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EFFECT OF POTASSIUM FERTILIZATION, *RHIZOBIUM* INOCULATION AND WATER DEFICIT ON THE YIELD AND QUALITY OF FENUGREEK SEEDS

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Abstract

The paper presents results on the effect of agronomic factors on the content of chlorophyll, the biometric parameters of plants and quality of fenugreek seeds. It has been shown that potassium fertilization and inoculation of fenugreek seeds had no significant effect on the plant's performance. The only trait influenced by those factors was the seed weight per plant; under higher doses of potassium fertilization $K_{2.5}$ and $K_{3.75}$, its value was about 10% higher, but fell down by 19.3% in treatments with inoculated seeds compared to non-inoculated ones. Soil water deficit caused reduction of plant height by 15.5%, number of pods by 18.3%, number of seeds per pod by 20%, weight of seeds by 28%, weight of the plant's aerial parts by 18.0%, and harvest index by 13.2%. In response to water deficit, the content of chlorophyll decreased significantly from 57.8 SPAD units at 43 DAS to 52.4 and 54.0 SPAD units in the next phases (51 and 58 DAS), when 40% and 80% of pods attained the final length. Increasing doses of potassium caused a significant increase in the crude protein (by 3.2-5.4%) and potassium content (by 7-8%) in seeds of fenugreek grown under water deficit. After seed inoculation, an increase in the protein content was found.

Key words: chlorophyll, drought, fenugreek, inoculation, potassium, yield.

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INTRODUCTION

Fenugreek (*Trigonellum foenum-graecum* L.), known as Greek hay, is an annual legume, commonly grown in south-western Asia and in the Mediterranean region. From countries in western Europe over to India, China, Iran or Canada, it is grown for aromatic seeds, but in some parts of Europe and Northern Africa it remains a fodder crop (Hussein, El-Dewiny 2011, Kinji, Rahdari 2012, Soori, Mohammadi-Nejad 2012). Fenugreek (*Trigonellum foenum-graecum* L.), is widely used for medicinal purposes because its seeds are rich in protein, oil, macronutrients, micronutrients, steroids, saponins, mineral salts and vitamins (Abdelgani et al. 1999, Madar, Stark 2002), like other legume species (Zuk-Golaszewska et al. 2010, Purwin et al. 2014). Amin et al. (2005) showed that fenugreek seed extract effectively prevents and inhibits the growth of breast cancer. It is suspected that the class of flavonoids which fenugreek contains may play a substantial role in the prevention of cancer.

In addition, fenugreek is environment friendly. Owing to a strain of the symbiotic bacteria *Rhizobium meliloti*, these plants use atmospheric nitrogen for the growth and development (Poi et al. 1991). Inoculation of fenugreek seeds with *Rhizobium meliloti* is important when this species is cultivated under elevated soil salinity conditions (Abd-Alla, Omar 1998). The salinity of 0.5-1% NaCl significantly decreases the enzymatic activity of soil. Introduction of cellulolytic fungi and wheat straw into soil improves the efficiency of symbiosis of *Rhizobium meliloti* with fenugreek plants. Such soil enrichment ensures a higher content of dry matter and nutrients including N, Ca, Mg and K in stems and roots of the plant. In general, the growth and yield of all plants are adversely affected by water stress (Alhadi et al. 1999). However, the investigations by Acharya et al. (2006*a*) and Makai et al. (2004) showed that fenugreek yielded significantly higher under water deficit and that this species was easily adaptable to arid growing conditions (Moyer et al. 2003). Another factor which influences the yield of this plant and enriches the content of nutrients in soil is the fertilization with nitrogen and phosphorus accompanied by potassium nutrition (Khirya, Singh 2003). The aim of this study was to determine the yield and yield components, chlorophyll content and quality of fenugreek seeds depending on different doses of potassium, seed inoculation and water deficit. Research in this area may be helpful in developing effective technologies of fenugreek cultivation for high-quality seeds.

