Effect of presowing treatment of seeds with insecticides on parameters related to nodulation and nitrate reduction in soybean [*Glycine max* (L.) Merr.]

Anna ILIEVA and Viliana VASILEVA*

Institute of Forage Crops, 89 “Gen. Vl. Vazov” Str., 5800 Pleven, Bulgaria, viliana.vasileva@gmail.com

ABSTRACT

Effect of presowing treatment of seeds with insecticides on parameters related to nodulation and nitrate reduction in soybean was studied in pot trial at the Institute of Forage Crops, Pleven, Bulgaria (2003-2004). It was found that insecticides Gaucho 600 FS (imidacloprid), Carbodan 35 ST (carbofuran) applied for presowing treatment of seeds at the doses of 1, 2 and 3 L/100 kg seeds, and Promet 400 CS (furathiocarb) (standard) at the dose of 3 L/100 kg seeds, had no suppressive effect on the root length, dry root mass and specific nodulating ability of plants. When used Gaucho 600 FS at the dose of 1 L/100 kg seeds, root mass was most developed, the largest number of nodules was formed, and specific nodulating ability was highest. The common tendency for decrease of nitrate reductase activity in leaves and significant increase in stems was found. Nitrate reductase activity increased in leaves, stems and roots in treatment with Carbodan 35 ST applied at the dose of 3 L/100 kg seeds. Chlorophylls a+b/carotenoids ratio exceeded this of the control only in treatment with Gaucho 600 FS at the dose of 1 L/100 kg seeds. However the analysis of the plant biomass did not include the amount of possible undegraded traces after using the insecticides tested.

Key words: nodulation, nitrate reductase, plastid pigments, presowing treatment, insecticides, soybean

INTRODUCTION

An application of insecticides as seed and soil treatments has become a common practice to combat insect pests in modern agriculture (Ahemad and Khan, 2010; 2011; Wahid and Farooq, 2012). It has been estimated that only 0.1% of applied pesticides reached the target pests but the remaining 99.9% affect the environment (Pimentel, 1995).

Presowing treatment of seeds with insecticides is an efficient, economic and ecological means of plant protection from pests (Epperlein et al., 1995; Dochkova et al., 2000; Anjum Suhail et al., 2000; Vasileva et al., 2003; Rotrekl and Cejtchaml, 2008). Some authors (Krohn and Hellpointner, 2002; Dewar et al., 2003) consider that the use of insecticides for presowing treatment of seeds even in the absence of attack by insects, leads to increase of aboveground biomass, roots and yield.

Legumes are key to building sustainable agriculture because the ability to nitrogen fixation (Zahran, 1999; Athar and Harding, 2000; Kot’s, 2001; Frame, 2005; Peyraud et al., 2009). When treated the seeds, the insecticides can be toxic to nitrogen-fixing bacteria of the genus *Rhizobium* and reduced the high symbiotic nitrogen fixation capacity (Athar and Harding, 2000; Khan et al., 2004). Therefore studies on the impact of this event on morphological, physiological and biochemical parameters are needed.

Soybean [Glycine max (L.) Merrill] is a nitrogen-fixing crop, valuable precursor as well as protein source for livestock (Lindemann and Glover, 1999). It is grown in more than 35 countries around the world and dominates international markets as main protein and oilseed crop. Opportunities based on nitrogen fixation from the air to absorb and accumulate large amounts of biological nitrogen in the soil, makes it in indispensable precursor to the main crop and an important source of free nitrogen and energy (King and Purcell, 2001). Currently there are three main products – seeds, oil and flour, which are widely used as food, both in animals and in humans (Todorova, 2003).

Soybean growers are increasingly shifting towards chemical applications to enhance yield, and the prophylactic use of fungicides and insecticides has recently been advocated as a means to optimize plant health and increase yields, even in the absence of significant pest pressure (Henry et al., 2011). Changes in soil micro flora after use of insecticides (with an active ingredient carbofuran) in soybean were studied and it was found nitrogen-fixing bacteria remained unaffected (Sarnaik et al., 2006).

