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Mineral composition of tef (*Eragrostis tef* (Zucc.) Trotter) – a new fodder crop in Bulgaria

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The effect of the sowing rate and nitrogen fertilization on the mineral composition of the tef biomass (*Eragrostis tef* (Zucc.) Trotter) was studied in 2021 – 2022 in the region of Central South Bulgaria. Three sowing rates – 10, 15 and 20 kg ha^{-1}, and four levels of nitrogen fertilization – 0, 30, 60 and 90 kg ha^{-1} were studied in two development phases (milk and dough maturity). Nitrogen content was higher in the milk maturity phase. The highest nitrogen content was found at fertilization with 90 kg ha^{-1} of nitrogen at a sowing rate of 15 kg ha^{-1} in both harvesting phases. The increase of nitrogen and sowing rates had no significant effect on Mg content, but there was a negative tendency in P, K and Ca content. A strong positive correlation was found between the N and Mg contents, a good positive correlation – between the Ca and Mg contents; between the N and P and Ca contents and between the P and Mg contents. Climatic factors had the strongest effect on the content of macroelements in the tef biomass. Nitrogen fertilization had a strong effect on N content, while the harvesting phases and the sowing rate influenced the K content. No regular influence of the tested sowing rates, nitrogen fertilizing rates and harvesting phases on the Ca:P and Ca:Mg ratios was found.

Key words: *Eragrostis tef* (Zucc.) Trotter, mineral composition, nitrogen rate, sowing rate, phase of harvesting

INTRODUCTION

Tef (*Eragrostis tef* (Zucc.) Trotter) is an annual cereal plant. Its grain is an important ingredient in the diet of the population in Ethiopia, Eritrea, South Africa and is used to produce traditional foods and beverages [1]. Due to the many dietary benefits and tolerance to extreme environmental conditions, this cereal crop is promising for cultivation and forage [2-4].

In the last decade, the demand for crops adaptive to the stress of climatic changes to provide biomass and grain in a short period under adverse environmental conditions has increased [5, 6]. The biological characteristics of the crop permit its use in compressing crop rotations - it can be successfully sown as a second crop on non-irrigated areas. Tef has a high drought tolerance. In the literature, it is presented as a crop that can replace or supplement some of the fodder crops - alfalfa, corn, sorghum, barley and wheat in the absence of sufficient moisture supply [7-9]. In comparison with other cereals, tef is less infested by diseases and pests [4, 9, 10].

The use of tef as a "healthy food" is due to the unique taste of the gluten-free grain and its biochemical composition [11]. The amylase content (20-26%) in the grain is comparable to other cereals [1] while the fiber is in higher percentage - 9.8% for tef [3].

The valuable biological qualities and the favourable chemical composition rank tef as an important crop for solving not only the food needs of the population, but also as a fodder crop. Studies on the mineral composition of tef biomass worldwide are quite scarce, especially for its cultivation as bulk forage. In recent years, some authors have reported the potential of the crop to produce silage and hay harvested in different phenological phases and studied the chemical and mineral composition of the tef biomass [4, 12]. The quality of tef forage is highly dependent on nitrogen fertilization, the maturity phase at harvest and the number of swaths [13, 14]. Currently, there is limited information on the nutritional value of the biomass and its potential for use as ruminant feed. According to literature, the content of crude protein in the tef biomass sources ranges from 8.5 to 21.5% [2, 4, 8, 14-17], the fiber content varies from 53 to 73% [2, 8] depending on the maturity phase. According to some authors, tef hay has the potential to replace corn silage in cattle rations and become a major forage source in the nutrition of bulls and dairy heifers [12, 16]. The maturity phase at harvest is one of the major factors affecting forage quality and digestibility [4, 16-18].

The objective of this study is to determine the influence of the sowing rate and nitrogen fertilization on the mineral composition and the

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quantitative ratios between the macroelements in two phases of harvesting the tef biomass grown for fodder under the conditions of Central South Bulgaria.

MATERIALS AND METHODS

The research was conducted in the period 2021-2022 in the area of the village of Tulovo, Stara Zagora county, located in the region of Central South Bulgaria. The field experiment was conducted with a white variety of tef of the Dutch company "Millets place". The experiment was based on the method of fractional plots and size of the harvest plot was 10 m^2, under non-irrigated conditions, after predecessor wheat. The soils in the area are alluvial, slightly to moderately enriched with humus (1.6% - 2.6%), with a slightly acidic to neutral reaction, slightly stocked with nitrogen (31.0 – 35.0 kg ha^{-1}) and phosphorus (8.0 – 27.0 ppm) and slightly to well stocked with potassium (93.0-136.0 ppm). The influence of sowing rate and nitrogen fertilization on the mineral composition of tef (*Eragrostis tef* (Zucc.) Trotter) biomass in two development phases (milk and dough maturity) were tested.

