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Teosinte crop growth, productivity and nutritive value as affected by NPK nano-fertilizers compared to conventional fertilizers

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SUSTAINABLE farming practices are critical for ensuring food security worldwide. NPK Nano fertilizers have the best effects of promoting nutrient absorption, so it has the potential to produce excellent fertilizers. (NPK.) nano forms of nitrogen, phosphorus, and potassium represent an effective alternative to conventional fertilizers. The current study assessed the effects of fertilizer compositions, nano and conventional NPK, on the forage quality, and growth of two teosinte varieties, (Gemmeiza 3, and Gemmeiza 4) aiming to identify sustainable options that enhance agricultural performance while reducing environmental impact. A split-plot design with three replicates was used in the field trial. The main plots representing varieties and sub-plots included five fertilizers treatments, i.e., F1: 100% conventional fertilizers, F2: 75% conventional fertilizers + 25% Nano particle fertilizers; F3: 50% conventional fertilizers + 50% Nano particle fertilizers; F4: 25% conventional fertilizers + 75% Nano particle fertilizers and F5: 100% Nano particle fertilizers during 2022 and 2023 seasons. Results revealed that Gemmeiza 4 variety exhibited superior growth performance and nutritional composition compared to Gemmeiza 3. Furthermore, F3 treatment resulted in improved plant height (112.9,126.2 and 103.2 cm across the three cuts), stem diameter, dry matter accumulation, and leaf area. The F3 treatment also enhanced fiber digestibility, while F1 fertilizer produced high protein (69.1 and 68.7) in the first and second seasons respectively. Plants treated with nanoparticles showed 100% genomic template stability (GTS), indicating the crucial role in maintaining genetic stability. These findings suggest that combining nano-fertilizers with conventional fertilizers, as in the F3 mixture, can enhance teosinte growth and forage quality. This highlights the importance of both nano and conventional NPK fertilizers in achieving sustainable agricultural outcomes.

Keywords: N.P.K Nano fertilizer , Teosinte Growth, Teosinte Productivity, Teosinte Nutritive Value, and GTS.

1. Introduction

Worldwide agricultural production is expected to quadruple by 2050 to meet the growing food demands resulting the world's expanding population (Murodsulton et al., 2024). In addition, the climate change and associated phenomena, such as rising global temperatures, evaluated atmospheric Co_2 levels, heat waves, flooding, severe storms, droughts, and other extreme weather occurrences, are the main focus of current scientific research and agronomy sciences. Teosinte (*Zea Mexicana* L.) 2n = 20 is one of the most important summer forage crops which are closely related to maize in most allometric trait and has the advantage of tillering and regeneration as a fodder crop (Salem et al. 2019; Abdel-Fattah et al., 2023). In Egypt, it is a promising summer feed crop that requires further study until farmers and producers may use it (Salem et al., 2024).

In the past, agricultural practices have contributed to soil degradation and the release of hazardous substances into the environment. In this regard, there is increasing interest in the use of nanomaterials as fertilizers for improving plant mineral nutrition (Hussein and Sabbour 2024). Fertilizers containing nanoparticles (NPK) have emerged as promising alternatives due to their efficiency and environmentally friendly properties. Nano-fertilizers (NFs) are widely used in plant nutrition as spray-based and soil-based applications. Because of their rapid and increased translocation to various plant parts (Soliman 2025). Nano- and bio-fertilizers are increasingly replacing traditional fertilizers. The

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following treatments were applied : F1: conventional fertilizers: 100%. F2: 75% conventional fertilizer + 25% nano. F3: 50% conventional fertilizer + 50% nano. F4: 25% conventional fertilizer + 75% nano and F5 100% nano fertilizers. Nano-fertilizers significantly enhance nitrogen uptake and crop yield (Mohamed and Awad 2024). Nanotechnology is being employed to address environmental issues and increase the value of agricultural outputs. Through the use of nanoparticles and nano-powders, fertilizers can be engineered for controlled or delayed nutrient release. High reactivity in nanoparticles can be attributed to either a larger density of reactive regions, a more specific surface area, or increased reactivity of specific locations on the particle surfaces. Nano-fertilizers are to delay the release of nutrients and extend the fertilizer's duration of action. Nanotechnology undoubtedly has the potential to have a major impact on energy, the economy, and the environment by improving fertilizers (Chhabra et al., 2025). Many stimuli, including heat, moisture, nano-fertilizers were designed to release their cargo in a controlled manner, either slowly or quickly. The biotic mineralization of P connected to soil inorganic colloids and N and P from soil organic matter is facilitated by the release of molecules carbonaceous in rhizosphere by crops during shortage nutrients. These root exudates can be thought of as environmental cues and utilized to develop nano biosensors that will be integrated into cutting-edge nano-fertilizers, reported by (Sharma et al., 2024). Foliar application of nano-fertilizer achieved better growth and yield (Nandy et al., 2025).

Foliar application of Nano fertilizers improved plant height, yield of wheat. (Muche et al., 2025) elucidated that combined applications of fertilizer improved the total nitrogen and agronomic mean values of the attributes. To provide the three primary nutrients nitrogen, phosphorous, and potassium (NPK) for a variety of crops and growth environments, fertilizers in artificial compounds designed the proper quantities or blending (Andualem et al., 2024). Nitrogen produces proteins and chlorophyll and stimulates the growth of leaves. Phosphorus promotes the growth of roots, flowers, and fruits. Protein synthesis, stem and root growth are induced by potassium Chawla and Kumar (2025). Mohamed and Awad (2024) studied Nano fertilizers versus traditional fertilizers for a sustainable environment and found that Nitrogen, Phosphorus, Potassium from conventional fertilizers are lost in environment and not taken up by plants. PCR based method known as RAPD is comparatively quick, easy, effective, dependable, and sensitive in identifying a variety of genetic changes and damaged DNA. Therefore, alterations in genomic materials that emerge in primer-specific DNA sequences can be detected using RAPD in genotoxicity research Aboulila and Galal (2019).

The purity of the template DNA is also necessary for effective RAPD analysis (Fadel et al., 2022). RAPD analysis was utilized to identify DNA alterations in bean cells after treatment with different doses (25, 50, and 75 mg per liter) in comparison to the untreated in order to evaluate genetic effects of n-SiO_2 and n-TiO_2. (Galal et al., 2020 and Ernst et al., 2024) indicated despite scant knowledge of their genotoxic effects on exposed plant tissue, the growing use of oxide nanoparticles has made it easier for them to enter the natural environment and cause biological interactions within ecosystems. Furthermore, the results showed that n–SiO_2 had a concentration-dependent genotoxic effect, but it seems to maintain the stability of genetic material, whereas n–TiO_2 showed a notable genotoxic effect.