MATERIAL AND METHODS

A pot experiment was performed in a greenhouse, at the University of Warmia and Mazury in Olsztyn, Poland (latitude 53°46′ N, longitude 20°25′ E). It was continued for two vegetation seasons. The experiment was set up in a completely randomized design with six replications. Fenugreek plants were grown in modified Kick-Braukmann pots filled with 10 kg of light soil composed of heavy loamy sand. The soil was slightly acid (pH = 5.5 in 1 M KCl) and abundant in available nutrients (P – 107, K – 124, and Mg – 19 mg kg^{-1} of soil). Treatments were as follows: potassium (form K_2SO_4) levels: K_0 (control), $K_{1.25}$, $K_{2.50}$, and $K_{3.25}$ g pot^{-1}, inoculation: (1) non-inoculated (control), (2) inoculated with *Rhizobium meliloti*; water deficit: (1) 50-60% of water capacity (control) – the plants were watered every day, (2) 30-35% of the water capacity from the flowering to seed formation phase (water deficit). Besides, all plants received 0.5g N ($CO(NH_2)_2$); 0.5g P ($Ca(H_2PO_4)$ H_2O); 0.3 g Mg ($MgSO_4$ H_2O). Also, during the whole growth of plants in 2005 and 2006, insects were effectively controlled.

The chlorophyll content (SPAD units) was measured at 7-day intervals from 43 DAS (flowering), through 51 DAS (40% of pods had reached the final length), 58 DAS (80 % of pods with the final length) until the beginning of the ripening of green seeds and filing the pod cavity (65 DAS). Green pigment was recorded with the use of a chlorophyll meter SPAD-502, Minolta, Japan (Peltonen et al. 1995).

Harvesting took place at full maturation stage. All the plants (12) from each pot were sampled to calculate the following parameters: plant height, weight of the aerial parts of a single plant, number of pods per plant, seeds per pods, thousand seed weight, seed weight per plant and the harvest index (HI). The HI was computed by dividing the seed weight per plant by the aerial plant weight. The crude fat content in fenugreek seeds was measured by weight after extraction with a naphthainthe Soxhlet apparatus. The plant material was mineralized in concentrated sulfuric acid (VI) with the use of hydrogen dioxide as an oxidant. The total nitrogen was determined calorimetrically using hypochlorite (Baethen, Alley 1989) and a conversion factor equal 6.25 was used to calculate the crude protein content. Phosphorus was determined by the vanadium-molybdenum method, potassium, calcium and sodium were analyzed with atomic emission spectrometry AES, and magnesium was assessed by AAS.

The results were statistically analyzed using a statistical software package Statistica v 9®. The statistical significance of the factors was evaluated according to an Anova model for a completely randomized design. The comparison of treatments was performed with the Tukey's HSD at $P < 0.01$.

RESULTS AND DISCUSSION

In the first year when the experiment was conducted, the temperature in the greenhouse averaged 11.0°C in April (10.4–16.0), 15.4°C in May (10.0–24.0), 16.7°C in June (13.2-22.1), 22.8°C in July (19.0-25.0) and 16.9°C in August (13.0-20.0). In the second year, the average temperature was 8.2°C in April (6.3–15.4), 22.2°C in May (15.0–32.0), 26.0°C in June (18.0-32.1), 32.3°C in July (29.3-33.2) and 26.9°C in August (25.1-30.6). Relative humidity ranged from 40% to 95%. The period of the growth and development of fenugreek plants was relatively short: 98 days (sown on 21 April and harvested on 28 July) in the first year and 110 days (sown on 28 April and harvested on 02 August) in the second year.

In our research, no significant differences were found in the chlorophyll content measured at the consecutive development phases of fenugreek that would be attributable to potassium fertilization (data not shown). In the first three growth phases (43, 51, and 58 DAS), all the plants, both inoculated and non-inoculated ones, had a similar content of chlorophyll. A significant difference appeared at the beginning of ripening phase (65 DAS), when plants grown from Nitragina-treated seeds contained less chlorophyll (Figure 1).

