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ORIGINAL RESEARCH

Enhancing Growth, Yield, and Forage Quality of Two Teosinte Genotypes Through NPK Nano-Fertilizer Application

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ABSTRACT: The overuse of synthetic fertilizers in agriculture has led to adverse effects on soil health, groundwater contamination, and the overall environmental sustainability of farming systems. Consequently, there is an urgent need for more innovative and environmentally friendly fertilizer approaches that can enhance agricultural productivity without compromising soil quality. Therefore, this study aimed to assess the impact of different fertilizer compositions, including conventional chemical fertilizers and nano nitrogen-phosphorous-potassium (NPK) fertilizers, on the growth, yield, and forage quality of two teosinte genotypes (G3 and G4) to identify more sustainable alternatives that could improve agricultural outcomes while minimizing environmental harm. A split-plot design field investigation with the main plots representing the teosinte genotypes and the sub-plots comprising five fertilizer formulations was conducted over two growing seasons (2021 and 2022) to investigate their effects on vegetative growth, yield attributes, and forage quality of two teosinte genotypes. The two teosinte genotypes were subjected to various fertilizer treatments (100% chemical fertilizers (CF) (F1), 75% CF + 25% nano NPK (F2), 50% CF + 50% nano NPK (F3), 25% CF + 75% nano NPK (F4), and 100% nano NPK (F5). Results indicated that G4 exhibited superior growth and nutritional composition compared to G3. Furthermore, F3 treatment resulted in enhancing shoot height, stem diameter, and dry matter accumulation. Additionally, F3 treatment improved fiber digestibility, but F1 treatment yielded the highest crude protein (CP). These findings suggest that integrating nano-fertilizers with traditional fertilizers, as exemplified by the F3 mixture, holds potential for optimizing teosinte growth and forage quality. In conclusion, this study underscores the importance of the research exploration of balanced fertilizer mixtures to enhance forage yield and quality in teosinte cultivation, advocating for a strategic integration of nano NPK and bulk chemical fertilizers for sustainable agricultural practices.

KEYWORDS: Chemical fertilizers, forage quality, growth performance, nanoparticles, NPK fertilizer, teosinte.

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1. Introduction

Chemical fertilizers play a crucial role in modern agriculture by providing essential nutrients to plants for optimal growth and productivity. These fertilizers are typically composed of macro and micronutrients that are readily available for plant uptake. They are produced through chemical processes and are commonly used in conventional farming systems. However, the excessive and indiscriminate use of chemical fertilizers has raised concerns regarding their negative environmental impacts, such as water pollution and soil degradation (Srivastav, 2020; Ali et al., 2020; Ali et al., 2022). To address these concerns, researchers have been exploring alternative approaches to fertilizer production and application. One such innovation is the development of nanofabricated chemical fertilizers. These nano-fertilizers are engineered at the nanoscale, with particle sizes ranging from 1 to 100 nm. They are designed to improve nutrient efficiency and reduce environmental risks associated with conventional chemical fertilizers (Babu et al., 2022).

Nanofabricated chemical fertilizers offer several potential advantages. Firstly, their nanoscale size allows for improved nutrient solubility and enhanced nutrient uptake by plants. Because of its higher efficiency, fertilizer application rates can be lowered, reducing the amount of nutrients lost to the environment. Furthermore, nano-fertilizers can be engineered to release nutrients gradually, providing a sustained nutrient supply to plants over an extended period (Abdelsalam et al., 2023). Moreover, some coatings or encapsulations can be added to nano-fertilizers to functionalize them and allow for the targeted delivery of nutrients to plant roots (Iqbal et al., 2019). Despite the promising advantages, the use of nanofabricated chemical fertilizers is still in its early stages, and further research is needed to assess their long-term effects on plant growth, soil health, and environmental safety. Concerns regarding the potential toxicity of nanoparticles and their accumulation in the soil and plants also need to be addressed through rigorous testing and risk assessment protocols.

Teosinte (*Zea mexicana* (Schrad.) Kuntze) is a wild grass species belonging to the genus *Zea* in the Poaceae family. Teosinte is known for its close genetic relationship with maize, making it a valuable tool for studying the evolution and domestication of this important cereal crop (Yang et al., 2019). It is native to Mexico and has been an important genetic resource for the improvement of maize (Sánchez et al., 2011). Teosinte grows in diverse habitats ranging from lowland tropical forests to highland areas. It has also been reported in other countries of Central America, including Guatemala, Honduras, and Nicaragua (Rivera-Rodríguez et al., 2023). Teosinte is distinguished from maize by unique morphological characteristics. Typically, it is taller, reaching a height of three meters, and has long, narrow leaves. The inflorescences are made up of several branches, each of which has a cluster of spikelets on it, with a glume enveloping 2-3 small seeds in each spikelet (González et al., 2018).

Teosinte is not commonly grown as a forage crop in Egypt or other parts of the world. However, it can potentially be used as a forage crop due to its high biomass production and nutritional value (Bondok et al., 2022). It can tolerate a wide range of environmental conditions, including drought and low soil fertility. These characteristics make teosinte a potential option for forage production in areas with limited water resources or poor soil quality (Sahoo et al., 2021). Teosinte adaptability, variety, implantation, and management, as well as nutritional value, are some of the factors that must be considered when growing it as a forage crop in Egypt. As fodder, teosinte is a highly nutritious forage with substantial levels of carbohydrates, proteins, and fiber (Bondok et al., 2022). Because of its great versatility as a viable fodder crop that can withstand harsh circumstances including acidic, drought- and flood-prone environments, and its capacity to produce biomass, it may also be produced in many advocating conditions (Devkota et al., 2015). In our previous study (Bondok et al., 2022), the performance of five teosinte genotypes was evaluated in Egypt to determine their resilience in the face of severe drought conditions. The findings indicated that genotypes G3 and G4 displayed strong tolerance to severe water stress, maintaining a high biomass yield because of their distinct biochemical and molecular characteristics. Consequently, the primary objective of this study was to evaluate how various fertilizer compositions, including both conventional chemical fertilizer and its nano form in various blend ratios, affect the vegetative growth criteria (plant height, stem diameter, and leaf area), yield characteristics (dry matter and fresh forage yield), and the assessment of forage quality parameters in G3 and G4 teosinte genotypes.

