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Effect of partial substitution of bulk urea by nanoparticle urea fertilizer on productivity and nutritive value of teosinte varieties

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Abstract. The integration of nanoparticle urea (NPU) in the fertilization scheme of forage crops with high nutrients' requirements, like teosinte (*Zea mexicana* L.), would help avoiding the environmental implications associated with the application of high rates of conventional bulk urea (BU), while not depriving the plant from its benefits. The effects of fertilization treatments composed of different percentages of NPU and/or BU, on yield, agronomic characteristics and quality attributes of three cuts of two teosinte local varieties were investigated in a split-split plot design during summers of 2018 and 2019. In general, the application of 50% NPU + 50% BU was similar to 100% BU in the production of highest amount of fresh yield, with the highest values for plant height and stem diameter, in addition to appreciable nutritive value, in terms of high crude protein (66.10 g kg^{-1}) and non-fiber carbohydrates (NFC), and low acid-detergent fiber (284.09 g kg^{-1}) and crude fat (36.97 g kg^{-1}) contents. While the 1st cut was characterized by the highest plant height (58.74 cm in average), stem diameter (7.64 mm in average) and leaf area (130.07 m^2 in average), the 3rd cut produced the highest amount of fresh yield (39.68 t ha^{-1} in average). Variations in quality measures among the three cuts were almost non-significant. Variations in yield and quality were detected between the two tested local varieties. In conclusion, the combined application of 50% NPU with 50% BU is recommended for the production of fodder teosinte in similar environments.

Key words: forage yield, forage quality, nanoparticle urea, nitrogen fertilizers, *Zea mexicana* L.

INTRODCUTION

Fertilization is a basic cultural practice in the farming systems worldwide, upon which the productivity of the different crop species is greatly dependent. Globally, the application of mineral fertilizers is a key management strategy that plays a significant role in enhancing crop productivity and, thus, maintaining sufficient food and feed supplies (Chaudhary et al., 2017).

Nitrogen (N) is the first and most important nutrient required for crop growth and development. As a constituent of chlorophyll, it greatly supports the photosynthesis process (Rathnayaka et al., 2018). In addition, N contributes for biosynthesis of many growth-promoting enzymes and proteins and, thus, plays a crucial role in regulating plant growth especially during the vegetative phase (Iqbal, 2019). Nonetheless, N is a precursor for several chemical compounds that protect the plant against diseases and parasites (Hoffland et al., 2000). Among the different commercial forms of mineral N fertilizers, urea [$CO(NH_2)_2$] is the most widely used, mainly due to its high N content (46% N), in addition to its compatibility with other nutrients (Elemike et al., 2019). However, around 75% of urea applied to the soil is lost (Chhowalla, 2017; Khalifa & Hasaneen, 2018). N fertilizer loss primarily occurs through nitrate leaching, that contaminates ground water leading to eutrophication, and volatilization that increases greenhouse gasses (i.e., nitrous oxides), contributing to the global warming. Nonetheless, the nitrogenous fertilizers' use efficiency in the modern farming systems is reported to be only 45–50% (Iqbal et al., 2019). The high N loss coupled with its low use efficiency forced the farmers to increase the amounts of applied N fertilizers in order to achieve better crop production (Rathnayaka et al., 2018), which resulted in rising the costs of the farming practice, meanwhile, increasing the consequent environmental implications (Chhowalla, 2017; Marchiol, 2019). Therefore, there is a pressing need to improve the N availability for plants, while reducing its harmful effects to the environment.

In this regard, the utilization of nanoparticle fertilizers, especially nanoparticle urea (NPU) was proposed by several researchers (e.g., Chhowalla, 2017; Kottegoda et al., 2017; Rathnayaka et al., 2018) to avoid the problems associated with the application of bulk urea, while not depriving the plant from its benefits. The main drive behind the low N use efficiency of the bulk chemical fertilizers is the lack of synchronization between nutrient release from the fertilizer and its demand by the plant (Marchiol, 2019). As the nanostructured fertilizers are advantaged by the controlled release of nutrients, this will allow for the effective duration of nutrient supply to the plant which would secure optimum fulfillment to its nutrients' needs (Liu & Lal, 2015) without any adverse environmental impacts (Kopittke et al., 2019). When used as foliar application, nanofertilizers have the ability to enter through the porous cell wall of plant cells due to their minute particle size (< 50 nm) allowing for high absorption compared to conventional fertilizers (Benzon et al., 2015). It is evident that applying nanoparticle N fertilizers in conjunction with reduced dosses of conventional N fertilizers can boost the productivity of several cereal crops, e.g., rice, maize and barley (Benzon et al., 2015; Gomaa et al., 2017; Iqbal, 2019). However, their potential with green forage crops is not yet exploited.