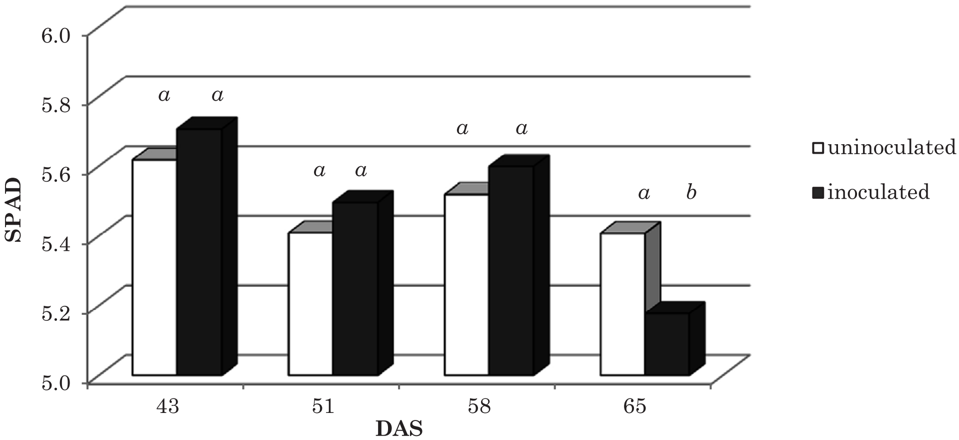

Fig. 1. Effect of inoculation on content chlorophyll (SPAD units) of fenugreek plants: DAS – days after sowing, *a*, *b* – the same letter at the means point to a significant difference

In the water deficit treatment with 50-60% of water capacity, the chlorophyll content was on the same level during the first three stages of growth (43, 51, and 58 DAS), but decreased at the beginning of ripening phase (65 DAS) to a significantly lower level of 50.4 SPAD (Figure 2). Water deficit affected the chlorophyll content during the growth of fenugreek plants. The study showed that chlorophyll decreased significantly from 57.8 SPAD units at 43 DAS to 52.4 and 54.0 SPAD units during the next phases, when 40% and 80% of pods had reached the final length (51 and 58 DAS). In a study by Al-Saady (2012), the chlorophyll content of 8 Omani fenugreek accessions ranged between 40-50 SPAD units. The presence of NaCl in soil significantly influenced the chlorophyll content, being positively correlated to that characteristic. The results reported by Pour el al. (2013) showed that salinity stress caused a significant reduction in the chlorophyll content of fenugreek, measured with the extraction method, versus the control. The mean total chlorophyll content was 1.917 mg g^{-1} FW (fresh weight) in all treated plants, ranging from 2.671 (the control) to 0.863 mg g^{-1} FW (at the highest short-term salinity stress).

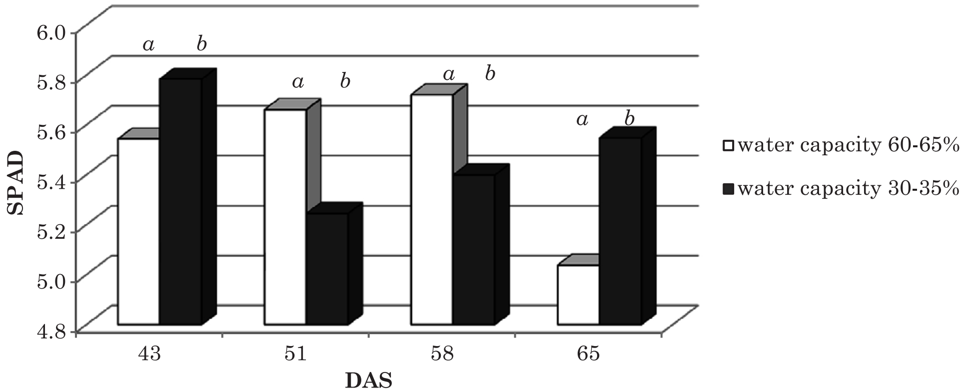

Fig. 2. Effect of water deficit on chlorophyll content (SPAD units) of fenugreek plants: DAS – days after sowing, *a*, *b* – the same letter at the means point to a significant difference

