Fertilizers with Protein Chelated Structures with Biostimulator Role

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The fast evolution of the methods and fertilization technologies using the extraradicular and liquid fertilizers was possibile due to controlled application of these in terms of the phases of vegetation, crop, nutritional deficiencies, as well as to the increase of the efficiency of the indicators as fertilization costs, crop increase rate and the promotion of ecological and durable agriculture. The use of the fertilizers of extraradicular and liquid type, because of the very little quantities of the applied active substance and because of the superior degrees of use by the plants of the nutritive, allows the reduction of the fertilization degree with the classic fertilizers and the reduction of the pollution of the soil and of the ground-water table through their administration. In the last 10 years, there have been used the hydrolysate proteins, of vegetal or animal nature, in composition of the fertilizers as the chelator substances for the important microelements in the metabolism of the plants [1-5]. A priority for Romanian agriculture research is the use of the hydrolyzates of collagen from animal protein for preparation of extraradicular fertilizer compositions [6-9].In this paper are presented the agrochemicals tests results of the new fertilizers with the high concentrations of microelements and of collagen hydrolyzate, which have the biostimulator role.

Keywords: fertilizers, hydrolyzate, collagen, biostimulator, chelates

The fertilizers with the extraradicular application are the homogenous solutions of chemical compounds, which contain the nutritive essential macro elements (N, P, K, Ca, Mg), as well as the microelements with the significant role in the biochemical processes in the metabolism of the plants (Fe, Cu, Zn, Mn, Co, S, Mo etc.). Microelements are stabilized as the metallic chelates or as some chemical compounds. Also other organic components of type of polycarboxylic acids, polysaccharides, surfactants and substances with the bioregulators make up the fertilizer compositions [3, 10-12].

The biostimulators for plants are a special category of synthetic or natural substances, which regulate the physiological processes from the plant. Generally, the products with bioregulator role are the organical substances which being applied in the reduced concentrations, participate at the physiological processes of growth of the plants, producing favorable quantitative and qualitative effects on the crops [1, 2, 13].

World-wide there is remarked the rising tendency for the production of the fertilizers which have in the composition the natural substances (the main or secondary products in the different technological processes) or the synthesis substances (which are accepted or not through the international regulations) from the category of the biostimulators, for example: heteroauxin, betaindoilacetic acid, triiodbenzoic acid, gibberellins and its derivatives, amino-acids (glycine, alanine, phenylalanine, proline, hydroxiproline, asparagine, glutamine, arginine, histidine, lysine, serine, threonine, valine), vitamins, peptides, ureides, phosphoproteins, protein hydrolysates, extracts from the algae's, as well as humic/fulvic acids. These substances in the reduced concentrations,sometimes even of of ppm order, manege the control of radicular system, and the foliar surface lead to the increase, of the new vegetative mass flowering rate and higher fruit crops. Also, they help the growth under conditions of climatic and/or technological stress [3, 5, 14, 15].

There are a lot of products and experimental data which present diverse solutions of fertilizers on the base of phosphates, potassium and ammonium polyphosphates and of protein hydrolyzates which are used as fertilizers in the high and intensive culture from the greenhouses.

It is well known that the using of the microelements as iron, copper, zinc, calcium, magnesium and manganese which are chelated with the protein hydrolyzates are easier assimilated by the plants and animals. Also, there is mentioned that the use of the protein hydrolyzate in the mixture with the polyphosphates of potassium through the increase of the agricultural production, for the increase of the absorption yields of phosphor and potassium. This effect was remarked for any source of protein in the hydrolyzate form which contained polypeptides, peptides and amino-acids including alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine, hydroxiproline, isoleucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine etc. [1,2,4,5,13].

Although, the protein hydrolyzate has a minor role in the absorption of the nitrogen by the plant, however this can assimilate the nitrogen from the protein in an easier way than under the form of ammonia gas, ammonia ion, or other sources of nitrogen.

The paper shows the new variants of fertilizers having a complex matrix of NPK type with a ratio $N:P_2O_5:K_2O$ of 1:1:1 (the primary and secondary phosphates of potassium and nitrogen under the nitric, ammonia and amide form), the microelements (boron, copper, iron, magnesium, molybdenum, zinc) in which a protein hydrolyzate (collagen) was introduced. The fertilizers were physico-chemically characterized and tested in the Vegetation House and in the experimental field on two vegetal cultures (sunflower and soya).