Since the area devoted to summer forage crops in Egypt is limited, due to the prioritization of other economic crops like rice, maize and cotton, there is a need to expand the production of high-yielding, high-quality fresh forage crops (Rady, 2018). Teosinte (*Zea mexicana* L.) was believed to be the ancestor of modern maize (*Zea mays* L.) that was indigenous to Mexico and Central America (Gaudin et al., 2011). As a multi-cut forage crop, teosinte is advantaged over other prominent summer forage grasses, like fodder maize, by its special ability to tolerate high temperatures and adverse environmental conditions, and yield high amounts of fresh fodder under stressed conditions (Devkota et al., 2017). In addition, it is suitable for fresh feeding, hay, and silage production (Mohan et al., 2017). However, for improved growth and high fodder productivity, teosinte is known with its high nutrient requirements, especially nitrogen (Kumar et al., 2016), which would entail the unfavorable consequences usually accompanying the application of high doses of conventional N fertilizers. It was therefore, worthwhile to investigate the effect of the integration of nanoparticle urea fertilizer in the fertilization scheme of teosinte, on its fodder productivity and quality. The current study was, thus, designed to evaluate the forage yield and some agronomic characteristics, in addition to the nutritive value of three cuts of two fodder teosinte varieties subject to varying applications of bulk and nanoparticle urea.

MATERIALS AND METHODS

Location

Field trials were conducted during two successive summer seasons (2018 and 2019) at Abis experimental farm, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Soil of the experimental farm is sandy loam in texture, with 1.80% organic matter, and 100, 30, and 389 ppm available nitrogen, phosphorous and potassium, respectively. The experimental location is arid with hot and dry Mediterranean summer seasons. Precipitation in the summer is zero, and average atmospheric temperature during the two successive seasons are presented in Table 1.

Table 1. Average monthly temperature during summer 2018 and 2019

Month	Average temperature (°C)	
	Summer 2018	Summer 2019
May	24.44	23.24
June	26.11	26.54
July	27.78	28.06
August	28.33	28.06
September	27.78	26.56

Design and treatments

A split-split plot experimental design with three field replications was adopted to investigate the variations in yield, dry matter content, some agronomic characteristics and forage quality parameters among three cuts (sub plots) of two teosinte (*Zea mexicana*) varieties; variety 1 and 2 (sub-sub plots), as affected by five bulk urea (BU) and/or nanoparticle urea (NPU) fertilizer treatments (main plots). The investigated fertilizer treatments were; 100% BU (F1), 75% BU + 25% NPU (F2), 50% BU + 50% NPU (F3), 25% BU + 75% NPU (F4), and 100% NPU (F5). The bulk urea (BU) under investigation was obtained from Abu Qir Fertilizers Company, Alexandria, Egypt, and contained 46% N by weight. Rates of the BU were calculated based on the recommended N fertilization for teosinte in the region, amounting to 280 kg N ha^{-1}.

The NPU was prepared by milling BU over two sieves of 2 mm and 51 µm diameter. Samples with particle size < 51 µm were grinded using Planetary Mono Mill as described by Elkhatib et al. (2015), to a particle size < 30 nm. Scanning electron microscope equipped with energy dispersive spectroscopy (SEM-EDS) (JSM-IT200 Series, JEOL, Japan) was used to determine the particle size of the NPU (Fig. 1). The NPU with diameter < 30 nm was suspended in de-ionized water to prepare a stock solution of 500 mg L^{-1}, which was dispersed by ultrasonic vibration (130 W, 20 kHz) for 25 minutes to avoid nanoparticles aggregation. Four concentrations of NPU, i.e, 200, 150, 100, and 50 mg L^{-1}, representing the 100, 75, 50, and 25% NPU treatment, respectively, were freshly prepared directly before application. To prevent NPU sedimentation, suspensions were continuously mixed using a magnetic stirrer.

