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Research Article

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Partial substitution of maize mineral fertilization with some organic and bio-fertilizers

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ABSTRACT

During the 2019 and 2020 summer seasons, field experiments were conducted at Shandaweel Agricultural Research Station in Sohag Governorate, Egypt, to investigate the effect of some organic and bio-fertilizers as partial N-fertilizer substitutes and their impact on yield components, N, P, K, carbohydrate and protein for the T.W.C.- 310 maize cultivar. A Randomized complete block design with three replications was used as the split-plot design. The applications of five treatments (control, K-humate, vinasse, bio-fertilizer, and vinasse + bio-fertilizer) were assigned to sub-plots, whereas nitrogen levels were assigned to the main plots. The results indicated that raising N-fertilizer doses from 90 to 120 kg N/fed. resulted in a considerable increase in all parameters under study. Vinasse treatment under application of N-fertilizer had a significant effect on plant height, leaf area, protein, carbohydrates, NP- content in grain and leaves, grain k- content, yield, and its components, then the treatments of K- humate, bio-fertilizer, or vinasse + bio-fertilizer, in both seasons. K- humate, vinasse, bio-fertilizer, and vinasse + bio-fertilizer are under application of 90 kg N/fed. caused a significant increase in most traits than those obtained under the recommended doses of N-fertilizer (120 kg N/fed.) without adding organic or bio-fertilizer, in both seasons. Therefore, under conditions of the present work, sowing of maize hybrid (T.W.C.- 310) with the application of 90 kg N/fed. combined with vinasse can be recommended to get the maximum grain yield and reduce the amount of chemical nitrogen fertilizer in agriculture.
Keywords: Bio-fertilizer, K- humate, Nitrogen fertilizer, Vinasse, Yield, Zea mays.

INTRODUCTION

Maize (*Zea mays* L.) belongs to the family Poaceae and is commonly grown all over the world (Ahmed *et al.*, 2020), it is an important cereal crop (Kandil *et al.*, 2020) having numerous applications, including food, industrial materials, and bioenergy, it is ranked third after wheat and rice (Wulandari *et al.*, 2019). The area of agricultural land planted with mays in Egypt is about 935778 hectares, producing 7.10 million tons of grain (FAO, 2017).

The most significant ingredient for most farmed crops is nitrogen (N). The presence of nitrogen in producing protein and nucleic acids, which are the most significant molecules in the plant cell, is one of the most important jobs of nitrogen in plants (Elmasry, 2017). Mineral fertilizers are used to improve soil fertility and plant nutrition, but crops benefit only with a portion of the nitrogen added, while the rest is lost as nitrous oxide emissions, nitrate leaching, or in agricultural drainage, which lowers the efficiency of nitrogen used in agriculture (Ngosong *et al.*, 2019). The sole use of chemical fertilizers degrades soil physicochemical and biological qualities, as well as is harmful to animals, plants, and human life (Jjagwe *et al.*, 2020). Alternative techniques of providing nutrients to the growing plant had to be developed. Organic and bio-fertilizers are considered promising alternative approaches for maize and other crop species production (Gao *et al.*, 2020).

Several investigations have been conducted on the effect of adding organic and bio-fertilizers on the growth and yield parameters of mays. Humic substances, known as humus, are decaying plant products with complicated structures and high molecular weights (Nalia and Sengupta, 2019 and Gao *et al.*, 2020). Humic compounds may absorb huge amounts of water and cations due to their chemical structures, improving the physical and chemical characteristics of soil, enhancing cation exchange capacity, and increasing the permeability of plant cell membranes, increasing germination, root system development, and yield (Jindo *et al.*, 2020; Nardi *et al.*, 2021 and Salih and Ali, 2021).

Vinasse is the liquid waste product of the fermentation process used to refine alcohol from crude sources, it is used in farming for cheap supplement sources (Reis and Hu, 2017 and Bastos *et al.*, 2021). Vinasse has more concentration of phosphate, sodium, sulfate, potassium, calcium, iron, carbon, micronutrients, and organic compounds and properly use of vinasse in irrigation and fertilization to improve the fertility of the soil and agricultural productivity (Buller *et al.*, 2021).

A biofertilizer is a material containing microorganisms that are added to the soil and makes certain critical nutrients available to plants for sustenance, either directly or indirectly. The provision of bio-fertilizer is an attempt to improve soil fertility on a physical, chemical, and biological level (Sondang *et al.*, 2019) because it has many important functions in the soil *ex;* It recycles organically existing soil nutrients, improves soil nutrient availability, and hence plant uptake, improves soil structure by creating various biochemicals, controls pathogens' negative effects on growth and alleviates soil pressures on plant growth and crop production. Therefore, using bio-fertilizers has become extremely important to obtain a high-quality yield and generate healthy products while preventing pollution (Salih and Ali, 2021).