Legumes have two sources of nitrogen nutrition - assimilation of soil nitrogen (mostly in the form of nitrate), and fixation of atmospheric molecular nitrogen in nodules in symbiosis with bacteria from genera *Rhizobium* and *Bradyrhizobium* (Arrese - Igor et al., 1990; Kretovich, 1997; Vance, 1998). The uptake of nitrate and subsequent reduction by nitrate reductase is the primary pathway of soil nitrogen utilization to soybeans. The utilization of nitrogen through the symbiotic relationship with *Rhizobium japonicum* (Kirchner) by the enzyme nitrogenase, affords a second major pathway of nitrogen input (Harper and Hageman, 1972).

Photosynthesis is a source of energy and electrons for the functioning of both, nitrogenase and nitrate reductase (O' Donohue et al., 1991; Welsh et al., 1996). 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.

Results from studies in legumes indicated a high positive correlation between the presence of nitrate reductase activity and the majority of the parameters related to nitrogen fixation, suggesting that nitrate reductase activity may play complementary functions to these of nitrogenase (Serrano and Chamber, 1990; Chamber-Perez et al., 1997). This is important in studies of application of insecticides, which may have toxic effect on the nodules.

The aim of this work was to study the effect of presowing treatment of seeds with insecticides on root length, dry root mass, specific nodulating ability, nitrate reductase activity and plastid pigments content in soybean (*Glycine max* (L.) Merr.).

MATERIALS AND METHODS

A pot trial with soybean cv. “Pavlikeni 121” was carried out in the greenhouse of the

Institute of Forage Crops, Pleven, Bulgaria (2003-2004). Pots of 10 L capacity and leached chernozem soil were used. Sowing was conducted at the depth of 2-3 cm and 4 well-developed plants were left in each pot. The insecticides Gaucho 600 FS (imidacloprid) and Carbodan 35 ST (carbofuran) at the doses of 1, 2 and 3 L 100/kg seeds, and Promet 400 CS (furathiocarb) (standard) at the dose of 3 L 100/kg seeds were used for presowing seed treatment. The seed treatment was made a day before sowing. Absorbent TZ 21 was used as a drying agent. The seeds were inoculated with Nitragin on the sowing day.

The following treatments were studied: 1. Control – dry; 2. Gaucho 600 FS – 1 L/100 kg seeds; 3. Gaucho 600 FS – 2 L/100 kg seeds; 4. Gaucho 600 FS – 3 L/100 kg seeds; 5. Carbodan 35 ST – 1 L/100 kg seeds; 6. Carbodan 35 ST – 2 L/100 kg seeds; 7. Carbodan 35 ST – 3 L/100 kg seeds; 8. Promet 400 CS – 3 L/100 kg seeds.

The insecticides used for seed treatment were allowed in country in the experimental period. We are taking into account the current position of insecticides included in research, i.e. in past 10 years situation has changed and furathiocarb used as a standard and carbofuran are prohibited for use in EU. Also, in order to minimize the exposure of bees the uses as seed treatment and soil treatment of plant protection products containing imidacloprid, thiamethoxam and clothianidin, EU Implementing Regulation (EU) 485/2013 dated on May 24rd 2013 is prohibited for crops attractive to bees (including soybean). This is not permanent prohibition and after two years EU will decide on those insecticides.

The plants were harvested at the early flowering stage of soybean. Root system was washed in laboratory conditions and root length (cm) and nodule number (nodules/plant) were recorded. Specific nodulating ability was determined as a ratio between nodule weight (g) and root mass weight (g). Nitrate reductase activity (µmol NO_2/g fresh weight) was determined *in vivo* in leaves, stems and roots of plants by method of Jaworski (1971), plastid pigments content (mg/100 g fresh weight) by Zelenskii and Mogileva (1980). Dry root mass (g) was determined after drying to constant weight (60^oC). Experimental data statistically processed, using software SPSS for Windows 2000.

RESULTS AND DISCUSSION

3.1. Effect of insecticides on root length and dry root mass of the plants

Data in Table 1 shows that the insecticides affected root mass length of the plants. When using Gaucho 600 FS at the dose of 1 L/100 kg seeds, the increase was by 14.3% as compared to the untreated control, when using Carbodan 35 ST at the doses of 2 and 3 L/100 kg seeds, by 9.1-10.4% respectively.

