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REVIEW ARTICLE

Effect of the cultivation technology elements on the activation of plant microbe symbiosis and the nitrogen transformation processes in alfalfa agrocoenoses

I. M. Didur [1], V. I. Tsyhanskyi [1], O. I. Tsyhanska [1], L. V. Malynka [1], A. O. Butenko [2*], Masik I. M. [2], T. I. Klochkova [2]

[1] Vinnytsia National Agrarian University, Soniachna Str. 3, 21008, Vinnytsia, Ukraine
[2] Sumy National Agrarian University, Gerasim Kondratyev Str. 160, 40021, Sumy, Ukraine; * andb201727@ukr.net

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Abstract

The effect of pre-sowing seed treatment with inoculant and its combination with a plant growth stimulant in terms of the optimization of the level of soil acidity on the formation of symbiotic plant productivity and the accumulation of biological nitrogen in the soil has been studied. On the basis of the results obtained it has been found that the use of pre-sowing treatment of alfalfa seeds with rhizobophyte in combination with Emistym S against the background of soil liming and the application of the full rate of lime subject to hydrolytic acidity provides the accumulation of the largest amount of biologically fixed nitrogen. This is on average over the years of research has run at 236.1 kg/ha.

Keywords: Alfalfa, soil liming, inoculation, growth stimulant, biological nitrogen

Introduction

One of the effective measures to increase the production of high-quality feed on forage lands while reducing the anthropogenic load on the environment and saving energy resources in the conditions of acute nitrogen deficiency is the use of biological factors of intensification. First and foremost, the potential of perennial legumes as a cheap natural source of symbiotic nitrogen (Bohovin 2005; Tarariko 2005). The leading role in solving the problem of production of feed protein is played by alfalfa (*Medicago sativa* L). This culture subject to the proper application of technological methods of cultivation maximizes the biological potential. Alfalfa reduces the cost of plant raw materials for the preparation of various types of feed (Petrychenko 2010).

The limiting factor in the formation of high and sustainable yields of leaf mass and the productivity of alfalfa is the acidic reaction of the soil solution, which inhibits the development of symbiotic microflora. This adversely affects the growth processes and plant development, especially in the early stages of organogenesis (Kvitko 2012). It has been found that in addition to the direct adverse impact of enhanced concentration of hydrogen ions and high content of mobile aluminum. Therein on plants, soil acidity also has a multilateral indirect effect. Hydrogen displaces calcium from soil humus. That results in an increase in its dispersion ability and mobility. The saturation of mineral colloidal particles with hydrogen leads to their gradual destruction. This is due to the low content of the colloidal fraction. In acidic soils in connection with which they distinct in adverse physical, and physical and chemical properties, poor structure, low absorption capacity. The microbiological processes, occurring in soils with high acidity and being extremely beneficial to plants, are suppressed. As a consequence of the absorption of nutrients occurs at an extremely low rate (Kiriyenko 1972).

Materials and Methods

Conditions for conducting research

The field studies were conducted in the experimental field of the Faculty of Agronomy of Vinnytsia National Agrarian University. Located in the central part of the Vinnytsia Region (Right-Bank Forest-Steppe). The territory of the experimental field has a smooth relief.

Soils: The soil cover of the experimental plot is represented by grey forest soils, which, according to the morphological characteristics, occupy an intermediate place between light and dark grey soils. The tilth top soil is 30 cm in depth with

the medium-loamy granulometric composition and crumby structure. Its density is in the range of 1.32-1.4 g/cm^3. According to the agrochemical survey, the tilth top soil has the following physical and chemical indicators: the content of humus (by Tiurin) is 2.06%, alkaline hydrolyzed nitrogen (by Cornfield) is equal to mg/kg, mobile phosphorus and exchangeable potassium (by Chyrykov) are 149 and 80 mg per 1 kg of soil, respectively, and pH of salt extract amounts to 5.9. Hydrolytic acidity is equal to 1.14 mg-eq per 100 g of soil.

