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Effect of presowing treatment of seeds with insecticides on nodulating ability, nitrate reductase activity and plastid pigments content of lucerne (*Medicago sativa* L.)

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Abstract. To determine the effect of presowing treatment of seeds on the nodulation ability, nitrate reductase activity and plastid pigments content of lucerne (cv. Obnova), a pot trial was carried out at the Institute of Forage Crops - Pleven, Bulgaria. The insecticides Promet 400 SK (furathiocarb) at the dose of 3 L, and Carbodan 35 ST (carbofuran) at the dose of 1, 2 and 3 L 100 kg^{-1} seeds were used. It was found that the insecticides tested did not depress the nodulation. Nodule number and specific nodulation ability when treated with Carbodan 35 ST at the dose of 3 L 100 kg^{-1} seeds exceeded that of the control by 23% and 7%, respectively. The root length for the variants with presowing treatment of seeds was higher as compared to the control by 7 to 26%. The variant with Carbodan at the doses of 2 and 3 L 100 kg^{-1} seeds and Promet increased nitrate reductase activity in roots, and that with Carbodan at the dose of 1 L 100 kg^{-1} seeds- in the leaves. Total content of plastid pigments increased in all variants with Carbodan and was lower than the untreated control in the variant with Promet.

Key words: lucerne, nodulation, root mass, nitrate reductase activity, plastid pigments

INTRODUCTION

Lucerne is a valuable protein forage crop. It is an economical source of biological nitrogen through its fixation by nodules (Peoples et al., 1995). The efficiency of symbiosis between plants and root nodule bacteria depends on numerous factors, as well as on the use of insecticides for controlling pests (Hartwig & Soussana, 2001). The presowing treatment of seeds with insecticides is considered an efficient, economical and ecological means of controlling insect pests in some forage crops (Schiffers & Copin, 1993; Dochkova et al., 2000; Ilieva et al., 2004).

The aim of this study was to determine the effect of presowing treatment of seeds with insecticides on nodulation ability, nitrate reductase activity and plastid pigments content of lucerne.

MATERIALS AND METHODS

A pot trial with leached chernozem from the region of Pleven with 4 replicates per treatment during 2003 and 2004 was carried out. Pots with capacity of 10 L were used. Four plants were grown in each pot. Lucerne cultivar Obnova was used. Promet 400 SK (furathiocarb) at the dose of 3 L (standard, and Carbodan 35 ST (carbofuran) at

the dose of 1, 2 and 3 L were applied per 100 kg seeds. Seeds were treated on the day of sowing. All variants were treated against a background of phosphorus and potassium (P, 110 mg P kg^{-1} soil; K, 110 mg K kg^{-1} soil). Phosphorus was applied as triple super phosphate and potassium as potassium chloride. The root length and number of nodules were recorded at early flowering stage of the plants after washing the roots in laboratory conditions. Specific nodulation ability was calculated as the ratio between the nodule weight and dry root mass weight. Nitrate reductase activity was determined *in vivo* by the method of Jaworski (1971). Plastid pigments content was determined by Zelenskii & Mogileva (1980). The experimental data were statistically analyzed using software SPSS for Windows 2000.

RESULTS AND DISCUSSION

Data given in Table 1 showed that the presowing treatment of seeds had a stimulating effect on nodulation of lucerne. The insecticides tested increased nodule number as compared to the control. The number of nodules varied from 57 (Carbodan 35 ST- 2 L 100 kg^{-1} seeds) to 64 (Carbodan 35 ST- 3 L 100 kg^{-1} seeds). The increase as compared to untreated control was 9 and 23%, respectively. We assume that Mg (2.54% in drying agent) contributed to the nodulating process.

Specific nodulation ability for the plants treated with Carbodan 35 ST at the dose of 3 L 100 kg^{-1} seeds was higher as compared to the control by 7%.

Table 1. Nodulation ability of lucerne after presowing treatment of seeds with insecticides.

Variants	Nodule number	+, increase %	Specific nodulation ability	+, increase %
Control	52	–	0.0402	–
Carbodan 35 S- 1 L	58	+ 11	0.0405	+ 1
Carbodan 35 S- 2 L	57	+ 9	0.0419	+ 4
Carbodan 35 S- 3 L	64	+ 23	0.0430	+ 7
Promet 400 SK- 3 L	58	+ 11	0.0418	+ 4
SE ($P = 0.05$)	2		0.0001	

The root system of the plants with the presowing treatment of seeds was healthier, longer and better developed as compared to that of the control. The root length was longer for the variants with Carbodan 35 ST, and the increase as compared to untreated control was from 8 to 26% (Table 2).