Fertilizer treatments were split into three equal doses, applied two weeks after sowing, and two weeks after each of the first and second cuts. At the time of fertilizer treatment application, BU was applied as top dressing, while NPU suspension was applied as foliar spray. The tested varieties were assigned to the sub-sub-plots and sown with the recommended seeding rate, amounting to 48 kg seeds ha^{-1}.

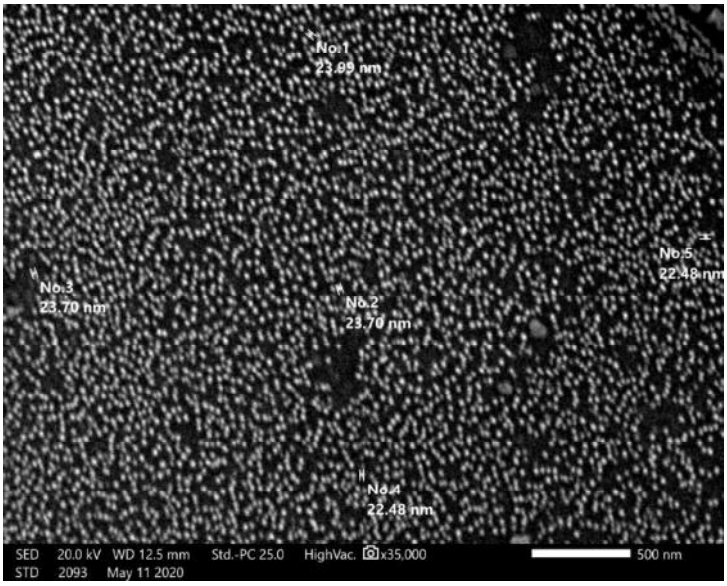

Figure 1. Scanning electron microscopy (SEM) image for nano particle urea (NPU).

Agronomy

Previous crop to teosinte cultivation in both experimental seasons, was wheat drilled on flat plots. After wheat harvesting, soil was plowed using a chisel plow in two perpendicular directions (20–25 cm depth), followed by land levelling, and ridging. Each experimental plot contained 4 ridges, 3 m long and 60 cm apart resulting in a total plot area of 7.2 m^2. A border of four ridges (7.2 m^2) was left between each two successive main plots to separate the fertilizer treatments. Sowing was done on 1st and 10th of May during 2018, and 2019, respectively. Seeds were drilled on the upper third part on one side of the ridge. Phosphorous fertilizer in the form of calcium monophosphate (15.5% P_2O_5) and potassium fertilizer as potassium sulphate (48% K_2O) were applied once before sowing with the recommended rates of 200 and 100 kg ha^{-1}, respectively. Flood irrigation was scheduled on weekly interval and hand weeding was practiced when necessary. At 45 days after sowing (DAS), first cut was taken, then 35 days interval was left before the second and third cuts.

Studied parameters

At each cut, plots were manually harvested using a garden sickle, leaving 5–7 cm above ground level to allow for regrowth. Total fresh yield per plot per cut was weighed on the field. Plant height (cm), stem diameter (mm), and leaf area per plant (cm^2) were calculated as an average of 5 randomly taken plants from the two ridges in the middle of each plot. A representative sub-sample per plot of approximately 750 g fresh matter was dried at 60 °C for 72 h until constant weight was reached to determine the dry matter (DM) content. The dried sub-samples were milled to a 1 mm particle size for forage quality evaluation. Nitrogen content (N) was determined using Kjeldahl procedure

(AOAC, 2012), then crude protein (CP) was calculated as N multiplied by 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF), representing the two prominent dietary fiber fractions, were sequentially analyzed using the semiautomatic ANKOM220 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) after Van Soest et al. (1991). Both fiber fractions were analyzed without heat stable amylase and expressed including residual ash content. Crude ash (CA) determination was done by incineration of Sub-samples in muffle oven at 550 °C for 3 h (AOAC, 2012). Soxhlet procedure was adopted to determine the crude fat (CF) content of the dried sub samples (AOAC, 2012). Finally, content of non-fiber carbohydrates (g kg^{-1}) was calculated using the following formula:

$$NFC = 1{,}000 - (CP + CF + NDF + CA) \qquad (1)$$

Statistics

Analysis of variance for the variations among cuts (C), fertilizer treatments (F), and varieties (V) was done using SAS 9.4 program (SAS Institute, Inc., 2012) - PROC MIXED. Studied factors and their interactions were statistically analyzed using the following model, with only replicates considered random:

$$\begin{gathered} Pijkl = \mu + Ri + Fj + eij + Ck + eijk + Vl + Cj \times Fk + Cj \times Vl \\ +Fk \times Vl + Cj \times Fk \times Vl + eijkl \end{gathered} \qquad (2)$$

where μ is the overall mean, R_i is the replicate effect (i = 1,2,3), F_j is the fertilizer treatment effect (j = 1,2,3,4,5), e_{ij} is the experimental error ‘a’, C_k is the cut effect (k = 1,2,3), e_{ijk} is the experimental error ‘b’, V_l is the variety effect (l = 1,2), and e_{ijkl} is the experimental error ‘c’.