The purposes of this study are to (1) Avoid the hazardous effect of mineral fertilizer by minimizing nitrogen doses for maize plants as the world moves toward clean agriculture, organic and bio-fertilizers are being used to replace a portion of mineral fertilization and (2) to study the impact of organic and bio-fertilizer under 90 and 120 kg N/fed. in some criteria in maize crop.

MATERIALS AND METHODS

During the summer seasons of 2019 and 2020, at Shandaweel Agricultural Research Station, in Sohag Governorate, two field experiments were done. to evaluate the impact of N-fertilizer levels and five treatments (control, K-humate, vinasse, bio-fertilizer, and vinasse + bio-fertilizer) on maize plant growth, yield, yield components, and chemical constituents. The experiments included 15 treatments resulting from the combinations of three nitrogen fertilizers levels; (control, 90, and 120 kg N/fed.) and five treatments (control, K- humate, vinasse, bio-fertilizer, and vinasse + bio-fertilizer). A split plot with three replications was used as the experimental design. The main plots were assigned to the three levels for N treatments, whereas the five treatments were assigned to sub-plots. The subplot area was 10.50 m^2 (3.0 x 3.50 m) containing five ridges, each was 3 m long and 70 cm wide. The maize hybrid was a Three-way cross-310 hybrid (T.W.C.- 310) that was planted in hills 25 cm apart in the first week of June in the 2019 and 2020 seasons. Before planting, surface soil samples (0-30 cm) were taken from the experimental sites in both seasons. At the Agricultural Research Center's (ARC), Soils and Water Lab., the chemical analyses and types for the soil of the experimental sites (Table 1) were performed according to the protocols given by Piper (1950) and Jackson (1967), also, the chemical composition of humic acid and vinasse used in the experiment were shown in Tables 2 and 3, respectively.

Nitrogen fertilizer (as urea fertilizer 46.5 % N) was added according to the treatment in two equal portions, the 1st half at the life irrigation and the 2nd at the following irrigation. At the time of land preparation, superphosphate (granules) was added as a single dose at a rate of 30 kg P_2O_5/fed. granulated single super phosphate (15.5 % P_2O_5). Also, potassium was added as potassium sulfate K_2SO_4 at the rate of 24 kg K_2O/ fed. (48 % K_2O = 50 kg potassium sulphate). Subplots contain soil application; Water (control), K- humate 10% (8 L/fed.), vinasse (4% = 300 L/fed.), bio-fertilizer (two package) and vinasse + bio-fertilizer (Table 2).

Table 1. Mechanical and chemical properties of the experimental soil sites

Season		2019	2020
Mechanical analysis	Fine sand %	21 %	37 %
	Coarse sand %	1.46 %	1.14 %
	Silt %	42 %	32 %
	Clay %	35.54 %	29.86 %
Soil texture		Clay	Clay loam
Chemical analysis	Organic	-	-
	Total N (%)	0.164	0.220
	$CaCO_3$ %	1.40	1.50
	Soluble ions (meq/100g soil (1:5))		
	CO_3^-	----	----
	HCO_3^-	0.26	0.33
	Cl^-	0.79	0.90
	SO_4^{-2}	1.00	1.15
	Ca^{++}	0.50	0.55
	Mg^{++}	0.24	0.34
	Na^+	1.17	1.33
	K^+	0.14	0.16
	EC, dS/m	0.21	0.24
	pH (1:1)	7.3	7.2

Table 2. Chemical composition of humic acid used in the first and second seasons of the experiment.

Analysis of Guaranteed		
Humic acid (HA)		80 %
Potassium as K_2O		10 %
Minerals		
N	P	K
0.51 %	9.6 %	8.33 %
Physical Data		
Appearance		Brown liquid
pH		8.5
Water solubility		> 98 %

Table 3. Some chemical characteristics of raw vinasse used in the experiment

Chemical composition	2019	2020
Total sugar % gm	6	6
Total soluble solids % Brix	32%	31
Nitrogen %gm	0.73	0.75
Organic matter % gm	5.32	5.38
Phosphorus %gm	0.08	0.08
Potassium % gm	2.00	2.03
Calcium % gm	1.20	1.21
Magnesium % gm	0.54	0.53
Ash % gm	6.5	6.4
Physical Data		
pH	4.5	4.5
Appearance	dark brown liquid	

Sugarcane vinasse is a byproduct of the sugar-ethanol manufacturing process, from a sugar and integrated industries company (Table 3).