The quantity of dry root mass increased – for Gaucho 600 FS, applied at the dose of 1 L/100 kg seeds, the increase was by 38.1%; for Carbodan 35 ST at the dose of 2 L/100 kg seeds, by 26.6%, and for Promet 400 CS at the dose of 3 L/100 kg seeds, by 35.0%.

Table 1. Root length and dry root mass of soybean after presowing seed treatment with insecticides

Treatments	Root length		Dry root mass	
	cm	+, increase, %	g/plant	increase in %
Control (C)	23.0	-	2.86	-
Gaucho 600 FS - 1 L	26.3	14.3	3.95	38.1
Gaucho 600 FS - 2 L	25.1	9.1	3.41	19.2
Gaucho 600 FS - 3 L	25.3	10.0	3.71	29.7
Carbodan 35 ST - 1 L	23.5	2.2	3.32	16.1
Carbodan 35 ST - 2 L	25.2	9.6	3.62	26.6
Carbodan 35 ST - 3 L	25.4	10.4	3.53	23.4
Promet 400 CS - 3 L	23.3	1.3	3.86	35
SE (P=0.05)	0.4		0.13	

3.2. Effect of insecticides on nodulation and specific nodulating ability

Nodulation of soybean was not suppressed under the influence of insecticides tested in our study (Table 2). Nodulation increased as compared to the untreated control when used Gaucho 600 FS – for dose of 1 L/100 kg seeds, by 106.0%; and for the doses of 2 and 3 L/100 kg seeds, by 77.5 and 29.6%, respectively. For Carbodan 35 ST the increase was by 15.5% for doses of 2 and 3 L/100 kg seeds.

Table 2. Nodulation of soybean after presowing seed treatment with insecticides

Treatments	Nodules/plant	increase in %
Control (C)	71	-
Gaucho 600 FS - 1 L	147	106.0
Gaucho 600 FS - 2 L	126	77.5
Gaucho 600 FS - 3 L	92	29.6
Carbodan 35 ST - 1 L	73	2.8
Carbodan 35 ST - 2 L	82	15.5
Carbodan 35 ST - 3 L	82	15.5
Promet 400 CS - 3 L	80	12.7
SE (P=0.05)	11	

Insecticides had no suppressing effect on specific nodulating ability and in all treatments it was higher than the control (Figure 1). The exceeding was higher when Gaucho 600 FS was used (on average by 27.7% compared to Carbodan 35 ST).

Specific nodulating ability had a highest value (39.320) for Gaucho 600 FS, applied at a dose of 1 L/100 kg seeds, when dry root mass was the most well developed and the largest number of nodules were formed (Table 1 and Table 2).

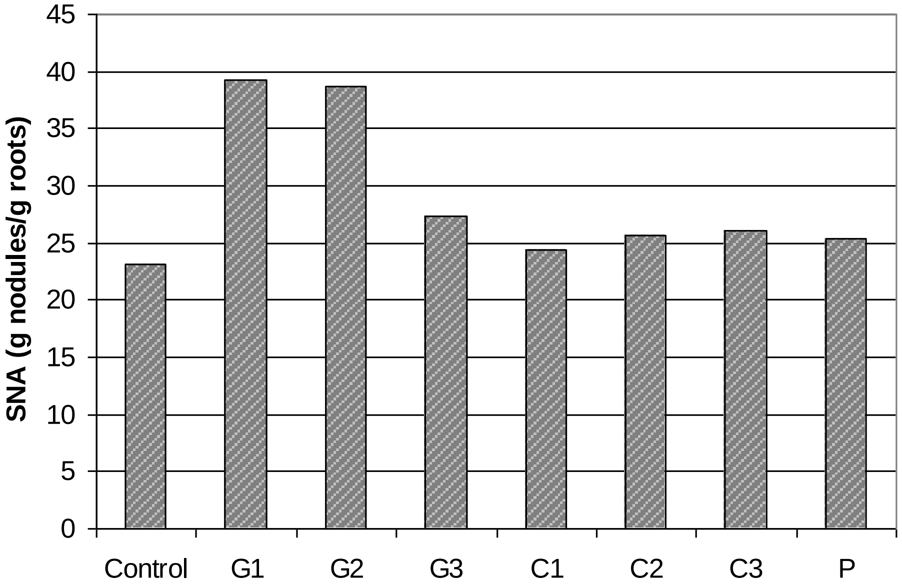

Figure 1. Specific nodulating ability of soybean after presowing treatment of seeds with insecticides