The results concerning yield and yield components of fenugreek are shown in Table 1. The applied doses of potassium had no significant impact on most of the analyzed traits except for the seed weight per plant. The height of fenugreek plants varied from 40.49 to 41.92 cm. On average, a single

Table 1

Yield and yield components of fenugreek

Factors	Plant height (cm)	Pods per plant (no)	Seeds per pod (no)	Thousand seeds weight (g)	Seeds weight per plant (g)	Above-ground parts of one plant (g)	Harvest index (%)
K dose (g pot^{-1})							
K_0	40.54	3.11	7.17	12.19	0.27	1.27	17.38
$K_{1.25}$	40.49	3.11	6.71	12.37	0.27	1.30	17.30
$K_{2.50}$	41.92	3.33	6.63	12.23	0.29	1.35	17.36
$K_{3.75}$	41.15	3.41	6.85	13.22	0.30	1.29	18.62
$HSD_{0.01}$	ns	ns	ns	ns	0.06	ns	ns
Rhizobium meliloti							
Non-inoculated	41.33	3.31	7.49	12.25	0.31	1.28	19.47
Inoculated	40.90	3.18	6.19	12.71	0.25	1.33	15.87
$HSD_{0.01}$	ns	ns	0.99	ns	0.04	0.07	2.14
Water capacity (%)							
50-60%	43.14	3.50	7.45	12.12	0.32	1.39	18.51
30-35%	36.47	2.86	5.96	11.85	0.23	1.14	16.07
$HSD_{0.01}$	1.40	0.40	0.99	ns	0.04	0.07	2.14

ns – non-significant

plant produced 3 pods with 7 seeds per pod. The seed weight per plant increased significantly under the higher doses of potassium: from 0.27 g at K_0 and $K_{1.25}$ to 0.29 g at $K_{2.50}$ and 0.30 g at the highest dose applied, i.e. $K_{3.75}$. At the same time, the thousand seeds weight (12.19-13.22 g plant^{-1}) was not significantly different within the range of potassium doses applied. The harvest index ranged from 17% to 18.6%. NEHRA et al. (2002) found that increasing levels of P_2O_5 up to 40 kg ha^{-1} and K_2O up to 45 kg ha^{-1} significantly raised the number of pods per plant, seeds per pod, length of pod and seed yield in comparison with the control. In another study by KHIRYA and SINGH (2003), it was shown that fertilization with nitrogen, phosphorus and potassium also affected the quantity of fenugreek seeds. In a field experiment conducted by KHAN et al. (2005), the harvest index varied from 25.50% to 27.87% depending on the phosphorus level.

Inoculation of fenugreek seeds with a bacterial strain *Rhizobium meliloti* had no significant effect on the plant weight, number of pods and thousand seed weight (Table 1). On the other hand, inoculated plants had a significantly lower number of seeds per pod (17.3% fewer), seed weight per plant (19.3% lower), and the harvest index (18.5% smaller), but a 3.9% higher weight of aerial parts of plants. The effect of inoculation on yield characteristics of fenugreek was also observed in earlier studies. Inoculation significantly increased pericarps weight. Besides, on plants inoculated with *Rhizobium meliloti* and treated with 0.5 g N per pot, the number of pods and seeds per plant was determined at 2.30 and 15.39 (WIERZBOWSKA, ZUK-GOLASZEWSKA 2014). ALAGAWADI and GUAR (1988) noticed that inoculation of fenugreek with microbes increased the dry matter content. ERUM and BANO (2008) suggested that increased dry matter production by inoculated plants may be attributed to enhanced nodulation, higher nitrogen fixation rate and general improvement of root development. In comparison with the non-inoculated control plants, nodulation increased the dry matter percentage in inoculated plants (POI et al. 1991). ABDELGANI et al. (1999) concluded that inoculation of seeds used for sowing did not differentiate significantly the thousand seed weight, whereas AKHTAR and SIDDIQUI (2009) stated that inoculation with *Rhizobium* sp. caused a bigger increase in the plant growth and yield. Contrary results were shown by POI et al. (1991), who stated that inoculation of fenugreek seeds increased the plant's yield by as much as 94% compared to non-inoculated seeds. FORAWI and ELSHEIKH (1995) showed that the dry matter of shoots and roots of inoculated fenugreek plants of cv. Berber cultivated under salt stress increased by 31% and 29%, respectively, in comparison with untreated seeds.

