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THE IMPACT OF NITROGEN FERTILIZATION AND *RHIZOBIUM* INOCULATION ON THE YIELD AND QUALITY OF *TRIGONELLA FOENUM-GRAECUM* L.

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Abstract

Fenugreek is one of the oldest known medicinal plants that have been used in traditional medicine in many parts of the world. The aim of this study was to determine the effects of nitrogen fertilization and *Rhizobium meliloti* inoculation on the yield, yield components and seed quality of fenugreek. The experiment was performed during two growing seasons. Chlorophyll content was determined with the use of the SPAD-502 chlorophyll meter. After harvest, biometric measurements were performed and the chemical composition of vegetative organs and seeds was determined in fenugreek plants. At 43 DAS, the chlorophyll content of control plant leaves was determined as 46 SPAD, and it decreased with plant growth. In treatments fertilized with nitrogen, the chlorophyll content of leaves increased from 48.5 ($N_{0.5}$) - 56.1 (N_1) SPAD (43 DAS) to 58.2 ($N_{0.5}$) - 60.6 (N_1) SPAD (58 DAS).Seed inoculation with *Rhizobium meliloti* was more highly correlated with seed quality than plant habitus and yield components. Inoculation decreased (by 11.5%) the crude fat content of fenugreek seeds, and increased phosphorus, calcium and sodium concentrations. Nitrogen fertilization significantly influenced morphological parameters and yield components, and it contributed to a significant increase in the total protein content of seeds.

Key words: Fenugreek, morphological parameters, nitrogen, quality, yield.

WPŁYW NAWOŻENIA AZOTEM I INOKULACJI *RHIZOBIUM* NA PLON I JAKOŚĆ *TRIGONELLA FOENUM-GRAECUM* L.

Abstrakt

Kozieradka pospolita jest jedną z najstarszych roślin leczniczych, była wykorzystywana w tradycyjnej medycynie w wielu częściach świata. Celem badań było określenie wpływu nawoże-

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nia azotem i inokulacji *Rhizobium meliloti* na plonowanie i zawartość składników w nasionach kozieradki. Eksperyment przeprowadzono w dwóch sezonach wegetacyjnych. Zawartość chlorofilu określono za pomocą chlorofilomierza SPAD-502. Po zbiorze wykonano pomiary biometryczne i oznaczono skład chemiczny nasion i wegetatywnych części roślin. W 43. dniu po siewie (DAS) zawartość chlorofilu w liściach roślin kontrolnych wynosiła 46 SPAD i zmniejszała się wraz ze wzrostem roślin. Na obiektach nawożonych azotem zawartość chlorofilu w liściach zwiększała się z 48,5 ($N_{0.5}$) - 56,1 (N_1) SPAD (43 DAS) do 58,2($N_{0.5}$) - 60,6(N_1) SPAD (58 DAS). Inokulacja materiału siewnego *Rhizobium meliloti* w większym stopniu wpływała na jakość nasion niż na pokrój roślin i komponenty plonu, zmniejszyła (do 11,5%) zawartość tłuszczu surowego i zwiększyła zawartość fosforu, wapnia i sodu w nasionach kozieradki. Nawożenie azotem istotnie różnicowało parametry morfologiczne roślin i komponenty plonu, co skutkowało istotnym wzrostem zawartości białka w nasionach.

Słowa kluczowe: kozieradka pospolita, cechy morfologiczne, azot, jakość, plon.

INTRODUCTION

Fenugreek (*Trigonella foenum-greecum* L.) is an annual herb of the family *Fabaceae*, which is commonly encountered in Southwest Asia and the Mediterranean region. Fenugreek is cultivated across Western Europe and China for its aromatic seeds, and it is grown for fodder in selected parts of Europe and Northern Africa (Kinji, Rhadi 2012, Kołodziej, Zejdan 2000, Manisha, Angoorabala 2013). This herbaceous plant is widely used for medicinal purposes, and its seeds are rich in protein, macronutrients, micronutrients, steroids, saponins, mineral salts and vitamins (Madar, Stark 2002). Amin et al. (2005) demonstrated that fenugreek extracts effectively prevent and inhibit the development of breast cancer.

