Effect of Organic and Mineral Fertilizers on Bird's-Foot Trefoil (*Lotus corniculatus* L.) Nodular Activity

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Abstract
Fertilising legume crops has been a very controversial cultivation technology measure because of the secondary effects on nitrogen-fixing ability. Numerous research have shown, as mentioned in literature, that an increase of the concentration of ammonia nitrate causes a decrease of the nodular activity in legumes and that the presence of $NO^{-}3$ ions for longer periods of time inhibit the development of nodules and the activity of nitrogenase. This paper presents the effect of the interactions between organic and mineral fertilisers on nodular activity in bird's-foot trefoil in a bifactorial trial setting.

Keywords: fertilising, *Lotus corniculatus* L., nitrogen fixing, nodular activity.

1. Introduction

The most important characteristic of the plant-bacteria symbiosis consists in their adaptation to an occasional lack of nutrients. In most cases, thus process also corresponds to the plant physiological processes. Nitrogen is indispensable in plants' though it is an outstanding element of the biosphere, nitrogen supply is limited: on one hand, plants do not have specific enzymes (nitrogenase) to fix molecular nitrogen, on the other hand, they can assimilate only soluble forms of inorganic nitrogen. Most soils are characterised by a lack of assailable nitrogen, phosphorus and some essential minerals. Many plants do get them by cooperating with certain symbiotic organisms that can fix molecular nitrogen or can mobilise insoluble nitrogen, phosphorus, etc., from the soil. The effect of the plant-bacterium interaction can also be pointed out through the nodosity-forming ability of the legumes [1-6]. This paper approaches the effects of applying organic and mineral fertilisers on nodosity-forming ability of bird's-foot trefoil (*Lotus corniculatus* L.).

2. Materials and methods

Research was carried out during 2013-2014 on the experimental field of the Pasture and Fodder Plant Research Centre of the Banat's University of Agricultural Science and Veterinary Medicine "King Michael I of Romania" from Timisoara, Romania. To do so, we set a bifactorial trial (3x5) with the following factors:

- **A.** organic fertilisers (**a1** = no fertilisation, **a2** = 20t/ha animal manure, **a3** = 40t/ha animal manure);
- **B.** mineral fertilisers (**b1** = no fertilisation, **b2** = ammonium nitrate N_{100}, **b3** = potassium nitrate N_{100}, **b4** = complexes 13:10:15 N_{100}, **b5** = superphosphate P_{100}).

The 15 trial variants had three replicates after the subdivided plot method. Fertilisation with animal manure was done in the fall of 2013 (November) by incorporating it in the soil during the basic ploughing. Fertilisation with mineral fertilisers

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was done every vegetation year, in early spring, at the beginning of vegetation in bird's-foot trefoil.
The biological material used in sowing was the native bird's-foot trefoil (*Lotus corniculatus* L.) cultivar Dragotim. Before sowing, we checked the seed germination ability and established the sowing rate depending on it. In the second half of March, we passed the ring borrower and then we sowed. During the trial, we made observations, measurements and analyses regarding the growth of the root system (the root/plant volume) and the symbiotic nodosities on the root system (number of nodosities/plant, nodosity/plant volume). These measurements were made in the second vegetation year, before budding, and before first mowing.
To make quantitative measurements, we used a digital scale of the KERN ABS type. We also took digital photos with a SONY – Cyber-shot, 7.2 mega pixels camera; we then archived the photos digitally for each plant apart (10 plants/variant). The data thus obtained were statistically processed through the variance analysis/F test, STUDENT test, DUNCAN test, and linear/square and multiple linear/square correlations.

3. Results and discussion

Applying organic fertilisers (animal manure) influenced positively the growth and development of bird's-foot trefoil plants at both root system and aerial vegetative system levels in both treatment variants. Data in Table 1 below show an increase of 55% of the plant weight (root system and aerial vegetative system) compared to the control variant in the variant treated with 20 t/ha animal manure and with 76% in the variant fertilised with 40 t/ha animal manure.

(a) (b) (a+b)

Figure 1. Nodosities on the root system of bird's-foot trefoil after applying organic (a) and mineral (b) fertilisers.