2. Materials and methods

2.1 Experimental location

This field investigation was conducted at the Gemiza experimental farm station, Gharbia, Egypt (30° 79′ N, 31° 12′ E) over two consecutive summer seasons in 2021 and 2022. The primary objective was to assess the effects of foliar sprays of NPK fertilizer nanoparticles compared to conventional chemical NPK fertilizers (ammonium nitrate), along with their combined effects, on the growth, productivity metrics, and nutritional value of two teosinte genotypes identified as Gemiza 3 and Gemiza 4 (G3 and G4). The soil's physiochemical properties and the chemical composition of the irrigation water utilized in this study are detailed in Tables 1 and 2, respectively.

2.2 NPK fertilizer nanoparticles synthesis via high-energy ball milling

The commercially available stoichiometric mineral NPK fertilizer (Egy Flex) comprising 20% nitrogen, 20% phosphorus, and 20% potassium, was purchased from Egyptchem International for Agrochemicals (Nubariyah, Alexandria, Egypt). The acquired NPK chemical fertilizer was introduced into the high-energy ball mill (Pulverisette-7, Fritsch, Germany) under standardized conditions of 200 rpm/min for approximately five hours, to warrant the fabrication of NPK fertilizer nanoparticles. After the milling operation, these nanoparticles were thoroughly collected for subsequent characterization.

2.3 Characterization of the synthesized NPK fertilizer nanoparticles

The characterization of the synthesized NPK fertilizer nanoparticles was conducted using scanning electron microscopy (SEM) equipped with an energy dispersive X-ray (EDX) analyzer (SEM–EDX, INCAx-Sight 6587, Oxford, UK) to identify the morphology and the size distribution of the produced nano-fertilizer.

During the application process, the NPK fertilizer nanoparticles were prepared in an aqueous solution for subsequent topical application as foliar treatments. Before application, the concentration of the nano NPK fertilizer was set at 1000 ppm. To create this solution, the nanoparticles were initially suspended in deionized water and then dispersed using ultrasonic vibration (100 W, 40 kHz) for an hour using magnetic bars for stirring to prevent particle aggregation.

2.4 Experimental setup and treatments

The field study was organized as a split-plot design, with a plot area of 12 m^2 (3 × 4 m) and replicated three times. The primary plots were assigned for the varieties (G3 and G4), while the subplots were designated for treatments involving NPK fertilizer nanoparticles and chemical fertilizers (CF). Before planting, the teosinte seeds were sterilized with a 0.1% $HgCl_2$ solution for eight minutes. Two teosinte seeds were sown in each hole, with holes spaced 35 cm apart and rows separated by 60 cm, on the 10th of May 2021 and the 18th of May 2022 for the respective seasons. Following planting, the plots were irrigated to field capacity (265 L/m^2), and after ten days, seedlings were thinned to one per hole once establishment was complete

Table 1. Physical and chemical properties of the soil at Gemiza experimental station.

Physical properties				
Depth (cm)	**Clay**	**Silt**	**Sand**	**Texture**
0-40	45.12	31.22	23.66	Clay-loamy
Chemical properties				
pH		7.75	Total macronutrients	
EC (dS m^{-1})		1.64	N (%)	0.143
Soluble ions (mmol l^{-1})			P (%)	0.031
Ca^{2+}		6.11	K (%)	0.355
Mg^{2+}		5.30	Available N (mg kg^{-1})	33.41
Na^{+}		7.44	Available P (mg kg^{-1})	10.62
K^{+}		0.21	Available K (mg kg^{-1})	315.71
CO_3^{2-}		0.00	Organic matter (%)	2.49
HCO_3^{-}		3.61	Organic carbon (%)	1.44
Cl^{-}		8.12	C/N ratio	10.06
SO_4^{2-}		7.38		
Extractable micronutrients (ppm)				
Fe^{2+}		3.82	Zn^{2+}	4.45
Mn^{2+}		3.14	Cu^{2+}	1.52

Note: EC, Electrical Conductivity (dS m^{-1}), Ca^{2+}, Calcium (mmol L^{-1}), Mg^{2+}, Magnesium (mmol L^{-1}), Na^{+}, Sodium (mmol L^{-1}), K^{+}, Potassium (mmol L^{-1}), CO_3^{2-}, Carbonate (mmol L^{-1}), HCO_3^{-}, Bicarbonate (mmol L^{-1}), Cl^{-}, Chloride (mmol L^{-1}), SO_4^{2-}, Sulfate (mmol L^{-1}), Fe^{2+}, Iron (ppm), Mn^{2+}, Manganese (ppm), Zn^{2+}, Zinc (ppm), Cu^{2+}, Copper (ppm).

Table 2. Chemical properties of water used in irrigation.

EC	Soluble cations (mmol l^{-1})				Soluble anions (mmol l^{-1})				SAR
(dS m^{-1})	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	SO_4^{2-}	Cl^{-}	
0.40	0.50	0.58	1.78	0.51	-	2.44	-	1.29	3.29

Note: SAR -sodium adsorption ratio, Ca^{2+},Calcium (mmol L^{-1}); Mg^{2+},Magnesium (mmol L^{-1}); Na^{+},Sodium (mmol L^{-1}); K^{+}- Potassium (mmol L^{-1}); CO_3^{2-} -Carbonate (mmol L^{-1}); HCO_3^{-}, Bicarbonate (mmol L^{-1}); Cl^{-}, Chloride (mmol L^{-1}); SO_4^{2-}, Sulfate (mmol L^{-1}); Fe^{2+}, Iron (ppm); Mn^{2+}, Manganese (ppm); Zn^{2+}, Zinc (ppm); Cu^{2+}, Copper (ppm).