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Table 1
PHYSICO-CHEMICAL FEATURES OF THE COLLAGEN HYDROLYZATE - HO_8

No. crt	Features	Experimental values
2	Colour	yellowish-white
3	Humidity (%)	8.04
4	Total Nitrogen* (%)	16.10 / 17.75*
5	Aminic Nitrogen* (%)	0.75 / 0.88*
6	Ratio (N total/N aminic) (RNH_2)	20.1/ 21.4*
7	Proteic Substance (%)	90.48/ 99.75*
8	Fat substances (%)	0.70 / 0.85*
9	Mineral substances (%)	0.15/ 0.25*
10	pH solution 5 %	4.3- 4.5
11	Intrinsec viscosity (dL/g),20 ^{0}C	0.060
12	Average molecular weight, Dalton (Da)	14500

*The values which are expressed at the dry substance (d.s.)

Experimental part

In the composition of the studied fertilizers, collagen hydrolysate acts as the chelator for the metallic ions (microelements). It was used a collagen hydrolyzate of HO_8 which was obtained through the bovine leather through the neutral hydrolyze, at 120°C, for 8 h, the product which is manufactured at INCDTP – ICPI Division, Bucharest [16, 17].

Structurally, the collagen is a scleroproteina representing about 30-35% from the total of the amine proteins; it contains the important quantities from all the amino-acids components of which proteins (especially glycine, proline and alanine), but cystine, cysteine and tryptophan are absent. Two from the amino-acids constituent, the proline and the hydroxiproline show a special importance for the formation of the triple helicoidally structure which is specific for the native collagen. Through the hydrothermal degradation of the triple helicoidally structure there are obtained the polypeptides with the different molecular weights which are dependent on the parameters of the used hydrolyses process. The polydispersity degree is very high and on this account the hydrolyzates are characterized through the average molecular weight which is determined through the chromatography on gel or viscometry [16].

The hydrolysis degree of the product can be determined through the content of amino nitrogen (NH_2). The ratio between the total nitrogen and the amino nitrogen is a measure of the hydrolyze degree and it shows the level of transformation of the total nitrogen in the free amino groups, respectively the free amino-acids. The value of the ratio N (total)/(NH_2) decreases when the hydrolyses degree increases, because the breakage of the chains of polypeptides advances and appeared more and more free amino groups, as a result of the their unlocking from the peptides connections The polydispersity degree of the partial collagen hydrolyzates. is very high because the cleavage of the polypeptides chains is promoted by the preliminary treatments of the hydrolyze proceeding which affect the intramolecular bounds. On this account the hydrolyzates are characterized through the average molecular weight which is determined through the chromatography on Sephadex gel or the viscometry [16].

The features of the powder collagen hydrolyzate (dried through atomization), used to obtain the fertilizers with the extraradicular application are showed in the table 1.

The collagen hydrolyzate is characterized through the high degree of purity (the protein substance of about 99.75%, which is formed in most polypeptides containing

Table 2
COMPOSITION IN THE AMINO ACIDS OF THE COLLAGEN HYDROLYZATE FROM THE FERTILIZERS

No.crt.	Amino acid	Concentration* (%)
1	Glycine	33.05
2	Proline	12.51
3	Alanine	12.03
4	Glutamic acid	8.2
5	Hydroxiproline	7.67
6	Aminosuccinic Acid	5.1
7	Arginine	4.54
8	Serine	3.48
9	Lysine	2.75
10	Valine	2.32
11	Leucine	2.31
12	Phenylalanine	1.8
13	Threonine	1.56
14	Isoleucine	1.05
15	Histidine	0.97
16	Tyrosine	0.38
17	Methionine	0.28

Note: * The values which are expressed at the dry substance (s.u.)

