

## Effect of Mineral Fertilization and Manure on Some Characteristics in Alfalfa (*Medicago sativa* L.)

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**Abstract:** Field trial was carried out at the Institute of Forage Crops, Pleven, Bulgaria (2000–2003) to study the effect of mineral fertilization and manure on nitrate reductase activity, plastid pigments content and dry root mass in alfalfa. The experiment was carried out on slightly leached chernozem soil subtype and no irrigation. Mineral fertilizer (ammonium nitrate) and manure (well rotted cattle manure) at the doses of 70, 140 and 210 kg N ha<sup>-1</sup> were applied. A tendency for increase of nitrate reductase activity in leaves compared to unfertilized control and decrease in roots regardless of dose for the both type of fertilizers was found. By increasing the dose of mineral fertilizer nitrate reductase activity in leaves and stems decreased and when manure was applied the values were similar. Mineral fertilizer and manure decreased the total chlorophyll content (chlorophyll a+b) and the decrease was less at high doses. Content of carotenoids increased for all treatments. Dry root mass formed after manure fertilization was from 25.2 to 51.7% greater than that after mineral.

**Key words:** Mineral fertilizer • Manure • Alfalfa • Nitrate reductase • Plastid pigments • Root mass

### INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the main source of proteinous forage and the most used legume crop in Bulgaria [1, 2]. The question for nitrogen fertilization of alfalfa is debatable in the scientific literature. According to some authors it is responsive of fertilization regardless its nitrogen fixing ability which allows for the provision of 40 to 70% from nitrogen [3-7]. According to other researchers mineral nitrogen inhibits the growth and nodulation of alfalfa [8-11]. Given that legumes used mineral nitrogen at the beginning the vegetation, but the maximum of symbiotic nitrogen fixation is the budding – beginning of flowering stage, it is necessary to apply nitrogen fertilizers [12-14].

From 30 to 50% from the applied mineral nitrogen is lost as elementary nitrogen in the atmosphere which strongly decreased the efficacy of mineral fertilizers [15]. The percent of losses of mineral nitrogen released from manure is significantly lower, which practically increased their efficiency [16, 17].

The question of fertilizing of alfalfa with manure is poorly studied but interest in organic fertilization increases steadily and it is seen as an alternative to intensive farming [5, 17-20].

Nitrate reductase is one of the key enzymes in nitrogen assimilation of legumes and the first enzyme in the chain in the recovery of nitrate [21, 22]. Source of energy and electrons for the functioning of both – nitrogenase and nitrate reductase, is plant photosynthesis. Changes in photosynthetic activity led to significant changes in the accumulation of recovered compounds, involved in the process of nitrogen fixation and reduction of nitrates [23-25].

In this work we aimed to study the effect of mineral fertilization and manure on the activity of nitrate reductase, plastid pigments content and quantity of dry root mass in alfalfa.

### MATERIALS AND METHODS

Field experiment was carried out at the Institute of Forage Crops, Pleven, Bulgaria (2000–2003) under no irrigation and soil subtype slightly leached chernozem. Block method was used, 4 replications and plot size of 10 m<sup>2</sup>. The next treatments were tested: 1. Control - N<sub>0</sub>P<sub>300</sub>K<sub>150</sub>; 2. MN<sub>70</sub>P<sub>300</sub>K<sub>150</sub>; 3. MN<sub>140</sub>P<sub>300</sub>K<sub>150</sub>; 4. MN<sub>210</sub>P<sub>300</sub>K<sub>150</sub>; 5. ON<sub>70</sub>P<sub>300</sub>K<sub>150</sub>; 6. ON<sub>140</sub>P<sub>300</sub>K<sub>150</sub>; 7. ON<sub>210</sub>P<sub>300</sub>K<sub>150</sub>. For mineral fertilizer ammonium nitrate was used (MN), for organic – well rotted cattle manure (ON).

Mineral nitrogen concentrations were calculated on the basis of the quantity of ammonium and nitrate nitrogen into the soil and mineral fertilizer. When applied manure we used the mineralization coefficients on the basis of total nitrogen (<http://www.ohioline.osu.edu>; [www.agriculture.purdue.edu](http://www.agriculture.purdue.edu)). [26, 27].

Sowing was made mechanized with sowing rate of alfalfa (cv. Victoria) 30 kg ha<sup>-1</sup> and interspaces of 11.5 cm. One cut for forage was harvested in the first year, four in the second and third year and three in the fourth.