The fertilizer treatments investigated included 100% chemical fertilizers (F1), 75% CF + 25% nano NPK (F2), 50% CF + 50% nano NPK (F3), 25% CF + 75% nano NPK (F4), and 100% nano NPK (F5). The chemical fertilizer ammonium nitrate (33.5%) utilized in the study was obtained from the Abu Qir fertilizers company, Alexandria, Egypt, and contained 33.5% nitrogen. At the time of teosinte fertigation, the chemical fertilizer ammonium nitrate was added as a top dressing at the rate of 280 kg ha^{-1}, according to the recommendations of the Agriculture and Land Reclamation Ministry, in three equal dosages administered two weeks after sowing, and two weeks after each of the first and second cuts. However, the nano NPK fertilizer was applied as a foliar suspension according to the design used. All agricultural activities were carried out promptly following the guidelines provided by the Egyptian Ministry of Agriculture and Land Reclamation. Phosphorus fertilizer in the form of calcium monophosphate (15.5% P_2O_5) at a rate of 283 kg ha^{-1} and potassium fertilizer in the form of potassium sulfate (48% K_2O) at a rate of 119 kg ha^{-1} were applied once before sowing following the recommended dosages.

In both growing seasons, three cuts were acquired under these conditions. The first cut was conducted approximately 45 days after planting, the second cut 32 days after the first, and the third cut 30 days after the second in each season.

2.5 Investigated parameters

During the harvest stages, a random selection of ten plants from each plot was undertaken to assess various parameters, including plant height (cm), main stem diameter (mm), as well as the dimensions of the third uppermost leaf blade on the main stem (length and maximum width) for the calculation of leaf area (multiplied by 0.75 cm). Dry matter yield was quantified as g/plant and the fresh fodder yield was quantified in ton fed^{-1}. The nitrogen content (N) was determined using Kjeldahl procedures following the guidelines outlined in AOAC (2012). Subsequently, the crude protein (CP) content was calculated by multiplying the nitrogen content by 6.25 (Sriperm et al., 2011).

The acid detergent fiber (ADF), representing key dietary fiber fractions, was analyzed sequentially utilizing the semi-automatic ANKOM 220 fiber Analyzer (ANKOM Technology, Macedon, NY, USA) following the method described by Van Soest et al. (1991). The fiber fraction was assessed without the use of heat-stable amylase and was expressed including residual ash content. The determination of crude fat (CF) content in the dried sub-samples was carried out using the Soxhlet method, in accordance with the procedures outlined in AOAC (2012).

2.6 Detection of genotoxicity by estimation of genomic template stability

The DNA extraction process involved using the GeneJET genomic DNA purification kit protocol (K0721, Thermo Fisher Scientific Inc., Waltham, MA, USA), comprising the steps of lysing cells with lysis solution and proteinase K, ethanol precipitation, and elution. The PCR master mixture preparation was conducted under biosafety conditions, utilizing the Ready-To-Go RAPD analysis kit with the investigated primers (Table S1). Agarose gel electrophoresis was employed to visualize and detect the amplification products. A 1.5% agarose solution was prepared, poured into a gel bed, and allowed to solidify. The gel was then subjected to electrophoresis at 80 V for 100 min. After electrophoresis, the gel was stained with ethidium bromide for 30 min. and destained in distilled water for 20 min.

The assessment of genotoxicity of the used fertilizers was performed through the assessment of genomic template stability (GTS). GTS was quantified for each primer using the equation (1) of Salarizadeh and Kavousi (2015).

$$GTS\ (\%) = \left(1 - \frac{a}{n}\right) x\ 100 \qquad (1)$$

Where "a" represent the average number of polymorphic bands in treated groups and "n" is the total number of bands in control samples. Polymorphic bands in the Random Amplified Polymorphic DNA (RAPD) analysis indicated variations compared to the control profile. The amplified bands were documented as 0 for absence and 1 for presence using Totallab software analysis (www.totalalb.com).

2.7 Statistical analysis

The data underwent one-way ANOVA analysis utilizing the SAS software package (Version 9.1.3, 2007) to determine the least significant differences (LSD) between genotypes at significance level of 5%. The assessment of variance in treatment discrepancies followed the methodology outlined by Steel and Torrie (1980).

