

Effect Of Bacterial Strains And Mineral Nitrogen Fertilizer On Fresh And Dry Forage Yield Of Two Maize (*Zea Mays* L.) Genotypes In Summer And Winter Seasons

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Abstract – A field experiment was carried out during two successive summer and two winter 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 fresh and dry forage yield. Treatments were distributed in Randomized Complete Block Design (RCBD) in four replicates, which consisted of two maize genotypes (Hudibi2 (C1) and ZML309 (C2)). The form of two types of bacterial strains *Bacillus megatherium* var. phosphorous + *Azotobacterspp* + *Azospirillum*spp (B1) and *Bacillus megatherium* var. phosphorous + *Azotobacterspp* + *Flavobacterium*spp (B2), nitrogen applied at rates of 80kg/f (N) plus control. Parameters which were mustered plant height (cm) , number of leaves /plant , fresh and dry forage yield The results showed that the combinations of B1 and B2 with N increased fresh and dry forage yield compared to B1, B2 and N each alone at all seasons. The general trend was that combinations (B2+N) had significant effect of plant height, number of leaves/ plant, fresh and dry forage yield in all seasons. Moreover, statistical analysis appeared that performance of genotype C2 is better than genotype C1 in both summer and both winter seasons.

Keywords – *Azotobacter*, *Azospirillum*, *Flavobacterium*, Maize, fresh and dry forage, yield.

I. INTRODUCTION

Maize or corn (*Zea mays* L.) belongs to the family Poaceae. The origin of Maize remains uncertain although is generally agreed what its evolution in to modern forms took place in Mexico. It is called “King of cereals” because of its productivity potential compared to any other cereal crop and it a remarkable adaptability in a wide range of climates, (Farnia and Keysam, 2015). It is cultivated globally being one of the most important cereal crops worldwide, superior position of maize is due to his very wide and variety utilization. During the centuries maize plant was known for it is multifariously use, it provides food for human, feed for animals and poultry, and fodder for livestock (ABPSD, 2008). Maize is the World’s primary source for animal feed. It is the only crop amongst non-leguminous combining high quantity of biomass along with better nutritional quality. Forage maize has become a major constituent of ruminant rations in recent years, where its inclusion in dairy cow diets improves forage intake and animal performance , crop has good reputation to increase milk production when fed as green forage (Mohammed and Mohammed, 2019). It is a promising cereal crop in Sudan with the potential usefulness for both human beings and livestock (Salih *et al.*, 2008). The major grass forage crops in Sudan include, Absabien (*Sorghum bicolor*), Sudan grass (*Sorghum sudanense*), Sorghum-Sudan grass hybrids and recently maize. Compared to others, maize performed very well in winter, so production of forage maize in winter solves the problem of livestock feed shortage during the cool season (Eltelib *et al.*, 2006).

Maize fodders contain relatively high concentration of soluble carbohydrates and yield a high quality biomass within a short period, making it attractive as hay and silage crops for tropical areas (Zubair *et al.*, 2015). The crop is nitro positive and needs ample quantity high yield. Nitrogen deficiency is a key factor for limiting maize yields (Alvarez and Grigera, 2005), its reduction due to

nitrogen deficiency is more than of other elements deficiency (Mohammadian *et al.*, 2010). Application of biofertilizers 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). The aim of the present study was to evaluate the effect of seed inoculation with bacterial strains, nitrogen fertilization and their combinations on fresh and dry forage yield of maize plants.

II. MATERIALS AND METHODS

Afield experiments were conducted during summers and winters 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 Toun, 2016).

Two open pollinated maize genotypes seeds namely Hudibi2 (C1) and ZML309 (C2) were obtained from Wad Madani Research Station, El-Gazzira State. Four bacterial strains *Bacillus megatherium varphosphors*, *Azotobacterspp*, *Azospirillum*spp, and *Flavo bacterium*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 have 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 40 cmx10 cm 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 at the last week of July in the two summer seasons and in second week of November in both winter seasons (first season 2016/17 and second season 2017/18). Seeds (5-7) coated by bacterial strains were sown. Nitrogen fertilizer 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 was used. Hand weeding was done each two weeks. All necessary agronomic practiced was done uniformly (Ukonze *et al.*, 2016).