The studied factors and their levels were as follows: factor A: sowing rate, kg ha^{-1} (A1 – 10; A2 – 15; A3 – 20); factor B: nitrogen fertilization rates, kg ha^{-1} (B1 – 0; B2 – 30; B3 – 60; B4 – 90). Variant 1 (A1B1) was adopted as a control – harvested at the milk maturity phase, with a sowing rate of 10 kg ha^{-1} without nitrogen fertilization. With the main tillage, background fertilization with 50 kg ha^{-1} P_2O_5 was made. Fertilization with nitrogen in the specified rates (factor B) was made immediately before sowing.

Plant materials and processing

Aerial parts of tef were harvested at a height of 6 cm from the base of the stem in two phases of development – milk and dough maturity. Average plant samples in 3 replicates were collected from each variant. Plant samples were pre-processed - cleaning from contaminants, drying in a dryer at 60°C and grinded in a mechanical grinder (final powder size less than 400 μm). The samples were stored in dark and cool rooms at 16 - 18 ºC prior to the analysis. The prepared samples were analyzed in the Chemical laboratory at the Faculty of Agriculture, Trakia University, Bulgaria.

Determination of mineral composition

The nitrogen content (N) was determined by the method of Kjeldahl (BDS – EN ISO 5983); phosphorus (P) – by colorimetry, measured at 470 nm on a SPEKOL 11 spectrophotometer; potassium (K); calcium (Ca), magnesium (Mg) with Perkin Elmer AANALYST-800 atomic absorption spectrometer [22].

Statistical analysis

The investigated parameters were measured in three replicates and the average results are presented as experimental results ± standard deviation (SD). To establish the influence of sowing rate, nitrogen fertilization rate and development phases on the mineral composition statistical procedures were applied by an ANOVA LSD test for statistical significance of the differences. After significant results were obtained by the ANOVA test, Tukey's HSD test was applied to all pairwise differences between means. The significant differences were tested and p values < 0.05 were considered statistically significant. To establish correlation dependencies and factor analysis, the software package for statistical data processing MS Excel software - 2010 was used.

RESULTS AND DISCUSSION

Nitrogen content for all three tested sowing rates was higher in the milk maturity phase (Table 1). Examining the results obtained on nitrogen content in the dry matter (DM) of tef biomass, an increase in its content was observed with an increase in nitrogen fertilization rates from 0 to 90 kg ha^{-1} in both analyzed phases, as established by some authors [4, 19]. The highest values were obtained by fertilizing with 90 kg ha^{-1} N and sowing rate of 15 kg ha^{-1}– 17.32 g kg^{-1} (milk maturity phase) and 16.72 g kg^{-1} (dough maturity phase).

In the milk maturity phase, the higher nitrogen content in the tef biomass compared to the control when using a nitrogen fertilization rate of 90 kg ha^{-1} was statistically very well proven at sowing rates of 10 and 15 kg ha^{-1} ($P<0.001$) and was not demonstrated at the highest sowing rate. At fertilization rate of 60 kg ha^{-1}, the differences in content were proven at all three sowing rates ($P<0.05$, $P<0.01$). For fertilization rate of 30 kg ha^{-1}, differences were proven only at a sowing rate of 15 kg ha^{-1} ($P<0.05$). In the dough maturity phase, the higher nitrogen content in the tef biomass compared to the control when using nitrogen fertilization rate of 90 kg ha^{-1} was statistically proven at sowing rates of 10 and 15 kg ha^{-1} ($P<0.05$, $P<0.001$). At a fertilization rate of 60 kg ha^{-1}, the differences in content were proven at the low sowing rate ($P<0.01$). For a fertilization rate of 30 kg ha^{-1}, no differences were statistically proven at all three sowing rates.