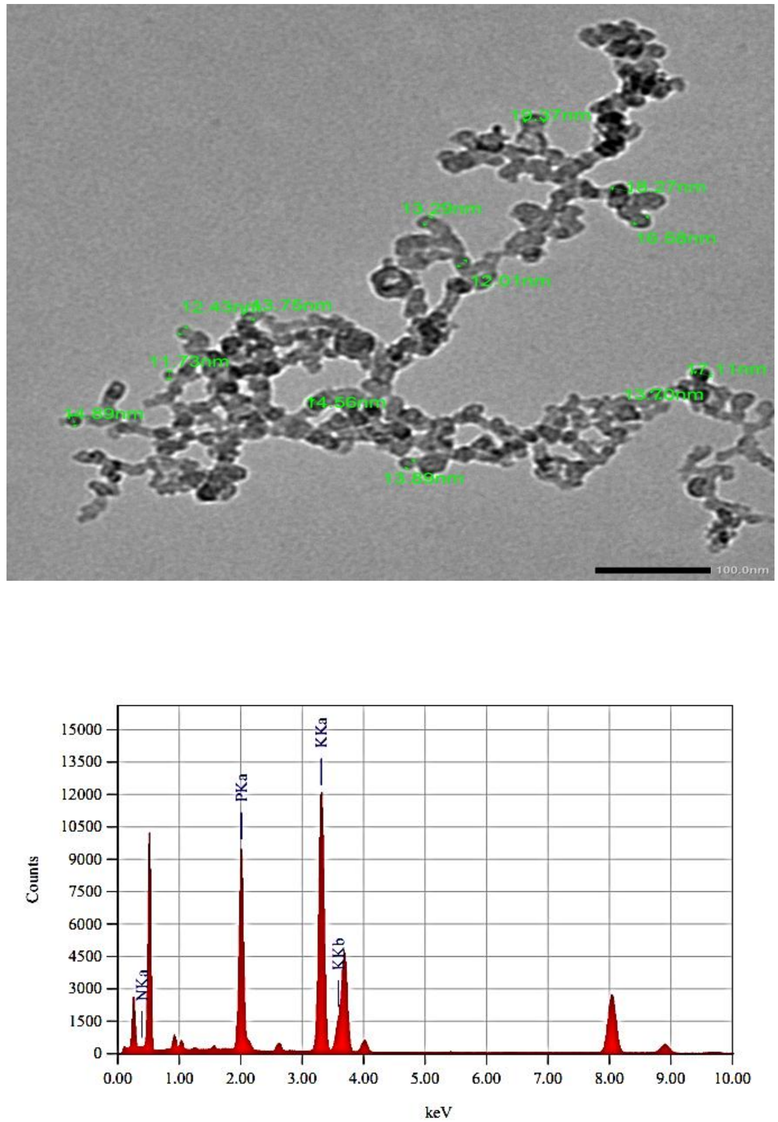
3. Results

3.1 Characterization of NPK nano-fertilizer

The NPK nano-fertilizer produced through high-energy ball milling exhibited a white powdery appearance, with its synthesis confirmed through scanning electron microscopy (SEM) analysis. SEM images depicted the nano-fertilizer particles as spherical, ranging in size from 11.73 to 19.37 nm, with an average size of 14.82 nm (Figure 1a). Furthermore, energy-dispersive X-ray (EDX) analysis (Figure 1b) unveiled the elemental composition of the nano-fertilizer, shedding light on the weight (Wt. %) and atomic (At. %) percentages of the constituent elements. These elemental ratios play a pivotal role in elucidating the chemical constitution and nutrient profile of the nano-fertilizer, critical for assessing its efficacy and nutrient balance in potential agricultural applications..

(a)

(b)



ZAF Method Standardless Quantitative Analysis
Fitting Coefficient : 0.5132

Element	(keV)	Mass%	Sigma	Atom%	Compound	Mass%	Cation	K
N K*	0.392	3.44	0.07	8.13				0.2840
P K	2.013	45.06	0.16	48.22				89.8997
K K	3.312	51.50	0.15	43.65				9.8163
Total		100.00		100.00				

Figure 1. Characterization of the synthesized NPK nano-fertilizer: a) SEM micrograph of NPK nano-fertilizer, b) energy dispersive X-ray (EDX) pattern with elemental percentages (Wt. & At. %) of NPK nano-fertilizer.

Table 3. Shoot height (cm) of two teosinte genotypes influenced by nano and bulk chemical fertilizer applications over three cuts during two growing seasons (Mean ± SD).

Genotype	1st Cut	2nd Cut	3rd Cut	Mean
G3	113.70±2.12[B]	133.8±0.57[A]	112.85±3.46[B]	120.05±2.05[B]
G4	120.35±4.31[A]	137.05±1.06[A]	115.15±3.46[A]	124.15±2.90[A]
Mean	117.00±3.25	135.40±0.85	113.95±3.46	122.10±2.47
Fertilizer treatments				
F1	118.65±2.90[a]	133.00±0.71[a]	109.05±0.78[a]	120.20±0.99[a]
F2	103.90±0.99[b]	120.25±2.05[b]	97.70±1.41[b]	107.25±0.78[b]
F3	118.35±2.76[a]	132.75±0.64[a]	108.90±0.85[a]	120.00±0.85[a]
F4	101.90±0.71[c]	115.00±0.57[c]	92.80±2.12[c]	103.20±0.71[c]
F5	99.75±1.91[d]	111.25±1.34[c]	93.40±2.83[c]	101.40±1.13[c]
Mean	108.45±0.78	122.45±0.21	100.35±0.78	110.41±0.13
LSD (5%)	2.45	2.2	2.12	1.025

Note: Different letters in the same column indicate significant statistical variances. Uppercase letters represent genotype discrepancies, while lowercase letters signify differences among the fertilizer formulations employed.

Notably, the atomic percentage composition of the synthesized NPK nano-fertilizer revealed the presence of 8.13% nitrogen (N), 48.22% phosphorus (P), and 43.65% potassium (K), offering valuable insights into its potential agricultural utility

3.2 Plant height

The data provided in Table 3 presents shoot height measurements for the two investigated teosinte genotypes and fertilizer treatments. In general, the two teosinte genotypes' peak shoot height was recorded in the second cut, resulting in mean shoot heights of 137.05 and 133.80 cm for G4 and G3, respectively. In terms of genotypes, G4 generally exhibited higher shoot heights compared to G3 across the three cuts, with a mean shoot height of 124.15 cm across the three cuts. This suggests that the G4 genotype exhibited a better growth response in terms of shoot height compared to the G3 genotype. Regarding fertilizer treatments, the investigation included five fertilizer treatments: F1 (100% chemical fertilizer), F2 (75% CF + 25% nano NPK), F3 (50% CF + 50% nano NPK), F4 (25% CF + 75% nano NPK), and F5 = (100% nano NPK).

The obtained data over the two growing seasons revealed that F1 and F3 displayed higher mean shoot heights of 120.0 cm, indicating that these treatments potentially have a positive impact on shoot height compared to the other fertilizers. Contrastingly, F5 showed the lowest mean shoot height (101.4 cm) over the two growth seasons, suggesting that this treatment may not be as effective in promoting teosinte shoot growth compared to the other fertilizer combinations. These findings highlight the importance of genotype selection and suitable fertilizer combinations in influencing the teosinte shoot height.

3.3 Stem diameter

The data provided in Table 4 shows the mean stem diameter measurements of G3 and G4 teosinte genotypes over two growing seasons in response to different fertilizer formulations across three cutting instances.