Data of fresh yield, dry matter content and agronomic characteristics will be presented and discussed separately for the two experimental seasons, upon the heterogeneity of variance’s error (Hartley, 1950), while data of forage quality parameters will be presented in a combined analysis over the two experimental seasons, upon homogeneity of the variance’s error. Least significant difference (L.S.D.) procedure - at 0.05 probability level - was used for means’ comparisons.

RESULTS

For all studied parameters, main effects will be presented and discussed only if interaction involving them is not significant.

Yield, dry matter and agronomic characteristics

Analysis of variance revealed that the fresh yield, DM content and agronomic parameters were significantly variable among the tested cuts for both 2018 and 2019 ($p < 0.01$). In addition, fertilizer treatments significantly ($p < 0.01$) affected all parameters except stem diameter, while fresh yield during 2018 and 2019, and agronomic parameters only during 2018 were significantly variable ($p < 0.01$) among the tested varieties. As for the significant interactions, during both seasons, fresh yield was significantly affected by the cut × fertilizer treatment interaction ($p < 0.01$), and agronomic parameters were significantly affected by the fertilizer treatment × variety interaction ($p < 0.01$). Meanwhile, the three-way interaction was declared non-significant for yield, DM and agronomic characteristics ($p > 0.05$).

Highest significant fresh yield was achieved for cut 3, amounting to 39.23 and 40.12 t ha^{-1}, for 2018 and 2019, respectively (Table 2). On the other hand, cut 1 was characterized with the lowest significant amount of fresh yield, 7.07 and 10.95 t ha^{-1}, for the two respective seasons. Applying 100% BU fertilizer resulted in the production of the highest significant fresh yield, which gradually decreased with the decrease in the percentage of BU and increase in the percentage of NPU. Variety 1 was superior to variety 2 concerning the amount of fresh yield, with 27.97 and 31.85 t ha^{-1}, during 2018 and 2019, respectively. Variations in DM content were non-significant among the three cuts and two tested varieties in 2018 and 2019 (Table 2). However, opposite to the fresh yield, the DM content was inversely proportional to the percentage of mineral N fertilizer. Lowest significant DM content was reported for 100% BU fertilizer application, and amounted to 146.05 and 150.23 g kg^{-1} for 2018 and 2019, respectively. On the other hand, the highest significant DM content was reported for the application of 100% NPU fertilizer which was non-significantly different from 75% NPU + 25% BU, and 50% NPU + 50% BU. Fresh yield was significantly affected by the cut × fertilizer treatment interaction during the two growing seasons (Table 3). For all studied fertilizer treatments, cut 1 was inferior to the cuts 2 and 3 concerning the amount of fresh yield production. The application of 100% BU fertilizer produced the highest significant amount of fresh yield for the three cuts. Similarly, partial substitution of BU with NPU fertilizer; i.e. 75% BU + 25% NPU, and 50% BU + 50% NPU, produced as high fresh yield amounts as the application of 100% BU.

Table 2. Means of fresh yield (t ha^{-1}) and dry matter content (g kg^{-1}) as affected by the fertilizer treatment, cut, and variety for the two growing seasons

	Fresh yield		DM	
	Summer 2018	Summer 2019	Summer 2018	Summer 2019
Cut				
C1	7.07^{c*}	10.95^{c}	158.33^{a}	160.52^{a}
C2	31.13^{b}	35.84^{b}	147.83^{a}	145.85^{a}
C3	39.23^{a}	40.12^{a}	161.76^{a}	158.95^{a}
Fertilizer				
F1	34.79^{a}	35.62^{a}	146.05^{b}	150.23^{b}
F2	29.69^{b}	31.48^{b}	153.98^{ab}	150.85^{b}
F3	28.87^{b}	29.74^{bc}	155.79^{ab}	152.69^{ab}
F4	22.04^{c}	27.86^{c}	159.21^{ab}	155.23^{ab}
F5	13.64^{d}	20.15^{d}	164.85^{a}	165.50^{a}
Variety				
V1	27.97^{a}	31.85^{a}	153.82^{a}	152.52^{a}
V2	23.65^{b}	26.09^{b}	158.13^{a}	157.68^{a}

* Means followed by different small letter(s) within the same column, are significantly different according to the L.S.D. test at 0.05 level of probability.