Rhizobium meliloti are nitrogen-fixing bacteria that lower the plants' demand for nitrogen fertilization and thus contribute to environmental protection (Abdelgani et al.1999). Nitrogen-fixing bacteria supply soil with nitrogen and other nutrients. Soil-dwelling microorganisms form a symbiotic relationship with fenugreek plants (Provorov, Tikhonovich 2003). The emergence of new production systems contributes to the popularity of fenugreek. The climatic, soil and nutritional requirements of fenugreek plants have been researched by numerous authors (Kirshnayya, Manjari 1995, Kinji, Rhadi 2012). In fenugreek, nitrogen fertilization contributes to intensified growth, deferred maturation, leaf health, stem development and luxuriant foliage with the desired dark-green color. Efforts are being made to improve the yield and qualitative composition of fenugreek to make it amenable to large -scale production. The aim of this study was to investigate the effects of nitrogen fertilization and *Rhizobium meliloti* inoculation on the yield, yield components and seed quality of fenugreek.

MATERIAL AND METHODS

A pot experiment in a completely randomized design with six replicates was performed in a greenhouse of the University of Warmia and Mazury in Olsztyn, Poland (latitude 53° 46'N, longitude 20° 25'E), during two growing seasons Fenugreek plants were grown in modified Kick-Braukmann pots filled with 10 kg of light soil developed from heavy loamy sand. The soil was slightly acidic (pH_{KCl} = 5.5) and abundant in available nutrients (P – 107, K – 124 and Mg – 19 mg kg^{-1} of soil). Greenhouse temperature ranged from 11.0°C to 22.8°C in the first season of the study and from 8.2°C to C 32.3°C in the second season. Relative humidity in the greenhouse ranged from 40% to 95%. The following treatments were applied: inoculation – (1) non-inoculated (control), (2) inoculation with *Rhizobium meliloti*; nitrogen fertilization (urea) rates: N_0 (control), $N_{0.5}$ and $N_{1.0}$ g pot^{-1}. All plants were fertilized with magnesium ($MgSO_4$ $7H_2O$) at 0.3 g per pot, phosphorus (KH_2PO_4) at 0.5 g per pot, and potassium (KH_2PO_4+K_2SO_4) at 2.5 g per pot. Regular pest control treatments were applied throughout the experiment.

The chlorophyll content of leaves was measured at 7-day intervals with the use of the SPAD-502 chlorophyll meter (Minolta, Japan). The samples were collected at 43 DAS (Days After Sowing), flowering, 51 DAS (40% of pods with final length), 58 DAS (80% of pods with final length), at the beginning of seed ripening and filling the pod cavity (65 DAS).

Harvesting took place at the full maturation stage. Every plant (12) from each pot was sampled, and the following parameters were determined: plant height, weight of aerial parts in individual plants, number of pods per plant, thousand seed weight, seed weight per plant. Plant material was mineralized in concentrated sulfuric acid (VI) with the use of hydrogen dioxide as an oxidant. Total nitrogen content was determined calorimetrically with hypochlorite. A conversion factor of 6.25 was applied to determine crude protein content by Soxhlet extraction. Phosphorus content was determined by the vanadium-molybdenum method, potassium, calcium and sodium levels were determined by flame spectrophotometry (AES), and magnesium concentrations– by atomic absorption spectrometry (AAS).

The results were statistically analyzed in the Statistica v9® application. The statistical significance of the factors was evaluated by Anova for a completely randomized design. The treatments were compared by Tukey's HSD at $P<0.01$. All results were expressed as mean values for the two-season experimental period.

RESULTS AND DISCUSSION

Seed inoculation had an insignificant influence on the chlorophyll content of fenugreek leaves (data not shown). Nitrogen fertilization contributed to significant differences in chlorophyll concentrations in the leaves of the analyzed species (Figure 1). At 43 DAS, chlorophyll concentrations were determined at 56.1 and 48.5 SPAD for fertilizer rates of 0.5 and 1.0 g N per pot, respectively. In control plants (N_0), chlorophyll content was significantly