Like shoot height, the second cut also reported the greatest stem diameter, with mean values of 18.45 and 16.65 mm for the G4 and G3 genotypes, respectively. Observing the genotypes, G4 consistently exhibited a larger stem diameter compared to G3 across all three cuts over the two growing seasons, culminating in a higher mean stem diameter of 14.60 mm. This suggests that G4 may have a superior growth response in terms of stem thickness compared to G3.

In the context of fertilizer treatments, treatments F2 and F3 displayed higher mean stem diameters of 12.40 and 13.45 mm, respectively. This indicates both fertilizer combinations exerted a positive impact on the teosinte stem diameter compared to the other fertilizer treatments. Conversely, F5 (100% nano NPK) showed the lowest mean stem diameter of 10.40 mm, showing that this treatment was less effective in promoting stem thickness compared to the other fertilizer combinations. This result provides insights into the importance of evaluating stem diameter across genotypes and fertilizer treatments as a criterion for assessing fertilizer efficacy.

3.4 Leaf area

The data in Table 5 presents the leaf area/plant (cm^2) for two teosinte genotypes (G3 and G4) as influenced by various fertilizer treatments across two growing seasons. When considering the genotypes, G4 consistently exhibited larger leaf areas compared to G3 across all three cutting instances, resulting in a higher mean leaf area of 46.49 cm^2. Furthermore, the leaf area at the second cut was the highest for both genotypes, measuring 60.15 cm^2 for G3 and 70.45 cm^2 for G4. This implies that G4 has a more robust leaf area development compared to G3 under the conditions studied.

Table 4. Stem diameter (mm) of two teosinte genotypes influenced by nano and bulk chemical fertilizer applications over three cuts during two growing seasons (Mean ± SD).

Genotype	1st Cut	2nd Cut	3rd Cut	Mean
G 3	9.45±2.76^B	16.65±0.92^B	12.00±1.13^B	12.65±1.63^B
G 4	11.25±2.90^A	18.45±2.90^A	14.05±1.77^A	14.60±2.55^A
Mean	10.35±2.76	17.55±1.91	13.05±1.48	13.63±2.09
Fertilizer treatments				
F1	7.85±2.19^c	13.25±2.62^d	10.45±1.77^c	10.50±2.26^d
F2	9.45±2.76^b	15.90±3.68^b	12.00±1.98^b	12.40±2.83^b
F3	10.55±3.04^a	16.95±3.46^a	13.05±2.19^a	13.45±2.90^a
F4	9.30±2.69 ^{b}	15.45±2.76^c	11.80±1.84^b	12.20±2.40^c
F5	7.80±2.40^c	13.25±2.76^d	10.30±1.70^c	10.40±2.26^d
Mean	8.95±2.62	14.95±3.04	11.50±1.84	11.79±2.53
LSD (5%)	1.02	1.02	1.05	1.03

Note: Different letters in the same column indicate significant statistical variances. Uppercase letters represent genotype discrepancies, while lowercase letters signify differences among the fertilizer formulations employed.

Table 5. Leaf area/plant (cm^2) of two teosinte genotypes influenced by nano and bulk chemical fertilizer applications over three cuts during two growing seasons (Mean ± SD).

Genotype	1st Cut	2nd Cut	3rd Cut	Mean
G 3	35.80±3.68^{B}	60.15±3.47^{B}	35.25±1.34^{B}	43.74±2.83^{B}
G 4	40.30±4.67^{A}	70.45±5.30^{A}	42.20±4.39^{A}	46.49±1.58^{A}
Mean	38.05±4.17	65.30±4.38	38.73±2.87	45.11±0.63
Fertilizer treatments				
F1	37.85±3.46^{a}	59.10±3.25^{a}	30.56±7.41^{a}	42.51±4.71^{a}
F2	34.19±1.38^{b}	49.25±1.20^{b}	18.10±0.42^{b}	33.85±1.00^{b}
F3	37.75±3.46^{a}	58.96±3.20^{a}	30.45±7.42^{a}	42.39±4.69^{a}
F4	33.23±1.30^{c}	46.35±4.03^{c}	16.60±1.41^{c}	32.06±2.25^{c}
F5	31.91±0.58^{d}	42.60±1.41^{d}	15.52±1.29^{c}	30.01±1.10^{d}
Mean	34.99±2.04	51.26±2.62	22.25±3.59	36.16±2.75
LSD (5%)	2.21	2.33	2.11	2.21

Note: Different letters in the same column indicate significant statistical variances. Uppercase letters represent genotype discrepancies, while lowercase letters signify differences among the fertilizer formulations employed.

Looking at the fertilizer treatments, like the results of shoot height, F1 and F3 treatments displayed higher mean leaf areas of 42.51 and 42.39 cm^2, respectively, demonstrating that both treatments potentially triggered a positive impact on leaf area/plant compared to the other fertilizer treatments. Conversely, F5 produced the lowest mean leaf area/plant (30.01 cm^2) among the investigated fertilizer treatments. In comparison to the other fertilizer combinations, this result implies that the F5 was less profitable at promoting the expansion of teosinte leaves. Therefore, genotype selection and the choice of fertilizer treatments play crucial roles in influencing leaf area development in teosinte plants.

3.5 Dry matter yield

The data presented in table 6 shows the mean dry matter content (g) of two teosinte genotypes (G3 and G4) as influenced by five fertilizer treatments across three cuttings over two growing seasons (2021/2022). Owing to the genotypes, G4 significantly exhibited higher dry matter/plant compared to G3 throughout all three cuttings, resulting in a mean dry matter content of 21.05 g/plant. Furthermore, for the three cuts, the second cut exhibited the greatest dry matter yield in the two genotypes, with values of 21.35 g/plant for G3 and 22.45 g/plant for G4. As a result, the G4 genotype demonstrates a greater potential for dry matter accumulation than the G3 genotype under the investigated conditions.

Table 6. Dry matter (g/plant) of two teosinte genotypes influenced by nano and bulk chemical fertilizer applications over three cuts during two growing seasons (Mean ± SD).