Weather conditions: The assessment of weather conditions during the years of research was carried out on the basis of the meteorological data. Obtained from the Vinnytsia Regional Hydrometeorological Center.

In 2011, the weather conditions were different from the multi-year indicators. But favorable enough for the formation of agrophytocenosis of alfalfa. During the growing season, the precipitation was 70 mm less than the rated value. While there was an increase in the average daily air temperature by 1.5°C in relation to the average multiyear indicators.

In terms of the weather conditions, the year 2012 was characterized by a high temperature. With the average daily temperature, which was +1.3°C higher than the multi-year value. And the insufficient amount of precipitation that was 14.2% less than the rated value.

The weather conditions of 2013 were quite different from the average multi-year data. But rather favorable for the formation of agrofitocenosis of alfalfa. As well as for the growth and development of alfalfa plants that turned out to be satisfactory.

Scheme of experiment: The placement of variants is systematic in one layer. The crop acreage of the experimental plot was 50 m^2, the registration plot amounted to 25 m^2. The variety of alfalfa (*Medicago sativa* L.) with the seed application rate of 8 million viable seeds/ha was used in the experiment. The treatment of soil was common to the Forest-Steppe Zone. The stubble cultivation with a disk harrow. Plowing to a depth of 25-27 cm was conducted in the autumn. Mineral fertilizers were applied in stock at the rate of $N_{30}P_{90}K_{90}$. In the spring, when the natural soil tilth was achieved, the fast-acting limestone fertilizer (at the rate of 0.5 and 1.0 under hydrolytic acidity) (Tab. 1) was applied with the subsequent cultivation.

Research methodology: The plant growth regulator Emistym S (TU U 88.264.021-95) at the rate of 15 ml per 1 ton of alfalfa seeds, and the liquid inoculant rhizobophyte (*Sinorhizobium meliloti*, 425a strain) were used in the experiment. The growing method we used is open with the application of the herbicide Picador (imazethapyr 100 g/l) at the rate of 1 l/ha. The common methods, namely "Methods of Field Experience" of B.A. Dospekhov (Dospekhov 1985), "Methods of Conducting Experiments on Feed Production" of the Institute of Feeds and Agriculture of Podillia of NAAS (Babych 1998) have also been used other official methodological publications (Yeshchenko 2005), were applied when conducting the research.

Table 1. Field trial design.

Factor A-soil liming	Factor B-pre-sowing seed treatment
Without liming (control);	Without treatment (control);
Liming (0.5 rate for h.a.);	Rhizobophyte;
Liming (1.0 rate for h.a.)	Rhizobophyte+Emistym S

The calculation of the number and mass (total and active) of nodules during the plant growing were carried out according to the methodological guidelines of H. S. Posypanov. Also made a determination of the total and active symbiotic potential and the amount of biologically fixed nitrogen (Posypanov 1991).

Results and Discussion

Conditions and dynamics of formation of biological nitrogen

According to the results of the conducted researches, a positive effect of the optimization of soil acidity level. Pre-sowing seed treatment with the biological preparation rhizobophyte and its combination with the plant growth regulator of natural origin Emistym S had the maximum dynamics on the formation of the number of nodule bacteria and their mass during the growing season of alfalfa was revealed (Tab. 2).

It should be noted that the formation of agrophytocenosis occurs during the sowing year. Due to the slow growth and development of plants, the number of nodules on the roots and their mass is significantly lower compared to the second and third growing years.

It was found that in the first growing year during the first hay mowing the largest number of nodules amounting to 46.9 pcs/plant, 35.3 pcs/plant of which being active with the mass of 101.1 and 73.4 mg/plant, respectively, were formed on the variants under the liming of soil with a full rate of lime and the seed treatment with rhizobophyte and Emistym S.