Table 1. Content of N, P, K, Ca and Mg in the tef biomass for 2021/2022 period, g kg^{-1} DM

Variant	N	P	K	Ca	Mg
Milk maturity phase					
1 A_1B_1 (control)	13.71±2.0	2.70±0.33	7.60±0.54	3.66±0.16	1.42±0.19
2 A_1B_2	14.79±1.6^{ba}	2.60±0.13^{a}	7.60± 0.31^{bc}	3.11±0.28^{ab**}	1.38±0.20
3 A_1B_3	15.52±1.8^{b*}	2.60±0.20^{a}	7.49±0.42^{b}	2.96±0.25^{a***}	1.49±0.21
4 A_1B_4	16.74±3.8^{cba***}	2.65±0.23^{a}	6.61±0.23^{a***}	3.19±0.32^{ab**}	1.49±0.19
5 A_2B_1	14.11±2.3^{a}	2.60±0.20^{a}	6.70±0.39^{a***}	3.03±0.09^{ab***}	1.27±0.14
6 A_2B_2	15.27±1.6^{ab*}	2.50±0.13^{a}	7.01±0.07^{a*}	2.87±0.37^{a***}	1.34±0.13
7 A_2B_3	16.28±2.1^{c**}	2.80±0.53^{ab}	7.09±0.29^{ab*}	3.00±0.19^{a***}	1.37±0.20
8 A_2B_4	17.32±1.4^{c***}	2.85±0.43^{ab}	6.84±0.33^{a**}	2.84±0.17^{a***}	1.47±0.16
9 A_3B_1	13.57±2.5^{a*}	2.65±0.03^{a}	7.65±0.25^{bc}	2.75±0.38^{a***}	1.45±0.30
10 A_3B_2	14.16±2.7^{a}	2.65±0.03^{a}	7.02±0.20*	2.86±0.48^{a***}	1.47±0.33
11 A_3B_3	15.91±2.3^{b**}	2.55±0.03^{a}	7.34±0.18^{ab}	2.96±0.35^{a***}	1.45±0.25
12 A_3B_4	15.03±3.1^{b}	2.50±0.07^{a}	6.91±0.15^{a**}	2.82±0.24^{a***}	1.58±0.27
Dough maturity phase					
13 A_1B_1	14.26±2.7^{a}	3.20±0.47^{b*}	8.40±0.18^{c**}	2.68±0.28^{a***}	1.34±0.22
14 A_1B_2	14.87±2.8^{b}	2.70±0.40^{ab}	8.25±0.17^{c**}	3.55±0.11^{b}	1.35±0.17
15 A_1B_3	16.05±2.0^{cb**}	2.70±0.33^{ab}	7.86±0.13^{bc}	3.01±0.18^{ab***}	1.32±0.16
16 A_1B_4	15.31±3.2$^{bc\ *}$	3.00±0.07^{b}	8.09±0.00^{c*}	3.04±0.30^{ab***}	1.36±0.23
17 A_2B_1	13.26±2.4^{a}	2.60±0.27^{a}	7.75±0.17^{bc}	3.19±0.04^{ab**}	1.40±0.14
18 A_2B_2	14.03±1.8^{a}	2.75±0.37^{ab}	8.04±0.28^{c}	3.23±0.21^{ab*}	1.48±0.17
19 A_2B_3	15.11±2.0^{ba}	3.10±0.00^{b}	7.26±0.48^{ab}	2.78±0.07^{a***}	1.47±0.11
20 A_2B_4	16.72±1.4^{c***}	2.70±0.07^{ab}	7.90±0.43^{bc}	3.22±0.12 ^{ab*}	1.52±0.11
21 A_3B_1	13.86±2.8^{a}	2.75±0.17^{ab}	7.74±0.44 bc	3.33±0.03^{ab}	1.55±0.04
22 A_3B_2	14.02±2.7^{a}	2.65±0.23^{a}	7.15±0.03 ab	3.48±0.03^{ab}	1.47±0.19
23 A_3B_3	14.15±2.8^{a}	2.65±0.23^{a}	7.32±0.12 ab	2.89±0.35^{a***}	1.37±0.17
24 A_3B_4	15.21±2.8^{b}	2.45±0.03^{a}	7.21±0.18 ab	2.87±0.10^{a***}	1.38±0.15
LSD, $P < 0.05$	1.50	0.44	0.47	0.33	15.76
LSD, $P < 0.01$	2.01	0.58	0.62	0.44	20.96
LSD, $P < 0.001$	2.63	0.76	0.82	0.58	24.67

The data are presented as mean ± SD; $^{*, **, ***}$ – Statistically significant differences between variants and control at $P<0.05$; 0.01 and 0.001, respectively; ^{a,b,c} – Different letters indicate statistically significant differences between variants at $P<0.05$.