Irrespective of the positive effect of the studied insecticidal preparations on the nodulation of lucerne, we also studied their action on the nitrate reductase activity. The nitrate reductase activity is one of the key enzymes participating in the nitrogen assimilation of plants. According to data of a number of authors (Sidorova et al., 1988; Kot's et al., 1990) inverse relation and compensation interaction exist between nitrogen fixation and nitrate assimilation in the pre-reproductive period.

Table 2. Root length of lucerne after presowing treatment of seeds with insecticides.

Treatments	Root mass	
	length cm	+, increase %
Control	23.9	–
Carbodan 35 S- 1 L	25.9	+ 8
Carbodan 35 S- 2 L	26.5	+ 11
Carbodan 35 S- 3 L	30.1	+ 26
Promet 400 SK- 3 L	25.6	+ 7
SE (*P*=0.05)	1.0	

Our data (Table 3) showed that the treatment of lucerne seeds with Carbodan at the dose of 1 L 100 kg^{-1} seeds increased nitrate reductase activity in the leaves by 21%, and decreased it in the roots by 9%, as compared to the untreated control. In the variants with treatment with Carbodan at the doses of 2 and 3 L 100 kg^{-1} seeds, nitrate reductase activity in the roots increased considerably (by 15% and 45%, respectively), and was considerably lower than the control in leaves. Lucerne belongs to a species accomplishing nitrate reduction in plant leaves, stems and roots (Arrese-Igor et al., 1991). Among all variants with Carbodan treatment there was low nitrate reductase activity in the stems (by 24% and 64%, respectively as compared to the control). The seed treatment with Promet increased nitrate reductase activity in the roots by 82%, and in the stems by 16%, and decreased it in the leaves by 42%.

Table 3. Nitrate reductase activity in lucerne plants after presowing treatment of seeds with insecticides.

Treatments	Leaves		Stems		Roots	
	µmol NO_2^- g^{-1} fresh weight	+, –, %	µmol NO_2^- g^{-1} fresh weight	+, –, %	µmol NO_2^- g^{-1} fresh weight	+, –, %
Control	17.4	–	5.0	–	1.32	–
Carbodan 35 S- 1 L	21.0	+ 21	1.8	– 64	1.20	– 9
Carbodan 35 S- 2 L	10.3	– 41	1.8	– 64	1.52	+ 15
Carbodan 35 S- 3 L	11.4	– 34	3.8	– 24	1.92	+ 45
Promet 400 SK- 3 L	10.1	– 42	5.8	+ 16	2.40	+ 82
SE (*P*=0.05)	2.2		0.8		0.2	

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

As a result of the existence of the inverse relation between the activities of nitrogenase and nitrate reductase and compensation complementation, the increase of nitrate reductase activity may be due to the lower nitrogen-fixing capacity of nodules.

Nitrate assimilation is a process dependent on photosynthesis (Kaiser et al., 1999). The data on plastid pigments content showed that only in the variant with Carbodan at the dose of 1 L 100 kg^{-1} seeds was there an increase of main chlorophyll *a* (by 13%), and in the other variants it was lower than the control (Table 4). Total chlorophyll content increased in the variants with Carbodan at the expense of the increase of accessory chlorophyll *b*. Presowing seed treatment with Promet resulted in a decrease of chlorophyll *a* and *b* content. That may be related to inhibition of

photosynthesis with regard to chlorophyll (Peneva, 2004).

Carotenoid content was lower than that in the control in the variants with Carbodan at the doses of 2 and 3 L 100 kg^{-1} seeds, and Promet (13%, 3% and 18%, respectively). Only in the variant with Carbodan at the dose of 1 L 100 kg^{-1} seeds was there an increase by 21%. The changes in carotenoid content were probably related to phytotoxicity and the defense reaction of plants. Carotenoids can prevent photodynamic damage of photosynthesizing apparatus molecules (Goodvin & Merser, 1986).

The total content of plastid pigments increased in all variants with Carbodan. When using Carbodan at the dose of 1 L 100 kg^{-1} seeds, the increase was by 24%, and at the dose of 2 and 3 L 100 kg^{-1} seeds it was by 6 to 8% (Fig. 1). This data corresponded to the high nitrate reductase activity in the leaves in the variant with Carbodan 1 L 100 kg^{-1} seeds and the low level in the other two variants. In the variant with Promet the total content of plastid pigments was 21% lower than that of the control. The coefficient of correlation between nitrate reductase activity and plastid pigments content was $r = 0.63$.