Data collection

Plant height (cm)

The height of the main stem was measured from ground level to the tip of the plant, using a tape meter from five selected plant each plot,

Number of leaves/plant:

The mean number of leaves was counted from randomly selected plant from each plot.

Fresh forage yield (tons/ha)

The average fresh weight was determined from one meter square harvested plant at milk stage from each plot. Sickle was used for clipping plants around five cm above the soil surface. The samples were weighed using a balance immediately in the field to get the fresh weight. Final fresh yield was calculated in tons per ha according to the following:

$$\text{Total Fresh forage yield (t/ha)} = \frac{\text{Fresh forage (kg/m}^2\text{)}}{100}$$

Dry forage yield (tons/ha)

Dry weight in grams was determined through dried fresh samples at sun light until a constant weight was reached and weighted to obtain the mean dry weight. Final dry yield was calculated in tons per ha according to the following:

$$\text{Total dry forage yield (t/ha)} = \frac{\text{dry forage (kg/m}^2\text{)}}{100}$$

Statistical analysis

The data collected were subjected to analysis using statistic8 computer program. Mean separations were made by Least Significance Difference test (LSD) at $P \leq 5\%$ (Gomez 1984).

III. RESULTS AND DISCUSSION:

Plant height

Statistical analyses showed that no different was deducted between cultivars in first summer season on plant height (Table 1), however in second summer C2 gained taller plant height 110.9cm compared to C1 which was 107.2cm also the same trend was obtained in winter seasons. Adding the companion of bio-fertilizers and nitrogen (B2+N) resulted in maximum values of plant height, which was increased over control by 20.7, and 29.3% in first and second season respectively. These results are obtained by Alnoaim *et al.*, (2004) Azab and Dewiny (2018), who reported that the application of bio-fertilizers using N fertilizer increased the plant height and leaf area of corn. That application of bio-fertilizers with nitrogen fertilizers to the soil not only increased the nutritious elements which the plant needed but also improved the physical conditions and living processes of the soil. These results can be due to the effect of bio-fertilizers on biological nitrogen fixation, production of growth hormone (auxin) and discharge of antibiotics leads to the development of root systems of corn, which increase the vegetative growth and yield, Garg, *et al.*, (2004), Azab and Dewiny (2018). In general C2 displayed good responded compared to C1 genotypes with all type of bacteria strains mixtures. Differences in plant height observed among maize varieties could be attributed to genetic variability.

Number of leaves/plant:

The effects of microbial inoculants, maize genotypes on number of leaves/plant were shown in (Table1). Maize genotypes inoculated with (B2+N) had the maximum number of leaves/plant as compared to other treatments. bacteria strains increasing leaves number specially in cereals by producing growth promoting nutrients and on the other hand, plant development is attribute that is influenced by growth hormones, especially oxine and activating this hormone has important role in increasing growth (Hoshang *et al.*, 2011). With respect to these results we can suggest that increasing in number of leaves enhance photosynthesis rate and this enhancement lead to increasing yield. Dordas and Sioulas (2009) suggested that nitrogen affects accumulation of dry matter in different parts of plants. Differences in dry matter accumulation is response to nitrogen is arises from difference in active solar radiation intake by plant canopy and plant performance in use of solar radiation

Fresh forage yield (tons/ha)

The data obtained in (Table1) indicated that C2 achieved huge fresh yield in the first summer season (34.3 tons/ha) and second winter season (34.1 tons/ha) compared to C1 which was recorded (27.4 tons/ha) at the second summer season. In contrast there was no significant different between C2, C1 in the second winter season. Application of combination bacteria strains mixtures plus nitrogen fertilizer (B2+N) resulted in significantly higher fresh forage yield 41.1, 32.8, 21.8 and 42.1 (tons/ha) at first and second summer and winter seasons respectively than others. (Table1).

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Interaction of (B1+N*C2) in first summer and first winter seasons evidence the greatest fresh forage yield, it increased over control by 79.2 and 125.8%, respectively, whereas (B2+N*C2) gained the best value in second summer and second winter seasons, it increased over control by 71.2 and 170.6% respectively (Table 2).

