ^{def}	316.5	108.8 ^g	232.2 ^g	340.6 ^g	277.2
	B1+N	217.6 ^{bc}	393.8 ^{bcd}	455.1 ^{bc}	355.5	143.3 ^d	322.0 ^b	385.0 ^c	283.4
	B2+N	250.2 ^{ab}	425.4 ^{ab}	481.7 ^{ab}	385.8	156.1 ^b	340.0 ^a	408.4 ^a	301.5
C2	Control	155.1 ^{fg}	312.2 ^g	366.2 ^{fg}	277.8	79.5 ^g	184.5 ^k	306.8 ⁱ	190.3
	B1	175.5 ^{def}	335.9 ^{efg}	396.6 ^{def}	302.7	96.2 ^h	195.7 ⁱ	334.0 ^h	208.6
	B2	209.6 ^c	388.7 ^{bcd}	428.8 ^{cd}	342.4	125.5 ^e	254.1 ^f	357.1 ^f	245.6
	N	201.6 ^{cde}	366.9 ^{cde}	398.5 ^{def}	322.3	114.8 ^f	217.5 ^e	332.6 ^h	233.8
	B1+N	227.2 ^{abc}	402.6 ^{abc}	469.2 ^{ab}	366.3	148.9 ^c	289.5 ^d	379.0 ^d	272.5
	B2+N	255.5 ^a	433.5 ^a	501.7 ^a	396.9	176.3 ^a	314.4 ^c	398.2 ^b	296.3
LSD		33.70	38.61	37.37		5.08	7.17	4.68	

Mean values bearing different superscripts within columns are significantly different ($P < 0.05$). Key: DAS; (Days After Sowing) C1; (Hudibi2) C2; (ZML309.) Control; (un-inoculated, unfertilized) B1; (*Bacillus megatherium var phosphorous + Azotobacter spp + Azospirillum spp*) B2; (*Bacillus megatherium var. phosphorous + Azotobacter spp + Flavobacterium spp*) N; (Nitrogen 80kg/F).

Leaf area:

First season: At 30 DAS, results revealed that maize genotypes inoculated with B2+N and

B1+N significantly ($p \leq 0.05$) increased leaf area as compared to the control. Furthermore, maize genotypes at 45 and 60 DAS followed the same aforementioned trend (Table 3). Overall means

showed that the combination of B2+N gains the highest leaf area irrespective to maize genotypes.

Second season: At 30, 45 and 60 DAS, inoculation of maize genotypes with the combinations of B2+N and B1+N significantly ($p \leq 0.05$) increased leaf area as compared to the other treatments and controls. Overall means showed that the combination of B2+N gain the highest leaf area irrespective to maize genotypes.

Number of leaves/plants

First season: All treatments achieved maximum number of leaves/ plants as compared to the control. At 30 and 45 DAS, inoculation of C1 and C2 with B2+N significantly ($p \leq 0.05$) increased leaves number per plant as the control (Table 4). at 60 (DAS), maize genotypes inoculated with B2+N followed by B1+N gave the

highest significant ($p \leq 0.05$) number of leaves/ plants. From overall means, the combination of B2+N gains the maximum number of leaves/ plants irrespective to maize genotypes.

Second season: At 30, 45 and 60 DAS, results showed that all treatments evidenced maximum number of leaves/ plants as compared to the controls. Among all treatments the combinations of B2+N and B1+N regardless to maize genotypes sustained the highest significant ($p \leq 0.05$) number of leaves per plant as compared to the other treatments and controls. Overall means showed that the combination of B2+ N gave the highest number of leaves/ plants irrespective to maize genotypes. Prajapati *et al.*, (2016) showed significant increase in number of leaves and grain yield over uninoculated control.