Table 3. Means of fresh yield (t ha^{-1}) as affected by the fertilizer treatment × cut interaction for the two growing seasons

Fertilizer	Summer 2018			Summer 2019		
treatment	Cut 1	Cut 2	Cut 3	Cut 1	Cut 2	Cut 3
F1	10.78^{bA}*	44.94^{aA}	48.66^{aA}	15.85^{bA}	48.95^{aA}	49.68^{aA}
F2	8.54^{bA}	36.88^{aA}	43.64^{aAB}	10.45^{bA}	40.47^{aA}	45.36^{aAB}
F3	8.23^{bA}	36.07^{aA}	42.32^{aAB}	12.85^{bA}	41.62^{aA}	44.95^{aAB}
F4	4.17^{cB}	23.37^{bB}	38.58^{aB}	6.95^{bB}	30.62^{aB}	37.12^{aB}
F5	3.61^{bB}	14.39^{aB}	22.92^{aC}	5.26^{bB}	20.15^{aB}	25.62^{aC}

* Means followed by different small letter(s) within the same fertilizer treatment, and different capital letter(s) within the same cut, for each growing season, are significantly different according to the L.S.D. test at 0.05 level of probability.

Means of the studied agronomic parameters among the three cuts are presented in Table 4. Cut 1 was characterized by the highest significant plant height, stem diameter, and leaf area, amounting to 56.80 m, 7.01 mm, and 129.69 m^2, for 2018, and 60.67 m, 8.26 mm, and 130.45 m^2, for 2019, respectively. Values of the three parameters significantly decreased with advanced cuts, reaching the lowest values for the cut 3.

Table 4. Means of agronomic parameters as affected by the cut for the two growing seasons

Agronomic parameter	Summer 2018			Summer 2019		
	Cut 1	Cut 2	Cut 3	Cut 1	Cut 2	Cut 3
Plant height (cm)	56.80^{a}*	51.68^{b}	42.43^{c}	60.67^{a}	54.30^{b}	48.22^{c}
Stem Diameter (mm)	7.01^{a}	5.50^{b}	4.46^{c}	8.26^{a}	5.92^{b}	4.56^{c}
Leaf area (cm^2)	129.69^{a}	110.76^{b}	94.54^{c}	130.45^{a}	115.85^{b}	101.42^{c}

* Means followed by different small letter(s) within the same row, for each growing season are significantly different according to the L.S.D. test at 0.05 level of probability.

Table 5. Means of agronomic parameters as affected by the fertilizer treatment × variety interaction for the two growing seasons

Agronomic Parameter	Season	Variety	Fertilizer Treatment				
			F1	F2	F3	F4	F5
Plant height (cm)	Summer 2018	V 1	57.71^{aA}*	56.41^{abA}	55.54^{abA}	52.96^{abA}	48.26^{bA}
		V 2	52.47^{aA}	42.46^{bB}	44.03^{abB}	49.84^{abA}	43.33^{bA}
	Summer 2019	V 1	58.26^{aA}	56.10^{aA}	57.01^{aA}	50.31^{abA}	45.67^{bA}
		V 2	55.36^{aA}	45.36^{bB}	50.03^{abA}	47.82^{abA}	40.39^{bA}
Stem diameter (mm)	Summer 2018	V 1	5.29^{bA}	6.85^{aA}	6.35^{abB}	6.20^{abA}	6.37^{abA}
		V 2	5.47^{aA}	5.20^{aB}	4.88^{aB}	4.67^{aB}	5.31^{aA}
	Summer 2019	V 1	6.36^{aA}	6.72^{aA}	7.20^{aA}	6.37^{aA}	7.01^{aA}
		V 2	5.37^{aA}	5.92^{aA}	5.02^{aB}	4.79^{aB}	4.99^{aB}
Leaf area (cm^2)	Summer 2018	V 1	134.22^{aA}	95.67^{bB}	92.68^{bB}	83.62^{bA}	86.97^{bA}
		V 2	149.74^{aA}	142.33^{aA}	143.41^{aA}	99.38^{bA}	88.60^{bA}
	Summer 2019	V 1	146.23^{aA}	100.30^{bB}	101.29^{bB}	99.33^{bA}	95.96^{bA}
		V 2	149.36^{aA}	140.20^{aA}	145.44^{aA}	100.09^{bA}	97.36^{bA}

* Means followed by different small letter(s) within the same variety, and different capital letter(s) within the same fertilizer treatment, for each studied parameter and growing season, are significantly different according to the L.S.D. test at 0.05 level of probability.