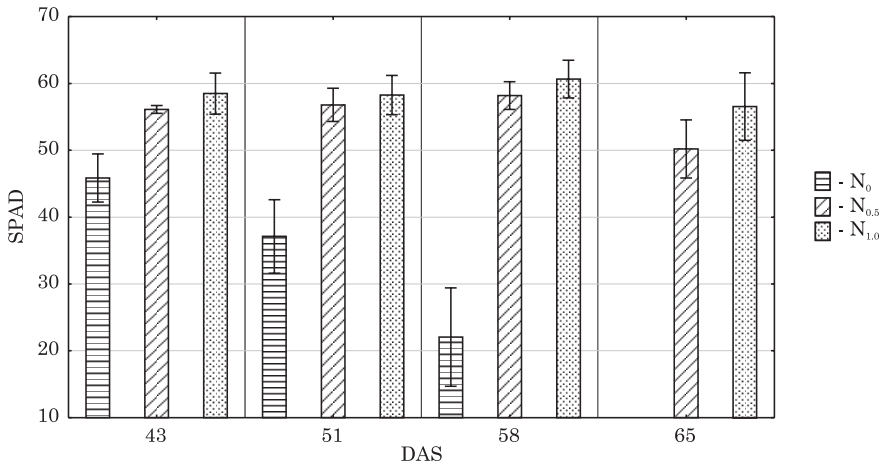

Fig. 1. Leaf chlorophyll content, subject to the applied nitrogen rate (mean values and confidence intervals, $P<0.01$ HSD = 6.55)

lower at 46 SPAD. On successive measurement days, chlorophyll concentrations continued to decrease in control plants, but remained fairly constant in leaves fertilized with nitrogen, because nitrogen is a structural element of chlorophyll and protein molecules, and thereby affects formation of chloroplasts and accumulation of chlorophyll in them (DAUGHTRY et al. 2000). At 58 DAS, control leaves contained significantly less chlorophyll (approximately 22 SPAD) than the leaves fertilized with nitrogen ($N_{0.5}$- 58.2 SPAD and $N_{1.0}$ – 60.6 SPAD). In control treatments, premature withering (65 DAS) significantly influenced plant habitus, the dry matter content of the analyzed vegetative organs and yield components (Figure 1, Table 1). N level also affected chlorophyll content, N concentration at anthesis, protein, and oil yield of Safflower *Carthamus tinctorius* L. (DORDAS, SIOULAS 2008). BOJOVIC, STOJANOVIC (2005), FRITSCHI, RAY (2007) and HOULES et al. (2007) demonstrated a positive correlation between N fertilization rates and leaf chlorophyll content is many plant species.

Seed inoculation and increasing nitrogen rates led to significant differences in selected morphological parameters, the weight of selected plant organs and yield components (Table 1). Inoculated plants were 9.5% shorter

Table 1

Selected morphometric parameters and yield components of fenugreek plants

Specification	Plant height (cm)	Number of		Weight of (g)			Number of seeds	Thousand seed weight (g)	Seed weight (g)
		branches	pods	stems	leaves	pericarps			
Non-inoculated									
$N_{0.0}$	25.90	0.692	1.94	0.140	0.157	0.112	16.04	7.70	0.123
$N_{0.5}$	48.95	1.163	1.97	0.933	0.502	0.105	11.50	14.39	0.170
$N_{1.0}$	41.77	1.567	2.36	0.857	0.550	0.128	16.36	12.95	0.211
Mean	38.87	1.141	2.09	0.643	0.403	0.115	14.63	11.68	0.155
Inoculated									
$N_{0.0}$	26.20	0.250	1.95	0.143	0.168	0.127	13.34	7.55	0.101
$N_{0.5}$	42.57	1.443	2.30	0.740	0.513	0.137	15.39	12.53	0.193
$N_{1.0}$	36.87	1.553	1.75	0.803	0.547	0.140	13.51	14.50	0.163
Mean	35.21	1.082	2.00	0.562	0.409	0.134	14.08	11.53	0.172
Mean for dose									
$N_{0.0}$	26.05	0.471	1.95	0.142	0.163	0.119	14.69	7.62	0.112
$N_{0.5}$	45.76	1.303	2.13	0.837	0.508	0.121	13.45	13.46	0.182
$N_{1.0}$	39.32	1.560	2.05	0.830	0.548	0.134	14.93	13.72	0.187
HSD $P<0.01$ for:									
N rate	1.39	0.352		0.052				0.48	0.029
Inoculated	1.13			0.043	0.059	0.012			
Interaction	1.96		0.38	0.074			2.18	0.68	

than control, which contributed to lower stem weight. Inoculation significantly increased pericarps weight. The highest number of pods (2.36) and seeds (16.36) per plant was reported in non-inoculated plants fertilized with 1 g N per pot. In 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, respectively (Table 1). The application of 0.5 and 1.0 g N per pot significantly influenced morphological parameters (plant height, number of branches, stem weight) and yield components (thousand seed weight, seed weight). The tallest plants (45.76 cm) were noted in treatments fertilized with 0.5 g N per pot. The number of branches increased significantly with a rise in nitrogen application rates. Stem branching was not observed in control plants (N_0) – Table 1. The application of 0.5 g N per pot increased leaf weight 3-fold and stem weight nearly 6-fold in comparison with unfertilized plants. A similar relationship was observed in a field experiment (Banafar et al. 1995) were the application of 60 N kg ha^{-1} and 40 P kg ha^{-1} significantly increased the weight of fenugreek leaves. In the study of (Daramola et al. 2013) increase an stem dry matter of other legumes crop (cowpea) accumulation was observed as N rates increased. Besides it, the content of macronu-

Table 2

Macronutrient content of aboveground parts of fenugreek (g kg^{-1} d.m.)