The current study aimed to compare the nutritional value of three cuts of two teosinte varieties under NPK Nano-fertilizers and conventional NPK fertilizers. The study also evaluated forage quality, growth performance, and selected agronomic traits, with the overarching goal of reducing environmental pollution and minimizing the use of chemical fertilizers**.**

2. Materials and methods:

2.1. Experimental Site and Soil Analysis

The field experiment was conducted during the 2022 and 2023 summer seasons at the Experimental Farm of Gemmeiza Research Station, Gharbia Governorate, Egypt (Agricultural Research Center, ARC). The objective was to assess the impact of NPK nanoparticle fertilizers on growth, productivity, and nutritive value of teosinte varieties, compared to conventional chemical fertilizers.

Soil physical and chemical properties were analyzed prior to planting and are presented in **Table 1**. The soil was classified according to Richards (1967) as clay loam, with moderate fertility, neutral pH (7.77), and EC of 1.66 dS m^{-1}. Micronutrients such as Fe, Mn, Zn, and Cu were present in moderate levels, and organic matter content was 2.46%.

Irrigation water was sourced from the Nile River, with its chemical composition detailed in **Table 2**. The water exhibited low salinity (EC = 0.40 dS m^{-1}) and a Sodium Adsorption Ratio (SAR) of 3.29, indicating good irrigation quality.

Table 1. The soil physical and chemical analysis.

Parameters.	**Values.**
Soil depth, Cm	0-40
Particle size distribution	
Coarse sand, %	5.22
Find sand %	18.44
Silt, %	32.05
Clay, %	44.29
Soil texture	Clay Loam
PH, 1:25 (Susp.)	7.77
EC. dSm^{-1}	1.66
Soluble ions meq^{-1}	
Ca^{2+}	6.10
Mg^{2+}	4.29
Na^{+}	7.40
K^{+}	0.22
CO_3^{-2}	0.00
HCO_3	3.58
CI^{-}	8.10
SO_4^{-2}	6.39
Total N.	0.144
Total P.	0.032
Total K.	0.356
Available N mg/kg	32.43
Available P mg/kg	9.60
available K mg/kg	312.69
Organic matter %	2.46
Organic carbon (O.C, %)	1.45
C/N Ratio	10.05
Extractable micronutrients ppm (DTPA)	
Fe.	3.50
Mn.	3.03
Zn.	3.41
Cu.	1.55

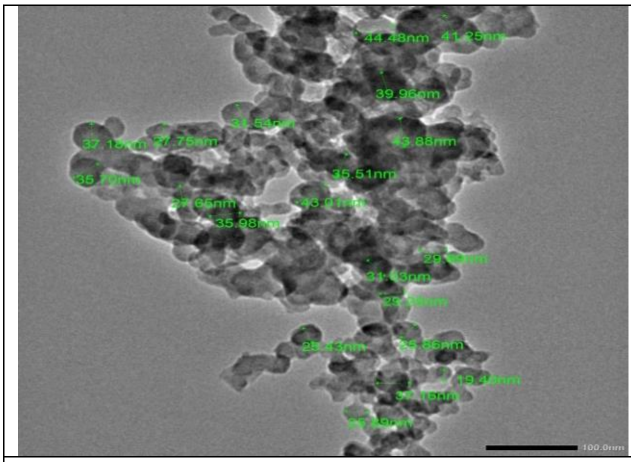
Table 2. Chemical composition of used the Nile water for irrigation.

(Water source)	**Cations, meq/L**				**Anions, meq/L**				**(EC)**	
	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	$CO3^{-2}$	HCO^{-3}	CI^{-}	SO_4^{-} 2	dSm^{-1}	SAR
The Nile water	0.52	0.57	1.75	0.53	-	2.40	1.30	-	0.40	3.29

SAR based on the **US Salinity Lab (1954)**
SAR: Sodium Adsorption Ratio

2.2. Nanoparticle Fertilizer Preparation and Characterization

Commercial NPK fertilizer (19-19-19) was obtained from Egyptchem International for Agrochemicals (Nubaria, Alexandria, Egypt). The fertilizer was milled using a high-energy ball mill (Pulverisette-7, Fritsch, Germany) at 200 rpm for 5 hours to produce nanoparticles. The morphology and elemental composition of the nano-fertilizer were analyzed using Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray (EDX) analysis (INCA-Sight, UK), as shown in **Figure 1a and 1b**.



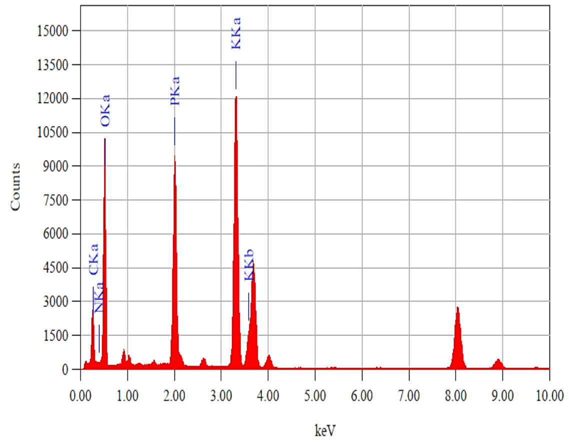


Fig. 1 a. The characterization of the produced Nano fertilizers NPK SEM micrograph of NPK Nano fertilizers, b energy dispersive x-ray pattern with elemental percentages Nano fertilizers NPK.

Fig. 1 b. Characterization of the produced NPK Nano fertilizers. a) SEM micrograph of NPK Nano fertilizers; b) energy dispersive x-ray pattern elemental percentages of nano NPK.

2.3. The experimental design.

This study used a split plot design with three replications. The main plots had the source varieties (Gemmeiza 3 and Gemmeiza 4), while the subplots contained NPK nanoparticle fertilizers and conventional fertilizer treatments. The study examined five fertilizers, as follows:

F1: 100% conventional fertilizers

F2: 75% conventional fertilizers + 25% NPK Nano particle fertilizers

F3: 50% conventional fertilizers + 50% NPK Nano particle fertilizers

F4: 25% conventional fertilizers + 75% NPK Nano particle fertilizers

F5: 100% NPK Nano particle fertilizers.

The ammonium nitrate (33.5%) contained 33.5% N by weight rates of N fertilization, equivalent to 280 Kg Nha^{-1}. Fertilizer treatments were divided into three equal dosages, applied two weeks after sowing and two weeks after the first and second cuttings. During fertilizer treatment, ammonium nitrate was applied as a top dressing. Meanwhile, the NPK nano fertilizer was applied as a foliar spray and the maximum concentration, which was equivalent to 100% NPK nanoparticle fertilizers, was 1500 ppm/liter.

2.4. Practices of Agronomy:

The two varieties were received from the Forage Crops Research Department, Field Crops Research Institute, Agricultural Research Center (ARC). Seeds were sowed by 20 kg/fed on May 10th 2022 and May 18th 2023, respectively. An experimental plot area was 12 m^2. Phosphorous monophosphate calcium (15.5% P_2O_5) 100 kg P_2O_5/fed as well as potassium sulphate (48% K_2O) were applied once before sowing at the recommended rate of 50 K_2O/fed. Three cuts were obtained under such conditions during the growing season, with cuts taken when the plant height reached 90 to 120 cm. Cuts were made 45 days after sowing for 1st cut, 32 days for 2nd cut, and 30 days for the 3rd cut. All cultural practices were completed on time.

