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Effect of Harvesting Time and Phosphate Bio fertilizers on the Nutritive Value of Two Maize Varieties

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Two Maize (Zea mays) varieties namely (Hudibia and Mugtama) and two harvesting times were used in this study to evaluate the effect of inorganic phosphorous(P_2O_5) and phosphate biofertilizers namely, Bacillus, mycorrhiza and the combination of them on maize forage quality. The evaluation was done by determining the percentage of CP, EE, OM, NDF, ADF, ADL, in vitro dry matter digestibility, and in situ degradability. A significant difference (P<0.05) was found between the two varieties for all parameters except for CP. Early time of harvest had the highest value of CP, EE, OM and DMD, and the lowest value of NDF, ADF and ADL. The highest percentage of organic matter (89.45%) was found for the treatment with bacteria alone, while the highest percentage of CP (12.9%) was found for the combination between bacteria and mycorrhiza, while

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the lowest percentage was found for the control. The highest value of DMD was recorded for the combination between bacteria and mycorrhiza while the lowest value was found for the control. The effect of inorganic phosphorous source (P_2O_5) and phosphorous biofertilizers on the fiber types was significant (P<0.05). For NDF the lowest value (52.52 %) was recorded in the combination between bacteria and mycorrhiza, while for ADF the combination between inorganic phosphorous and bacteria secured the lowest value (39.98). The highest readily degradable faction (a) was found in the Hudibia variety when fertilized by inorganic phosphorous + mycorrhiza, while the highest slowly degradable fraction (b) was found in the *Mugtamaa* Varity when fertilized by phosphorous+ bacteria. The highest potential degradability (PD) was found in *Mugtamaa* variety when fertilized by inorganic phosphorous+ mycorrhiza. From this study, it could be concluded that the combination between bacteria and mycorrhiza improved the nutritive value of the forage by increasing CP and DMD content and decreasing the NDF content.

Keywords: Inorganic phosphorous; phosphate biofertilizers; nutritive value; maize forage.

1. INTRODUCTION

The agroecosystems are under significant pressure due to the need to expand agricultural production on a global scale in the face of increasingly diminishing and deteriorating land resources [1]. Mineral phosphorus fertilizers need a significant amount of energy to produce, and their use has long-term environmental implications relating to decreased soil fertility and carbon release [2]. "Excessive use of chemical fertilizers is not only cost intensive but also creates the problem of environmental issues such as nitrate pollution and loss of soil carbon. Such environmental considerations have prompted a search for new environmentally friendly ways to feed plants. Currently, ecological farming is receiving special attention on a global scale. Phosphate bio fertilizers were viewed as the most effective ecological measure in this situation" [3].

"Numerous synthetic fertilizers containing acids radicals, like sulfuric and hydrochloride tend to increase soil acidity, adversely affect soil health and the population of beneficial organisms, and interfere with plant growth" [4]. "Phosphorous is the most important key element in the nutrition of the plant, next to nitrogen. It plays an important role in virtually all major metabolic processes in plant including photosynthesis and energy transfer" [5]. "The Bio fertilizers are considered as the most favorable natural compounds to enhance the micro-organism activities in the soil" [6]. The main objectives of the current research are to determine the effect time of harvesting and types of phosphate biofertilizers (Bacillus as phosphorous solublizing bacteria and mycorrhiza as phosphorous mobilizer) on the chemical composition of two maize varieties.

2. MATERIALS AND METHODS

2.1 Samples Collection

The samples were brought from the Department of Botany and Agric. Biotechnology, Faculty of Agriculture, University of Khartoum. The whole plant of two forage maize varieties (Hudibia and Mujtamaa) were used in the study. The samples included forage harvested at two stages of maturity. Different treatments were applied in the study included: inorganic phosphorous fertilizer (P_2O_5) , and phosphorous solubilising microorganisms microorganisms (*Bacillus* sp.) and phosphorous mobilizer (mycorrhiza) and their combinations as a source of phosphate biofertilizers.

2.2 Location and Site Characterizations

Soil samples were collected from the topsoil (0- 30 cm) from experiments sites at the University of Khartoum Farm Shambat area by using the standard procedure recommended by [7]. "These soil samples were used for soil pH was measured on the soil paste by using an Analogue pH meter WAP, the electrical conductivity of soil extract (ECe) was obtained by conductivity meter WPA C M 35. Calcium and magnesium were determined volumetrically with Ethylene diamine tetra acetate (E.D.T.A), sodium and potassium were determined photometrically by corning-EEL flame photometer, the carbonate and bicarbonate and chloride were determined according to the procedure" outlined by [8]. Available phosphorus was determined by sodium bicarbonate method [9]. Total N will be determined by a Kjeldahl method according to [10], organic carbon was determined according to the modified procedure [11]., and the organic carbon was then multiplied by a factor 1.72 to obtain organic matter % according to [12].