Genotype	1st Cut	2nd Cut	3rd Cut	Mean
G3	18.15±0.07[B]	21.35±0.07[B]	21.05±0.07[B]	20.20±0.00[B]
G4	18.60±0.14[A]	22.45±0.07[A]	22.05±0.07[A]	21.05±0.07[A]
Mean	18.35±0.07	21.85±0.07	21.55±0.07	20.63±0.04
Fertilizer treatments				
F1	13.65±0.07[e]	14.35±0.07[e]	15.30±0.14[e]	14.45±0.07[e]
F2	15.35±0.07[d]	15.70±0.14[d]	16.20±0.14[d]	15.70±0.07[d]
F3	18.55±0.07[c]	19.45±0.07[c]	19.75±0.07[c]	19.25±0.07[c]
F4	18.65±0.07[b]	20.15±0.07[b]	20.55±0.07[b]	19.75±0.07[b]
F5	18.85±0.07[a]	21.55±0.07[a]	21.75±0.07[a]	20.65±0.07[a]
Mean	17.05±0.07	18.25±0.07	18.65±0.07	17.96±0.07
LSD (5%)	0.17	0.20	0.19	0.18

Different letters in the same column indicate significant statistical variances. Uppercase letters represent genotype discrepancies, while lowercase letters signify differences among the fertilizer formulations employed.

Regarding fertilizer treatments, F5 exhibited the highest mean dry matter content of 20.65g/plant, suggesting that this treatment could positively influence dry matter accumulation when compared to the other treatments. In contrast, F1 showed the lowest mean dry matter content (14.45 g/plant), suggesting that the bulk chemical fertilizer treatment was less effective in promoting dry matter production when compared to the alternative fertilizer combinations. These results underscore the importance of genotype and fertilizer treatment choice for maximizing dry matter production in teosinte cultivation.

3.6 Fresh forage yield

The mean fresh yield (ton/fed) at different cutting times for G3 and G4 teosinte genotypes under the influence of various fertilizer treatments across the 2021 and 2022 growing seasons is presented in Table 7. The data manifested that the teosinte G4 genotype relatively displayed higher fresh yields compared to the G3 genotype across the three cuttings, averaging a total fresh yield of 37.65 ton/fed, slightly surpassing the average fresh yield of the G3 genotype total yield (36.30 ton/fed). Nevertheless, the fresh yield of the two teosinte genotypes peaked at the second cut, producing 21.25 and 22.50 ton/fed for the G3 and G4 genotypes, respectively.

Regarding the fertilizer applications, the conventional bulk chemical fertilizer (F1) demonstrated the maximum total fresh yield (34.10 ton/per fed), while the fertilizer composition comprising 50% bulk chemical fertilizer with 50% nano NPK (F3) yielded a comparatively similar total fresh yield (34.00 ton/fed) compared to the other fertilizer mixtures. In contrast, the fertilizer treatment of 100% nano NPK (F5) exhibited the lowest total fresh yield (22.85 ton/fed), indicating its comparatively inferior efficacy in enhancing the fresh yield of teosinte when compared to the other fertilizer formulations. These data necessitate the genotype selection and fertilizer treatments for optimizing fresh yield production besides enhancing overall forage productivity in teosinte farming practices.

3.7 Forage quality parameters

The data presented in Figure 2 outlines the average contents of crude protein (CP), acid detergent fiber (ADF), and crude fat (CF) for the G3 and G4 teosinte genotypes under the influence of various fertilizer treatments. Looking at the teosinte genotypes, G4 exhibited higher mean values for CP (66.23 g kg^{-1}) compared to G3 (63.00 g kg^{-1}). In terms of ADF, G4 (290.95 g kg^{-1}) had slightly lower ADF compared to G3 (297.85 g kg^{-1}), indicating that G4 possesses a slightly better fiber digestibility profile. Moreover, G3 displayed a higher mean CF content (40.02 g kg^{-1}) compared to G4 (37.25 g kg^{-1}), implying differences in fat content between the two genotypes.

Regarding fertilizer treatments, the bulk chemical fertilizer (F1) resulted in the highest mean CP content (69.07 g kg^{-1}) among the investigated fertilizer treatments, followed closely by F2 (68.10 g kg^{-1}) and F3 (67.30 g kg^{-1}). This suggests that the integration of nano-fertilizers with the bulk chemical fertilizer causes a non-touchable impact on the protein content in teosinte plants. This indicates that the incorporation of nano-fertilizers alongside conventional chemical fertilizers exerts a non-discernible influence on the protein content in the teosinte forage. In terms of ADF, F3 exhibited the lowest mean value (285.04 g kg^{-1}), indicating potentially better fiber digestibility compared to the other treatments. Regarding CF, F1 had the lowest mean content (30.72 g kg^{-1}), while F5 showed the highest (44.94 g kg^{-1}), suggesting varying effects of the fertilizer treatments on fat content in teosinte. The data underscores the intricate relationship between genotype selection and fertilizer treatments influencing the nutritional composition of teosinte plants.

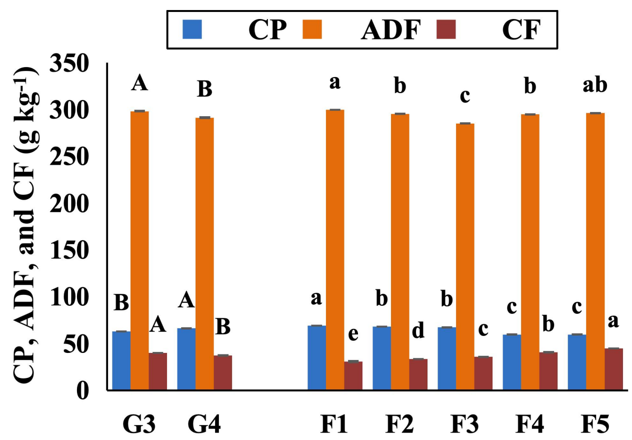


Figure 2: Means of crude protein, acid detergent fiber, and crude fat contents (g kg^{-1}) of two teosinte genotypes influenced by nano and bulk chemical fertilizer applications over two growing seasons (Mean ± SD). Different letters indicate significant statistical variances. Uppercase letters represent genotype discrepancies, while lowercase letters signify differences among the fertilizer formulations employed.