Table 1. Effects of bacterial strains, nitrogen fertilizer and their combination of fresh forage yield and dry forage yield of maize genotypes in summer and winter seasons 2016/2017-2017/2018

Summer								
Treatments	Fresh forage yield (t/ha)		Dry forage yield (t/ha)		Plant height/cm)		Number of leaves	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
Genotypes								
C1	27.4b	24.6a	25.8b	11.7a	115.8A	107.2B	13.6B	12.6B
C2	34.3a	20.6b	28.6a	9.2b	112.43A	110.9A	14.0A	13.2A
LSD	2.13	1.17	1.18	0.81	4.00	0.285	0.187	0.998
Fertilizers								
Control	19.0d	16.9e	20.1e	9.7d	103.0D	12.1E	12.1E	103.0D
B1	27.4c	19.8d	24.7d	10.0c	108.2CD	13.2D	13.2D	108.2CD
B2	32.9b	23.5c	30.3b	10.3c	116.0B	13.8C	13.8C	116.0B
N	29.1c	24.4c	27.4c	10.8c	112.7BC	13.2D	13.2D	112.7BC
B1+N	35.6b	28.0b	32.2b	13.0b	118.3B	15.1B	15.1B	118.3B
B2+N	41.1a	32.8a	35.4a	14.9a	126.8A	15.6A	15.6A	126.8A
LSD	3.70	2.03	2.04	1.41	11.3E	12.1E	94.8F	103.0D
Winter								
Treatments	Fresh forage yield (t/ha)		Dry forage yield (t/ha)		Plant height/cm)		Number of leaves	
Genotypes	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
C1	13.5b	33.7a	12.5b	15.2b	12.6B	13.6B	107.2B	115.8A
C2	17.5a	34.1a	14.9a	16.6a	13.2A	14.0A	110.9A	112.43A
LSD	1.64	1.25	0.93	0.82	0.285	0.187	0.998	4.00
Fertilizers								
Control	8.2d	19.1e	6.6d	8.1d	94.8F	12.1E	334.3E	103.0D
B1	13.8c	30.9d	13.4c	15.0c	104.8E	13.2D	356.9D	108.2CD
B2	15.1c	35.9c	13.6c	15.4c	109.5C	13.8C	403.8C	116.0B
N	15.1c	36.6c	13.8c	15.8c	107.3D	13.2D	354.7D	112.7BC
B1+N	18.8b	38.8b	16.5b	18.1b	115.4B	15.1B	443.5B	118.3B
B2+N	21.8a	42.1a	18.2a	19.9a	122.6A	15.6A	477.2A	126.8A
LSD	2.84	2.16	1.60	1.41	1.729	0.324	2.612	6.930

Means in the same column with same letter are not significantly different. 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/).

Dry forage yield (tons/ha):

The data obtained in (Table1) revealed that C2 displayed maximum values at all seasons, except second summer whereas C1 achieved (11.7 tons/ha) (Table 1).

Increasing dry forage yield was occurred when (B2+N) applied at all seasons, which were 35.4, 14.9, 18.2 and 19.9 (tons/ha) in first and second summer and winter seasons respectively.

(B2+N*C2) interaction displayed the greatest dry forage yield at all seasons except second winter season (B2+N*C1) recorded the best value, it increased over control by 87.7, 54.9, 127.4 and 102.9% first and second seasons respectively (Table 2).

Data illustrated in table 1 and 2 showed that the high fresh and dry yield was positively correlated with winter seasons especially second winter, however first winter the crop was attacked with worm crowd in early stage of growth controlled by spraying Hitec 44%EC.

Both fresh and dry forage yields were significantly influenced by the application of companion of bacteria strains mixtures with nitrogen B2+N in all seasons. Increased in plant fresh and dry weight may be due to the increase of N in the root zone as a result of nitrogen application and fixed N by bacteria, besides the solubilization of mineral nutrient synthesis of vitamins, amino acids and gibberellins, which stimulate growth and yield. Thus production of more dry matter as a result of improved photosynthetic activity at higher level of N (Ayub *et al.*, 2009; Tariq *et al.*, 2011). These results are in agreement with other reports of Ayub *et al.*, (2007), and Mahfouz *et al.*, (2015).

In conclusion the increment in plant fresh and dry weight may be attributed to a greater increase of root biomass resulting in the higher absorption of nutrients and water from the soil leading to production of higher vegetative biomass (Ahmed *et al.*, 2013).