Specification	Stems						Leaves						Pericarps					
	N	P	K	Na	Ca	Mg	N	P	K	Na	Ca	Mg	N	P	K	Na	Ca	Mg
Non-inoculated																		
$N_{0.0}$	8.59	2.70	32.74	3.85	11.32	2.85	16.22	7.53	64.15	5.40	36.42	4.93	10.37	4.70	35.15	3.87	23.92	5.13
$N_{0.5}$	7.60	2.91	29.50	4.11	11.41	2.78	23.20	6.87	54.98	6.34	42.02	5.64	9.72	5.33	38.82	4.29	21.40	4.96
$N_{1.0}$	10.26	2.91	33.71	4.29	10.22	3.06	27.22	7.70	48.91	5.81	29.69	5.78	10.14	7.40	37.52	5.09	16.20	5.94
Mean	8.82	2.84	31.98	4.08	10.99	2.90	22.21	7.37	56.02	5.85	36.05	5.45	10.08	5.81	37.16	4.41	20.51	5.34
Inoculated																		
$N_{0.0}$	4.87	2.62	19.43	5.03	9.45	3.95	16.00	7.51	33.15	6.78	23.30	7.13	10.00	6.15	30.85	5.67	13.61	5.60
$N_{0.5}$	5.14	2.16	27.74	5.45	8.56	3.58	19.87	7.22	45.55	6.74	26.30	8.74	12.19	8.04	32.37	5.56	10.90	6.21
$N_{1.0}$	7.43	1.75	11.49	5.22	8.06	3.58	23.99	6.70	33.38	7.27	29.16	8.16	14.24	5.81	22.64	4.98	10.83	4.56
Mean	5.82	2.18	19.55	5.23	8.69	3.71	19.95	7.14	37.36	6.93	26.25	8.01	12.14	6.67	28.62	5.40	11.78	5.46
Mean for N dose																		
$N_{0.0}$	6.73	2.66	15.52	5.22	8.37	3.64	16.11	7.52	48.65	6.09	29.86	6.03	10.18	5.42	33.00	4.77	18.76	5.36
$N_{0.5}$	6.37	2.54	28.62	5.23	8.53	3.67	21.54	7.05	50.27	6.54	34.16	7.19	10.96	6.68	35.60	4.92	16.15	5.59
$N_{1.0}$	8.85	2.33	22.60	5.23	8.45	3.66	25.60	7.20	41.14	6.54	29.43	6.97	12.19	6.60	30.08	5.03	13.51	5.25
HSD α=0.01 for:																		
N dose	0.94				0.92		1.03		5.69			0.82	0.56				3.88	
Inoculated	0.77	0.26	7.86	0.54	0.75	0.43	0.84		5.36		6.81	0.67	0.46		4.20	0.59	3.17	
Interaction		0.45					1.45		9.28				0.80			1.01		

trients in green forage of red clover dependent on from phase development (Zuk-Golaszewska et al. 2010). Nitrogen fertilization contributed to a significant increase in thousand seed weight and seed weight (by 76.6% and 60%, respectively) compared with the control one. In the study of Detroja et al. (1996), the application of 30 kg nitrogen per ha significantly influenced thousand seed weight (14.32 g) in comparison with unfertilized fenugreek plants (11.38 g). Sharma (2000) reported the highest number of branches (4.4) and pods per plant (64), and the highest seed yield per plot (725 g) and per ha (18.2 g) in treatments fertilized with 60 kg per ha in comparison with control. In a study by Datta et al. (2005), the yield of fenugreek seeds also increased significantly at fertilization rates of 0, 15, 25 and 45 kg N ha^{-1}. Seed yield increased to 1.07 t ha^{-1} in treatments fertilized with 45 kg N ha^{-1} in comparison with control (0.66 t ha^{-1}). Chaudhary (1999) demonstrated that the application of 40 kg nitrogen per ha and 40 kg phosphorus per ha contributed to an increase in the thousand seed weight (12.11 g) of fenugreek plants relative to control (11.76 g). In the other research application of organic manures and *Rhizobium* inoculation enhanced the seed yield (Naimuddin et al. 2014). In this study, an increase in nitrogen fertilization rates ($N_{1.0}$) had no significant effect on the mentioned parameters (Table 1).