Table (4). Effects of bacterial strains and nitrogen fertilizer on two maize genotypes number leaves/plant

Genotypes	Treatments	1 st Season 2016/2017				2 nd Season 2017/2018			
		DAS			Overall mean	DAS			Overall mean
		30	45	60		30	45	60	
C1	Control	8.6 ^g	10.8 ^g	11.9 ^h	10.4	6.0 ^g	9.0 ^{ef}	10.8 ^{gh}	8.6
	B1	9.1 ^{fg}	11.2 ^{fg}	12.4 ^f	11.1	6.3 ^{efg}	9.4 ^{ef}	11.8 ^{ef}	9.2
	B2	9.4 ^{defg}	12.2 ^{de}	13.7 ^{cd}	11.8	6.6 ^{ef}	10.4 ^{bcd}	12.5 ^{cde}	9.8
	N	9.3 ^{efg}	11.8 ^{ef}	13.0 ^{ef}	11.4	6.3 ^{fg}	9.8 ^{cde}	11.8 ^{ef}	9.3
	B1+N	10.2 ^{ef}	12.4 ^{cde}	14.9 ^b	12.5	8.0 ^b	11.2 ^{abc}	13.3 ^{bc}	10.8
	B2+N	10.6 ^{abc}	13.3 ^{ab}	15.4 ^{ab}	13.1	8.8 ^a	12.0 ^a	14.3 ^a	11.7
C2	Control	9.1 ^{fg}	11.6 ^{efg}	12.4 ^f	11.0	6.3 ^{efg}	8.8 ^{ef}	10.6 ^h	8.6
	B1	9.7 ^{cde}	12.2 ^{de}	13.5 ^{de}	11.8	6.7 ^{df}	9.3 ^{ef}	11.6 ^{efg}	9.2
	B2	10.3 ^{bcd}	12.9 ^{bcd}	14.0 ^c	12.4	7.5 ^c	10.2 ^{bcd}	12.3 ^{def}	10.0
	N	10.3 ^{bcd}	12.8 ^{bcd}	13.4 ^{de}	12.2	7.1 ^d	9.6 ^{def}	11.6 ^{fg}	9.4
	B1+N	11.1 ^{ab}	13.0 ^{abc}	15.3 ^b	13.0	8.3 ^b	11.0 ^{abc}	13.1 ^{cd}	10.9
	B2+N	11.3 ^a	13.8 ^a	15.8 ^a	13.5	8.8 ^a	11.8 ^{ab}	14.1 ^{ab}	11.6
LSD		1.00	0.817	0.458		0.41	1.48	0.90	

Mean values bearing different superscripts within columns are significantly different ($P < 0.05$). Key: DAS; (Days After Sowing) C1; (Hudibi2) C2; (ZML309.) Control; (un-inoculated, unfertilized) B1; (*Bacillus megatherium var phosphorous* + *Azotobacter* spp + *Azospirillum* spp) B2; (*Bacillus megatherium var phosphorous* + *Azotobacter* spp + *Flavobacterium* spp) N; (Nitrogen 80kg/F).

Chlorophyll content

Results revealed that chlorophyll content level declined towards maturity (Table 5). Two seasons were trended the same line, maize genotypes inoculated with B2+N and B1+N recorded highest level of chlorophyll content as compa-

red to other treatments and controls. Overall means showed that the combination of B2+N wins the highest level of chlorophyll content irrespective to maize genotypes.

Meanwhile, Goswami *et al.*, (2019) reported that combination of inorganic source and bacterial

strains significantly enhanced plant height, number of leaves, stem width and chlorophyll content at 30, 60 and at harvest of crop over uninoculated control. Ghetya *et al.*, (2018) revealed that application of 45 kg K₂O/ha + KSB seed inoculation+KSB soil application enhanced leaf chlorophyll content of maize crop. The

obtained results for increasing chlorophyll content may be attributed to the microorganism's effect on nutrients release in soil in available forms leading to the rise of nitrogen content in the plants (Shanthi *et al.*, 2012; Mahato and Neupane, 2017).

Table (5). Effects of bacterial strains and nitrogen fertilizer on two maize genotypes chlorophyll content