2.3 Chemical Analysis

The proximate components of the sample were determined, Ash, EE, and CP according to AOAC methods [13]. NDF, ADF, and ADL were determined according to [14].

2.4 *In situ* **Dry Mater Degradability**

In situ degradability of the samples was determined according to the procedure described by [15]. " Each forge sample of maize weighing 5 g was transferred into nylon bags with a dimension of 8 x 14 cm and a porosity of 40-45mm and incubated in duplicate in two rumen fistulated steers. The samples containing nylon bags were incubated for 4, 8, 16, 24, 48 and 72 hours, and there after hand washed using tap water. Zero-hour solubility was also determined by hand washing samples contained in nylon bags in a similar way to the incubated feed samples. The washed samples were air dried in an oven at 105° c to constant weight to determine dry matter degradability. The *in situ* degradability parameters were fitted using the equation $P = a + b$ (1- e^{-ct}), where P is the DM disappearance at time t, an immediately soluble (wash loss), b is the slowly degradable fraction and c the rate of degradation" [15].

2.5 *In vitro* **Two Stage Digestibility**

The dry matter digestibility of the samples was determined using the procedure 'two-stages *in vitro'* described by [16].

2.6 Statistical Analysis

Data obtained from the study was subjected to analysis of variance (ANOVA) according to the statistics 8 program. The data were statistically analysed in a completely randomized design (CRD) to compare treatment means.

3. RESULTS AND DISCUSSION

A significant difference (P<0.05) was found between the two varieties Hudibia and Mugtamaa in OM, EE and digestibility, whereas no significant difference was found for CP. These results could be comparable to the results obtained by [17] who stated that the digestible dry matter and crude protein yield did not show a significant variation among the different varieties. As general evaluation, [18] reported that the feeds which have <12%, 12- 20% and >20% CP are classified as low, medium and high protein sources, respectively. Based on this classification, the two maize varieties evaluated for forage purposes are classified as low protein feed sources. However, [19] reported that forages whose CP contents could range between 9–12% can be regarded as highly palatable. [19] noted that the means of protein percentage in the 16 forage maize genotypes ranged from 6.98% to 10.09%, obtained by the genotypes TEEI 5 and Hudiba-2, respectively. Moreover, they stated that, For the 16 maize genotypes, the Fats content means ranged from 0.61% to 1.21% obtained by the genotypes TEE1 20 and TEEI 21, respectively. The Ash content % means ranged from 0.81% to 2.85% obtained by the genotypes TEE1 1 and TZ STR 166, respectively [20]. Observed that the variation in OM, CP, EE, CF, Total ash and NFE content often related to the cultivars of maize including some varieties and their crosses.

Table 1 noted the variation in fiber fractions NDF, ADF and ADL for the two maize varieties. A significant difference (P<0.05) was observed between the two varieties. Same results were obtained by [17] who stated that Acid detergent fiber (ADF) was significantly influenced by genotype at silage harvesting stage (P < 0.01) [21, 22] reported that "ADF and NDF are composed of cellulose plus lignin and cellulose plus hemicellulose plus lignin, respectively and are the structural carbohydrates in plants, which play an important role in the digestibility of the forage. The decrease in ADF and NDF (cell wall components) is an indication of improvement in the quality of the fodders". These results in comparison to the results obtained by [23]. who reported that "Genotype did not significantly (P>0.05) affect NDF at both harvesting stages. Forage grasses, which have < 50% NDF are considered high quality and > 60% as low-quality forage" [24]. related "the digestibility of feeds to the fiber because the indigestible portion has a proportion of ADF, and the higher the value of ADF the lower the feed digestibility" [25] reported "the minimum recommended value of ADF for forage should be 17-21% and according to these results the evaluated maize genotypes for forage purposes exceeded this ADF value recommended for forage".