Characteristic factors for the development of tuber bacteria

When carrying out the second mowing of the leaf-stem mass, the tendency towards the formation of nodules on the alfalfa roots remained. Under such growing conditions the total number of nodules on the alfalfa roots was 54.9 pcs/plant. Of which were active 41.3 with a mass of 120.9-92.7 mg/plant, respectively (Tab. 3).

In the second growing year, the largest number of nodules was formed during the first and second mowing of grass stand. As a result, further decline followed by a decrease in the intensity of their formation in the second half of the summer period. The greatest number of total and active nodules and their mass was received on the variants with a full rate of lime. As a result of the combined pre-sowing seed treatment with rhizobophyte and Emistym S.

During the first mowing, the total number of nodules on these variants was, respectively, 104.9 pcs/plant with a mass of 235.6 mg. Active nodules were equal to 83.5 pcs/plant with a mass of 189.2 mg. However, their number was decreasing from mowing to mowing. During the fourth mowing it amounted to, respectively 63.4 and 46.5 pcs/plant. Their mass was 142.4 and 105.9 mg.

The inoculation of seeds before sowing has contributed to the growth of the total number of nodules by 6.1-13.8. Active nodules- by 4.7-13.5 pcs/plant during the first mowing, but its effectiveness decreased up to 1.1-5.9 and 1.7-5.4 pcs/plant during the fourth mowing. The combined treatment with rhizobophyte and Emistym S increased the total number of nodules by 8.2-15.7 pcs/plant during the first mowing and by 2.4-8.8 pcs/plant during the fourth mowing. The number of

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Table 2. Dynamics of the number of nodules on the roots of alfalfa plants depending on pre-sowing seed treatment and soil liming, pcs/plant (2011-2013).

Soil liming	Seed treatment*	1st growing year		2nd growing year hay mowing				3rd growing year			
		1st	2nd	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Without liming	1	21.4	26.2	63.2	55.1	41.8	33.7	69.2	61.3	51.6	38.1
		14.3	17.8	42.7	39.6	29.3	21.1	47.1	43.5	34.9	24.8
	2	27.8	31.8	69.3	57.2	43.7	34.8	73.8	63.6	53.5	39.6
		18.9	21.7	47.4	42.3	30.8	22.8	51	45.7	36.4	26.3
	3	31.6	35.4	71.4	60.3	42.6	36.1	76.6	64.9	53.9	40.1
		22	24.3	50.2	45.8	32.1	23.6	54	49.8	39.3	28.3
0.5 of the rated value under h.a.	1	28.1	32.9	87.4	76.4	62.5	49.5	95.4	84	76.3	64.9
		18.6	23	58.9	56.1	43.2	33.6	65.3	62.2	51.4	43.6
	2	37.9	44.6	98.5	84.6	68.9	54.3	103.8	87.9	79.9	66.1
		25.4	30.2	68.6	63.4	47.4	36.8	71.6	68.1	53.6	44.4
	3	41.2	46.4	98.7	84.1	70.4	56.2	108.5	93	84.2	69.9
		28.6	32.5	71.8	65.7	50.5	39.4	77.2	71.8	59.1	48.6
1.0 of the rated value under h.a	1	30.5	36.3	89.2	80.8	68.8	54.6	99.7	91.7	84.3	70.5
		21.2	25.4	65.7	59.8	48.3	37	72.9	69.3	57.9	51.1
	2	41.6	48.5	103	91.2	77.3	60.5	109.4	96	88.5	72.4
		29.9	35.2	79.2	68.9	55.6	42.4	81.7	76.5	62.4	53.2
	3	46.9	54.9	104.9	92.3	81.5	63.4	113.6	101.5	93.8	78.4
		35.3	41.3	83.5	74.5	59.4	46.5	88.6	80.9	68.4	60.9

Note: *1. Without treatment; 2. Rhizobophyte; 3. Rhizobophyte+Emistym S. Seed treatment in the numerator there is the total number of nodules, pcs/plant in the denominator there is the number of active nodules, pcs/plant

Table 3. Dynamics of the mass of nodules on the roots of alfalfa plants depending on pre-sowing seed treatment and soil liming, mg/plant (2011-2013).