Genotypes	Treatments	1 st Season 2016/2017			2 nd Season 2017/2018		
		DAS		Overall mean	DAS		Overall mean
		45	60		45	60	
C1	Control	37.6 ^f	31.8 ^d	34.7	31.8 ^{fg}	28.7 ^e	30.3
	B1	40.1 ^{ef}	37.2 ^{cd}	38.7	34.3 ^{efg}	33.2 ^d	33.8
	B2	44.8 ^{bcd}	42.0 ^{abc}	43.4	38.9 ^{bc}	36.7 ^{bc}	37.8
	N	42.5 ^{bc}	32.1 ^d	37.3	36.0 ^{def}	34.6 ^{bcd}	35.3
	B1+N	45.7 ^{bc}	44.4 ^{abc}	45.1	40.0 ^b	38.3 ^b	38.7
	B2+N	51.2 ^a	46.5 ^{ab}	48.9	44.8 ^a	43.1 ^a	43.5
C2	Control	43.2 ^{cde}	39.4 ^{bcd}	41.3	31.0 ^g	28.7 ^e	29.9
	B1	45.0 ^{bcd}	42.9 ^{abc}	44.0	33.5 ^{efg}	33.2 ^d	33.4
	B2	46.6 ^{bc}	44.9 ^{ab}	45.8	38.1 ^{bcd}	36.7 ^{bc}	37.4
	N	46.6 ^{bc}	42.9 ^{abc}	44.8	35.2 ^{ef}	34.8 ^{cd}	34.9
	B1+N	47.7 ^b	44.4 ^{ab}	46.1	39.2 ^{bc}	37.3 ^{bc}	38.3
	B2+N	52.0 ^a	49.4 ^a	49.0	44.0 ^a	42.3 ^a	43.1
LSD		3.541	8.005		3.63	3.26	

Mean values bearing different superscripts within columns are significantly different ($P < 0.05$). Key: DAS; (Days After Sowing) C1; (Hudibi2) C2; (ZML309.) Control; (un-inoculated, unfertilized) B1; (*Bacillus megatherium var phosphorous*+*Azotobacter* spp + *Azospirillum* spp) B2; (*Bacillus megatherium var phosphorous*+*Azotobacter* spp + *Flavobacterium* spp) N; (Nitrogen 80kg/F).

Table (6). Effects of bacterial strains and nitrogen fertilizer on two maize genotypes root dry weight, cubs Length, number of cubs/ plant and number of rows/ cobs

Genotypes	Treatments	1 st Season 2016/2017				2 nd Season 2017/2018				
		Root dry weight (gm)	Cub length (cm)	Cubs /plant	Rows /cub	Root dry weight (gm)	Cub length (cm)	Cubs /plant	Rows /cub	
C1	Control	56.8 ^f	13.3 ^f	1.1 ^d	10.2 ⁱ	28.7 ^h	10.9 ^e	1.1 ^c	9.6 ^g	
	B1	62.5 ^{ef}	14.5 ^{de}	1.1 ^d	10.8 ^h	32.5 ^g	9.5 ^g	1.1 ^c	9.3 ^g	
	B2	62.5 ^{ef}	15.2 ^d	1.2 ^{cd}	12.6 ^{ef}	40.0 ^f	12.0 ^{cd}	1.2 ^{bc}	11.2 ^e	
	N	71.1 ^{cd}	15.9 ^c	1.2 ^{cd}	13.3 ^{cd}	42.6 ^f	10.1 ^f	1.2 ^{bc}	10.3 ^f	
	B1+N	73.6 ^{cd}	16.1 ^c	1.4 ^b	12.8 ^{de}	50.5 ^{de}	12.4 ^c	1.4 ^{ab}	12.1 ^c	
	B2+N	78.2 ^c	16.3 ^c	1.4 ^b	13.8 ^{bc}	52.9 ^{cd}	11.1 ^e	1.4 ^{ab}	11.4 ^{de}	
C2	Control	66.8 ^{de}	14.2 ^e	1.1 ^d	11.5 ^g	48.1 ^e	11.0 ^e	1.1 ^c	10.7 ^f	
	B1	67.9 ^{ed}	14.9 ^d	1.1 ^d	12.3 ^f	50.8 ^{de}	11.0 ^e	1.1 ^c	11.1 ^e	
	B2	93.9 ^b	16.6 ^c	1.5 ^b	13.4 ^c	55.0 ^c	12.9 ^b	1.4 ^{ab}	12.6 ^b	
	N	97.8 ^b	17.5 ^b	1.4 ^{bc}	14.1 ^b	59.7 ^b	11.6 ^d	1.3 ^{ab}	11.8 ^{cd}	
	B1+N	98.2 ^{ab}	17.4 ^b	1.5 ^b	14.1 ^b	60.0 ^b	13.4 ^a	1.4 ^{ab}	13.6 ^a	
	B2+N	106.4 ^a	18.2 ^a	1.7 ^a	15.3 ^a	66.7 ^a	12.1 ^c	1.5 ^a	12.7 ^b	
LSD		8.51	0.651	0.651	0.54	3.04	0.602	0.602	0.46	

Mean values bearing different superscripts within columns are significantly different ($P < 0.05$). Key: C1; (Hudibi2) C2; (ZML309.) Control; (un-inoculated, unfertilized) B1; (*Bacillus megatherium var phosphorous*+*Azotobacter* spp + *Azospirillum* spp) B2; (*Bacillus megatherium var phosphorous*+*Azotobacter* spp + *Flavobacterium* spp) N; (Nitrogen 80kg/F).