Fenugreek is sensitive to drought, especially during flowering. Distribution of rainfall during the growing season has a reverse impact on yield. Fenugreek is a hydrophilic plant, which should be grown in areas with optimal rainfall or on irrigated land (ALHADI et al. 1999, HUSSEIN, EL-DEWINY 2011). The water deficit (30-35% of water capacity) in the soil under fenugreek determined the analyzed biometric traits (Table 1). Significant differences were observed in the plant height, number of pods per plant, seeds per pod, seeds weight per plant, aerial parts of a single plant and the harvest index. Only the weight of a thousand seeds was similar in both of the soil moisture variants. Soil water deficit caused reduction of the plant height by 15.5%, number of pods by 18.3%, number of seeds per pod by 20%, weight of seeds by 28%, aerial weight by 18.0% and the harvest index by 13.2%. In a study conducted by AKHALKATSI and LOSCH (2005), drought-exposed *Trigonella coerulea* plants produced a much lower seed mass per plant when compared to the control. Optimal moisture conditions during the growth and development of plants led to the formation of a higher number of seeds per plant. The cited studies confirm our results in that the weight of a thousand seeds is not significant. The effect of this trait in soybean exposed to water shortage reported by MASOUMI et al. (2011) was different. These authors showed that the thousand seed weight significantly decreased under more intense water stress in all the analyzed cultivars. Water deficit during the stage of flowering differentiated significantly seed yields of fenugreek. In our study, the harvest index was 18.51% in control and 16.07% under water deficit (Table 1). As shown by AKHALKATSI, LOSCH (2005), plants of *Trigonella coerulea* L. growing under water deficit were significantly shorter (90±9.5 cm) than the control or the plants watered twice a day. When there is water deficit in soil, plants produce a lower mass of seeds per plant compared to the control. In the present study, an increase in the soil's moisture content did not differentiate significantly the size and quality of fenugreek seeds in comparison with the control. The highest seed weight per plant (7.7 g) was obtained under control conditions; the seed weight per plant from fenugreek exposed to drought was 2.1 g and it reached 6.7 g from plants grown on soil with the highest moisture content (AKHALKATSI, LOSCH 2005). In another research, the correlation coefficient between seed yield obtained from arid land and from irrigated soil was positive and significant: $r = 0.54$, $p < 0.05$. This finding may be a starting point for demonstrating that fenugreek can quickly adapt itself to water deficit in soil (ACHARYA et al. 2006*b*).

The effects of K fertilization, inoculation and water deficit on the content of chemical composition are presented in Table 2. The content of crude protein was significantly higher in the variants fertilized with potassium with inoculated seeds and exposed to water deficit. With respect to potassium fertilization, the content of crude fat ranged from 51.5 in the control to 54.8 g kg^{-1} DM at the highest potassium dose. Among the analyzed minerals, the content of potassium was higher in response to potassium fertilization with the higher doses $K_{2.50}$ and $K_{3.75,}$ but the lowest content of potassium occurred under the water deficit conditions. Irrespective of the analyzed factor, the seeds contained similar concentrations of phosphorus (7.83), sodium (0.831), calcium (1.35) and magnesium (2.28). The protein content in seeds of fenugreek varied from 270.5 to 285.2 g kg^{-1} DM. The crude protein content increased together with an increasing rate of K fertilization. Our study demonstrated that incrementally higher doses of potassium (1.25, 2.50 and 3.75 g pot^{-1}) caused a significant increase in the content of crude protein in