Soil liming	Seed treatment*	1st growing year		2nd growing year hay mowing				3rd growing year			
		1st	2nd	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Without liming	1	43.8	49.5	122.5	109.4	80.3	65.3	141.7	122.9	102.4	75.3
		31.4	35.8	87.1	80.7	58.7	42.4	96.7	88.5	71.7	50.9
	2	55.6	61.2	130	114.6	83.2	65.3	147.6	128.3	106.3	78.3
		39.8	43.8	93.3	86.8	61.3	45.7	101.1	91.4	73.4	51.9
	3	64.2	69.2	141.3	122	83.5	71.4	155.7	132.4	106	78.4
		47.3	49.6	103.8	94.7	65.4	48.2	108.9	100.8	78.3	55.4
0.5 of the rated value under h.a.	1	58.1	66.7	180.5	160.6	121.9	102.2	197.4	177.8	167.1	141.6
		41.2	47.7	123.7	121	85.3	70.9	137.2	130.9	110.6	95.2
	2	78.6	95.1	202.3	180.9	141.5	111.5	215.3	193.4	173.4	143.2
		55	67.5	143.4	138.7	98.3	77.1	149.6	145	114.5	96.1
	3	86.2	99.1	212.7	188.6	151.7	121.1	226.9	204.6	189.4	156.7
		62.1	72.5	157	150.4	109.8	86.4	162.5	157.2	131	108.9
1.0 of the rated value under h.a.	1	63.1	76.1	187.3	175.1	134.5	114.6	206.4	199.8	171.2	142.7
		45.8	56.4	140	132.7	95.4	79.2	153	150.3	118.5	103.5
	2	86.4	108	217.7	197.3	155.9	127.9	227.2	218.7	176.3	143.8
		64.1	78.7	169.4	152.2	113.1	91.1	170.8	165.6	123.2	105.7
	3	101.1	121	235.6	206.9	172.4	142.4	244.9	229	184.9	154.1
		73.4	92.7	189.2	170.1	126.7	105.9	192.1	181.7	135.7	119.6

Note: *1. Without treatment; 2. Rhizobophyte; 3. Rhizobophyte+Emistym S. Seed treatment in the numerator there is the total number of nodules, pcs/plant in the denominator there is the number of active nodules, pcs/plant.

active nodules, respectively, by 7.5-17.8 and 1.7-9.5 pcs/plant.

We have been installed that the treatment of seeds with the plant growth regulator Emistym S and rhizobophyte not only increases the number of nodules compared to the control but also significantly increases their mass due to the formation of largely sized nodules. The nature of the effect of the methods

Table 4. The value of biological nitrogen accumulation by alfalfa sowing depending on pre-sowing seed treatment and soil liming, kg/ha (2011-2013).

Soil liming	Seed treatment*	Growing years			
		1st	2nd	3rd	Average
Without liming	without treatment	42.8	138.5	142.1	107.8
	with rhizobophyte	53.3	148	146.4	115.9
	with rhizobophyte+ Emistym S	61.7	160.7	157.8	126.7
0.5 of the rated value under h.a.	without treatment	56.7	208.1	223.6	162.8
	with rhizobophyte	78.2	236.7	236.6	183.8
	with rhizobophyte+ Emistym S	85.8	260.8	263	203.2
1.0 of the rated value under h.a.	without treatment	65.2	232.4	246.9	181.5
	with rhizobophyte	91.1	272.7	263.6	209.1
	with rhizobophyte+ Emistym S	106.6	307.8	293.9	236.1

of pre-sowing seed treatment and soil liming on the formation of the total mass and the mass of active nodules is similar to the formation of their number.