3.8 Biosafety NPK nano-fertilizers

The data presented in Table 8 and supplementary Figure S1 evaluates the genotoxic effects of nano-fertilizers on teosinte forage by assessing genomic template stability (GTS) values. The investigation utilized the RAPD-PCR fingerprinting technique of five primers (OPA-11, OPD-18, CB-21, OPV-07, and OPA-03) to determine the GTS percentage for various combinations of bulk and nano-fertilizers in comparison to the bulk NPK fertilizer. The results indicated that all tested combinations of bulk and nano-fertilizers showed no polymorphic bands, suggesting no detectable genetic alterations at the molecular level when compared to the control group. The average number of polymorphic bands found in each treated group was recorded as zero, further supporting the absence of genetic variability induced by the fertilizers.

The comparison of total bands in the control sample to the absence of polymorphic bands in the treated groups resulted in a ratio of 0, indicating no observable genetic changes in the treated samples. Consequently, the GTS percentage for all fertilizer combinations was calculated as 100%, signifying complete genomic stability in the teosinte plants exposed to the different fertilizer treatments. These findings suggest that the application of the tested combinations of bulk and nano-fertilizers did not induce genotoxic effects on the teosinte forage based on the assessment of GTS values.

Table 7. Fresh yield (ton/fed) of two teosinte genotypes influenced by nano and bulk chemical fertilizer applications over three cuts during two growing seasons (Mean ± SD).

Genotype	1st Cut	2nd Cut	3rd Cut	Total
G3	9.30±0.14^{A}	21.25±1.48^{B}	5.75±0.07^{A}	36.30±1.41^{B}
G4	9.40±0.14^{A}	22.50±1.70^{A}	5.75±0.07^{A}	37.65±1.63^{A}
Mean	9.30±0.14	21.85±1.63	5.75±0.07	36.98±1.52
Fertilizer treatments				
F1	7.35±0.64^{a}	21.70±0.00^{a}	5.05±0.21^{a}	34.10±0.85^{a}
F2	6.85±0.49^{a}	19.35±0.21^{b}	4.65±0.07^{b}	30.85±0.78^{b}
F3	7.25±0.64^{a}	21.70±0.00^{a}	5.05±0.21^{a}	34.00±0.85^{a}
F4	6.55±0.35^{a}	17.80±0.85^{c}	3.80±0.00^{c}	28.15±0.49^{c}
F5	6.55±0.21^{a}	13.20±0.28^{d}	3.10±0.42^{c}	22.85±0.92^{d}
Mean	6.85±0.49	18.75±0.07	4.30±0.14	29.99±0.58
LSD (5%)	0.07	0.17	0.09	0.11

Different letters in the same column indicate significant statistical variances. Uppercase letters represent genotype discrepancies, while lowercase letters signify differences among the fertilizer formulations employed.

Table 8. Genomic template stability (GTS) using the RAPD-PCR technique based on five primers data for teosinte crop.

Character	Fertilizer formulations			
	F1	F2	F3	F4
No of polymorphic bands	0.00	0.00	0.00	0.00
Average polymorphic bands (a)	0.00	0.00	0.00	0.00
Total number of bands in the control (n)	29	29	29	29
a/n	0.00	0.00	0.00	0.00
1-a/n	1.00	1.00	1.00	1.00
GTS (%)	100	100	100	100

4. Discussion

Nanoparticles characterized by reduced particle dimensions and large surface areas represent a promising candidate for utilization as a fertilizer in teosinte and various other cultivars. The utilization of macronutrients in the form of nanostructure stands as a pivotal strategy for the gradual and regulated release of essential nutrients, thus addressing the persistent concerns of soil contamination stemming from the overapplication of conventional fertilizers (Haydar et al., 2024). Notably, as elucidated by Yadav et al. (2023), the advantageous slow-release features possessed by nano-fertilizers offer a solution to the soil's limited capacity for native fertilizer retention. Building upon this concept, Kopittke et al. (2019) proposed that a blend comprising 50% NPK nano-particle fertilizer and 50% chemical fertilizer supplies a dependable regulatory mechanism for optimal growth.

The current investigation revealed that the growth (shoot height and leaf area) of two teosinte genotypes (G3 and G4) under various fertilizer treatments across three successive cuts along two growing seasons. Throughout the three cuts, both genotypes exhibited their maximum shoot height and leaf area during the second cut. Notably, G4 consistently displayed a higher growth rate compared to G3 throughout the study across all cuts. This trend underscores the superior growth response of the G4 genotype relative to G3. Several specific mechanisms could contribute to the differences in the growth pattern observed among the two genotypes like genetic variations, hormonal regulation, response to environmental stimuli, and epigenetic marks (Dar et al., 2022; Agarwal et al., 2020; Abdulraheem et al., 2024).

In terms of fertilizer treatments, the investigation encompassed five distinct treatments: F1 (100% chemical fertilizer), F2 (75% chemical fertilizer + 25% nano NPK), F3 (50% chemical fertilizer + 50% nano NPK), F4 (25% chemical fertilizer + 75% nano NPK), and F5 (100% nano NPK). Analysis of the data spanning two growing seasons unveiled that F1 and F3 exhibited elevated mean shoot height and leaf area, indicating their potential efficacy in enhancing teosinte growth compared to the other fertilizer combinations. Conversely, F5 demonstrated the lowest mean teosinte growth rate, signifying its comparatively less effectiveness in promoting teosinte growth when juxtaposed with alternative fertilizer formulations. The enhancement effect of nano NPK fertilizers has been attributed to their small dimensions, which allow for the retention of numerous ions due to a high surface area, facilitating a gradual release that aligns with crop demand (Helaly et al., 2021). Additionally, nano-fertilizers are readily absorbed by the leaf epidermis and transported to the stems, enhancing the assimilation of active compounds, as they exhibit a slow-release mechanism, hence promoting the growth and yield of the crop species (Qureshi et al., 2018; Reddy et al., 2024).