Moreover, the three agronomic parameters were significantly variable as affected by the fertilizer treatment × variety interaction during both seasons (Table 5). Plant height for the two varieties gradually decreased with decreasing the percentage of BU fertilizer, however, the application of 50% BU + 50% NPU fertilizer gave similar plant heights to 100% BU fertilizer. Only in case of 75% BU + 25% NPU (during both seasons) and 50% BU + 50% NPU (during 2018) was the variety 1 superior to the variety 2 in plant height. Concerning the stem diameter (Table 5), variety 1 produced thinner stems when 100% BU was applied compared to the other fertilizer treatments during 2018. Meanwhile, variations among the five fertilizer treatments were non-significant for variety 2 during 2018 and both varieties during both seasons. During 2018, when mixtures of BU and NPU fertilizers were used, variety 1 produced thicker stems than variety 2. Moreover, during 2018, variety 1 gave thicker stems, with BU: NPU ratios 50:50% and 25:75% and with 100% NPU as well. Leaf area means of both growing seasons, presented in Table 5, showed that for variety 1, application of 100% BU resulted in significantly higher leaf area values than the other fertilizer treatments, while in case of variety 2, application of 100% BU as well as 75:50% and 50:50% BU: NPU ratios, resulted in higher leaf area values than the other fertilizer treatments with increased proportion of NPU. For the two growing seasons, leaf area of both varieties was significantly similar at 100% BU, 100% NPU and 25% BU + 75% NPU, while, at 75% and 50% BU + complementary NPU percentages, variety 2 gave significantly higher leaf area than variety 1.

Forage quality parameters

Combined analysis of variance for the studied forage quality parameters revealed that CP, ADF and CF contents were significantly variable among the tested fertilizer treatments and varieties ($p < 0.01$). In addition, ADF content was also significantly affected by the cut ($p < 0.01$). Moreover, the three-way interaction cut × fertilizer treatment × variety was significant in case of NFC and NDF contents ($p < 0.01$). Average CP and CF contents for the three cuts were 64.74 and 38.06 g kg^{-1}, respectively (Table 6). Cuts 2 and 3 were characterized by increased ADF contents than cut 1. Fertilizer treatments with 100, 75, and 50% BU resulted in significantly higher CP contents. On the contrary, CF content was higher with fertilizer treatments with increased NPU percentage (100 and 75%). Despite the statistical significance, little variations in ADF content were observed among the five tested fertilizer treatments, where the difference between the highest and lowest values was only 1.87%. Observably, variety 2 was characterized by higher CP content, yet lower ADF and CF contents than variety 1.

Table 6. Means of crude protein (CP), acid detergent fiber (ADF) and crude fat (CF) contents (g kg^{-1}) as affected by the fertilizer treatment, cut and variety combined over the two growing seasons

	CP	ADF	CF
Cut			
C1	64.64^{a*}	267.83^{b}	38.31^{a}
C2	66.13^{a}	315.01^{a}	38.27^{a}
C3	63.45^{a}	303.49^{a}	37.61^{a}
Fertilizer			
F1	68.22^{a}	302.78^{a}	30.63^{c}
F2	67.82^{a}	297.34^{a}	34.21^{b}
F3	66.10^{a}	284.09^{b}	36.97^{b}
F4	60.74^{b}	295.82^{ab}	42.83^{a}
F5	60.86^{b}	297.19^{a}	45.69^{a}
Variety			
V1	62.99^{b}	299.90^{a}	40.09^{a}
V2	66.50^{a}	290.99^{b}	36.04^{b}

* Means followed by different small letter(s) within the same column, are significantly different according to the L.S.D. test at 0.05 level of probability.