Table 2

Chemical composition of fenugreek seeds

Factors	Crude protein (g kg $^{-1}$ DM)	Crude fat (g kg $^{-1}$ DM)	Macroelements (g kg^{-1} DM)				
			P	K	Na	Ca	Mg
K dose (g pot^{-1})							
K_0	270.5^b±3.24	51.55^a±1.95	7.84^a±0.17	15.32^a±0.41	0.820^a±0.17	1.34^a±0.14	2.11^a±0.14
$K_{1.25}$	279.2^a±2.35	53.91^{ab}±2.26	7.78^a±0.15	15.44^a±0.34	0.835^a±0.24	1.35^a±0.18	2.34^a±0.24
$K_{2.50}$	280.1^a±2.79	54.27^{ab}±2.03	7.79^a±0.27	16.16^b±0.38	0.902^a±0.25	1.40^a±0.20	2.28^a±0.21
$K_{3.75}$	285.2^a±6.75	54.82^b±2.12	7.90^a±0.17	16.20^b±0.49	0.755^a±0.23	1.30^a±0.19	2.38^a±0.22
Rhizobium meliloti							
Non-inoculated	275.9^a ±3.47	54.48^a±1.85	7.79^a±0.14	15.86^a±0.31	0.808^a±0.17	1.39^a±0.13	2.28^a±0.15
Inoculated	281.7^b±5.09	52.80^a±1.72	7.87^a±0.13	15.70^a±0.31	0.874^a±0.15	1.30^a±0.12	2.28^a±0.15
Water capacity (%)							
50-60%	272.6^a±2.88	55.75^b±1.42	7.78^a±0.14	16.09^a±0.33	0.830^a±0.12	1.41^a±0.15	2.28^a±0.17
30-35%	284.9^a±4.87	51.52^a±1.72	7.88^a±0.13	15.48^a±0.36	0.826^a±0.14	1.29^a±0.09	2.28^a±0.11

All values are the mean from six replicates ± standard deviation; *a*, *b* the same letter at the means point to a significant difference comparison to the control K_0. This parameter was higher by 3.2% (279.2 g kg^{-1} DM) to 5.4% (285.2 g kg^{-1} DM) for 1.25 and 3.75 g pot^{-1}, respectively.

Significant differences were observed in the potassium concentration depending on various potassium doses. Potassium in the doses of 1.25 and 2.50 g K pot^{-1} significantly increased the potassium content in seeds. The average content of potassium changed in the range from 15.32 to 15.44 g kg^{-1} DM in response to the lower fertilizer levels, up to 16.16 to 16.20 g kg^{-1} DM under the higher fertilization doses. Generally, the content of nitrogen, phosphorus and potassium in young plants is high. On the other hand, as the plant ages, the content of calcium and manganese usually increases while the concentration of phosphorus declines (Kinji, Rahdari 2012). It was proven that potassium is very mobile in plants; the element participates in several physiological processes and is responsible for the uptake of other nutrients. In addition, potassium affects the plant's vigour and ability to tolerate drought and disease (Sadanandan et al. 1998). In our study, the use of the strain *Rhizobium meliloti* for inoculation of seeds caused a significant increase in the protein content in seeds of growing plants, where it reached 281.7 g kg^{-1} DM, thus being 2.1% higher than in seeds of plants from non-inoculated seeds. The inoculation with *Rhizobium meliloti* slightly decreased the fat content in seeds. However, in a study by Abdelgani et al. (1999), inoculation with two *Rhizobium* strains significantly increased the crude fat content in seeds of two fenugreek varieties - Baladi and Rubatab (by 7.5 and 6.4%, respectively, compared to the control). In some other experiments reported by Hemavathy and Prabhakar (1989) and Rao and Sharma (1987), seeds of fenugreek contained 7.5%, and 7.9% of crude fat, respectively. Inoculation did not affect the accumulation of P, K, Na, Ca and Mg in fenugreek seeds. Similar results were obtainedby Abdelgani et al. (1999).