The content of phosphorus in the above-ground biomass of tef was 2.70 g kg^{-1} DM in the control variant. A comparison between the harvesting phases shows that its content is higher in the dough maturity phase. The variation of the values depending on the sowing rates is within small limits. The highest values weere recorded at a sowing rate of 15 kg ha^{-1} in the first phase and at a sowing rate of 10 kg ha^{-1} in the second phase on average for the experimental period. Nitrogen fertilization with increasing rates did not lead to increased phosphorus content in tef biomass. The highest values were most often observed in the unfertilized variants. On average for the period of the experiment, higher phosphorus content in the tef biomass was statistically proven in the non-fertilized variant at a sowing rate of 10 kg ha^{-1} in the dough maturity phase, compared to the control, but with a low level of reliability ($P<0.05$). A lower phosphorus content was not statistically proven either in the milk phase or in the dough phase.

Compared to the macronutrients phosphorus, calcium and magnesium, potassium is in the largest amount. Compared by phases, potassium content is higher in the dough maturity phase. Fertilization with increasing nitrogen rates from 0 to 90 kg ha^{-1} did not increase the potassium content in the tef biomass. The lowest potassium content on average for the study period was found in the milk maturity phase, in the variant fertilized with 90 kg ha^{-1} N at a sowing rate of 10 kg ha^{-1} (6.61 g kg^{-1} DM) and in the non-fertilized variant at a sowing rate of 15 kg ha^{-1}

(6.70 g kg^{-1} DM). These lower values of potassium content in the above-ground biomass of tef were statistically very well proven (P<0.001). The lower values of potassium in the same phase at sowing rates of 15 and 20 kg ha^{-1} and the highest nitrogen fertilization rates were also well proven (P<0.01). The highest average potassium content was found in the dough maturity phase, at asowing rate of 10 kg ha^{-1}, in the non-fertilized variant and those fertilized with 30 and 90 kg ha^{-1} N (8.40, 8.25 and 8.09 g kg^{-1} DM, respectively). The higher values compared to the control variant were well proven statistically (P<0.01 and P<0.05).

The highest value of the Ca content depending on the sowing rates, was recorded at a sowing rate of 10 kg ha^{-1} in the milk maturity phase. No strict regularity was observed, according to which fertilization with increasing nitrogen rates leads to an increased content of calcium in the biomass of tef. The highest value (3.66 g kg^{-1} DM) was found for the control variant. For the experimental period, compared to the control, the lower content of calcium in the biomass of tef was statistically very well proven for all variants in the milk maturity phase, regardless of sowing rate (P < 0.001, P < 0.01). This also applies to the dough maturity phase in the variants fertilized with 60 and 90 kg ha^{-1} N (P < 0.001, P < 0.05).

The content of Mg in the biomass of tef varied in a narrow range – from 1.27 to 1.58 g kg^{-1} DM. When applying nitrogen fertilization with increasing doses from 0 to 90 kg ha^{-1} a slight increase in the magnesium content of tef biomass was observed. The highest value (1.58 g kg^{-1} DM) was in the variant fertilized with 90 kg ha^{-1} N, and the lowest (1.27 g kg^{-1} DM) - in the non-fertilized variant in the milk maturity phase. Compared to the control variant, no differences in magnesium content were statistically proven.

Vinyard *et al.* [4] and Ream *et al.* [12] found a decrease in crude protein content in the tef biomass when harvested in early-heading stages of maturity compared to late-heading stages of maturity. For the other macroelements, the changes were within narrow limits.

Significant positive correlations were established between the nitrogen content and the content of magnesium, phosphorus and calcium in the tef biomass, and negative - to the potassium content (Table 2). Well-proven positive correlation dependences were established between the calcium content and the magnesium content; between the phosphorus content and the magnesium and calcium content and negative between the potassium content and magnesium content. Authors [21] reported a highly significant correlation between Mg and Ca (r = 0.9272) in wheat, indicating a similar pattern for the effect of fertilization in tef.

Table 2. Correlation (r) dependences between the contents of macroelements .

	N	P	K	Ca	Mg
N	1	0.572**	-0.443**	0.555**	0.855**
P		1	-0.056	0.349**	0.575**
K			1	-0.135	-0.406**
Ca				1	0.686**
Mg					1

** – Statistical significance at P < 0.01

Factor analysis shows that the year conditions had the strongest and very well proven (P<0.001) effect on N, P, Ca and Mg content in the tef biomass (Table 3), while the K content was most influenced by the harvesting phase. Nitrogen fertilization rate had a strong effect on the N content (P<0.001). Sowing rate influenced the K content (P<0.01). Harvesting phase influenced the P content, while the Mg content was influenced by the year*sowing rate and year*phase (P<0.05).