2.5. Measured parameters:

At cutting time, ten plants were randomly selected from each plot to estimate plant height (cm) on the main stem, main stem diameter (mm), and leaf area multiplied by 0.75, as well as fresh fodder and dry yields. Nitrogen content was evaluated using the Kjeldahl technique **AOAC (2012).** The crude protein (CP) was calculated as N. 6.25 times, (NDF) neutral detergent fiber and acid detergent fiber are the two important components. Fiber fractions were evaluated sequentially using a semiautomatic ANKOM 220 fiber analyzer (**Van Soest et al., 1991).** Both fiber fractions were evaluated without heat-stable amylase and measured with residual ash content. Sub-samples were incinerated in a muffle oven at 550°C to 3 hours to determine crude ash **AOAC (2012).** To determine crude fat (CF) were used the soxhlet device.

2.6. Detecting genotoxicity by estimating genomic template stability (GTS)**:**

Genomic template stability was calculated for each primer using the equation as reported by **Salarizadeh and Kavousi (2015)**:

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

a is the average number of polymorphic bands detected in each treatment group, and **n** is the total number of bands in the control samples. The polymorphic bands detected in the RAPD study were defined as band gains or losses when compared to the control profile.

2.7. RAPD (random amplified polymorphic DNA) technique:

DNA extraction procedure for total genomic analysis of four plant leaves following the manufacturer's procedure for the Gene JET Genomic DNA Purification Kit (K0721/Thermo Fisher).

Data analysis: Bands amplified were recorded 0, 1 for presence and absence using total lab software analysis (www.totalalb.com). **Statistics:** Data were subjected to analysis of variance (ANOVA), using SAS and SPSS software. Means were compared by Duncan's Multiple Range Test (DMRT) (1965) at 5% significance. Variance analysis followed **Steel and Torrie (1980)**.

3. Results

3.1. NPK Nano fertilizers characterization:

Data presented in **Table 3** showed that the variety Gemmeiza 4 recorded the highest plant height during the second cut, with mean values of **126.2 cm** and **124.5 cm** in the 2022 and 2023 seasons, respectively. In comparison, **Gemmeiza 3** recorded mean heights of **125.5 cm** and **123.0 cm** in the same seasons. Across all cuts and both seasons, Gemmeiza 4 consistently exhibited taller plants than Gemmeiza 3, suggesting a superior growth response in terms of plant height. Results from both seasons demonstrated that the **F1** and **F3** treatments significantly enhanced plant height compared to other treatments. In contrast, the **F5** treatment (100% nano) consistently resulted in the shortest plants, indicating that nano NPK alone may be less effective in promoting vertical growth compared to mixed or conventional fertilization strategies. These findings highlight the importance of selecting **Gemmeiza 4** and utilizing balanced fertilizer combinations (particularly F1 and F3) to maximize plant height in teosinte.

Table 3. Plant height affected by Nano and conventional fertilizer applications across three cuts at both seasons.

Treatments	2022			2023		
A. Varieties	1^{st} Cut	2^{nd} Cut	3^{rd} Cut	1^{st} Cut	2^{nd} Cut	3^{rd} Cut
Gemmeiza 3	111.2 b	125.5b	101.3b	109.7a	123.0a	103.5a
Gemmeiza 4	112.9 a	126.2a	103.2a	108.0b	124.5b	103.8b
B. Fertilizer						
F1	120.3 b	136.0 a	111.5 a	114.8 b	134.7 a	116.2 a
F2	119.4 a	132.8 b	103.8 b	109.9 c	127.1 c	108.3 c
F3	120.4 a	133.1 b	109.3 b	116.4 a	132.3 b	108.4 b
F4	101.8 c	115.2 c	91.3 c	102.4 d	114.6 d	94.1 d
F5	98.5 d	112.1 d	95.4 d	101.0 e	110.3 e	91.3 e

F1: conventional fertilizers: 100%. F2: 75% conventional fertilizer + 25% nano. F3: 50% conventional fertilizer + 50% nano. F4: 25% conventional fertilizer + 75% nano and F5 100% nano.

Respecting the interaction effects between the Teosinte varieties and fertilizer treatments on plant height in both seasons (**Figure 2 A and B**). It can be observed that there was a significant increase due to the interaction. The highest values were produced by Gemmeiza 4 when treated with F1 followed by F2 and F3. Gemmeiza 3 gave shortest plant at F5.

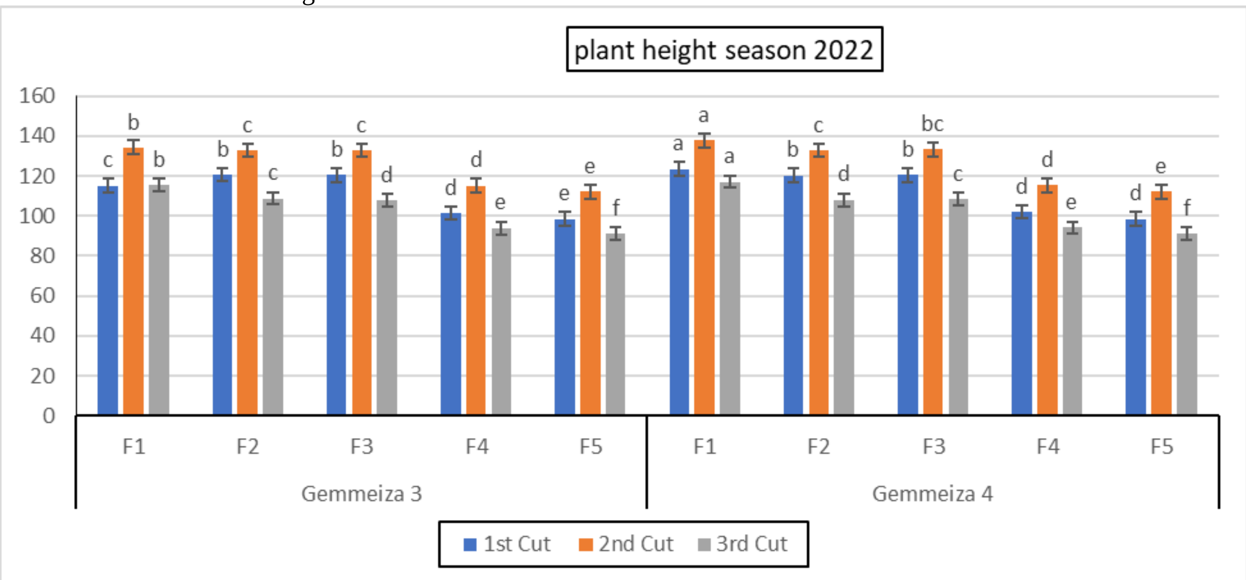


Fig. 2 A. Plant height as impacted by the interactions of two varieties and fertilizers treatments during both seasons.