Forage variety	ОM	СP	EЕ	DMD	NDF	ADF	ADL
Hudubia	88.443 ^a	11.655°	0.6804°	77.219^{a}	58.593°	38.27°	$18,714^{\circ}$
Mugtamaa	87.825 ^p	11 726 a	0.7189^{a}	73.500 ^b	62.555°	47.7°	19.34^{a}
SEM	0.0778	0.0626	0.006	1.2385	0.0377	0.0371	0.0468
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Table 1. Effect of Plant Varieties on the Proximate Composition and Digestibility of Maize forage

Means with different superscriptions are statistically different at (p<0.05)

The effect of time of harvest on the proximate composition and dry matted digestibility is shown in Table 2. The data revealed that there was a significant effect between early and late harvesting time. Early harvesting time recorded the highest value of OM, CP and dry matter digestibility which were found to be 86.96, 12.5 and 79.08 respectively whereas for EE the late time of harvest secured the highest value. These results were consistent with the results obtained by [26] who reported that the advance of maturity of maize reduces the crude protein content and increased in vitro true digestibility. Similar results were also obtained by [27] who found that the apparent total digestibility of dry matter, organic matter, crude protein, crude fat, starch and gross energy for growing dairy cows significantly decreased with maturity of maize crop. These results moreover, were in line with the results obtained by [28] who determined the chemical composition of three maize hybrids harvested at the beginning of six reproductive stages of maturity, concluded that, the advancement in maturation was linearly related to the crude protein (CP) content of the stem, whole plant, and leaves, and there was a difference among the hybrids [29] described Harvesting maize green forage at 65 days, as advanced maturation which reflected in a reduction of crude protein (CP), and a significant increase in the neutral and acid detergent fiber fractions which resulted in a decline in the digestible organic matter (DOM).

The effect of harvest time on the fiber fractions (NDF, ADF and ADL) is noted in Table 2. A significant increase (P<0.01) between significant increase (P<0.01) between parameters were observed with the advanced maturation. The results indicated that with advanced maturity, the cell wall constituent was increased which was reflected in the nutritive value of maize forage. In the same context, many previous authors approved that delaying in maturity had a negative consequence on the forage maize nutritive value as a result of increasing ADF, NDF and ADL of the forage [26] and [30] reported that a significant increase in the NDF content was detected with advancement

in harvesting time under all tested plant densities. Different results were found by [31] and [32] who observed "an increase in ear percentage, dry matter yield and a decrease in fresh forage yield, NDF and ADF of maize as maturity advanced. Other different results were noted by [33] wherein, the delay in harvest time provides an opportunity to accumulate higher amounts of dry matter, and decreases NDF and ADF due to the dilution effect of the increasing amounts of starch)".

Table 3 shows the effect of inorganic phosphorous and phosphate bio fertilizers on the proximate composition and digestibility of maize forage. Significant differences (P<0.05) were noted between all parameters under study. The highest organic matter (89.45%) was found for Bacteria bacillus while the lowest value (84.6%)was found for phosphorous+ fungi, the highest value of CP (12.9%) was found for the combination between bacteria and fungi while the lowest value was found for the control. For the dry matter digestibility, the highest value was recorded for the combination between bacteria and fungi while the lowest value was found for the control. From this study, the combination between bacteria and fungi improved the nutritive value of the forage to the best, which shows the synergetic effect between these microorganisms and phosphorous in the soil. It could be stated that the worldwide Maize quantity and quality could be increased by the utilization of fertilizer (bio fertilizers especially). "Biological phosphate fertilizers containing beneficial bacteria and fungi increased phosphate solutions by increasing soil acidity or alkaline phosphatase enzyme, which can be absorbed by plants easily. Soil chemical and biological characteristics improved by bio fertilizer; moreover, due to the use of low doses of chemical fertilizers, agricultural production will be free from contaminants" [34] The results obtained in this study were not in line with the results obtained by [6] who stated that, no significant effect was found for the application of phosphorus fertilizer, bacterial strains or Mycorrhiza and their interaction on the percentage of digestible dry matter (DMD) and CP, however, seeds inoculation by bacteria strain 41, could increase the amount of DMD to an acceptable level of 66.34%. and the maximum amount of crude protein (14.41%) was obtained by co-application of 30 kg/ ha of triple super phosphate1 fertilizer, bacterial strain 41 and Mycorrhiza, these results can indicate the synergetic effect between bacterial strains 41; Mycorrhiza and chemical phosphorus fertilizer (up to 30 kg ha level). The same results were obtained by [35] noted that the effect of phosphorous fertilization on the nutritive value of *Zea mays* revealed that plots applied with P2 had a significant influence (P>0.05) on the CP content with the highest value recorded for P2 level of fertilization for *Zea mays* in pure stand and in the mixture. Moreover, [36] illustrated that the main effect of phosphorus fertilizer application was insignificant but effect of bio fertilizer application was a significant on the CP. [37] report that the crude protein, crude fiber and ash contents in maize fodder increased with an increase in the P application rate. The maximum crude protein, crude fiber and ash contents were 10.55, 21.63 and 7.26 % respectively. The increase in crude fiber contents was due to more dry matter accumulation with P application. Similarly, [38] Rreported that crude fiber content was increased with P application along with N. Increase in ash % is due to an increase in mineral matter. [39] Noted that the maximum amount of ash (8.12%) was obtained when sole Mycorrhiza was applied but, with no significantly different from treatments of co application of Mycorrhiza and bacteria strain 9 and sole application of bacterial strains. they concluded that the application of Mycorrhiza can provide favorable conditions for more phosphorus uptake. In phosphorous deficient soils, the effect of Mycorrhiza in providing phosphorous to the plants is equal to the role of *Rhizobium* sp. in supplying nitrogen for the leguminous family.