(Treatments: Control; G1, Gaucho 600 FS - 1 L; G2, Gaucho 600 FS - 2 L; G3, Gaucho 600 FS - 3 L; C1,Carbodan 35 ST - 1 L; C2, Carbodan 35 ST - 2 L; C3, Carbodan 35 ST - 3 L; P, Promet 400 CS - 3 L)

3.3. Effect of insecticides on nitrate reductase activity of the plants

Nitrate reductase activity in the organs of plants was changed compared with untreated control (Table 3). There was no linear relationship between nitrate reductase activity and the concentration of insecticides tested.

Nitrate reductase activity in the control was the highest in leaves (14.7 µM NO_2^{-}/g fr.wt.), followed by roots (10.7 µM NO_2^{-}/g fr.wt.) and stems (2.5 µM NO_2^{-}/g fr.wt.).

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

Treatments	Leaves		Stems		Roots	
	µmol NO_2^{-}/ g fresh weight	+, -, %	µmol NO_2^{-}/ g fresh weight	+, -, %	µmol NO_2^{-}/ g fresh weight	+, -, %
Control (C)	14.7	-	2.5	-	10.7	-
Gaucho 600 FS - 1 L	14.5	- 1.4	2.9	+ 16	3.2	- 70.1
Gaucho 600 FS - 2 L	8.1	- 44.9	3.3	+ 32	15.2	+ 42.1
Gaucho 600 FS - 3 L	14.2	- 3.4	3.4	+ 36	2.8	- 73.8
Carbodan 35 ST - 1 L	9.5	- 35.4	5.7	+ 128	24.0	+124.3
Carbodan 35 ST - 2 L	13.9	- 5.4	6.5	+ 160	4.4	- 58.9
Carbodan 35 ST - 3 L	28.3	+ 92.5	2.8	+ 12	16.7	+ 56.1
Promet 400 CS - 3 L	12.6	- 14.3	2.5	-	10.9	+ 1.9
SE (P=0.05)	2.1		0.5		2.6	

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

Data showed a common tendency for the decrease of nitrate reductase activity in leaves (where the reduction of nitrate mainly occurred) and significant increase in stems. Only for Carbodan 35 ST, applied at the dose of 3 L/100 kg seeds, nitrate reductase activity in leaves increased by 92.5%. In the treatment with Carbodan 35 ST, applied at the doses of 1 and 2 L/100 kg seeds, the greatest increase in the activity of this enzyme was observed. Our data confirm the opinion of other authors that plants can accumulate and reduced nitrates in stems due to the various factors (Andrews et al., 1984; Ligero et al., 1987).

Nitrate reductase activity in roots varied depending on the dose of insecticides. For Gaucho 600 FS it decreased by 70.1% and 73.8 %, respectively for doses of 1 and 3 L/100 kg seeds, and increased by 42.1% for dose of 2 L/100 kg seeds. For Carbodan 35 ST it increased significantly (by 124.3% and 56.1%) for the doses of 1 and 3 L/100 kg seeds, but decreased by 58.9% for the dose of 2 L/100 kg seeds. Nitrate reductase activity in the stems and roots were at the level of untreated plants for Promet 400 CS.

We assume that the variation of nitrate reductase activity was associated with effect of insecticides tested on nodulation. As a result of compensatory interaction of two systems of inorganic nitrogen assimilation the activity of nitrate reductase was changed. Nodules used the products of photosynthates as a source of energy for molecular nitrogen reduction. This consumption leads to decrease in energy and reduction potential, which are necessary for nitrate reduction in leaves and roots (Remmler and Campbell, 1986; Campbell, 1989).

Nitrate reductase activity increased in all plant organs when applied Carbodan 35 ST at the dose of 3 L/100 kg seeds despite increasing nodule number. A possible explanation is that insecticide in this dose affects nitrogen-fixing bacteria strains in the nodules and they become ineffective. We found a negative correlation between nodule number and nitrate reductase activity (in leaves r = - 0.26, and in roots r = - 0.36).