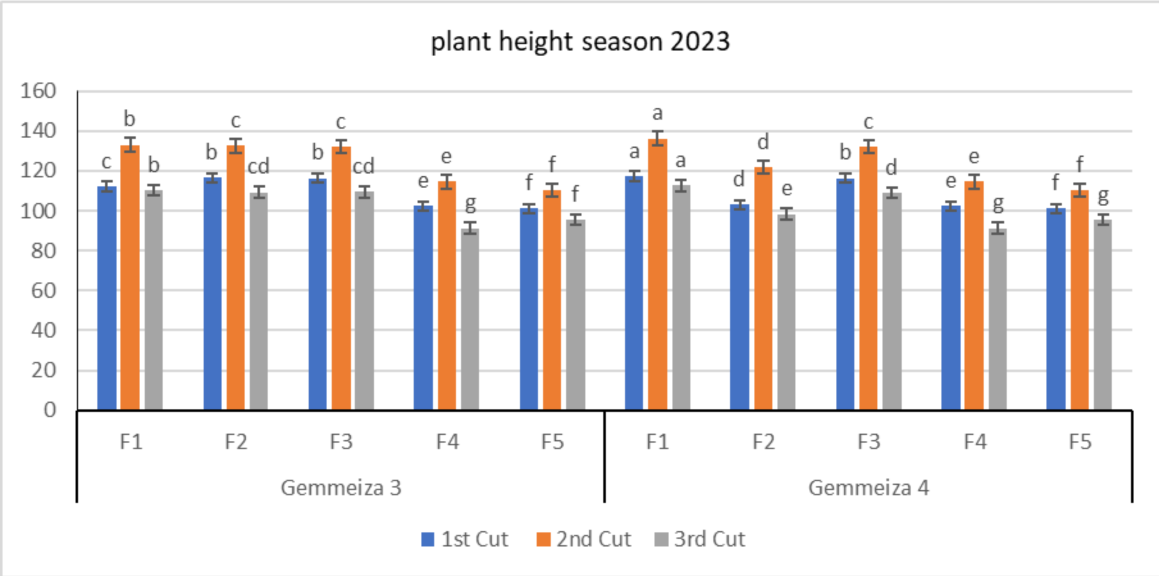


Fig. 2 B. Plant height as impacted by the interactions of two varieties and fertilizers treatments during both seasons.

Data presented in **Table 4** showed the stem diameter as affected by conventional fertilizer and nano across cuts in 2022 and 2023 seasons. The variety Gemmeiza 4 recorded thick stem diameter 7.73 mm in first cut and 14.58 mm in second cut and 10.85 in last cut comparing other variety Gemmeiza 3 which recorded thin stem diameter. The Gemmeiza 4 variety surpassed Gemmeiza 3 on the other hand F3 recorded significant values for stem diameter in all cuts through both seasons.

Table 4. Stem diameter as affected by conventional fertilizer and nano across cuts in 2022 and 2023 seasons.

Treatments	2022			2023		
A. Varieties	**1st Cut**	**2nd Cut**	**3rd Cut**	**1st Cut**	**2nd Cut**	**3rd Cut**
Gemmeiza 3	7.15 b	14.12 b	10.31 b	11.24 b	17.56 b	13.07 a
Gemmeiza 4	7.73 a	14.58 a	10.85 a	11.61a	18.20 a	13.59 a
B. Fertilizer						
F1	8.35 a	16.20 b	11.85 a	12.35 b	18.90 b	14.03 b
F2	6.90 c	12.35 d	9.88 d	11.40 c	18.50 c	13.40 c
F3	8.40 a	14.50 c	11.50 b	12.70 a	19.40 a	14.60 a
F4	7.30 b	17.40 a	10.50c	11.20 c	17.40 d	13.12 d
F5	6.23 d	11.30 e	9.17 e	9.48 d	15.20 e	11.50 e

Values the same column came next by the same letters are not significant different, Duncan's Multiple Range Test at 0.05.
F1: conventional fertilizers: 100%. F2: 75% conventional fertilizer + 25% nano. F3: 50% conventional fertilizer + 50% nano. F4: 25% conventional fertilizer + 75% nano and F5 100% nano.

As shown in **(Figure 3 A, B**) highly significant diameter of stem was reported for by used F3 fertilizer for all studied fertilizer treatments, cut 1 was inferior to second cut and third cuts concerning yield production in fresh weight.

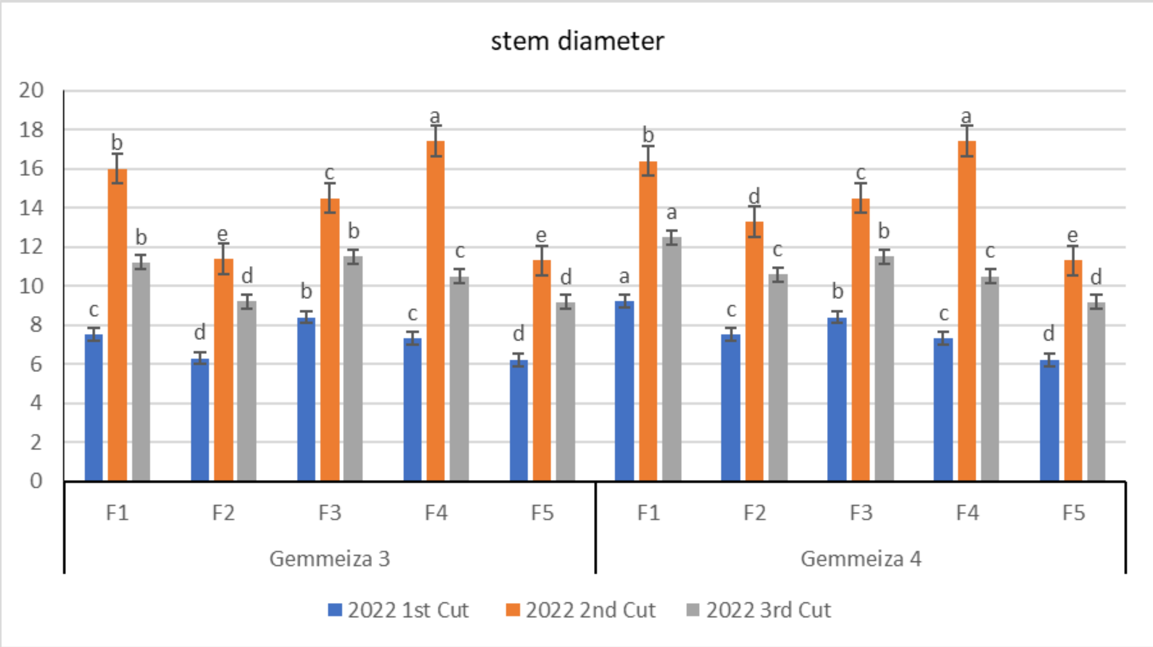


Fig. 3 A. Stem diameter interactions between varieties and fertilizers treatments during 2022 season.