Table 3. Influence of factors on the mineral composition of the tef biomass, %

Factors	N	P	K	Ca	Mg
Year	83.01***	41.88***	19.84***	41.54***	81.63***
Nitrogen rate	7.57***	1.33	5.58	6.10	0.83
Sowing rate	1.16	3.77	11.55**	2.94	0.88
Phase of maturity	0.55	3.38*	24.80***	1.68	0.08
Year*nitrogen rate	0.47	10.86	1.77	1.07	0.28
Year*sowing rate	1.94	0.99	1.68	1.23	2.39*
Year*phase	0.13	0.00	1.69	4.32	2.00*
Other factors	5.17	37.79	33.09	41.12	11.91

*, **, *** – Statistical significance at P<0.05; 0.01 and 0.001, respectively

Table 4. Ca:P ratio and Ca:Mg ratio of the tef biomass for the 2021/2022 period

Variant	Ca:P	Ca:Mg	Variant	Ca:P	Ca:Mg
Milk maturity phase			Dough maturity phase		
1 A_1B_1 (control)	1.4:1	2.6:1	13 A_1B_1	0.8:1	2.0:1
2 A_1B_2	1.2:1	2.2:1	14 A_1B_2	1.3:1	2.6:1
3 A_1B_3	1.1:1	2.0:1	15 A_1B_3	1.1:1	2.3:1
4 A_1B_4	1.2:1	2.1:1	16 A_1B_4	1.0:1	2.2:1
5 A_2B_1	1.2:1	2.4:1	17 A_2B_1	1.2:1	2.3:1
6 A_2B_2	1.1:1	2.1:1	18 A_2B_2	1.2:1	2.2:1
7 A_2B_3	1.1:1	2.2:1	19 A_2B_3	0.9:1	1.9:1
8 A_2B_4	1.0:1	1.9:1	20 A_2B_4	1.2:1	2.1:1
9 A_3B_1	1.0:1	1.9:1	21 A_3B_1	1.2:1	2.1:1
10 A_3B_2	1.1:1	1.9:1	22 A_3B_2	1.3:1	2.4:1
11 A_3B_3	1.2:1	2.0:1	23 A_3B_3	1.1:1	2.1:1
12 A_3B_4	1.1:1	1.8:1	24 A_3B_4	1.2:1	2.1:1

In the present study, the Ca:P ratio varied from 0.8:1 to 1.4:1 and the Ca:Mg ratio varied from 1.8:1 to 2.6:1 in the tef biomass (Table 4). The average values of these ratios during the two harvesting phases were equal. No regular influence of the studied sowing rates and nitrogen fertilizer rates on the Ca:P and Ca:Mg ratios was found.

Fujihara *et al.* [19] found high Ca:P ratio values (over 6:1, even over 12:1) in wheat straw and green-pea straw, while in wild grass, this ratio was below 6:1. Optimum Ca:P ratio in green feed for ruminants is 2:1, although that ratio can vary a lot [20]. In the present experiment, balanced ratios were found between Ca:P and Ca:Mg.

CONCLUSION

Nitrogen fertilization has a positive effect on the nitrogen content in the tef biomass. Nitrogen content is higher in the milk maturity phase. The highest nitrogen content was found at fertilization with 90 kg ha^{-1} of nitrogen at a sowing rate of 15 kg ha^{-1} in both harvesting phases.

With an increase in the sowing rate and nitrogen fertilization, a decrease in phosphorus, potassium and calcium content was observed. The use of different sowing rates and nitrogen fertilization doses does not significantly change the magnesium content of the tef biomass.

A strong positive correlation (r = 0.855) was found between the N and Mg contents, a good positive correlation – between the Ca and Mg contents (r = 0.686); between the N and P and Ca contents (r = 0.572; 0.555) and between the P and Mg contents (r = 0.575).

Climatic factors have the strongest effect on the content of macroelements in the tef biomass. Nitrogen fertilization has a strong effect on N content, while the harvesting phase and the sowing rate influence the K content.

No regular influence of the tested sowing rates, nitrogen fertilizer rates and harvesting phases on the Ca:P and Ca:Mg ratios was found.

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