The dry matter content and fresh forage yield of the teosinte genotypes G3 and G4 under various fertilizer treatments across three cuttings over two growing seasons showed that G4 consistently displayed higher dry matter/plant and fresh forage yield compared to G3, with the second cut yielding the highest dry and fresh matter, indicating G4's superior dry matter and fresh fodder production potential. As for fertilizer treatments, F5 (100% nano NPK) treatment showed the highest mean dry matter content but the lowest fresh yield, while F1 (100% chemical NPK) exhibited the lowest dry matter content and the highest total fresh yield, emphasizing the impact of fertilizer selection on dry matter and fresh fodder production. The efficacy of nano-fertilizers in boosting forage yield is ascribed to their enhanced capacity to provide essential nutrients, as well as their ability to enhance the absorption and transport of available nutrients, hence facilitating superior crop growth and yield (Reshma Anjum et al., 2024). To elucidate the enhanced crop growth and yield observed in response to the synergistic application of nanoparticle and conventional fertilizers, Benzon et al. (2015) attempted to clarify the underlying mechanisms. They ascribed these favorable results to the concept of sink strength, denoting the sink's capacity to efficiently harness photosynthetic products for their growth and function, contingent upon its size and metabolic vigor.

The results of the nutritional quality attributes of the teosinte genotypes G3 and G4 under the investigated fertilizer treatments showed that G4 exhibited higher crude protein (CP) content compared to G3. Conversely, G4 displayed slightly lower acid detergent fiber (ADF) content than G3, indicating better fiber digestibility in G4. Moreover, G3 demonstrated a higher crude fat (CF) content compared to G4, suggesting genotype-specific differences in fat composition. Regarding the impact of fertilizer treatments, the bulk chemical fertilizer (F1) led to the highest CP content, followed closely by F2 and F3, implying a positive influence of these fertilizer mixes on protein levels in teosinte. F3 exhibited the lowest ADF content, indicating improved fiber digestibility compared to other treatments. In terms of CF, F1 had the lowest content, while F5 recorded the highest, highlighting varied effects of fertilizer treatments on fat content in teosinte plants. These findings underscore the intricate interplay between genotype selection and fertilizer treatments in manipulating the nutritional profile of teosinte. The results suggest that specific fertilization strategies, especially those combining nano-fertilizers with conventional chemicals, can have discernible impacts on protein, fiber, and fat content in teosinte forage, highlighting the importance of tailored approaches to optimize the nutritional quality of teosinte crops for enhanced livestock feed or other agricultural applications. According to the findings of Payghan (2016), the enhancement in the nutritive value of fodder millet is characterized by higher CP and lower ADF contents due to the combined application of nanoparticles and chemical fertilizers. Conversely, the CF content of the herbage exhibited an opposing trend, increasing in response to the NPK nanoparticle fertilizer treatments.

The enhancement of crude protein content and the decrease in acid detergent fibers in teosinte due to NPK nano-fertilizer can be attributed to improved nutrient absorption efficiency and increased nutrient availability by the applied nano-formulated NPK fertilizer. Nano-fertilizers have a higher surface area to volume ratio, which enhances the efficiency of nutrient absorption by plants, leading to increased protein synthesis in plants (El-Saadony et al., 2021). Also, nano-fertilizers can release nutrients gradually and in a more controlled manner compared to traditional fertilizers. This sustained nutrient release can ensure a continuous supply of essential elements, promoting better protein synthesis in plants (Channab et al., 2024).

Owing to the content of ADF, nano-fertilizers may influence the plant's cell wall structure and composition, potentially leading to alterations in fiber content and digestibility, modifying the plant's structural components, making the fibers more easily digestible (Garg et al., 2023). In summary, the application of NPK nano-fertilizer can enhance CP content and decrease ADF in teosinte through improved nutrient absorption efficiency, increased nutrient availability, potential modifications in plant cell wall composition, and genotype-specific responses.

5. Conclusion

The evaluation of teosinte growth responses and forage quality under diverse fertilizer treatments underscores the significance of tailored nutrient management strategies in agricultural practices. Genotype-specific variations in growth metrics such as shoot height, stem diameter, leaf area, and dry matter content highlight the genetic influence on teosinte productivity. Additionally, the varying effects of different fertilizer compositions on fresh yield and nutritional composition emphasize the need for precision in fertilizer selection to optimize teosinte cultivation. The integration of nano NPK fertilizer with bulk chemical fertilizer at a 1:1 ratio (F3) notably increased teosinte shoot height. Furthermore, F2 (75% bulk chemical fertilizer, 25% nano NPK) and F3 demonstrated higher mean stem diameters. Leaf area was significantly greater in treatments involving bulk chemical fertilizer (F1) and F3. Notably, 100% nano NPK fertilizer (F5) exhibited the highest mean dry matter accumulation. The application of bulk fertilizer (F1) resulted in the maximum total fresh yield, while F3 produced a comparable yield. Regarding crude protein (CP) content, F1 treatment yielded the highest CP, followed by F2 and F3. Notably, F3 displayed the lowest mean acid detergent fiber (ADF) value, while F5 exhibited the highest crude fat (CF) content. Thus, the study's findings provide valuable insights into the potential of nano-fertilizers in combination with the bulk fertilizer in enhancing the teosinte growth parameters and forage quality. Moving forward, a deeper understanding of genotype-fertilizer interactions and their implications on teosinte productivity will be crucial for sustainable forage production and livestock feed quality enhancement in agricultural systems. To enhance the study's practical relevance, it is recommended to investigate the long-term effects of nano-fertilizers on soil health, thereby providing significant insight for future research activities.

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