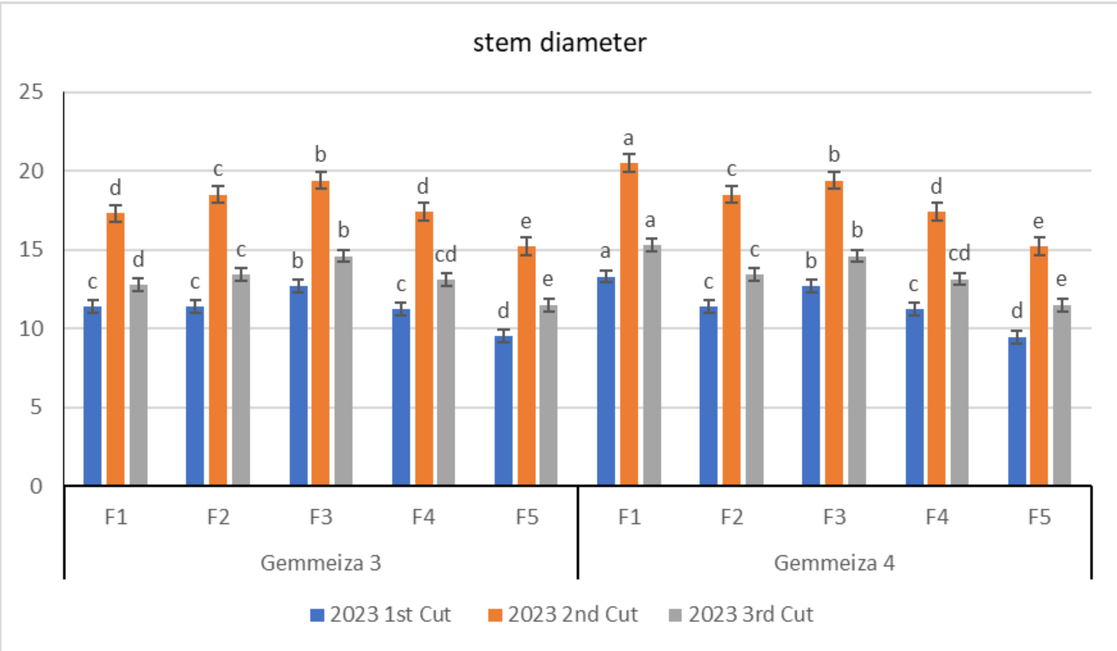


Fig. 3 B. Stem diameter interactions between varieties and fertilizers treatments during 2023 season.

Table 5. Fresh weight as affected by conventional and nano fertilizers in the two growing seasons.

Treatments	2022			2023		
A. Varieties	**1st Cut**	**2nd Cut**	**3rd Cut**	**1st Cut**	**2nd Cut**	**3rd Cut**
Gemmeiza 3	7.43 a	19.15 a	4.55 a	7.02 a	18.42 b	4.08 a
Gemmeiza 4	7.39 a	18.95 b	4.31 a	6.99 a	18.78 a	4.02 a
B. Fertilizer						
F1	9.25 a	23.00 a	5.33 a	9.45 a	20.75 b	5.55 a
F2	7.34 c	20.44 c	4.77 b	6.50 b	19.20 c	4.60 b
F3	7.70 b	21.16 b	5.20 a	6.32 b	21.30 a	4.33 b
F4	6.33 d	15.35 d	3.44 c	6.35 b	18.53 d	3.44 c
F5	6.44 d	15.30 d	3.41 c	6.40 b	13.21 e	2.35 d

F1: conventional fertilizers: 100%. F2: 75% conventional fertilizer + 25% nano. F3: 50% conventional fertilizer + 50% nano. F4: 25% conventional fertilizer + 75% nano and F5 100% nano

Data in **Table 5** shows the effect of teosinte varieties and five fertilizers on fresh yields. Data illustrates that the differences were significant in all cuts in both seasons. Adding F1 fertilizer in conventional fertilizers markedly increased fresh weight yield followed by F3 when compared with the other different fertilizers. Application of 100 % conventional fertilizers gave 9.30,23.70, and 5.20 ton/fed in the first, second and third cut, respectively in 2022 season. The lowest value of fresh forage yields Gemmeiza 3 were observed in comparing to Gemmeiza 4. The highest total forage yield can be achieved from the plots which received high portion of F1 fertilizer. The variety Gemmeiza 4 superior other variety in fresh weight, on the other hand F1 and F3 recorded significant values in fresh weight in comparing other fertilizers.

Table 6. Fresh weight as influenced by the interaction between varieties and fertilizers treatments during both seasons.

Treatments		2022			2023		
A. Varieties	B. Fertilizers	1st Cut	2nd Cut	3rd Cut	1st Cut	2nd Cut	3rd Cut
Gemmeiza 3	F1	9.20 a	22.30 b	5.47 a	9.40 a	20.20 b	5.70 a
	F2	7.47 bc	21.70 c	5.20 a	6.50 b	19.20 c	4.60 b
	F3	7.70 b	21.16 d	5.20 a	6.50 b	21.10 a	4.27 b
	F4	6.35 d	17.20 f	3.47 c	6.30 b	18.40 d	3.50 c
	F5	6.42 d	13.40 g	3.40 c	6.40 b	13.21 e	2.35 d
Gemmeiza 4	F1	9.30 a	23.70 a	5.20 a	9.50 a	21.30 a	5.40 a
	F2	7.20 c	19.17 e	4.34 b	6.50 b	19.20 c	4.60 b
	F3	7.70 b	21.16 d	5.20 a	6.14 b	21.50 a	4.38 b
	F4	6.31 d	13.50 g	3.40 c	6.40 b	18.67 d	3.37 c
	F5	6.47 d	17.20 f	3.41 c	6.40 b	13.21 e	2.35 d

Values the same column came next by the same letters are not significant different, Duncan's Multiple Range Test at 0.05.
F1: conventional fertilizers: 100%. F2: 75% conventional fertilizer + 25% nano. F3: 50% conventional fertilizer + 50% nano. F4: 25% conventional fertilizer + 75% nano and F5 100% nano

Significant increases were detected in fresh weight, from plants which received F1 and F3 treatments with Gemmeiza 4 variety, as compared to the control, as presented in **Table 6** in both seasons. Data illustrates that the differences between teosinte fresh weight as affected by varieties and fertilizers were significant in all cuts in both seasons. Adding F1 fertilizer to Gemmeiza 4 markedly increased fresh weight yield when compared with the other variety i.e. 23.70 and 21.30 in the second cut in both seasons, respectively. The lowest value of fresh weight yields (13.40 and 13.21) were observed under the Gemmeiza 3 in the second cut when receiving F5.

Table 7. Dry weight as influenced by the interaction between varieties and fertilizers treatments two seasons.