Table 1. Influence of organic fertilisation on the volume of bird's-foot trefoil (*Lotus corniculatus* L.) plants

Treatment variants	Plant weight (g)	Aerial vegetative system weight (g)	Root system weight (g)
a1 - Control	**4.59**	**1.96(100%)**	**2.63**
a2 - 20 t/ha animal manure	7.15*(155%)	3.36***(171%)	3.79**(144%)
a3 - 20 t/ha animal manure	8.09***(176%)	3.71***(189%)	4.38***(166%)
DL5%	1.4	0.62	0.75

Nodosity forming ability materialised in the number of nodosities/plant and the volume of the root system differentiates depending on the amount of animal manure applied on bird's-foot trefoil (Table 2). Compared to the control variant (no treatment), in the variants treated with 20 t/ha animal manure there was a diminution of both nodosity number and nodosity weight. The strongest diminution was when applying 40 t/ha of animal manure: the number of nodosities decreased with 38% and the weight of the nodosities decreased with 50%.

Table 2. Influence of organic fertilisation on the nodosity formation ability in bird's-foot trefoil (*Lotus corniculatus* L.) plants

Treatment variants	Number of nodosities/plant	Weight of nodosities (mg)	Number of nodosities/plant : root system weight
a1 - Control	**101.14(100%)**	**41.53(100%)**	**38.46(100%)**
a2 - 20 t/ha animal manure	84.51°°(84%)	28.13°°°(68%)	22.30 (58%)
a3 - 20 t/ha animal manure	62.54°°°(62%)	20.6°°°(50%)	14.28(37%)
DL5%	10.17	6.01	8.0

This decrease can be determined by the content of total nitrogen of the 40 t/ha (about 190 kg/ha) of animal manure that can influence the nitrogen fixation process. The ratio between the number of nodosities/plant and the root weight is lower than that of the control: 40% less in the variant treated with 20 t/ha animal manure and 63% in the variant treated with 40 t/ha animal manure (Table 2). Applying chemical fertilisers alone influenced positively the growth and development and the nodosity formation ability in bird's-foot trefoil. Of the four variants of fertilisation, treating with ammonium nitrate caused the lowest growth (insignificant) compared to the variant treated with complex fertilisers (N:P:K), where there was the highest growth (statistically significant) (Tables 3 and 4).

Table 3. Influence of organic fertilisation on vegetal weight in bird's-foot trefoil (*Lotus corniculatus* L.) plants

Treatment variants	Plant weight (g)	Aerial vegetative system weight (g)	Root system weight (g)
b1 - Control	**3.60(100%)**	**1.43(100%)**	**2.17(100%)**
b2 - ammonium nitrate	4.33(120%)	1.74(121%)	2.59(119%)
b3 - potassium nitrate	6.04**(167%)	2.38*(166%)	3.66**(168%)
b4 - N:P:K	7.43***(206%)	2.87***(200%)	4.56***(210%)
b5 - superphosphate	6.67***(185%)	2.55**(178%)	4.12***(189%)
DL5% (g/plant)	1.53	0.83	0.87

Table 4. Influence of organic fertilisation on nodosity formation ability in bird's-foot trefoil (*Lotus corniculatus* L.) plants

Treatment variants	Number of nodosities/plant	Weight of nodosities/plant (mg)	Number of nodosities/plant : root system weight
b1 - Control	**46.19(100%)**	**11.26(100%)**	**21.28(100%)**
b2 - ammonium nitrate	54.09(117%)	26.03**(231%)	20.88(98%)
b3 - potassium nitrate	106.05***(230%)	55.86***(496%)	29.09(137%)
b4 - N:P:K	125.17***(271%)	39.08***(347%)	27.44(130%)
b5 - superphosphate	82.17***(178%)	18.21(162%)	19.94(94%)
DL5% (g/plant)	11.02	10.01	7.2

The number of nodosities/plant was, in the control variant, 46.19, i.e. an increase of only 17% in the variant treated with ammonium nitrate and 2-3 times in the other variants (130% when treating with potassium nitrate, 171% when treating with complex fertilisers and 78% when treating with superphosphate).

Among variants of chemical fertilisation, we noted the treatment with potassium nitrate when there was the highest ratio between the number of nodosities and the weight of the root system (29.09, i.e. 37% more than in the control variant), while in both variants of treatment (ammonium nitrate and superphosphate) the ratio was about 20, as in the control variant. The presence of K and P in the chemical composition of the two fertilisers can be a positive factor favouring the growth of the nodosity formation ability in this legume.

Data presented in Tables 5 and 6 show the combined effect of applying organic and chemical fertilisers on the growth and development of the plants of bird's-foot trefoil,

as well as on their nodosity formation ability. As for the weight of the plants of bird's-foot trefoil, there were significant growths only in the combinations between the rate of 20 t/ha animal manure and complex fertilisers (N:P:K) or with superphosphate (Table 5).