Yield components

Root dry weight: Results showed that all treatments increased root dry weight as compared to the corresponding control (Table 6). At both seasons, inoculation of C2 and C1 with B2+N followed by B1+N, N and B2 significantly ($p \leq 0.05$) increased root dry weight as compared to the corresponding control.

Cub length: Results showed that all treatments significantly ($p \leq 0.05$) increased cub length at both seasons as compared to the corresponding control except the inoculation of maize C2 with B1 (Table 6).

Number cubs/plant: Result in table (6) revealed that all treatments significantly ($p \leq 0.05$) increased number of cubs/plant as compared to the corresponding control, except B1. Among all treatments the combinations of B2+N and B1+N, irrespective to maize genotypes sustained the highest number of cubs per plant.

Number of rows/cub: Inoculation by B2+N and B1+N significantly ($p \leq 0.05$) increased numbers of rows/cob at both seasons as compared to the corresponding control (Table 6).

Number of seeds/row: At first season, results appeared that un-inoculated, unfertilized genotypes (C1 and C2) gave 19.3 and 20.4 number of grains /row, respectively (Table 7). All treatments increased number of grains /row as compared to the corresponding control. C1 appeared non-significant different between application of B1 and B2 (Table 7). At second season result showed that un-inoculated, unfertilized genotypes (C1 and C2) gave 17.5 and 20.8 numbers of grains /row, respectively. All treatments increased number of grains/rows as compared to the corresponding control. (table7). C1 showed non-significant different between application of B1+N and B2+N, however C2 cleared non-significant different between application of B2 and N. Among all treatments the combination between B2+N and B1+N, at both seasons irrespective to maize genotypes sustained

enhanced the number of grains/rows as compared to the other treatments.

Harvest index %: At first season, results showed that un-inoculated, unfertilized genotypes (C1 and C2) gave 20.5 and 23.9 harvest index, respectively (Table 7). All treatments increased harvest index as compared to the corresponding control. C1 showed non-significant different among application of control, B1, B2 and N, whereas C2 appeared non-significant different between application of B2 and N (Table 7). At second season, results showed that un-inoculated, unfertilized genotypes (C1 and C2) gave 10.0 and 14.8 harvest index, respectively. All treatments increased number of grains/row as compared to the corresponding control. C1 cleared non-significant different between application of B2, N and between B1, B2. Among all treatments the combination between B2+N and B1+N, at two seasons irrespective to maize genotypes sustained greater the harvest index as compared to the other treatments.

100 seeds weight/gm: At first season, results showed that un-inoculated, unfertilized genotypes (C1 and C2) gave 13.9 and 15.9 100 grains weight, respectively (Table 7). All treatments increased number of 100 grains weight as compared to the corresponding control. At second season result cleared that un-inoculated, unfertilized genotypes (C1 and C2) gave 8.8 and 10.2 gm 100 grains weight, respectively. All treatments increased number of 100 grains weight as compared to the corresponding control. C2 cleared non-significant different between application of B1, B2 and between B1+N and B1+N. Among all treatments the combination between B1+N and B1+N. at both seasons irrespective to maize genotypes sustained better the 100 grains weight as compared to the other treatments.

Yield (t/ha): At first season, results showed that un-inoculated, unfertilized genotypes (C1 and C2) gave 2.4 and 3.1 yield (t/ha), respectively (Table 7). All treatments increased yield (t/ha)

as compared to the corresponding control. C1 showed non-significant different between application of control, B1 and between B2, N while C2 appeared non-significant different between application of B1+N and B1+N. At second season result showed that un-inoculated, unfertilized genotypes (C1 and C2) gave

0.9 and 1.8 yield (t/ha), respectively. All treatments increased yield (t/ha) as compared to the corresponding control. Among all treatments the combination between B2+N, B1+N and N, at both seasons irrespective to maize genotypes sustained improved the yield (t/ha) as compared to the other treatments.