3.4. Changes in plastid pigments content

Photosynthesis is a process closely related to nitrogen fixation and nitrate assimilation. It is known that plastid pigments content (chlorophylls and carotenoids) is one of the indicators of the response of plants to environmental changes due to various factors. Treatment with insecticides Gaucho 600 FS at the doses of 2 and 3 L/100 kg seeds, and with Carbodan 35 ST at the doses of 1 and 3 L/100 kg seeds, increased the content of chlorophylls a and b, carotenoids and total pigment content compared to untreated control (Table 4).

When applied Gaucho 600 FS at the dose of 1 L/100 kg seeds, Carbodan 35 ST at the dose of 2 L/100 kg seeds, and Promet 400 CS at the dose of 3 L/100 kg seeds, plastid pigments content decreased. In the treatment with Gaucho 600 FS at the dose of 1 L/100 kg seeds, decrease of content of chlorophylls a+b was insignificant (by 4.4%), while in the treatment with Promet 400 CS at the dose of 3 L/100 kg seeds, by 14.9%.

Carotenoids play an important role in protecting the photosynthetic apparatus of plants. In our study the content of carotenoids increased when applied insecticides, except treatment with Gaucho at the dose of 1 L/100 kg seeds and Promet CS at the dose of 3 L/100 kg seeds. The largest increase of carotenoids was found in treatment with Carbodan 35 ST at the dose of 3 L/100 kg seeds (by 40.1%). Significant increase of nitrate reductase activity in leaves, stems and roots was observed in this treatment only. This is probably a protective reaction of plants resulting from activation of oxidative processes (Hristov and Abrasheva, 2001). Correlation between nitrate reductase activity and plastid pigments content was found (r =0.63).

Table 4. Plastid pigments content in leaves of soybean after presowing treatment of seeds with insecticides

Treatments	Plastid pigments mg/100 mg fr.wt.				
	chl. a+b	carote noids	total content	chl.a/chl. b	chl.a+b/ carotenoids
Control (C)	288.5	65.3	353.8	1.47	4.42
Gaucho 600 FS - 1 L	275.9	54.6	330.5	1.53	5.05
Gaucho 600 FS - 2 L	311.9	73.6	385.5	1.48	4.24
Gaucho 600 FS - 3 L	297.6	68.9	366.5	1.38	4.32
Carbodan 35 ST - 1 L	290.5	72.4	362.9	1.43	4.01
Carbodan 35 ST - 2 L	278.2	65.5	343.7	1.41	4.24
Carbodan 35 ST - 3 L	354.3	91.5	445.8	1.49	3.87
Promet 400 CS - 3 L	245.4	63.0	308.4	1.61	3.90
SE (P=0.05)	11.3	3.79	14.5	0.02	0.13

It is believed that the ratio of chlorophyll a/chlorophyll b and chlorophylls a+ b/carotenoids indicate the physiological status of plants (Petkova and Poryazov, 2007). Ratio of chlorophyll a/chlorophyll b in our study varied from 1.38 to 1.61 as compared to untreated control (1.47).

Chlorophylls a+b/carotenoids ratio in all treatments had lover values as compared to control, except for Gaucho 600 FS, applied at the dose of 1 L/100 kg seeds. The lowest value (3.87, and 4.42 for the control) was found in Carbodan 35 ST at the dose of 3 L/100 kg seeds due to the increased part of carotenoids.

CONCLUSIONS

Insecticides Gaucho 600 FS and Carbodan 35 ST, applied for presowing treatment at the dose of 1, 2 and 3 L/100 kg seeds, and Promet 400 CS at the dose of 1 L/100 kg seeds, had no suppressive effect on root length, dry root mass and specific nodulating ability in soybean. When used Gaucho 600 FS at the dose of 1 L/100 kg seeds, the root mass of plants was most developed, the largest number of nodules was formed, and specific nodulating ability was highest.

The common tendency for decrease of nitrate reductase activity in leaves and significant increase in stems was found. Nitrate reductase activity increased in leaves, stems and roots for Carbodan 35 ST applied at the dose of 3 L/100 kg seeds. Chlorophylls a+b/carotenoids ratio exceeded this of the control only in the treatment with Gaucho 600 FS at the dose of 1 L/100 kg seeds.