The effect of inorganic phosphorous and phosphorous biofertilizers on the fiber fractions is noted in Table 3. Significant differences were found between treatments. For NDF the lowest value (52.52%) was recorded in the combination between Bacteria and fungi, while the highest value (64.17%) was attained in the control, for ADF the combination between inorganic phosphorous and bacteria secured the lowest value (39.98%). Similar results were reported by [6] who noted that the NDF was significantly affected by the interaction between phosphorus and bacteria treatments. The minimum NDF value was obtained (57.27%) by

the sole application of 60 kg/ ha supper phosphate triple which its effect was not significantly different from sole application of bacterial strain 41. The above results indicated that there is an antagonistic effect between inorganic phosphorus fertilizer and bacteria activity regarding NDF value in barley forage. They also stated that, the bacterial strain 41 could substitute inorganic phosphorous fertilizers to provide the best conditions for decreasing NDF in barley Karoon x Kavir cultivar and increase the quality of forage [36] illustrated that the effect of inorganic phosphorus fertilizer on NDF content was insignificant, Whereas, the effect of biofertilizer application was significant. Biofertilizer-applied plots had higher ADF content than alone phosphorus applied [35] noted that the effect of phosphorous fertilization on the CF content and the NDF content, was found to be nonsignificant among all various levels of phosphorous fertilization. The results in this study disagreed with those of [40] who reported that ADF and NDF contents of sorghum fodder did not change significantly with P application [41] reported that P and K fertilizers application did not affect the ADF and NDF contents in forage sorghum.

Table 4 shows the degradability kinetics of maize forage as affected by different treatments of inorganic phosphorous and phosphate biofertilizers. The application of inorganic phosphorus secured the highest (34.2) readily degradable fraction (a), while the highest value of potential degradability (PD) and effective degradability at an outflow rate (ED 0.2%) obtained with the application of inorganic phosphorous+ *Bacillus sp.,* while the highest value of degradation rate (c) was obtained by bacillus + mycorrhiza. These results were supported by [35] for the effect of phosphorous fertilization on Rhodes grass and *Clitoria ternatea*. They reported that phosphorous fertilization affects the potential degradability (PD) and the effective degradability (ED) at all outflow rates significantly (P <0.05). Level 2 of phosphorous fertilization recorded the highest mean value followed by level 1 and zero levels for (PD) and (ED). Readily degradable fraction (a), slowly degradable fraction (b) and degradation rate did not affect significantly by phosphorous fertilization, however, the ranking of phosphorous fertilization was as follows $2 > 1 > 0$ >3. These results were in conformity of [42] who worked on fertilizer application on the nutritive value of durum wheat Straw, and found that

Table 2. Effect of time of harvest on the proximate composition and digestibility of maize forage

Means with different superscriptions are statistically different at (p<0.05)

Table 3. Effect of inorganic phosphorous and phosphate biofertilizers on the Proximate Chemical Composition and Fiber Fractions of Maize Forage

Means with different superscriptions are statistically different at (p<0.05)

Table 4. Effect of Different Treatments of inorganic phosphorous and phosphate biofertilizers on Rumen Degradability Kinetics (%)

Means with different subscription in the same column differ significant; a= readily degradable fraction; b= slowly degradable fraction; c= degradation rate; PD=potential degradability; ED=effective degradability.