The bio-fertilizer utilized in this study was created by the Ministry of Agriculture's General Organization for Agricultural Equalization Fund. Microbial containing nitrogen fixers (namely, *Azospirllum* sp.) and phosphate dissolving bacteria (namely, *Bacillus megatherium* var. phosphonium). All the treatments were added as a soil application to maize plants twice at the elongation stage, the first was done after 30 days from sowing, whereas the second was used two weeks after the first (45 days from sowing). A constant volume (1 liter per subplot) was added twice in all cases before irrigation. In addition to plants that had been treated with water (control). Plots were irrigated according to the optimized recommendations for maize and the weeds were controlled by hand.

The tested growth characters which were recorded are plant height (cm) was recorded at harvest, The ear leaf area (LA - cm^2) was calculated using the method of McKee (1964), it involved measuring the length of the leaf blade from its base to the tip of the leaf (leaf $_L$) and the width of the leaf at its widest point (leaf $_W$) and multiplying them by a correction factor (0.75) as illustrated in the equation:

Leaf Area (LA) = $Leaf_L \times Leaf_W \times 0.75$

Plants leave at 60 days from sowing were gently washed with water and dried at 70 ºC in an oven, also, at full maturity, grain samples were taken for the estimation of biochemical and nutrient contents. According to Fales (1951), the anthrone sulphuric acid technique was used to estimate total carbohydrate (%) and according to Lowry *et al.*, (1951) the protein content was assessed.

A mixture of concentrated sulphuric acid and perchloric acid was used to digest leaves and grain (Wicks and Firminger, 1942) to determine plant nutrients content N, P and K as follows:

The method of micro-Kjeldahl was used to determine the total N% (A.O.A.C. 1995).

In the H_2SO_4 system, phosphorus was measured by using the chlorostannous reduced molybdophosphoric blue color technique and colorimetrically determined using the method introduced by Jackson (1958) and potassium was measured photometrically by using a flame photometer, as reported by Jackson (1958).

After 120 days after sowing, the harvest is done manually (at maturity stage). Grain samples of five plants were randomly chosen from each plot to investigate the following characters; ear length and diameter, kernels/ ear, 100- grain weight, and yield (ard./feddan).

The data were statistically evaluated by using the computer MSTAT-C statistical analysis package, according to Freed *et al.,* (1989). The least significant difference (LSD) at 5% was used to compare mean values (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Morphological characters

A-Effect of nitrogen fertilization

The variation in height and leaf area of the maize crop for the different nitrogen levels is shown in Tables 4 and 5, respectively. With increasing nitrogen fertilizer up to the lightest amount (120 kg N/fed.), plant height and leaf area rose dramatically. This result is by the findings of Iqbal et al., (2014) who noted that the recommended doses of nitrogen (120 kg N/fed.) is important in metabolism and energy production, and it is necessary for plant growth and development., In addition, nitrogen application causes a rise in internode length and, as a result, plant height.

B-Effect of organic and bio-fertilizer

In terms of the influence of organic and bio-fertilizers, the findings revealed considerable differences in maize height and leaf area between treatments. Application of K-humate, vinasse, bio-fertilizer, and vinasse + bio-fertilizer, gave the greatest mean plant height and leaf area than those obtained by control, in both seasons.

C-Effect of interaction between N-levels, organic, and bio-fertilizer (AxB)

As a result of the combined impacts of nitrogen fertilizer levels, organic fertilizer and bio-fertilizer plant height have shown in table 4 which indicate that, application of 120 kg N/fed. with K-humate, vinasse, bio-fertilizer or bio-fertilizer + vinasse gave the highest values of plant height than those obtained by recommended dose without adding activators, in both seasons. Also, application of 90 or 120 kg N/fed. with adding any of K-humate, vinasse or vinasse + bio-fertilizer gave highest values of leaf area than those obtained by recommended dose only in both seasons (Table 5). This finding is consistent with that of Abiodun *et al.,* (2016), who discovered that applying vinasse to the soil induces short changes in the population of microorganisms in the soil, increasing their activity in the agglutination of soil particles, and improves the structure of the soil. A beneficial effect of K- humate on plant growth is confirmed in the study by Zaghloul *et al.,* (2009) who stated that the growth parameters were increased by HA application, this rise could be attributed to the beneficial effects of K-humate on soil structure which lead to increased root growth, enhance uptake of macronutrients and micronutrients from the soil and increase the permeability of plant membranes resulted in accelerate cell division and plant development (Gomaa *et al.,* 2014). Beneficial bacteria create phytohormones that promote plant growth, which could explain the rise in plant growth with the bio-fertilizer application. (Spaepen, 2015).