Table 5. Combined influence of organic and mineral fertilisation on the growth and development of bird's-foot trefoil (*Lotus corniculatus* L.) plants

Treatment variant		Plant weight (g)	Aerial system weight (g)	Root system weight (g)
a1 – no organic fertilisation	b1 - Control	**3.67(100%)**	**1.56(100%)**	**2.11(100%)**
	b2 - ammonium nitrate	5.22(142%)	2.47(158%)	2.75(130%)
	b3 - potassium nitrate	6.19(168%)	2.78(178%)	3.41(161%)
	b4 - N:P:K (13:10:15)	6.79*(185%)	3.13*(200%)	3.66*(173%)
	b5 - superphosphate	6.32*(172%)	2.92(187%)	3.40(161%)
a2 – 20 t/ha animal manure	b1 - Control	**3.98(100%)**	**1.72(100%)**	**2.26(100%)**
	b2 - ammonium nitrate	5(126%)	2.55(148%)	2.45(108%)
	b3 - potassium nitrate	6.52(164%)	3.01(175%)	3.51(155%)
	b4 - N:P:K (13:10:15)	7.01*(176%)	3.44*(92%)	3.57(158%)
	b5 - superphosphate	6.65*(167%)	3.17*(184%)	3.48(154%)
A3 – 40 t/ha animal manure	b1 - Control	**4.42(100%)**	**1.88(100%)**	**2.59(100%)**
	b2 - ammonium nitrate	4.79(108%)	2.46(131%)	2.33(90%)
	b3 - potassium nitrate	6.6(149%)	3.22(171%)	3.38(131%)
	b4 - N:P:K (13:10:15)	7.11*(161%)	3.52*(187%)	3.59(139%)
	b5 - superphosphate	6.78(153%)	3.38*(180%)	3.4(131%)
DL5%		2.65	1.44	1.51

Of the 15 combinations of fertilisations none was above the fertilisation with chemical fertilisers alone. The same was in the nodosity formation ability and in the ratio between the number of nodosities/plant and the root system weight (Table 6). Our findings regarding effect of organic and mineral fertilizers on bird's-foot trefoil (*Lotus corniculatus* L.) nodular activity are supported by other authors reporting comparable results in other plants such as arabidopsis or soybean [7,8].

Table 6. Combined influence of organic and mineral fertilisation on nodosity formation ability in bird's-foot trefoil (*Lotus corniculatus* L.) plants

Treatment variant		Nodosities/ plant (N)	Weight of nodosities/ plant (g)	Nodosities/ plant : weight of nodosities/plant
a1 – no organic fertilisation	b1 - Control	**53.86(100%)**	**7.68(100%)**	**25.52(100%)**
	b2 - ammonium nitrate	52.36(97%)	35.95**(468%)	19.04(75%)
	b3 - potassium nitrate	123.14***(229%)	51.51***(671%)	36.11(141%)
	b4 - N:P:K (13:10:15)	172.00***(319%)	68.97***(898%)	46.99(184%)
	b5 - superphosphate	104.36***(194%)	43.56***(567%)	30.69(120%)
a2 – 20 t/ha animal manure	b1 - Control	46.71(87%)	16.59(216%)	20.67(81%)
	b2 - ammonium nitrate	71.57(133%)	38.59***(502%)	29.21(114%)
	b3 - potassium nitrate	99.57***(185%)	43.88***(571,4%)	28.36(111%)
	b4 - N:P:K (13:10:15)	127.57***(237%)	38.69(504%)	35.73(140%)
	b5 - superphosphate	77.14*(143%)	2.89***(38%)	22.16(87%)
A3 – 40 t/ha animal manure	b1 - Control	38.00(71%)	9.52(123,9%)	14.67(57%)
	b2 - ammonium nitrate	38.36(71%)	3.55(46%)	16.46(65%)
	b3 - potassium nitrate	95.43***(177%)	72.18(940%)	28.23(111%)
	b4 - N:P:K (13:10:15)	75.93*(141%)	9.57***(124,6%)	21.15(83%)
	b5 - superphosphate	65.00(121%)	8.17(106%)	19.11(75%)
DL5%		19.1	17.35	12.5

4. Conclusions

Organic fertilisation, particularly when applying 40t/ha animal manure, had a negative influence of nodosity formation in bird's-foot trefoil, when the root system of the plants increased significantly compared to the control variant.

Chemical fertilisation, except for the fertilisation with ammonium nitrate, favoured the nodosity formation ability: there was a twofold increase of the number of nodosities/plant in the variants treated with potassium nitrate, complex fertilisers or superphosphate.

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