Table 2. Interaction effect of maize genotypes, bacteria strains, nitrogen fertilizer and their combination on forage at summer and winter seasons 2016/2017-2017/2018

Summer									
Treatments		Fresh forage yield (t/ha)		Dry forage yield (t/ha)		Plant height /cm)		Number of leaves	
genotypes	Fertilizers	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
C1	Control	13.6G	19.8F	10.7G	10.2F	90.25A	60.9K	10.8GB	8.8I
	B1	23.0F	22.1D	11.3EF	12.1BC	71.2FG	71.7I	11.2FG	9.2H
	B2	30.2CD	26.1C	14.9CDE	11.4C	79.0BCDE	74.3G	12.2DE	10.2F
	N	26.2EF	25.6D	12.8DEF	11.1CD	77.0CDEF	73.3H	11.8EF	9.6G
	B1+N	33.1BCD	29.8B	16.3CD	14.4A	82.0BCD	74.8F	12.4CDE	11.0DE
	B2+N	38.2B	33.9A	18.7BC	15.8A	90.3A	81.7B	13.3A	11.8C
C2	Control	24.5f	3.9g	13.8fg	7.1g	72.0EFG	62.5J	11.6EFG	9.9FG
	B1	31.8cd	17.5e	16.4cd	7.8e	76.0DEF	73.3H	12.2DE	10.8E
	B2	35.6bc	20.8d	15.2cde	9.2de	86.0AB	75.8D	12.9BCD	11.8C
	N	32.1cd	23.5cd	17.4bc	10.4cd	82.2BCD	74.9E	12.8BCD	11.2D
	B1+N	37.9b	26.2c	21.3ab	11.6c	83.4ABC	76.3C	13.0ABC	12.1B
	B2+N	43.9a	31.5ab	24.9a	13.9ab	89.4A	83.3A	13.8A	13.3A
LSD		5.23	2.88	4.0	2.0	7.08	0.034	0.817	0.389
Winter									
C1	Control	10.2i	16.0h	4.8g	6.0f	92.9j	97.7h	11.0j	11.6h
	B1	23.9g	31.0f	11.4e	13.0d	102.9h	104.1g	12.1ghi	12.6f
	B2	28.3ef	34.8e	12.7de	14.4cd	107.7fg	112.7e	12.8ef	12.8ef
	N	26.1efg	37.7bcd	13.4cde	15.6bc	105.5g	110.1f	11.9hi	12.8ef
	B1+N	31.2cd	39.4bc	15.3bc	16.8b	113.6d	116.3cd	13.5cd	14.2c
	B2+N	34.8ab	43.3a	17.4ab	19.2a	120.8b	123.0b	14.5ab	15.0b

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C2	Control	15.9h	22.2g	8.4f	10.2e	96.7 ⁱ	98.8 ^h	11.6 ^{ij}	12.1 ^g
	B1	25.6fg	30.7f	15.3bc	17.1b	106.7 ^{fg}	108.5 ^f	12.7 ^{fg}	12.9 ^{ef}
	B2	32.2bc	37.1cde	14.6cd	16.3bc	111.3 ^{de}	116.9 ^c	13.4 ^{de}	13.4 ^d
	N	28.6de	35.5de	14.3cd	16.0bc	109.1 ^{ef}	114.4 ^{de}	12.6 ^{fgh}	13.1 ^{de}
	B1+N	33.3abc	39.2bcd	18.8a	20.4a	117.2 ^c	122.3 ^b	14.1 ^{bc}	14.9 ^b
	B2+N	35.9a	40.9ab	19.1a	20.7a	124.4 ^a	127.0 ^a	15.1 ^a	15.6 ^a
	LSD	2.88	3.06	2.27	2.00	2.446	2.32	0.698	0.445

Means in the same column with same letter are not significantly different. 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/).

IV. CONCLUSIONS

From the above discussion, it is concluded that bacteria strains considered natural products that increase fresh and dry forage yield without damaging the environment. Application of (B2+N, B1+N) recorded the best fresh and dry forage yield compared with a different treatment. Performance of cultivar C2 is better than cultivar C1 in all growth and yield parameters in both summer and both winter seasons.

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