In the third year of the alfalfa growing, the smallest number of nodules on the plant roots was observed on the experiment variants without soil liming. Thus, in case of the open growing, their number amounted to 69.2-47.1 pcs/plant and 38.1-24.8 pcs/plant during the first and fourth mowing. The liming with the full rate of lime under h.a. and the composition of rhizobophyte and Emistym S have increased the number of nodules, respectively, up to 113.6-88.6 and 78.4-60.9 pcs/plant.

Conditions of accumulation of biological nitrogen

It was found that in case of the open growing, in the first year of alfalfa life, the amount of biologically fixed nitrogen ranged from 42.8 to 106.6 kg/ha, in the second year from 138.5 to 307.8 kg/ha, and in the third year-from 142.1 to 293.9 kg/ha (Tab. 4).

The soil liming with the full rate of lime and combined seed treatment before sowing with rhizobophyte and Emistym S under the open growing with the application of herbicide on average during the years of research have provided the maximum amount of biologically fixed nitrogen equal to 236.1 kg/ha. It's on 54.6 kg/ha, or 30.1% more than on the variants without seed treatment and 128.3 kg/ha more in relation to the control without liming and seed treatment.

Discussion

In order to improve soil fertility, increase the productivity of agricultural crops, including alfalfa, and to make the best use of mineral fertilizers it is expedient to carry out liming in the range of 1.0-1.5 under hydrolytic acidity. It provides an increase in the amount of 1.2-1.8 tons of feed units per hectare of rotation area (Yatsentiuk 2010). The mobility of toxic elements of aluminum and manganese decreases in the limed soil. These processes contribute to nodule formation and an increase in the nitrogen fixation of alfalfa (Brauer 2002).

Recently, the problem of increasing plant productivity has been solved not only by applying the selection and genetic methods, fertilizers, pesticides, but also by using biological preparations and plant growth regulators (Yavorska 2004).

The intensified activity of nitrogen fixation processes occurring in the root zone of plants can be achieved with the use of physiologically active substances characterized by auxin-cytokine activity (Berdnikov 2005). Both associative and symbiotic nitrogen fixation processes are enhanced by virtue of plant growth stimulants. These preparations are the analogs of exogenous phytohormones of cytokine, gibberellin and auxin effect, and unsaturated fatty acids, carbohydrates, amino acids. They are used for pre-sowing seed treatment and top dressing of plants (Makrushyn 2003).

The conceptual direction of the development of biotechnologies and biological nitrogen fixation in agriculture is the development of preparations in order to stimulate growth, increase the production of legumes. Creation is important the original complex compositions of multifactorial action, which combine the properties of plant growth regulators, fertilizer elements, means of plant resistance to stress and diseases (Provorov 2013; Kots 2011). In light of this, the study of agrobiological methods of growing forage crops. Including alfalfa, with the use of plant growth regulators, as well as their combination with biological preparations based on active strains of nodule bacteria. It aims to improve the nitrogen nutrition of plants in the monoculture. Important is also a significant increase in the level of the biological conversion of atmospheric nitrogen into organic nitrogen-containing compounds. Is particularly relevant for the creation of sustainable production of feed raw materials and maximization of the biological potential of plants under the unstable soil and climatic conditions.

When growing agricultural crops, biologically fixed nitrogen is extremely important for legumes since this reduces the use of mineral fertilizers and the cost of production. Perennial legumes, in particular, alfalfa, are not only a source of high-protein feed, but they are the best precursors, especially for grains, cereals, and industrial crops. They not only increase their yields but also improve the soil structure and fertility (Bohovin, 2005).

Conclusion

According to the results of our research, under the conditions of Right-Bank Forest-Steppe, the best conditions for the symbiotic activity of alfalfa seedlings were created under the conditions: Non-perishable growing method. The introduction of the herbicide in the year of sowing. Conduct calcining the full norm. Use of pre-sowing seed treatment with bacterial preparation risobofit with plant growth regulator Emistim S. Under these conditions, the maximum indicator of biologically fixed nitrogen is formed-236.1 kg/ha.

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