After taking of soil monoliths (20x30x40 cm) from every replication and after washing of root system of the plants, dry root mass weight was determined, drying at 60 °C (g plant<sup>-1</sup>) [28]. Nitrate reductase activity (μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> fresh weight) was determined *in vivo* in leaves, stems and roots of plants by method of Jaworski [29], plastid pigments content (mg 100 mg<sup>-1</sup> fresh weight) by Zelenskii and Mogileva [30]. Experimental data were averaged for the period of study and statistically processed using SPSS<sup>®</sup> software version 10.0 (SPSS Inc., Chicago, IL, USA).

**RESULTS AND DISCUSSION**

Nitrate reductase activity in organs of alfalfa changed depending on the forms and doses of fertilizers (Figure 1).

Alfalfa belongs to the species assimilating nitrates mainly in the leaves [14, 31]. In our study nitrate reductase activity for the plants from unfertilized control were 18.6 μmol NO<sub>2</sub><sup>-</sup>g<sup>-1</sup> fresh weight in leaves, 10.8 μmol NO<sub>2</sub><sup>-</sup>g<sup>-1</sup> fresh weight in stems and 8.20 μmol NO<sub>2</sub><sup>-</sup>g<sup>-1</sup> fresh weight in roots. Experimental data indicated similar trends in treatments with nitrogen fertilizer, both mineral and manure. An increase of nitrate reductase activity in leaves was observed and decrease in the roots as comparing to the control. Nitrate reductase activity in the leaves increased by 53.2% – 140.3% when applied mineral nitrogen fertilizer and by 122.0% – 148.9% when manure was applied (Table 1).

Nitrate reductase activity in stems increased compared to the control only in treatment with 70 kg N ha<sup>-1</sup> mineral nitrogen (11.6 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> fresh weight against 10.8 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> fresh weight in control) and in

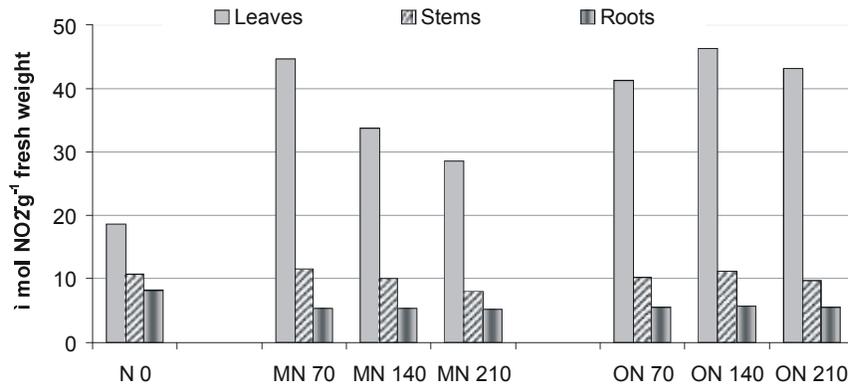


Fig. 1: Nitrate reductase activity in lucerne after applying of mineral fertilizer and manure

Table 1: Nitrate reductase activity in lucerne after applying of mineral fertilizer and manure

Treatments	Leaves		Stems		Roots	
	μmol NO <sub>2</sub> <sup>-</sup> g <sup>-1</sup> fresh weight	+, -, %	μmol NO <sub>2</sub> <sup>-</sup> g <sup>-1</sup> fresh weight	+, -, %	μmol NO <sub>2</sub> <sup>-</sup> g <sup>-1</sup> fresh weight	+, -, %
N <sub>0</sub> P <sub>300</sub> K <sub>150</sub> (C)	18.6	-	10.8	-	8.20	-
MN <sub>70</sub> P <sub>300</sub> K <sub>150</sub>	44.7	+140.3	11.6	+ 7.4	5.40	- 34.2
MN <sub>140</sub> P <sub>300</sub> K <sub>150</sub>	33.7	+ 81.2	10.1	- 6.5	5.40	- 34.2
MN <sub>210</sub> P <sub>300</sub> K <sub>150</sub>	28.5	+ 53.2	8.10	- 25.0	5.20	- 36.6
ON <sub>70</sub> P <sub>300</sub> K <sub>150</sub>	41.3	+122.0	10.2	- 5.6	5.50	- 32.9
ON <sub>140</sub> P <sub>300</sub> K <sub>150</sub>	46.3	+148.9	11.3	+4.6	5.70	-30.5
ON <sub>210</sub> P <sub>300</sub> K <sub>150</sub>	43.2	+132.3	9.8	- 9.3	5.50	-32.9
SE (P=0.05)	0.38		0.04		0.04	