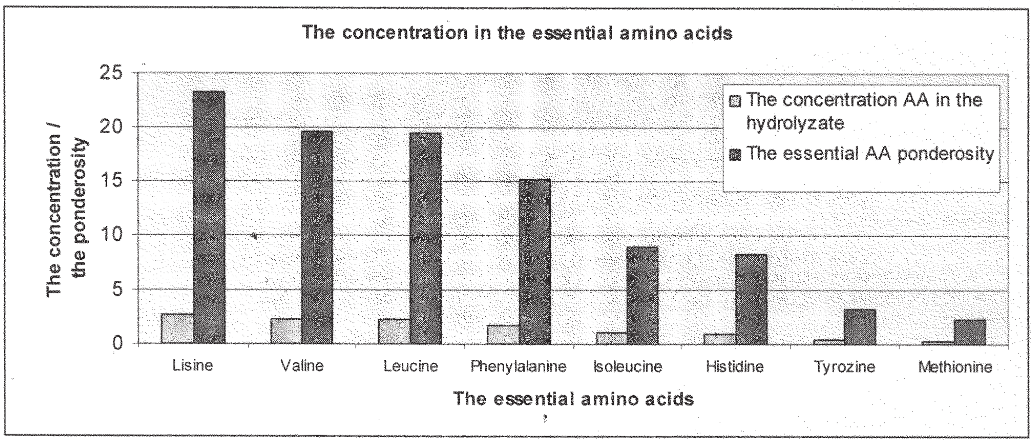

Fig. 1. Concentration and the weight of the essential amino acids from the proteic hydrolyzate of collagen

about 10 amino acids in polypeptide chain, with the molecular weight of 14 500 Da.

The composition in amino acids (AA) of the protein hydrolyzate used to obtain the extraradiculari fertilizers, determined through the HPLC (Jasco LC Net II/ADC with a reversed phase cartridge colum Nucleosil C18, 250x4 mm – Merck) chromatography is presented in the table 2. The collagen contains less essential amino acids in contrast with the other animal proteins, about 80% from those which are in the casein (fig. 1).

The technological process to obtain of the fertilizers of extraradicular type is shown in the figure 2 and it comprises the following technological operations:

- preparation of the solution of primary phosphates and of the secondary phosphates of potassium through the neutralization of the phosphoric acid (concentration 85% H_3PO_4) with the potassium carbonate or the potassium hydroxide (the solution of concentration 8 -10% K_2O);

- preparation of the solution of NPK type through the adjunction of nitrogen under the nitric, ammonia and amide form (the mass report N_{NO3}:N_{NH4}:N_{NH2}: = 1:1:35);

- preparation of the solution of chelated microelements (Fe, Cu, Zn, Mn, Mg, Ni, Co, B, Mo, S) using as agents of chelating - EDTA and respectively complexing, citric acid - $C_6H_8O_7$;

- preparation of the solution of 20% of protein hydrolyzate through the dissolution of the powder in the water;

- the mixture, the homogenization of the two solutions (b and c) and the getting of the final solution of witness fertilizer 111, 131 and 111;

- the mixture/homogenization of the NPK solution with the microelements (e) with the solution of protein hydrolyzate (d) and the getting of the experimental fertilizant 111 AH1, 111 AH2 and 111 AH4;

- the analysis of the physical-chemical features of the variants of fertilizers.

For avoiding the division of the microelements in the component of the solutions there are introduced the substances which form the chelates/stable compounds with these (EDTA, DTPA, EDDHA, HEEDTA, $C_6H_8O_7$, $C_4H_6O_6$, $C_6H_{12}O_7$, $C_4H_6O_4$, polyphosphates, lignite sulphonates, humates, amino acids) conferring them a higher mobility