With reference to the significant three-way interaction in case of NFC and NDF, Table 7 shows that, generally, variety 2 was characterized with significantly higher NFC, yet lower NDF contents than variety 1 across all cuts and fertilizer treatments. Application of 50% BU + 50% NPU fertilizer, was a common treatment among all tested cuts, that gave significantly high NFC values. Same fertilizer treatment gave low NDF values for cuts 1 and two, while for cut 3, it resulted in high NDF content. The different direction of variation as well as the variable magnitude were probably the main reasons behind the significant three-way interaction.

Table 7. Means of non-fiber carbohydrates (NFC) and neutral detergent fiber (NDF) contents (g kg^{-1}) as affected by the fertilizer treatment × cut × variety interaction combined over the two growing seasons

NFC						
Fertilizer	Cut 1		Cut 2		Cut 3	
treatment	Variety 1	Variety 2	Variety 1	Variety 2	Variety 1	Variety 2
F1	236.53	309.88	207.54	225.35	263.12	307.04
F2	311.68	350.66	221.41	232.55	270.15	299.54
F3	314.85	340.93	233.77	252.92	255.92	296.24
F4	294.49	313.19	247.62	275.39	261.71	276.89
F5	296.82	318.86	247.36	272.63	245.26	280.01
L.S.D.$_{0.05}$	12.58					
NDF						
Fertilizer	Cut 1		Cut 2		Cut 3	
treatment	Variety 1	Variety 2	Variety 1	Variety 2	Variety 1	Variety 2
F1	655.64	578.78	684.43	662.36	631.63	584.64
F2	578.82	533.34	661.10	647.25	621.59	588.36
F3	568.62	548.26	657.39	653.84	630.13	596.30
F4	596.38	578.33	633.05	610.71	622.90	607.67
F5	587.10	567.69	634.40	613.87	634.69	605.49
L.S.D.$_{0.05}$	15.75					

DISCUSSION

Nutrient management is among the most important agronomic practices that needs to be accurately adjusted in order to achieve optimum productivity with satisfactory nutritional value from fodder teosinte (Mohan et al., 2017). The success of using nano fertilizers is highly dependent on the crop species (Elemike et al., 2019). In addition, texture of the experimental soil plays an important role in determining the effectiveness of the nano fertilizer application. As explained by Chhowalla (2017), the examination of nano fertilizer application is recommended in the sandy loam soils, like the experimental location of the current study, where the slow release nature of the NPU becomes an advantage due to the poor native fertilizer retention of the soil. As suggested by Kopittke et al. (2019), including the bulk-sized form (BU) with the nano-sized form (NPU) of the nitrogenous fertilizer under investigation, in the current study, provided a reliable control measure and allowed for a valid interpretation of the results.

The highest significant fresh yield, plant height and leaf area in the current study were observed for the 100% BU treatment. The linear positive effect of nitrogen on crop yield and agronomic characteristics has been previously reported by several researchers (e.g., Bahmaniar & Sooaee Mashaee, 2010; Pannacci & Partolini, 2018; Adamovics et al., 2019), which was attributed to extensive increase in cell growth rate with higher N rates, which resulted in taller plants (Rathnayaka et al., 2018) and higher herbage yield. Observably the application of 50% BU with 50% NPU produced as high fresh yield and tall plants as 100% BU. Similar observations were reported by Liu & Lal (2014), Benzon et al. (2015), and Al-Juthery et al. (2018) for rice, soybean, and wheat respectively, where researchers documented an increase in yield and plant height for mixtures of nano and conventional fertilizers. They attributed this to the improved ability of nano fertilizers to provide the essential nutrients, in addition to enhancing the transportation and absorption of the available nutrients, resulting in better crop growth. An attempt was made by Benzon et al. (2015) to clarify the promoted crop growth and development, noted by the high fresh yield and agronomic characteristics resulting from the combined treatment of nanoparticle and conventional fertilizers. They attributed this to the 'sink strength', known as the ability of a sink to utilize photosynthates towards its own benefit based on its size and activity. As described by Taiz & Zaiger (2006), sink size is known as the total biomass of sink tissue, while sink activity is the uptake rate of photosynthates per unit biomass of sink tissue. As previously mentioned, the ability of nanoparticle fertilizer to enhance the transportation and absorption of nutrients, will be positively reflected on these processes, resulting in better crop growth.