	2022			2023		
	1st Cut	2nd Cut	3rd Cut	1st Cut	2nd Cut	3rd Cut
A. Varieties						
Gemmeiza 3	17.35 a	19.51 a	19.65 a	17.71 a	19.55 b	19.73 a
Gemmeiza 4	17.93 a	18.55 a	19.86 a	17.93 a	19.80 a	20.02 a
B. Fertilizer						
F1	18.40 a	21.85 a	21.60 a	18.13 a	21.95 a	21.50 a
F2	16.80 b	18.55 b	18.60 bc	15.40 b	15.67 e	16.40 d
F3	16.10 b	16.88 c	17.28 c	18.53 a	19.50 d	19.60 c
F4	18.35 a	16.28 c	19.83 ab	18.53 a	20.10 c	20.50 b
F5	18.57 a	21.60 a	21.47 a	18.50 a	21.17 b	21.37 a

Data illustrated in **Table 7** shows the variety Gemmeiza 4 superior other variety in dry weight, on the other hand F1 and F5 recorded significant values in dry weight yield in comparing other fertilizers of two seasons of dry yield. The highest mean of dry weight can be observed in the third cut which recorded 19.86 and 20.02 in both seasons respectively in the third cut from Gemmeiza 4 while the Variety Gemmeiza 3 recorded lowest means. Plants received F1 and F5 fertilizers recorded highest dry weight yield compared to other fertilizers.

As shown in **Table 8 highly** significant increases were detected in dry weight yield, from plants which received F5 nano fertilizers treatments with Gemmeiza 4 variety, as compared to the other fertilizers in both seasons. Data shows the effect of the interactions effect of two seasons of dry yield of teosinte under nano and conventional fertilizers. The highest mean of dry weight can be observed in the third cut which recorded 21.47 and 21.37 in both seasons respectively.

Table 8. Dry weight as influenced by the interaction between varieties and fertilizers treatments during both seasons.

		2022			2023		
G	**F**	**1st Cut**	**2nd Cut**	**3rd Cut**	**1st Cut**	**2nd Cut**	**3rd Cut**
Gemmeiza 3	F1	18.20 a	21.30 ab	21.10 ab	18.10 a	21.40 b	21.00 bc
	F2	18.20 a	21.30 ab	21.10 ab	15.30 a	15.60 e	16.30 f
	F3	13.70b	14.30de	15.20c	18.27 a	19.50 d	19.47 e
	F4	18.10 a	19.07 c	19.40 b	18.37 a	20.10 c	20.50 cd
	F5	18.57 a	21.60 ab	21.47 ab	18.50 a	21.17 b	21.37 ab
Gemmeiza 4	F1	18.60 a	22.40 a	22.10 a	18.17 ab	22.50 a	22.00 a
	F2	15.40 b	15.80 d	16.10 c	15.50 b	15.73 e	16.50 f
	F3	18.50 a	19.47 bc	19.37 b	18.80 a	19.50 d	19.73 de
	F4	18.60 a	13.50 e	20.27 ab	18.70 a	20.10 c	20.50 cd
	F5	18.57 a	21.60 ab	21.47 ab	18.50 a	21.17 b	21.37 ab

F1: conventional fertilizers: 100%. F2: 75% conventional fertilizer + 25% nano. F3: 50% conventional fertilizer + 50% nano. F4: 25% conventional fertilizer + 75% nano and F5 100% nano

Data presented in **Table 9** shows the variety Gemmeiza 4 superior other variety in leaves area, on the other hand F1, F2 and F5 recorded significant values in leaves area in comparing other fertilizers. Gemmeiza 4 teosinte variety recorded 5430.4 and 5567.7 leaves area in the second cuts in both seasons respectively comparing to other variety Gemmeiza 3. Plants received F1 fertilizers recorded highest leaves area in second cut (6220.3 and 6840.4) in both seasons.

Table 9. Leaves area as influenced by the interaction between varieties and fertilizers treatments during two seasons.

	2022			2023		
A. Varieties	**1st Cut**	**2nd Cut**	**3rd Cut**	**1st Cut**	**2nd Cut**	**3rd Cut**
Gemmeiza 3	3354.5b	5126.4b	2300.8b	3604.9b	5334.8b	2486.9b
Gemmeiza 4	3430.5a	5306.4a	2397.3a	3708.7a	5567.7a	2669.0a
B. Fertilizer						
F1	3510.1 c	6220.3 a	3670.3 a	4100.4 a	6840.4 a	4075.5 a
F2	3540.3 a	5680.4 b	2532.3 b	3516.2 c	5011.5 c	1840.3 c
F3	3530.4 b	5670.5 c	2521.9 c	4020.1 b	6122.4 b	3571.2 b
F4	3231.2 d	4350.2 d	1560.4 d	3415.3 d	4920.3 d	1760.4 d
F5	3150.4 e	4160.4 e	1460.3 e	3231.8 e	4361.5 e	1642.6 e

F1: conventional fertilizers: 100%. F2: 75% conventional fertilizer + 25% nano. F3: 50% conventional fertilizer + 50% nano. F4: 25% conventional fertilizer + 75% nano and F5 100% nano

Leaves area parameter was highly significantly variable as affected by the fertilizer treatment and variety interaction during both seasons (**Table 10**). Leaves area for the two varieties significantly increased when treated with 75% F1: conventional fertilizers: 100%. F2: 75% conventional fertilizer +

25% nano. F3: 50% conventional fertilizer + 50% nano. F4: 25% conventional fertilizer + 75% nano and F5 100% nano fertilizer + 25% nan fertilizers with Gemmeiza 4 variety compared to the other fertilizer treatments during 2022 and 2023. The variety of Gemmeiza 4 recorded high leaves area when treated with F1 in both seasons during the three cuts.

Table 10. Leaves area as influenced by the interaction among varieties and fertilizers treatments during both seasons.

		2022			2023		
Varieties	**Fertilizers**	**First Cut**	**Second Cut**	**Third Cut**	**First Cut**	**Second Cut**	**Third Cut**
Gemmeiza 3	F1	3320.1 d	5770.3 b	3430.2 b	3840.6 c	6260.5 b	3620.3 b
	F2	3540.3 b	5680.4 c	2532.3 c	3516.2 d	5010.2 d	1840.3 d
	F3	3530.4 c	5670.5 d	2520.6 d	4020.1 b	6122.4 c	3570.2 c
	F4	3231.2 e	4350.2 e	1560.4 e	3415.3 e	4920.3 e	1760.4 e
	F5	3150.4 f	4160.4 f	1460.3 f	3232.1 f	4360.5 f	1643.4 f
Gemmeiza 4	F1	3700.2 a	6670.4 a	3910.3 a	4360.3 a	7420.4 a	4530.6 a
	F2	3540.3 b	5680.4 c	2532.3 c	3516.2 d	5012.9 d	1840.3 d
	F3	3530.4 c	5670.5 d	2523.3 d	4020.1 b	6122.4 c	3572.2 c
	F4	3231.2 e	4350.2 e	1560.4 e	3415.3 e	4920.3 e	1760.4 e
	F5	3150.4 f	4160.4 f	1460.3 f	3231.4 f	4362.5 f	1641.7 f

F1: conventional fertilizers: 100%. F2: 75% conventional fertilizer + 25% nano. F3: 50% conventional fertilizer + 50% nano. F4: 25% conventional fertilizer + 75% nano and F5 100% nano

3.2. Forage quality Parameters:

Figures 4 A, B and C illustrated the averages of the crude protein, and acid detergent fiber as well as crude fat for two teosinte varieties as effected by fertilizers treatments in both seasons.