+, -, %- increase, decrease to the control

Table 2: Plastid pigments content in leaves of lucerne after applying of mineral fertilizer and manure

Treatments	Plastid pigments (mg 100 mg <sup>-1</sup> fresh weight)							
	chl. a+b	% to C	carotenoids	% to C	total content	% to C	chl.a/ chl.b	chl.a+b/ carotenoids
N <sub>0</sub> P <sub>300</sub> K <sub>150</sub> (C)	305.69	-	30.55	-	336.24	-	1.53	10.0
MN <sub>70</sub> P <sub>300</sub> K <sub>150</sub>	264.17	- 13.6	54.40	+78.1	318.57	-5.3	1.79	4.85
MN <sub>140</sub> P <sub>300</sub> K <sub>150</sub>	254.33	-16.8	39.89	+30.6	294.22	-12.5	1.67	6.37
MN <sub>210</sub> P <sub>300</sub> K <sub>150</sub>	279.99	- 8.4	86.42	+182.9	366.41	+9.0	1.81	3.24
ON <sub>70</sub> P <sub>300</sub> K <sub>150</sub>	289.17	-5.4	50.01	+63.7	339.18	+0.9	1.64	5.78
ON <sub>140</sub> P <sub>300</sub> K <sub>150</sub>	262.39	-14.2	49.75	+62.8	312.14	-7.2	1.62	5.27
ON <sub>210</sub> P <sub>300</sub> K <sub>150</sub>	302.21	-1.1	56.80	+85.9	359.01	+6.8	1.70	5.32
SE (P=0.05)	7.6		6.6		9.7			

+, -,%- increase, decrease to the control

treatment with 140 kg N ha<sup>-1</sup> manure (111.3 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> fresh weight against 10.8 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> fresh weight). There was a significant decrease of nitrate reductase activity in the roots (by 30.5% – 36.6%) and the values were similar in all treatments with fertilization.

With increase of dose of mineral nitrogen nitrate reductase activity in leaves and stems decreased. The strongest decrease in nitrate reductase activity was for the dose of 210 kg ha<sup>-1</sup> mineral nitrogen (28.5 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> fresh weight in leaves and 8.10 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> fresh weight in stems). Some authors suggest that it was associated with rapid accumulation of ammonia or the products of its metabolic which inhibited the nitrate reductase [32, 33].

When applied manure the values of nitrate reductase activity in leaves and stems for the three doses were similar, but the highest nitrate reductase activity (46.3 μmol NO<sub>2</sub><sup>-</sup> g<sup>-1</sup> fresh weight) was found in treatment with 140 kg ha<sup>-1</sup> manure.

In all treatments with mineral nitrogen and manure the values of nitrate reductase activity in roots of plants were similar. Our data concerning low reduction of nitrates in roots of alfalfa regardless the applied nitrogen fertilizers confirmed the results obtained from Vance and Heichel [31]. They suggested that root nitrate reductase activity may be regulated differently than other tissue.

Data for plastid pigments content (Table 2) showed that the applying of nitrogen fertilizer (mineral and manure) decreased content of total chlorophyll (chlorophyll a + b) in alfalfa.

The control plants showed higher photosynthetic capacity, because of their greater number of active nodules, which need photo assimilates [34-36].

When compared total chlorophyll content depending on the dose, it shown that the decrease was lower when applied high doses of nitrogen fertilizers. At the dose of 210 kg ha<sup>-1</sup> mineral nitrogen, total chlorophyll content decreased as compared to unfertilized control by 8.4% and when applied 210 kg ha<sup>-1</sup> manure, by 1.1%. The higher

content of chlorophylls (a and b) after high doses of nitrogen fertilizer were a protective reaction of the plants against a strong increase of the nitrogen concentration in plant issues [37].

The content of carotenoids significantly increased compared to the control in all treatments and the strongest increase was found at the dose of 210 kg ha<sup>-1</sup> – for mineral nitrogen fertilizer the increase was by 182.9% and for manure – by 85.9%.