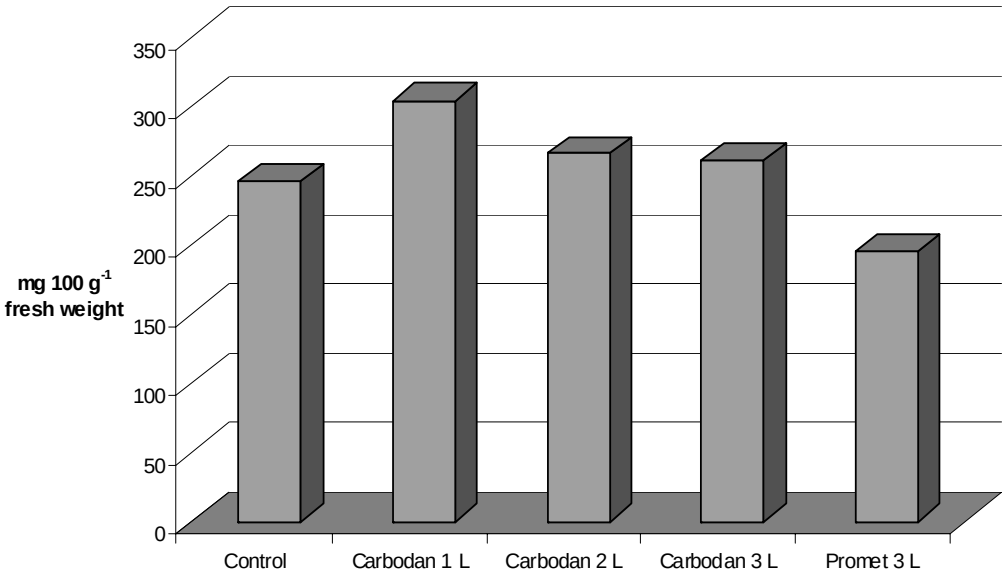

Fig. 1. Total content of plastid pigments in lucerne plants after presowing treatment of seeds with insecticides.

Table 4. Plastid pigments content in lucerne plants after presowing treatment of seeds with insecticides.

Treatments	Plastid pigments						Carotenoids		Total pigment content	
	chlorophyll *a*		chlorophyll *b*		chlorophyll *a*+*b*					
	mg 100 g^{-1}	+, –, %	mg 100 g^{-1}	+, –, %	mg 100 g^{-1}	+, –, %	mg 100 g^{-1}	+, –, %	mg 100 g^{-1}	+, –, %
Control	133.4	-	70.2	-	203.6	-	42.3	-	245.9	-
Carbodan 35 S- 1 L	151.0	+ 13	102.0	+ 45	253.1	+ 24	51.2	+ 21	304.3	+ 24
Carbodan 35 S- 2 L	119.2	- 11	110.2	+ 57	229.4	+ 13	36.7	- 13	266.1	+ 8
Carbodan 35 S- 3 L	130.5	- 2	89.4	+ 27	220.0	+ 8	41.1	- 3	261.0	+ 6
Promet 400 SK- 3 L	97.7	- 27	62.4	- 11	160.1	- 21	34.6	- 12	194.8	- 21
SE (*P*=0.05)	8.8		9.1		15.5		2.9		17.7	

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

CONCLUSIONS

Presowing treatment of seeds of lucerne with the insecticides Promet 400 SK at the dose of 3 L 100 kg^{-1} seeds, and Carbodan 35 ST at the dose of 1, 2 and 3 L 100 kg^{-1} seeds did not depress the nodulation. The application of Carbodan 35 ST at the dose of 3 L 100 kg^{-1} seeds resulted in an increase of nodule number and specific nodulation ability by 23% and 7%, respectively. The root length for the variants with presowing treatment of seeds exceeded that of the control by 7 to 26%. The treatment with Carbodan (2 and 3 L 100 kg^{-1} seeds) and Promet increased nitrate reductase activity in roots, and that with Carbodan at the dose of 1 L 100 kg^{-1} seeds - in the leaves. Total content of plastid pigments increased in all variants with Carbodan and was lower than the untreated control in the variant with Promet.

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