The water deficit increased the protein content but decreased the level of potassium in fenugreek seeds (Table 2). The protein content under water deficit (30-35% of water capacity) was significantly higher compared to plants grown under control conditions (50-60% water capacity), that is 284.9 and 272.6 g kg-1 DM, respectively. The crude fat content in seeds decreased by 7.5% in plants grown at water deficit compared to these with an optimum water supply. The data showed that water deficit during the flowering phase did not change the content of other macronutrients like P, Ca, Mg, and Na in fenugreek seeds (Table 2). Akhalkatsi and Losch (2005) showed that during drought seeds accumulate more phosphorus: 8.12 g in the control up to 14.27 g under water deficit conditions. The content of nitrogen and potassium was the same, independently of the soil's moisture. Hussein and El-Dewiny (2011) found that water stress caused a decrease in the content of N and P in fenugreek varieties, but K concentration was almost constant under water stress. Kinji and Rahdari (2012) stated that a relative water content increased in plants deficient in potassium 'compared to' the control. Whole fenugreek seeds contain 25.4 g 100 g^{-1} protein, 368 mg 100 g^{-1} P, 70.2 mg 100 g^{-1} Ca, 160 mg 100 g^{-1} Mg (Rao, Sharma 1987).

CONCLUSIONS

It has been shown that potassium fertilization and inoculation of seeds fenugreek had no significant effect on the plant's performance. The only trait influenced by these factors was the seed weight per plant; in response to higher doses of potassium fertilization, i.e. $K_{2.5}$ and $K_{3.75}$, its value was about 10% above the control K_0 and $K_{1.25}$, but 19.3% lower in treatments with inoculation versus plants grown from non-inoculated seeds. Soil water deficit depressed the plant height by 15.5%, number of pods by 18.3%, number of seeds per pod by 20%, weight of seeds by 28%, aboveground weight by 18.0% and the harvest index by 13.2%.

At water deficit, the content of chlorophyll decreased non-significantly from 57.8 SPAD units at 43 DAS to 52.4 and 54.0 SPAD units in the next phases (51 and 58 DAS), when 40% and 80% of pods had reached the final length. Besides, at 65 DAS there was less chlorophyll in plants from treatments with *Rhizobium meliloti*. The chlorophyll content in leaves depended more strongly on the moisture conditions and seed inoculation than on the different K doses. The analyzed factors slightly modified the content of macronutrients in fenugreek seeds. An increase in nitrogen and phosphorus in plants from inoculated seeds and grown under conditions of water deficit was connected with a reduction in seed yield.

Increasing doses of potassium (1.25, 2.50 and 3.75 g pot^{-1}) caused a significant increase of crude protein (by 3.2-5.4%) and potassium (by 7-8%) in seeds of fenugreek. In treatments with water deficit, the seed content of protein increased, while the content of potassium decreased. Using a strain of *Rhizobium meliloti* for seed inoculation caused a significant increase in the protein content in seeds of growing plants. This value was 281.7 g kg^{-1} DM, that is 2.1% higher than in seeds of plants from non-inoculated seeds.

The content of crude fat in treatments with potassium fertilization ranged from 51.5 in the control to 54.8 g kg^{-1} DM at the highest potassium dose. Under the water deficit conditions, there was more crude fat than under water stress-free conditions, and the inoculation with *Rhizobium meliloti* slightly decreased the fat content in seeds. In general, all agrochemical factors somewhat affected the accumulation of P, K, Na, Ca and Mg in fenugreek seeds.

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