However the analysis of the plant biomass did not include the amount of possible undegraded traces after using the insecticides tested.

REFERENCES

Ahemad, M., Khan, M.S. (2010) Comparative toxicity of selected insecticides to pea plants and growth promotion in response to insecticide-tolerant and plant growth promoting *Rhizobium leguminosarum.* Crop Protection, 29, 325-329.

Ahemad, M., Khan, M.S. (2011) Pesticide Interactions with Soil Microflora: Importance in Bioremediation. In: Ahmad, I. et al. (Eds.), Microbes and Microbial Technology: Agricultural and Environmental Applications, Chapter 15, Springer Science+Business Media, LLC 2011, p. 393.

Andrews, M., Sutherland, J., Thomas, R., Sprent, J. (1984) Distribution of nitrate reductase activity in six legumes: The importance of the stem. New Phytol., 98, 301-310.

Anjum Suhail, M., Jalal Arif and Muhammad Shahid Yazdani. (2000) Comparative Efficacy of Some Insecticides Against Insect Pest Complex of Maize. Pakistan Journal of Biological Sciences, 3, 1052-1053.

Arrese-Igor, C., Garcia-Plazaola, J., Hernandez, A., Aparicio-Tejo, P. (1990) Effect of low nitrate supply to nodulated lucerne on time course of activities of enzymes involved in inorganic nitrogen metabolism. Physiologia Plantarum, 80, 185-190.

Athar, M., Harding, J. (2000) Nodulating legumes from the Tahoe Basin, California. Sida, 19, (1), 205-211.

Campbell, W. (1989) Structure and regulation of nitrate reductase in higher plants. In: Wray, J.L., Kinghorn, J.R. (Eds.), Molecular and Genetic Aspects of Nitrate Assimilation, Oxford, Science Publications, Oxford, pp. 125-154.

Chamber-Perez, M., Camacho-Martinez, M., Soriano-Niebla, J. (1997) Nitrate-reductase activities of *Bradyrhizobium* sp. in tropical legumes: effects of nitrate on O_2 diffusion in nodules and carbon costs of N_2 fixation. J. Plant Physiol., 150, 92-96.

Dewar, A.M., May, M.J., Woiwod, I.P., Haylock, L.I., Champion, G.T., Garner, B.H., Sands, R.J.N., Qi, A., Pidgeon, J.D. (2003) A novel approach to the use of genetically modified herbicide tolerant crops for envinronmental benefit. Proc. R. Soc Lond. B 270, 335-340.

Dochkova, B., Vasileva, V., Ilieva, A. (2000) Effect of presowing treatment of seeds with Promet 400 SK on nodule-feeding weevils of *Sitona* genus and nodulation in spring forage pea. Plant Science (Bg), 37, 645-649.

Epperlein, K., Fuschs, E., Gruntzig, M., Kuntze, L. (1995) Influence of a seed treatment of maize with imidacloprid on the colonization of aphids as well as on the infestation with viruses transmitted by aphids. Arch. Phytopathol. Pl. Protect., 29, 401-415.

Frame, J. (2005) Forage legumes for temperate grasslands. Food and Agriculture Organization of the United Nations.

Harper, J., Hageman, R. (1972) Canopy and Seasonal Profiles of Nitrate Reductase in Soybeans (*Glycine max* L. Merr.). Plant Physiol., 49, 146-154.

Henry, R., Johnson, W., Wise, K. (2011) The impact a fungicide and an insecticide on soybean growth, yield and profitability. Crop Protection, 30, 1629-1634.

Hristov, I., Abrasheva, P. (2001) Influence of *Grapevine fanleaf* virus *Grapevine leafroll* associated virus 3 on the vine in terms of in vitro cultivation. Plant Science (Bg), 38, 269-274.

Jaworski, E. (1971) Nitrate reductase assay in plant tissues. Biochem. Biophis., Res. Commun., 43, 1274-1279.

Khan, M., Zaidi, A., Aamil, M. (2004) Influence of herbicides on Chickpea *Mesorhizobium* symbiosis. Agronomie, 24, 123-127.