The effect of NPU application, in the current study, was more pronounced in case of dry matter accumulation of the tested varieties, where the DM content significantly increased with increasing the percentage of NPU in the applied fertilizer treatment. Similar results were reported by Rathnayaka et al. (2018) and Manikandan & Subramanian (2016) for rice and maize crops, respectively. This might be partially attributed to the increase in chlorophyll production and rate of photosynthesis accompanying the application of NPU, which allows for better translocation of assimilates and photosynthates to different plant parts resulting in higher DM accumulation (Singh et al., 2017)

In line with the current results, Payghan (2016) reported an increase in the nutritive value of fodder millet, in terms of more CP and less NDF and ADF contents with the combined application of nanoparticle and chemical fertilizers. On the other hand, CF content of the herbage followed an opposite trend in response to the fertilizer treatments, with treatments with increased BU percentage significantly decreasing CF content. This could be attributed to the increase in CP content with increasing N fertilization, which led to decreasing the other chemical components, including CF. A similar negative association between CP and CF contents was reported by Nawar et al. (2020) for sunflower and soybean.

The integration of NPU with BU in the fertilization management of teosinte, allowed to reduce the amount of urea required to reach the optimum productivity and quality from the crop. Similar findings were reported by researchers at the Sri Lankan Institute of Nanotechnology for rice crop (Kottegoda et al., 2017). In agreement with the current results, the researchers stated that rice productivity was significantly enhanced when 50% less conventional urea fertilizer was used in presence of nanoparticle fertilizer application. Nonetheless, it was clear from the current results that complete reliance on NPU would not support the production of teosinte, and that best results on forage yield and quality were achieved with the combined application of BU and NPU. Similar findings were reported by Payghan (2016) for fodder millet.

In comparison to other summer forage relatives, like maize, teosinte is known for its high genetic diversity (Niazi et al., 2015), which partially explains the variations in yield, agronomic characteristics and quality between the two tested varieties. The high yielding variety 1 was characterized by the tallest stems. The positive correlation between high fresh yield and plant height was confirmed for other forage grasses like fodder pearl millet (Habiba et al., 2018) and oat (Amanullah et al., 2004). In an opposite trend to the fresh yield production, variety 2 was characterized by higher nutritive value, in terms of high CP, CP, NFC, yet lower NDF, ADF and CF, thus, might be more recommended for feeding purposes.

Teosinte is characterized by its slow initial growth (Radwan et al., 2000), which might explain the very low amount of fresh yield achieved in the current study on the 1st cut, taken at 45 DAS compared to the 2nd and 3rd cuts. The fresh yield amounts reported by Tarrad et al. (2010) for several teosinte varieties on the 2nd and 3rd cuts, were similar to the current results, while they reported much higher fresh yield on the 1st cut. In their study, the 1st cut was taken at 64 DAS; thus, the plants were allowed to stay longer in the soil and had a better chance to build up a vigorous vegetative growth resulting in a higher amount of fresh yield. Estimation of the yield on dry matter basis is useful for a meaningful comparison among cuts and varieties especially for feeding purposes (Frandsen, 1986). Based on the reported highly significant positive correlation between fresh and dry yields of forage grasses (Knight et al., 1996), the non-significant variations among the three cuts in DM content, suggests that the variations in dry yield will follow the same trend of the fresh yield, with the 1st cut being inferior to the 2nd and 3rd cuts. In partial agreement with the current study, Tarrad et al. (2010) observed that the leaf area of teosinte varieties was the lowest on the 3rd cut, where also the shortest stems were produced. However, same researchers reported the highest leaf area and tallest plants on the 2nd cut, while the current results showed that these were characteristics of the 1st cut.

CONCLUSION

Based on the current results, the integration of nanoparticle urea in the fertilization scheme of fodder teosinte allowed for 50% reduction in the applied conventional urea fertilizer, without sacrificing the yield and quality of the produced forage. The combined application of 50% NPU with 50% BU, resulted in the production of high yield with satisfactory nutritive value, in terms of high crude protein (CP) and non-fiber carbohydrates (NFC), and low fiber and crude fat contents, similar to the addition of 100% BU. This practice was, however, less expensive and safer for the environment. While the 1st cut was characterized by the highest plant height, stem diameter and leaf area, the 3rd cut produced the highest amount of fresh yield. Variations in quality measures among the three cuts were almost non-significant. Variations in yield and quality were detected between the two tested local varieties. In conclusion, the combined application of 50% NPU with 50% BU is recommended for the production of fodder teosinte in similar environments.

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