Table (7). Effects of bacterial strains and nitrogen fertilizer on two maize genotypes number of grains/rows, harvest index %, 100 grains weight and yield

Geno- types	Treatments	1 st Season 2016/2017				2 nd Season 2017/2018			
		Seeds /row	Harvest index %	100 seeds weight (gm)	Yield (t/ha)	Seeds /row	Harvest index %	100 seeds weight (gm)	Yield (t/ha)
C1	Control	19.3 ⁱ	20.5 ^d	13.9 ^h	2.4 ^g	17.5 ^f	10.0 ^f	8.8 ^g	0.9 ^j
	B1	20.9 ^h	21.2 ^d	14.2 ^{gh}	2.6 ^g	18.7 ^{ef}	12.6 ^g	9.9 ^f	1.0 ⁱ
	B2	20.8 ^h	22.9 ^d	15.2 ^{fgh}	2.7 ^{fg}	20.2 ^d	17.0 ^e	10.3 ^{cd}	1.3 ^h
	N	21.3 ^{gh}	22.8 ^d	15.8 ^{efg}	2.8 ^{fg}	19.3 ^e	17.0 ^e	10.2 ^{ef}	1.4 ^g
	B1+N	22.8 ^{ef}	25.0 ^c	16.3 ^{cdef}	3.5 ^{cd}	21.9 ^c	18.8 ^d	10.4 ^e	1.9 ^{ef}
	B2+N	23.9 ^{de}	26.4 ^{abc}	17.5 ^{bcde}	3.7 ^c	22.7 ^c	19.0 ^d	11.8 ^b	2.0 ^d
C2	Control	20.4 ^{hi}	23.9 ^c	15.9 ^{defg}	3.1 ^{ef}	20.3 ^{de}	14.8 ^e	10.2 ^{ef}	1.8 ^f
	B1	22.2 ^{fg}	24.6 ^{bcd}	16.9 ^{bcdef}	3.2 ^{ed}	20.6 ^d	21.6 ^c	10.9 ^d	1.9 ^e
	B2	24.7 ^{cd}	25.6 ^{bc}	17.6 ^{bcd}	4.5 ^b	23.1 ^c	22.3 ^c	11.1 ^d	2.4 ^c
	N	25.6 ^{bc}	25.7 ^{bc}	17.9 ^{bc}	4.7 ^{ab}	22.6 ^c	21.8 ^{cb}	11.6 ^{bc}	2.6 ^b
	B1+N	26.2 ^{ab}	27.0 ^{ab}	18.5 ^{ab}	4.7 ^a	25.2 ^a	22.3 ^c	12.3 ^a	2.8 ^a
	B2+N	27.1 ^a	28.1 ^a	19.9 ^a	5.0 ^a	24.0 ^b	23.4 ^a	12.5 ^a	2.6 ^b
	LSD	1.13	1.83	1.805	0.38	0.60	1.00	0.429	0.090

Mean values bearing different superscripts within columns are significantly different ($P < 0.05$). Key: C1; (Hudibi2) C2; (ZML309.) Control; (un-inoculated, unfertilized) B1; (*Bacillus megatherium* var *phosphorous*+*Azotobacter* spp + *Azospirillum* spp) B2; (*Bacillus megatherium* var *phosphorous*+*Azotobacter* spp + *Flavobacterium* spp) N; (Nitrogen 80kg/F).

Crozier *et al.*, (1988) reported that, in addition to the nitrogen partition, bacterial strains produce hormones that stimulate root growth, the main one being auxin, indole acetic acid (AIA). By stimulating root growth, it promotes higher absorption of water and nutrients, also favoring aerial growth and increased dry matter production, resulting in productivity increases.