Carotenoids protect chlorophylls from the oxidative effect of the light, enter as components of antioxidant protection of plants [21, 38]. Increased content of carotenoids was related to the increased phytotoxicity and probably was a protective reaction of plants – resulting from activation of oxidative processes [39].

The ratio of chlorophyll a/chlorophyll b in the treatments with fertilization had higher values than the control. For the fertilization with mineral nitrogen fertilizer were 1.67 – 1.81 and for manure – 1.62–1.70.

The values of the ratio chlorophyll a+b/carotenoids decreased in treatments with nitrogen fertilization compared to unfertilized control. The decrease was as a result of increased content of carotenoids and confirms the phytotoxic effect of nitrogen fertilizers in alfalfa plants. The strongest decrease of chlorophyll a+b/carotenoids ratio was found in treatment with 210 kg ha<sup>-1</sup> mineral nitrogen fertilizer (3.24, in control 10.0).

Data for plastid pigments content and correlations between them showed a strong phytotoxic effect of mineral nitrogen fertilizer.

Mineral fertilization and manure influenced on dry root mass quantity (Figure 2). Manure releases nitrogen slowly as the highest rate of mineralization of nitrogen (30%) becomes during the first year [40]. This way of releasing of nitrogen fully answered to the needs of alfalfa development. Released from manure mineral nitrogen which is in small quantities stimulated roots development and reduced the inhibitory effect of mineral nitrogen.

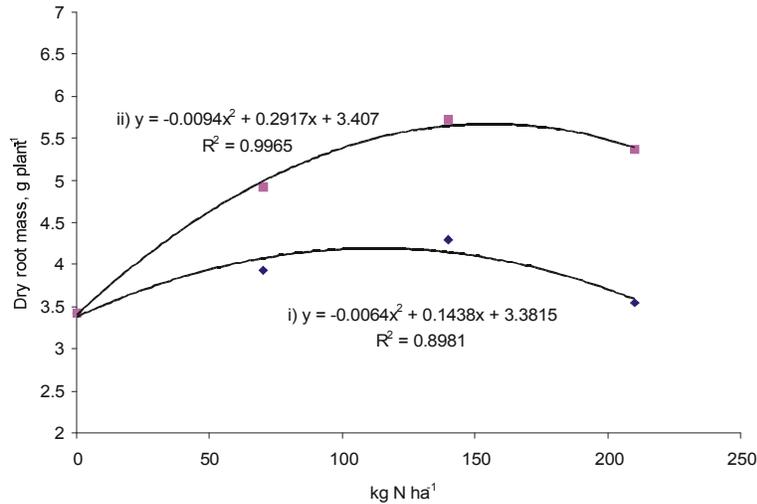


Fig. 2: Curve, prognostic equation and relationship between dry root mass and mineral fertilizer (i) and manure (ii) in lucerne

When applied mineral fertilizer at the doses of 70 and 140 kg N ha<sup>-1</sup>, dry root mass increased compared to the unfertilized control by 14.5 and 25.0%, respectively. Mineral fertilizer at the dose of 210 kg N ha<sup>-1</sup> did not stimulate the root system development. When applied manure dry root mass was from 43.5 to 66.7% higher than the control. Root system of these plants was stronger and better developed.

Manure contains humic and fulvic acids which stimulated the growth of root system, improved physical properties of soil, increased coefficient of use of both, water and nutrients [41]. This explains significantly greater quantity of root mass (from 25.2 to 51.7%) in the treatments with manure as compared to mineral.

### CONCLUSIONS

When applied mineral fertilizer and manure at the doses of 70, 140 and 210 kg N ha<sup>-1</sup> a tendency for increase of nitrate reductase activity in leaves and decrease in roots regardless of dose were found. By increasing the dose of mineral fertilizer nitrate reductase activity in leaves and stems decreased. The values of nitrate reductase activity were similar when manure was applied. Mineral fertilizer and manure decreased the total chlorophyll content (chlorophyll a+b) and the decrease was less at high doses. The content of carotenoids significantly increased and the strongest increase was found at a dose of 210 kg ha<sup>-1</sup> (for mineral nitrogen fertilizer by 182.9% and for manure by 85.9%). Dry root mass formed after manure fertilization was from 25.2 to 51.7% greater than that after mineral.

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