King, C., Purcell, L. (2001) Soybean nodule size and relationship to nitrogen fixation response to water deficit. Crop Science, 31, 1376-1378.

Kot's, S. Ya. (2001) Physiological bases of highly efficient functioning of alfalfa symbiotic systems in agrocenoses. Thesis for the degree of a Doctor of Sciences (Biology). Institute of Plant Physiology and Genetics, NAS of Ukraine, Kyiv.

Kretovich, V. (1997) Biochemistry assimilation of atmospheric nitrogen by plants, M., p. 486.

Krohn, J., Hellpointner, E. (2002) Environmental fate of imidacloprid. Pflanzenschutz Nachrichten Bayer, Special Edition, 55, 1-26.

Ligero, F., Lluch, C., Hervas, A. (1987) Effect of nodulation on the expression of nitrate reductase activity in pea cultivars. New Phytol., 1, 53-61.

Lindemann, W.C., Glover, C.R. (1999). Nitrogen fixation by legumes. (Available on-line at http://www.cahe.nmsu.edu/pubs/_a/a-129.html).

O'Donohue, M., Moriarty, D., McRae, I. (1991) Nitrogen fixation in sediments and the rhizosphere *Zostera capricornia*. Microb Ecol., 22, 53-64.

Petkova, V., Poryazov, I. (2007) Results from application of organic regulator and stimulator Humustim in garden bean and Brussels cabbage. In: Sengalevich, G. et al., (Eds.), Humustim – gift of nature, 119-125.

Peyraud, J. L., Le Gall, A., Lüscher, A. (2009) Potential food production from forage legume-based-systems in Europe: An overview. Irish J. Agric. Food Res. 48, 115–135.

Pimentel, D. (1995) Amounts of pesticides reaching target pests: environmental impacts and ethics. J. Agric. Environ. Ethics, 8, 17-29.

Remmler, J., Campbell, W. (1986) Regulation of corn leaf nitrate reductase: II. Synthesis and turnover of the enzyme's activity and protein. Plant Physiol., 80, 442-447.

Rotrekl, J., Cejtcham,l J. (2008) Control by seed dressing of leaf weevils of the genus *Sitona* (Col.: Curculionidae) Feeding on Sprouting Alfalfa. Plant Protect. Sci., 44, 61–67.

Sarnaik, S.S., Kanekar, P.P., Raut, V.M., Taware, S.P., Chavan, K.S., Bhadbhade, B.J. (2006) Effect of application of different pesticides to soybean on the soil microflora. Journal of Environmental Biology, 27, 2, 423-426.

Serrano, A., Chamber, M. (1990) Nitrate reduction in *Bradyrhizobium* sp (*Lupinus*) strains and its effects on their symbiosis with *Lupinus luteus*. J Plant Physiol., 136, 240–246.

Todorova, R. (2003) Research and development of new soybean genotypes, resistant to abiotic stress. Ph.D. thesis, S.

Vance, C. (1998) Legume symbiotic nitrogen fixation: agronomic aspects. In: Spaink, H.P., Kondorosi, A., Hooykaas, P.J.J. (Eds.). The Rhizobiaceae: molecular biology of model plant-associated bacteria. Kluwer Academic Publishers, pp. 509-530.

Vasileva, V., Ilieva A., Dochkova, B. (2003) Possibilities for control of injurious insects in forage pea by presowing treatment of seeds with insecticides. II. Nodulation and nitrogenous compounds. Acta Entomologica Bulgarica,9, 22-28.

Wahid, A., Farooq, M. (2012) Is seed invigoration economical and practical? Journal of Agriculture and Social Sciences, 8, 79-80.

Welsh, D., Bourgues, S., de Wit, R., Herbert, R.A. (1996) Seasonal variations in nitrogen fixation (acetylene reduction) and sulphate reduction rates in the rhizosphere of *Zosteranoltii*: nitrogen fixation by sulphate reducing bacteria. Mar Biol.,125, 619-628.

Zahran, H.H. (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. Rev., 63, 968-989.

Zelenskii, M., Mogileva, G. (1980) Comparative evaluation of photosynthetic ability of agricultural crops by photochemical activity of chloroplasts. VIR, Leningrad, pp. 36 (in Russian).