2- Biochemical analysis

A-Effect of nitrogen fertilization

The effects of nitrogen fertilizer levels and the application of K-humate, vinasse, bio-fertilizer, and vinasse + bio-fertilizer on total carbs and protein percent in grain in 2019 and 2020 seasons are shown in tables 6 and 7. Data show that the highest values of carbohydrate and protein % in grain were obtained under the level of 120 kg N/fed., in both seasons. These findings are consistent with those of Elmasry (2017), who discovered that nitrogen fertilizer treatment enhanced total carbs and protein percent in grain. Nitrogen is a component of plant compounds such as nucleotides, amides, and amines, as well as an essential component of amino acids (protein building blocks) and nucleic acids (DNA and RNA). (Rizwan *et al.*, 2003) and it has a direct impact on maize grain quality and growth behavior. This, in turn, improves photosynthesis and dry matter accumulation leading to higher total carbohydrates and protein % (Tranavičiené *et al.*, 2008).

Table 4. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on plant height (cm) of maize, in 2019 and 2020 seasons.

	Season	2019				2020			
	Nitrogen levels	0	90	120	Mean	0	90	120	Mean
Treatments	Water	255.	264.0	272.6	263.8	265.0	267.6	272.6	268.4
	K- humate	262.	273.0	281.6	272.4	267.6	276.3	281.6	275.2
	Vinasse	263.	272.3	284.3	273.2	271.0	277.3	286.0	278.1
	Bio-fertilizer	261.	275.3	282.3	273.1	270.6	272.0	278.3	273.6
	Vinasse + Bio-fertilizer	262.	273.3	284.0	273.2	268.3	276.3	280.6	275.1
	Mean	260.	271.6	281.0		268.5	273.9	279.8	
	LSD at 5%								
	Nitrogen (A)		0.92				2.99		
	Treatments (B)		3.00				3.55		
	A x B		5.19				6.15		

Table 5. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on leaf area (cm^2) of maize, in 2019 and 2020 seasons.

	Season	2019				2020			
	Nitrogen levels	0	90	120	Mean	0	90	120	Mean
Treatments	Water	450.	549.8	693.1	564.6	640.3	733.3	675.4	683.0
	K- humate	645.	734.0	791.6	723.6	781.4	812.6	816.1	803.4
	Vinasse	666.	741.1	824.4	744.0	774.7	779.5	827.9	794.0
	Bio-fertilizer	504.	640.5	752.0	632.3	718.8	760.3	772.8	750.6
	Vinasse + Bio-fertilizer	642.	737.8	819.9	733.3	792.9	799.6	811.7	801.4
	Mean	581.	680.6	776.2		741.6	777.1	780.8	
	LSD at 5%								
	Nitrogen (A)		16.01				33.45		
	Treatments (B)		30.62				37.19		
	A x B		53.04				64.41		

B-Effect of organic and bio-fertilizer

Application of organic and bio-fertilizer to maize had a highly significant influence on total carbohydrate and protein content in leaves and grain when compared with the control treatment, in the 2019 and 2020 seasons (Table 6 and 7). It was shown a pronounced difference detected in carbohydrate and protein content in grain due to vinasse and K- humate application, in both seasons.

C-Effect of the interaction between N-levels, organic, and bio-fertilizer (AxB)

The combined effects of nitrogen fertilizer levels, organic and bio-fertilizer on carbohydrate % in grain were significant in both seasons. Application of 90 or 120 kg N/fed. combined with vinasse application gave highest values of the carbohydrate % in grain than the control, in 2020 season (Table 6). Also, the highest protein % in grain were was recorded by application 90 or 120 kg N/fed. with vinasse application, in both seasons followed by K- humate then bio-fertilizer (Table 7). These findings are in line with Junqueira *et al.*, (2009), who reported that the utilization of vinasse in fertigation frameworks is very important because, its application brought about increasing nontoxic concentrations of Na, K, and Mn in the soil, as well as the ability to contribute a substantial amount of water and mineral supplements, an improvement in soil quality and crop productivity.

The positive effect of HA was explained by Chattha *et al.*, (2015) they noted that foliar spray of HA enhanced carbohydrate and protein contents in maize crops. The rise could be related to the HA role as a growth regulator for plants, which causes it to act as a sink for various nutrients involved in the formation of new tissues in plants, as well as to improve the photosynthetic process and protein synthesis. (Haghighi Teixeira Da Silva, 2013).

The outcomes also showed that the bio-fertilizer increased significantly carbohydrate and protein % in maize crop, the data are compatible with Abo-Marzoka *et al.*, (2017) who found that bio-fertilizer help in the availability of mineral and their forms in the soil and increase chlorophyll content and photosynthesis process, also, according to Gomaa *et al.*, (2013), photosynthetic activity is raised in inoculated plants by symbiosis with microbes, which promotes three efficiencies of photosynthesis.