Maize variety	Treatment	a	b	C.	PD	ED 0.2	ED0.5	ED 0.8
Hudibia	Control	36.10^{a}	35.900i	0.018a	72.0^9	53.07 ^h	45.60^{b}	42.67°
	Inorganic Phosphorous (P_2O_5)	36.100^{a}	37.833 ⁿ	0.0170^{b}	74.03 ^t	53.30^{9}	45.57^{b}	42.63^a
	(P_2O_5) + bacillus	31.800^t	48.300^{9}	0.0150°	80.10^e	52.60 ^I	43.00°	39.50°
	(P_2O_5) + mycorrhiza	35.400 ^b	61.30^{de}	0.0110 ⁹	96.63^{b}	56.80^{a}	46.20 ^a	42.60^{a}
	Bacillus	34.500°	59.700 ^e	0.011^{19}	94.27°	55.73^{b}	45.30°	41.77^{b}
	Mycorrhiza	30.200	68.433 ^b	0.0110^{9}	98.70^{a}	55.2 ^{cd}	43.00°	38.8^{19}
	Bacillus+mycorrhiza	30.100	68.700 ^b	0.0120 ^t	98.80^{a}	55.23°	43.00°	38.73^{9}
Mugtamaa	Control	30.90^{9h}	65.700°	0.0120^{t}	96.63^{b}	55.10°	43.30 ^e	39.30 ^e
	Inorganic phosphorous (P_2O_5)	32.300 ^e	62.433°	0.0110q	94.73bc	54.90^e	43.87°	40.07 ^d
	(P_2O_5) + Bacillus	30.300	70.533^{a}	0.0110^{9}	100.00a	55.30°	43.00°	38.80fg
	(P_2O_5) +mycorrhiza	30.700 ^h	68.033 ^b	0.0110 ⁹	98.70^{a}	54.80^e	43.00°	38.90fg
	Bacillus	31.100 ⁹	48.900^{9}	0.0160°	80.03 ^e	53.00 ^h	43.07 ^t	39.37^e
	Mycorrhiza	30.800gh	49.600 ⁹	0.0160°	80.40°	52.80	42.80^{9}	39.00°
	Bacillus+ mycorrhiza	32.900°	51.967	0.0130^e	84.90°	53.80	43.92^d	40.40 ^c
	Se	0.189	0.834	4.378e-04	0.9705	0.065	0.079	0.107

Table 5. Rumen degradability kinetics (%) of two maize varieties as affected by inorganic phosphorous and phosphate biofertilizers treatments

Means with different subscriptions in the same column differ significant; a= readily degradable fraction; b= slowly degradable fraction; c= degradation rate; PD=potential degradability; ED=effective degradability.

phosphorous fertilization had no significant effect on dry matter degradability. The effect of the application of different types of phosphate biofertilizers on the *in situ* degradation characteristics of the two maize varieties is shown in Table 5. The readily degradable faction (washing loss) for Hudibia variety when fertilized by inorganic phosphorous + mycorrhiza was the highest. The insoluble, but slowly degradable fraction was found to be the highest in Mugtamaa variety when fertilized by phosphorous+ bacillus. The highest potential degradability (PD) was found in Mugtamaa when fertilized by inorganic phosphorous+ mycorrhiza, while the highest value of effective degradability for all outflow rate was attained by Hudibia when fertilized by phosphorous+ mycorrhiza [43]. The forage with high soluble and degradable fraction content reflected the ability of the forage to supply sufficient quantities of N to meet rumen microbial requirements [35] [44] Justified these differences in degradation characteristics to the level of ADF and cellulose content in leaves of the different varieties [45] stated that a fast rate of degradation of feed may not always be desirable particularly with concerning the rate of N degradation. Extensive ruminal degradation of feed N to ammonia above the level that can be utilized for microbial protein synthesis or observed in the rumen is converted into urea and excreted in urine; this will put a limit on animal production [42] studied the effects of variety, and fertilizer application on in sacco DM degradability characteristics of wheat straw, reported that, the soluble fraction (washing loss) was the highest in Tikur sinde and the lowest in Arendeto. The ED was the highest inTikur sinde followed by Arendeto and it was the lowest in the improved varieties.

4. CONCLUSION

Based from the results of this study it could be concluded that:

Variety Mugtamma had the highest values of CP, OM, EE and DMD.

Early time of harvest secured the highest value of CP, OM, EE and DMD.

The combination of *Bacillus sp* and mycorrhiza recorded the highest value of CP and DMD and the lowest value of NDF.

For ADF the combination between inorganic phosphorous and *Bacillus sp.* secured the lowest value.

5. RECOMMENDATIONS

Delaying harvest time had a negative impact on the nutritive value of maize, so it's recommended for silage making to put this factor into consideration.

Variety Mugtamma exhibited the best nutritive value, so it is recommended to conduct additional research for i1mproving this variety.

Additional research is also recommending a better combination of phosphate biofertiluzers which contributed better to maize production and quality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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