Javeed *et al.*, (2019) reported that the combination of phosphorus solubilization bacteria (PSB) + RNPK improved the yield and yield-related characteristics of spring maize grown in sandy soil. Moreover, it also enhanced dry matter characteristics and maize grain quality. In the present study, inoculation of the bacterial strains B2, B1 in combination N could potentially be

used as bio-inoculants to improve maize yield. Adoption of microbial consortia as plant growth promoting microorganisms instead of single-strain inoculants is as an important approach to increase the production strategies. The role and importance of seed inoculation with bacterial strains, nitrogen fertilization and their combinations on different plant species in sustainable crop production has been reviewed by several authors (Dakhly *et al.*, 2004; Azab and Dewiny, 2018). Increased the plant height, stem thickness and leaf area of maize due to companion bacterial strains and nitrogen fertilizer B2+N and B1+N can be explained by the fact that application of bacterial strains with nitrogen fertilizer not only increased the nutritious elements which the plant needed but also incre-

ase of N in the root zone and the synergistic effect of these microorganisms on the physiological and metabolic activities of the plant. This enhancing effect may induce exudates of some hormonal substances like cytokinins and auxins, which encourage plant height, stem thickness and leaf area. This also may be attributed due to more atmospheric nitrogen fixed

in the soil, which was probable due to mobilization of bacteria, providing favorable conditions and discharge of antibiotics leads to the development of root systems of maize, which increase the vegetative growth (Garg *et al.*, 2005; Akbari *et al.*, 2009; Azab and Dewiny, 2018).

Table (8). Effects of bacterial strains and nitrogen fertilizer on biochemical characters of two maize genotypes

Genotypes	Treatments	1 st Season 2016/2017			2 nd Season 2017/2018		
		Protein%	Nitrogen %	Fiber%	Protein%	Nitrogen %	Fiber%
C1	Control	7.6G	1.2I	3.0H	8.0E	1.3E	2.7G
	B1	9.3D	1.5F	3.7E	9.3CD	1.5CD	3.3D
	B2	9.7BC	1.55CD	3.8CD	9.5BC	1.6A	3.4C
	N	9.5CD	1.52DE	3.7E	10.2A	1.5BC	3.3D
	B1+N	10.0AB	1.6BC	3.9B	10.2A	1.6A	3.5B
	B2+N	10.2A	1.64A	4.1A	10.4A	1.7A	3.7A
C2	Control	7.3H	1.2J	2.9I	7.7E	1.2E	2.5H
	B1	8.5F	1.36H	3.5G	8.9D	1.4D	3.1F
	B2	8.9E	1.4G	3.6F	9.3CD	1.5CD	3.2E
	N	8.8E	1.4G	3.5G	9.0D	2.4D	3.1F
	B1+N	9.3D	1.5EF	3.7E	10.0AB	1.6AB	3.3D
	B2+N	10.1A	1.6AB	3.8C	10.2A	1.6A	3.4BC
	LSD	0.247	0.038	0.06	0.471	0.075	0.080

Mean values bearing different superscripts within columns are significantly different ($P < 0.05$). Key: C1; (Hudibi2) C2; (ZML309.) Control; (un-inoculated, unfertilized) B1; (*Bacillus megatherium var phosphorous*+*Azotobacter* spp. + *Azospirillum* spp.) B2; (*Bacillus megatherium var phosphorous*+*Azotobacter* spp. + *Flavobacterium* spp.) N; (Nitrogen 80kg/F).

Effects of bacterial strains and nitrogen fertilizer on biochemical characters of two maize genotypes:

In term of grain quality, results displayed that all treatments increased nitrogen%, crude protein and fiber as compared to the corresponding control (Table 8). In among all treatments the combination of B2+N increased nitrogen%, crude protein and fiber irrespective to maize genotypes. Moreover, C2 showed the highest values as compared to C1. This finding was supported by Saber and Sharaf (2013) who reported that application bio-fertilizers increase the protein% in wheat cultivar. Moreover, Helmy *et al.*, (2014) found that using the bio-fertilizer with urea increase protein content in barley (*H. vulgare*. L). Hellal *et al.*, (2011)

indicated that applying bacteria strains treatment alone or in combination with chemical N fertilizer increased the chemical constituents of dill (*A.graveolens* L.) plant compared to the untreated control. Therefore, the inoculation with these bacterial strains may be an ecological alternative for decreasing the dependence on chemical fertilizers.

Conclusions

The combination of nitrogen and bacterial strains *Bacillus megatherium var. phosphorous* + *Azotobacter* spp. + *Flavobacterium* spp. gave the highest significant results in plant growth parameters and yield components, irrespective to maize genotypes. Maize genotype ZML309 showed the highest values as compared to genotype Hudibi2.

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