Table 6. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on total carbohydrate in grain (%) of maize, in 2019 and 2020 seasons.

	Season	2019				2020			
	Nitrogen levels	0	90	120	Mean	0	90	120	Mean
Treatments	Water	50.92	54.00	63.67	56.20	59.61	60.89	63.27	61.26
	K- humate	57.97	63.51	75.58	65.68	64.60	69.00	72.95	68.85
	Vinasse	65.65	71.69	77.59	71.64	68.95	74.45	76.16	70.30
	Bio-fertilizer	58.70	68.43	72.25	66.46	61.98	69.53	71.27	67.59
	Vinasse + Bio-fertilizer	56.22	65.82	72.67	64.90	63.73	64.48	72.04	66.75
	Mean	57.89	64.69	72.35		63.78	67.67	71.14	
LSD at 5%									
Nitrogen (A)			1.21				0.60		
Treatments (B)			0.65				0.96		
A x B			1.13				1.66		

Table 7. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on protein in grain (%) of maize, in 2019 and 2020 seasons.

	Season	2019				2020			
	Nitrogen levels	0	90	120	Mean	0	90	120	Mean
Treatments	Water	8.00	10.44	12.00	10.15	7.57	8.67	9.67	8.63
	K- humate	12.29	12.51	13.79	12.87	11.79	13.47	14.02	13.09
	Vinasse	11.49	13.97	15.43	13.63	10.07	14.34	14.78	13.06
	Bio-fertilizer	10.28	11.15	13.77	11.73	9.11	12.40	13.21	11.57
	Vinasse + Bio-fertilizer	11.74	12.00	14.63	12.79	10.10	12.13	12.65	11.63
	Mean	10.76	12.02	13.93		9.73	12.20	12.87	
LSD at 5%									
Nitrogen (A)			1.34				0.62		
Treatments (B)			0.95				0.83		
A x B			1.64				1.43		

3- Plant nutrients content

A-Effect of nitrogen fertilization

Results are given in Table 8-13 clear that NPK % in leaves and grain was affected by nitrogen applications. NPK % in leaves and grain was increased gradually and significantly by increasing nitrogen levels. The most effective dose of nitrogen fertilizer on nitrogen % in leaves and grain were found under 120 kg N/fed. in both seasons, also, application of 90 kg N/fed. resulted in the greatest values for leaf P % and grain K % with no significant changes when compared to an application of 120 kg N/fed. in both seasons.

The beneficial effect of nitrogen on grain quality could be attributed to the fact that nitrogen enhances photosynthetic pigment content and photosynthesis rate, increasing the number of metabolites synthesized and minerals, resulting in higher dry matter accumulation and NPK content in grain (Elmasry, 2017). These findings could also be explained by nitrogen's important function in protein activation and synthesis, and other compounds including starch, sugar, cellulose, cell wall, and vitamins, also, potassium encourages various enzymes and photosynthesis as well as plant root development which encourage NPK uptake and plant growth (Abo-Marzoka *et al.,* 2017).

B-Effect of organic and bio-fertilizer

In terms of the influence of organic and bio-fertilizers on NPK % in leaves and grain, the results in table 8-13 reveal that vinasse application increases NPK % in leaves and grain, in both seasons.

C-Effect of the interaction between N-levels, organic, and bio-fertilizer (AxB)

The combined effects of nitrogen fertilizer levels, organic and bio-fertilizer on NPK % in leaves and grain were significant in both seasons. The application of 120 kg N fed. coupled with vinasse, followed by KH, and finally, bio-fertilizer, achieved the maximum NPK-content, the highest values of NPK % in leaves and grain were obtained using 90 kg N/fed. or 120 kg N/fed. in combination with vinasse treatment than those obtained by recommended dose only, in 2019 and 2020 seasons.

These observations are corroborated by Abiodun *et al.,* (2016), who demonstrated that vinasse is used in water systems, mostly in sugar cane culture it enhanced quality in soil organic, physical, and chemical properties. Following the application of vinasse, increased Mg, Ca, and K concentrations, improved soil macro aggregate, and superior advancement of the radicular framework in sugar cane have all been reported (Mo *et al.,* 2009). Vinasse in fertigation increased cation concentration in the soil, particularly potassium, and increased agriculture productivity (Ao *et al.,* 2009). Because vinasse is a vital source of potassium, its use could reduce the need for inorganic potassium fertilizers while also reducing environmental contamination (Tejada *et al.,* 2007).