Data in **Figure (4 A, B, C**) indicate that Gemmeiza 4 with F1 and achieved high total nitrogen, phosphorus and total protein content were higher in pods produced from plants primed with water or Mo solution than those of pods produced from untreated plants in the two seasons. There were no significant effects on the potassium content in pods of plants treated or untreated.

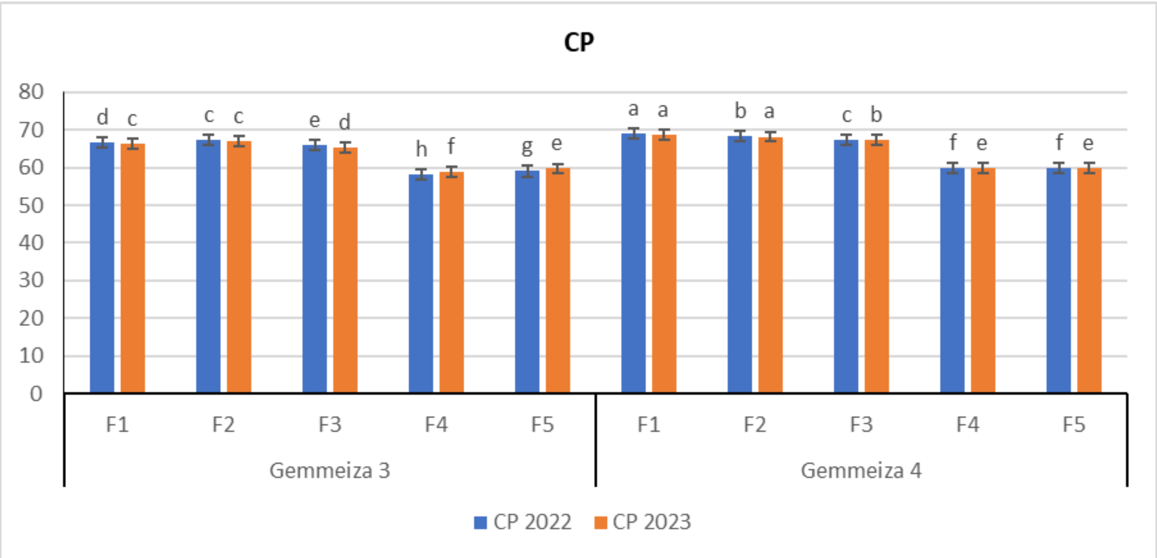


Fig. 4 A. Averages crude protein for two varieties as effected by fertilizers treatments in both seasons.

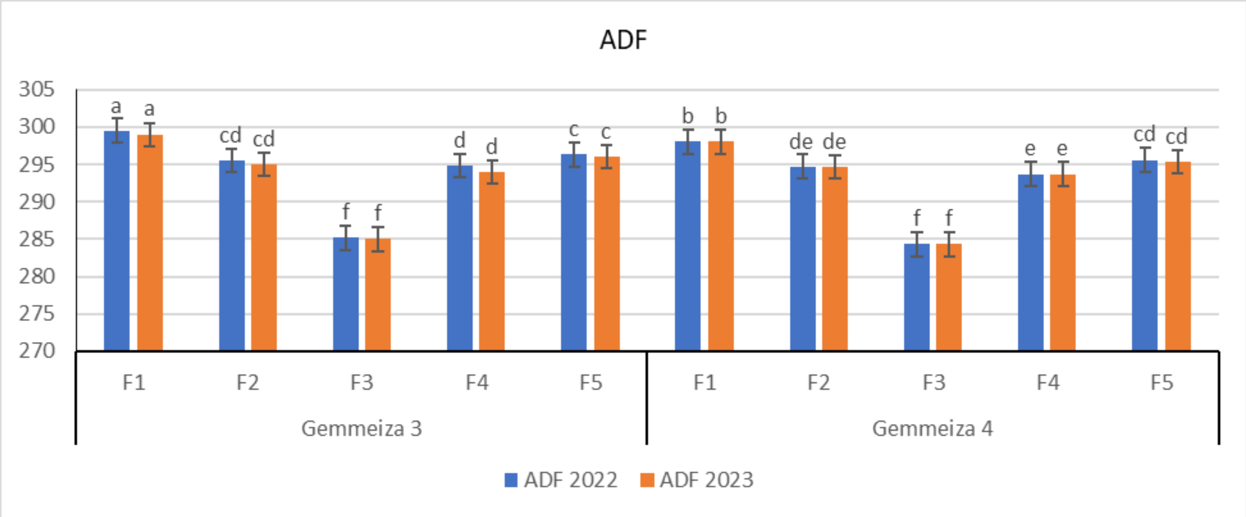


Fig. 4 B. Averages acid detergent fiber for two varieties as effected by fertilizers treatments in both seasons.

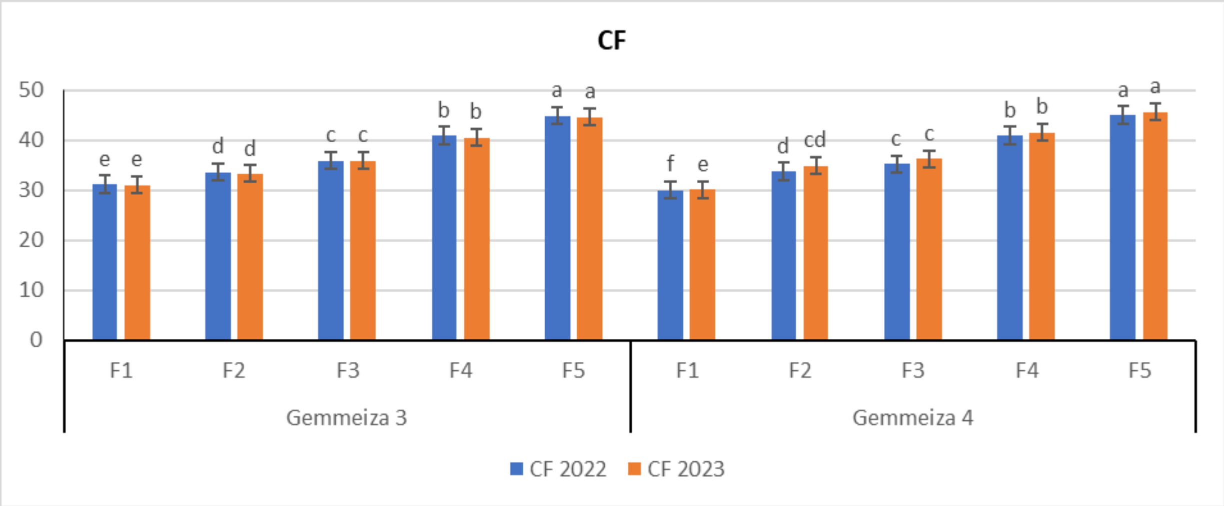


Fig. 4 C. Averages crude fat for two varieties as effected by fertilizers treatments in both seasons.