The nitrogen content of vegetative organs in fenugreek plants was correlated with both seed inoculation and fertilization rate (Table 2). The highest nitrogen concentrations were reported in the stems and leaves of non-inoculated plants fertilized with 1.0 g N per pot (10.26 and 27.22 g kg^{-1} d.m., respectively), which contributed to a higher nitrogen content of pods (10.14 g kg^{-1} d.m.). The vegetative organs of inoculated plants contained less potassium and calcium, but more sodium and magnesium (Table 2). Seed inoculation with *Rhizobium meliloti* increased the nitrogen content of pods by 41.3%. The highest nitrogen concentrations (14.24 g kg^{-1} d.m.) were determined in the pods of plants that were inoculated and fertilized with a high nitrogen rate.

Seed inoculation with *Rhizobium meliloti* significantly lowered (by 11.5%) crude fat content and increased phosphorus, calcium and sodium concentrations (by 6.1%, 28.2% and over 130%, respectively) in fenugreek seeds (Table 3). In other study reported by Hemavathy and Prabhakar, (1989) seeds of fenugreek contained 7.5% crude fat. Potassium and magnesium levels remained fairly constant regardless of inoculation and nitrogen fertilization. The application of nitrogen contributed to a significant increase in the total protein content of fenugreek seeds (Table 3). The highest total protein content (333.9 g kg^{-1} d.m.) was reported in the seeds of inoculated plants supplied with the highest nitrogen rate. In the research Gendy (2013) *Rhizobium* inoculation increased nitrogen, carbohydrates, potassium and protein percentages of fenugreek.

Table 3

Total protein, crude fat and macronutrient concentrations in fenugreek seeds

Specification	Total protein	Crude fat	P	K	Na	Ca	Mg
	(g kg^{-1} d.m.)						
Non-inoculated							
$N_{0.0}$	241.5	57.10	7.65	23.20	0.442	0.96	2.74
$N_{0.5}$	311.5	56.30	7.73	16.07	0.580	1.12	2.32
$N_{1.0}$	315.1	53.10	8.03	14.21	0.866	1.42	2.25
Mean	289.4	55.50	7.80	17.83	0.629	1.17	2.44
Inoculated							
$N_{0.0}$	222.1	49.30	8.23	22.68	1.842	1.41	2.45
$N_{0.5}$	295.9	50.60	8.08	15.63	1.448	1.42	2.13
$N_{1.0}$	333.9	47.30	8.55	14.18	1.111	1.68	2.15
Mean	283.9	49.07	8.28	17.50	1.467	1.50	2.24
Mean for N rate							
$N_{0.0}$	231.8	53.20	7.94	22.94	1.14	1.19	2.60
$N_{0.5}$	303.7	53.45	7.90	15.85	1.01	1.27	2.23
$N_{1.0}$	324.5	50.20	8.29	14.20	0.99	1.55	2.20
HSD P=0.01 for:							
N rate	13.40						
Inoculated		3.59	0.34		0.25	0.23	
Interaction	18.95				0.43		

CONCLUSIONS

1. Nitrogen fertilization significantly affected the chlorophyll content of fenugreek leaves. In the leaves of plants fertilized with nitrogen, chlorophyll concentrations were determined in the range of 48.5 - 56.1 SPAD (43 DAS) to 58.2 - 60.6 SPAD (58 DAS), subject to the applied nitrogen rate. At 43 DAS, the chlorophyll content of control plant leaves was determined at 46 SPAD, and it decreased along plant growth.

2. Nitrogen fertilization had a greater influence on the weight of vegetative organs and the seed yield of fenugreek than seed inoculation. Fertilization improved morphological parameters (plant height, number of branches, stem weight), yield components (thousand seed weight, seed weight), and total protein content.

3. Seed inoculation with *Rhizobium meliloti* had a greater effect on seed quality than on plant habitus or yield components. Inoculation significantly decreased the crude fat content of fenugreek seeds, but it increased phosphorus, calcium and sodium concentrations.

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