Other studies had shown that there was a significant increase in the uptake of the elemental content and increased NPK % with an application of K- humate this may be due to that KH increase root membrane permeability and root expansion leading to better absorption of nutrients and increases shoot growth (Sajadian and Hokmabadi, 2015).

Also, bio-fertilizer caused an increase in NPK %, this increase may be because bio-fertilizer led to nitrogen fixation, growth-promoting substances, and enhancing nutrient uptake. Using nitrogen fertilizer plus bio-fertilizer resulted in enhancing growth characteristics of maize plants rather than an application of mineral nitrogen alone (Abo-Marzoka *et al.*, 2017). Also, according to Gomaa *et al.*, (2013), plants treated with N_2 fixing microorganisms have higher photosynthetic capability due to enhanced nitrogen intake.

Table 8. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on leaf N % of maize, in 2019 and 2020 seasons.

	Season	2019				2020			
	Nitrogen levels	0	90	120	Mean	0	90	120	Mean
Treatments	Water	1.92	2.24	2.31	2.16	1.96	2.34	2.81	2.37
	K- humate	2.39	3.16	3.34	2.96	3.14	3.42	3.64	3.40
	Vinasse	2.50	3.39	3.58	3.16	3.28	3.84	4.21	3.77
	Bio-fertilizer	2.04	2.21	2.68	2.31	3.12	3.63	3.94	3.56
	Vinasse+Bio-fertilizer	2.12	2.47	3.07	2.55	2.46	3.31	3.51	3.09
	Mean	2.20	2.70	3.00		2.79	3.31	3.62	
	LSD at 5%								
	Nitrogen (A)		0.24				0.42		
	Treatments (B)		0.17				0.37		
	A x B		0.29				0.64		

Table 9. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on grain N % of maize, in 2019 and 2020 seasons.

	Season	2019				2020			
	Nitrogen levels	0	90	120	Mean	0	90	120	Mean
Treatments	Water	1.24	1.79	2.17	1.73	0.98	1.65	2.21	1.61
	K- humate	1.83	2.23	2.42	2.16	1.26	2.28	2.49	2.01
	Vinasse	1.66	2.33	2.46	2.14	1.27	2.47	2.52	2.09
	Bio-fertilizer	1.63	2.03	2.26	1.97	1.13	1.70	2.34	1.72
	Vinasse + Bio-fertilizer	1.68	2.23	2.46	2.12	1.27	2.41	2.47	2.05
	Mean	1.61	2.12	2.35		1.18	2.10	2.41	
	LSD at 5%								
	Nitrogen (A)		0.19				0.09		
	Treatments (B)		0.07				0.09		
	A x B		0.13				0.16		

Table 10. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on leaf P % of maize, in 2019 and 2020 seasons.

	Season	2019				2020			
	Nitrogen levels	0	90	120	Mean	0	90	120	Mean
Treatments	Water	0.213	0.260	0.264	0.246	0.200	0.222	0.243	0.222
	K- humate	0.251	0.281	0.293	0.275	0.209	0.285	0.302	0.265
	Vinasse	0.269	0.292	0.301	0.288	0.237	0.289	0.311	0.279
	Bio-fertilizer	0.237	0.252	0.269	0.252	0.214	0.232	0.237	0.228
	Vinasse + Bio-fertilizer	0.251	0.276	0.299	0.275	0.243	0.278	0.312	0.278
	Mean	0.244	0.272	0.285		0.221	0.261	0.281	
	LSD at 5%								
	Nitrogen (A)		0.021				0.008		
	Treatments (B)		0.012				0.008		
	A x B		0.021				0.013		

Table 11. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on grain P % of maize, in 2019 and 2020 seasons.

Season		2019				2020			
Nitrogen levels		0	90	120	Mean	0	90	120	Mean
Treatments	Water	0.343	0.518	0.558	0.473	0.429	0.522	0.557	0.503
	K- humate	0.529	0.590	0.653	0.591	0.543	0.603	0.665	0.604
	Vinasse	0.557	0.593	0.648	0.599	0.507	0.626	0.655	0.596
	Bio-fertilizer	0.446	0.528	0.560	0.511	0.436	0.521	0.562	0.506
	Vinasse + Bio-fertilizer	0.571	0.577	0.652	0.600	0.443	0.597	0.658	0.566
	Mean	0.489	0.561	0.614		0.471	0.574	0.619	
LSD at 5%									
Nitrogen (A)			0.013				0.059		
Treatments (B)			0.009				0.061		
A x B			0.016				0.106		

Table 12. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on leaf K % of maize, in 2019 and 2020 seasons.