3.3. Genotoxicity of Nanoparticles:

Biosafety of Bulk N.P.K nanoparticle fertilizer:

In this study for teosinte crop to evaluate genotoxicity of applied nanoparticles, Genomic template stability value was applied. In our investigation, Random Amplification of Polymorphic DNA (RAPD) fingerprinting technique was used to detect Genomic template stability (%) among applied nanoparticles. Based on our findings, genome template stability GTS value 100% in plants treated with nanoparticles. This indicates that the largest genome stability was nanoparticles concentration presented in **Table 11.**

Table 11. Genomic template stability for each random primer for three bulk NPK nano fertilizers for teosinte.

	NPK Nano fertilizers.			
	Control	**A1**	**A2**	**A3**
Polymorphic bands in every group that was treated.	0.00	0.00	0.00	0.00
The average number of polymorphic bands discovered for each group a	0.00	0.00	0.00	0.00
Bands in control sample (n)	21	21	21	21
(a/n)	0.00	0.00	0.00	0.00
1- a/n	I	I	I	I
GTS (1- a/n) *100	100	100	100	100

Table 12. A linear pearson correlations coefficient for two teosinte varieties as effected by fertilizers.

	Plant	Stem diameter	Fresh weight	Dry weight	Leaves area
Plant height	1				
Stem diameter	0.830**	1			
Fresh weight	0.847**	0.915**	1		
Dry weight	-0.122ns	-0.198ns	-0.178ns	1	
Leaves area	0.8522	0.8024**	0.794**	0.318**	1

As shown in **Table 12** which displayed the correlation coefficient of the various measures of the two teosinte varieties and five fertilizers in the second cut, height, diameter, fresh, dry weight, and leaves area were highly significant positively correlated with the parameters under study. Significant and highly significant negative correlations were found with dry weight and studied traits, respectively. Regarding the higher plant, diameter of the stem, fresh, dried weight, and leaves area of teosinte varieties have made progress in genetic improvement.

4. Discussions

NPK nano-fertilizers have demonstrated strong potential in promoting nutrient absorption and improving plant performance due to their small particle size and large surface area. These properties enable efficient uptake through the leaf epidermis, followed by transport to stems and other tissues, ultimately enhancing assimilation and boosting crop growth, productivity, and quality. As reported by Haydar et al. (2024), excessive reliance on conventional fertilizers contributes to soil contamination, highlighting the need for innovative alternatives such as nano-structured macronutrient fertilizers. These nano-formulations provide a controlled and gradual release of nutrients, a strategy endorsed by Yadav et al. (2023) to address soil limitations in nutrient retention.

Kopittke et al. (2019) demonstrated that fertilizer combinations such as 50% conventional with 50% nano (F3) offer a balanced nutrient supply, promoting optimal plant development. The current study investigated the effects of nano-NPK on plant height and leaf area in two teosinte varieties, Gemmeiza 3 and Gemmeiza 4, across three cuts. Notably, plant height and leaf area peaked in the second cut for both varieties, with Gemmeiza 4 consistently outperforming Gemmeiza 3 in growth rate across all cuts. This growth advantage may be attributed to genetic differences, hormonal regulation, environmental response mechanisms, and epigenetic factors (Agarwal et al., 2020; Dar et al., 2022; Abdulraheem et al., 2024).

Among fertilizer treatments, F1 (100% conventional) and F3 (50% conventional + 50% nano) were most effective in enhancing plant height and leaf area, suggesting these formulations are well-suited for teosinte cultivation. In contrast, F5 (100% nano) resulted in the lowest growth, indicating that nano-fertilizers alone may not be sufficient for optimal crop performance. Helaly et al. (2021) highlighted the advantage of nano-particles in ion retention due to their high surface area, which supports their role in nutrient delivery.

Gemmeiza 4 also outperformed Gemmeiza 3 in dry matter accumulation and fresh forage yield across all cuts, with the second cut being the most productive. Interestingly, F5 produced the highest dry matter content but the lowest fresh weight, whereas F1 achieved the highest fresh yield despite having the lowest dry matter content. These results suggest that different fertilizer combinations influence water content and biomass composition differently.

The improvement in yield due to nano-fertilizers is supported by findings from Reshma Anjum et al. (2023), who attributed the increase to enhanced nutrient availability, absorption efficiency, and internal transport processes. Cao et al. (2025) further explained that the synergistic effect of combining nano- and conventional fertilizers enhances photosynthetic efficiency, sink strength, and metabolic activity.

Regarding nutritional quality, Gemmeiza 4 consistently exhibited higher protein content and lower acid detergent fiber (ADF) compared to Gemmeiza 3, indicating superior digestibility. Fertilizer treatment F1 yielded the highest crude protein content, followed by F2 and F3, while F3 showed the lowest ADF values, suggesting improved fiber digestibility. Conversely, F5 had the highest crude fiber content, while F1 had the lowest. These results underscore the importance of fertilizer formulation in shaping the nutritional profile of teosinte.

Payghan (2016) and Channab et al. (2024) also reported that combining nano- and conventional fertilizers improves fodder quality by reducing ADF and increasing protein content. The slow and targeted release of nutrients from nano-formulations enhances protein synthesis and modifies plant cell wall composition, which can affect fiber structure and digestibility (Garg et al., 2023).

Lastly, Sompark et al. (2024) and Silprasit et al. (2016) highlighted the role of genotoxicity studies in environmental safety assessments. Though beyond the scope of this study, these insights stress the importance of understanding potential unintended effects of agricultural inputs, including nano-materials, on genetic stability in ecosystems.

5. Conclusions

Overall, the findings of this study demonstrate that combining nano-NPK fertilizers with conventional fertilizers significantly enhances teosinte growth and forage quality under the conditions of the Gemmeiza Research Station. The application of nano-fertilizers positively influenced key growth traits such as plant height, stem diameter, leaf area, and dry matter content, reflecting the genetic potential of teosinte for high biomass production. Among all treatments, nano-NPK combinations yielded the best performance across growth stages, with F2 (75% conventional + 25% nano) and F3 (50% conventional + 50% nano) proving most effective in improving both agronomic traits and feed quality. Notably, the F5 (100% nano) treatment resulted in the highest crude fat content, while F3 showed the lowest mean acid detergent fiber (ADF), indicating improved fiber digestibility.

Given that all applied nano-materials were deemed safe for human health and the environment, the study supports the sustainable use of nano-fertilizers in forage systems. Therefore, the combined application of conventional and nano-NPK fertilizers—particularly F2 and F3—is recommended for enhancing teosinte growth and fodder nutritive value. To ensure long-term agricultural sustainability and practical relevance, further research is recommended on the long-term effects of nanoparticle application on soil health and crop productivity. The outcomes of this study provide valuable insights into the role of nano-fertilizer technology in improving livestock feed quality and advancing sustainable forage production systems.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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