Season		2019				2020			
Nitrogen levels		0	90	120	Mean	0	90	120	Mean
Treatments	Water	1.680	1.903	2.421	2.002	1.920	2.039	2.339	2.099
	K- humate	2.199	2.940	3.060	2.733	2.458	3.025	3.192	2.892
	Vinasse	2.063	2.740	2.894	2.566	2.334	2.824	2.881	2.680
	Bio-fertilizer	2.730	2.806	3.038	2.858	2.417	2.773	3.153	2.781
	Vinasse + Bio-fertilizer	2.470	2.626	3.008	2.702	1.927	2.058	2.563	2.183
	Mean	2.228	2.603	2.884		2.211	2.544	2.826	
LSD at 5%									
Nitrogen (A)			0.070				0.090		
Treatments (B)			0.092				0.084		
A x B			0.160				0.145		

Table 13. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on grain K % of maize, in 2019 and 2020 seasons.

Season		2019				2020			
Nitrogen levels		0	90	120	Mean	0	90	120	Mean
Treatments	Water	0.363	0.381	0.418	0.387	0.438	0.458	0.484	0.460
	K- humate	0.430	0.539	0.556	0.508	0.508	0.559	0.580	0.549
	Vinasse	0.507	0.546	0.568	0.540	0.489	0.557	0.587	0.544
	Bio-fertilizer	0.451	0.505	0.522	0.492	0.513	0.548	0.568	0.543
	Vinasse + Bio-fertilizer	0.435	0.464	0.485	0.461	0.474	0.521	0.532	0.509
	Mean	0.437	0.487	0.510		0.484	0.528	0.55	
LSD at 5%									
Nitrogen (A)			0.023				0.025		
Treatments (B)			0.025				0.022		
A x B			0.042				0.039		

4- Yield and its components

A-Effect of nitrogen fertilization

Results presented in Table 14-18 reveal that increasing nitrogen fertilizer rates significantly increased ear length, ear diameter, kernels no./row, 100- grain weight, and grain yield, in both studied seasons. The highest values for ear length, ear diameter, kernels no./row and grain yield were observed by application of 90 kg N/fed. and 120 kg N/fed., in 2019 and 2020 seasons. This results in accordance to Hammad *et al.*, (2012) who said that nitrogen accelerates the formation of chlorophyll which leads to an increase of photosynthesis, which in turn resulted in an increase in metabolic processes and more filling for grain.

B-Effect of organic and bio-fertilizer

Results in Tables 14-18 clear that application of the organic and biofertilizers to maize plants had a significant influence on yield and its components in both seasons. Maize hybrid obtained the highest values by the application of vinasse, K- humate or vinasse + bio-fertilizer in both seasons.

C-Effect of the interaction between N-levels organic and bio-fertilizer (AxB)

In both the 2019 and 2020 seasons, the adding of 90 kg N/fed. combined with the spraying of vinasse produced the highest mean values of ear length and diameter, kernels number in row, 100-grain weight, and grain yield than the recommended dose without adding K- humate or bio-fertilizers. The highest values of ear length, ear diameter, kernels no./row, 100- grain weight and grain yield were recorded under 120 kg N/fed. combined with vinasse without significant differences with the values of adding 90 kg N/fed. combined with the application of vinasse. Also, 120 kg N/fed. c combined with K- humate or bio-fertilizer or vinasse + bio-fertilizer significantly increased yield and its components (Tables 14-18).

As for the role of vinasse in increasing the yield and yield component, these data are by those of Armengol *et al.*, (2003), who revealed that using vinasse in agriculture improves soil characteristics for stable aggregate, structure formation, water retention, and porosity, enhanced nutrient availability, hydraulic conductivity use efficiency, increased the soil water content, improve soil organic matter content and soil biochemical properties eventually on crop yield and development (Hati *et al.*, 2006).

K-humate's beneficial influence on grain yield has been thoroughly shown by Gomaa *et al.*, (2014) and Elmasry, (2017) they revealed that humic acid supplementation improved early maize development, improved dry matter accumulation, and encouraged the formation of metabolic products that translocate to grain.

Also, grain yield and yield components increase due to better transfer of photosynthetic substances due to bio-fertilizer treatment these results clarify with those of Rusecki *et al.*, (2019) who noted that improved microbial activity is required to turn nutrients and organic materials into the soil and make them available to the crops.

Table 14. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on ear length (cm) of maize, in 2019 and 2020 seasons

Season		2019				2020			
Nitrogen levels		0	90	120	Mean	0	90	120	Mean
Treatments	Water	17.67	18.17	19.50	18.44	15.50	15.90	17.73	16.38
	K- humate	20.27	21.23	21.73	21.08	20.93	21.00	22.00	21.31
	Vinasse	21.00	22.43	23.07	22.17	19.40	21.37	22.10	20.96
	Bio-fertilizer	20.50	20.53	20.57	20.53	19.27	20.90	21.53	20.57
	Vinasse + Bio-fertilizer	20.23	20.67	20.90	20.60	17.93	21.60	21.73	20.42
	Mean	19.93	20.61	21.15		18.61	20.15	21.02	
LSD at 5%									
Nitrogen (A)			1.06				1.91		
Treatments (B)			0.99				1.26		
A x B			1.71				2.19		

Table 15. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on-ear diameter (cm) of maize, in 2019 and 2020 seasons.

Season		2019				2020			
Nitrogen levels		0	90	120	Mean	0	90	120	Mean
Treatments	Water	4.23	4.30	4.37	4.30	2.97	3.70	4.07	3.58
	K- humate	4.33	4.40	4.57	4.43	4.13	4.73	4.77	4.54
	Vinasse	4.03	4.53	4.57	4.38	4.17	4.77	4.83	4.59
	Bio-fertilizer	4.43	4.50	4.53	4.49	4.27	4.63	4.70	4.53
	Vinasse + Bio-fertilizer	4.30	4.33	4.40	4.34	4.33	4.70	4.73	4.59
	Mean	4.27	4.41	4.49		3.97	4.51	4.62	
LSD at 5%									
Nitrogen (A)			0.30				0.31		
Treatments (B)			0.25				0.27		
A x B			0.43				0.47		

Table 16. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on kernels number/ ear of maize, in 2019 and 2020 seasons.

Season		2019				2020			
Nitrogen levels		0	90	120	Mean	0	90	120	Mean
Treatments	Water	408.6	449.3	496.0	451.3	423.3	498.0	536.0	485.7
	K- humate	510.6	540.0	616.0	555.5	604.0	652.6	710.0	655.5
	Vinasse	524.0	658.0	666.6	616.2	544.0	686.0	766.3	665.4
	Bio-fertilizer	490.0	525.3	560.0	525.1	594.0	644.0	681.0	639.6
	Vinasse + Bio-fertilizer	464.6	521.3	584.0	523.3	558.6	681.3	700.0	646.6
	Mean	479.6	538.8	584.5		544.8	632.4	678.6	
LSD at 5%									
Nitrogen (A)			52.16				78.14		
Treatments (B)			61.56				48.68		
A x B			106.63				84.31		

Table 17. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on 100- grain weight (g) of maize, in 2019 and 2020 seasons.

	Season	2019				2020			
	Nitrogen levels	0	90	120	Mean	0	90	120	Mean
Treatments	Water	29.10	31.00	34.82	31.64	27.44	27.63	35.67	30.25
	K- humate	36.16	37.81	41.55	38.51	35.99	36.11	38.78	36.96
	Vinasse	35.56	38.86	42.30	38.91	37.45	39.55	40.31	39.10
	Bio-fertilizer	32.34	37.72	38.29	36.11	30.53	34.94	37.96	34.48
	Vinasse + Bio-fertilizer	33.60	37.26	39.94	36.94	34.87	38.71	39.04	37.54
	Mean	33.35	36.53	39.38		33.26	35.39	38.35	
	LSD at 5%								
	Nitrogen (A)		1.23				2.52		
	Treatments (B)		1.19				3.09		
	A x B		2.07				5.36		

Table 18. Effect of N-fertilizers, organic, and bio-fertilizer and their interactions on grain yield (ard./fed.) of maize, in 2019 and 2020 seasons.

	Season	2019				2020			
	Nitrogen levels	0	90	120	Mean	0	90	120	Mean
Treatments	Water	6.73	17.32	18.60	14.22	7.02	18.54	20.71	15.42
	K- humate	11.65	22.91	23.07	19.21	10.85	23.69	24.18	19.57
	Vinasse	11.27	24.02	25.11	20.13	11.51	23.73	24.89	20.04
	Bio-fertilizer	9.67	21.19	22.62	17.83	11.29	21.53	23.20	18.67
	Vinasse + Bio-fertilizer	8.43	22.54	23.66	18.21	9.82	23.04	24.44	19.10
	Mean	9.55	21.60	22.61		10.09	22.10	23.48	
	LSD at 5%								
	Nitrogen (A)		1.78				1.71		
	Treatments (B)		1.54				1.36		
	A x B		2.67				2.35		

CONCLUSION

Through this study, it can be recommended to use vinasse with the application of N-fertilizer at the rate of 90 kg N/fed. could substitute 30 kg N/fed. which contributes to saving production costs by reducing the amount of chemical fertilizer addition and to reduce the potential risk of environmental pollution coupled with the chemical fertilizers manufacturing as well as minimize the harmful effect of nitrogen on human life.

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