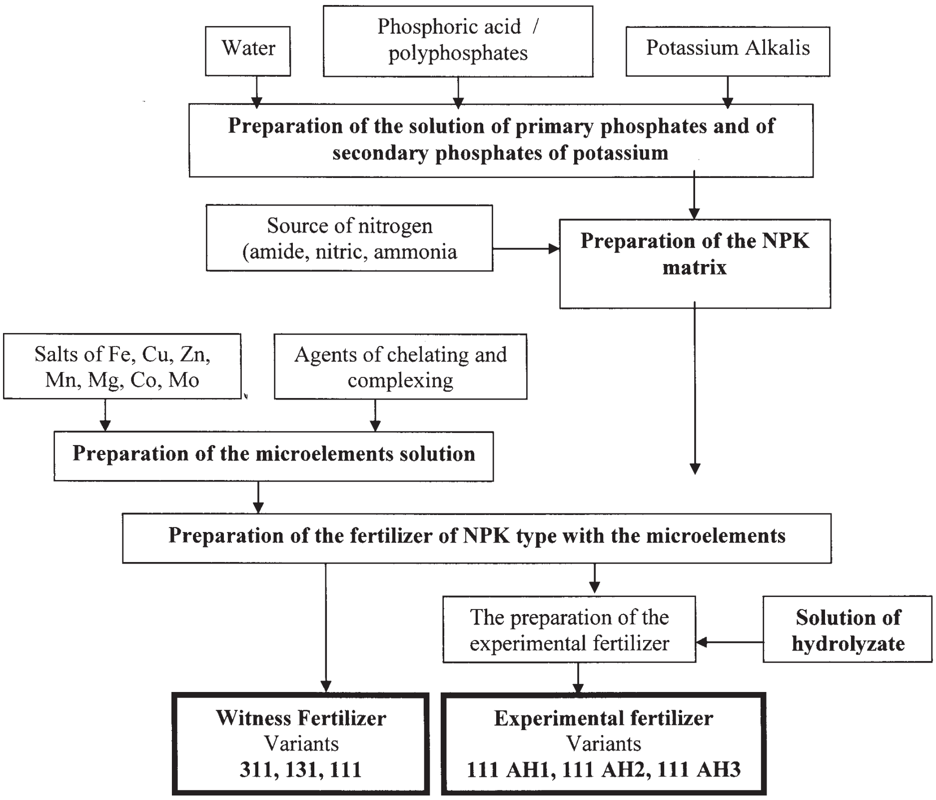

Fig. 2. Technological scheme for obtaining of the fertilizers

Table 3
COMPOSITIONAL VARIANTS OF PERFORMED AND EXPERIMENTED FERTILIZERS

No. crt.	Physico-chemical features	Classic (witness) extraradicular fertilizer			Extraradicular fertilizer with the protein hydrolyzate		
		311	111	131	111 AH1	111 AH2	111 AH4
1	Total Nitrogen (N), (g/l)	180	90	60	90	90	90
2	Phosphor (P_2O_5), (g/l)	60	90	180	90	90	90
3	Potassium(K_2O), (g/l)	50	80	60	80	80	80
4	Boron (B), (g/l)	0.2	0.2	0.2	0.2	0.2	0.2
5	Copper (Cu), (g/l)	0.08	0.08	0.08	0.08	0.08	0.08
6	Iron (Fe), (g/l)	0.4	0.4	0.4	0.4	0.4	0.4
7	Zinc (Zn), (g/l)	0.06	0.06	0.06	0.06	0.06	0.06
8	Molybdenum (Mo), (g/l)	0.01	0.01	0.01	0.01	0.01	0.01
9	Sulphur (S), (g/l)	0.6	0.6	0.6	0.6	0.6	0.6
10	Magnesium (Mg), (g/l)	0.15	0.15	0.15	0.15	0.15	0.15
11	Manganese (Mn), (g/l)	0.2	0.2	0.2	0.2	0.2	0.2
12	Cobalt (Co), (g/l)	0.01	0.01	0.01	0.01	0.01	0.01
13	Protein substances, (%)	-	-	-	1	2	4
14	pH (units of pH)	6.6 – 6.8	6.6 – 6.8	6.6 – 6.8	6.6 – 6.8	6.6 – 6.8	6.6 – 6.8

in the vegetable tissues, the relative efficiency of these fertilizers being dependent of the rate and the size of the transport of the nutritive constituents through the extraradicular surface of the plant.

The polypeptides from the collagen hydrolyzate form the chelate compounds with the metallic ions, especially Fe, Ca, Mg, Cu, Zn through the reactive carboxylic, hydroxylic and nitrogenophore groups of NH- pirolidinic type and through the peptides -CO-NH- connections [18]. The formed chelates are more stable as the average molecular weight of the hydrolyzate is less or the concentration of the metallic ions is more reduced. For assuring the transmembranous transport of the chelate microelements it was opted for the neutral collagen hydrolyzate of HO_8 type, which it is used as chelated support for the microelements used in the food of the animals, having a biostimulating action [16].

The physico-chemical features of the variants of fertilizers of extraradicular type used in the agricultural chemical tests are showed in the table 3.

The agricultural chemical tests achieved on the wheat cultures (ROMULUS type) and soya (AG0801RR type), the experiments from the field were achieved on a soil of chernozem with 3.18 – 3.55 % humus, 0.18 – 0.26% nitrogen, mobile phosphor (soluble P in AL) 37.6 – 138 ppm, well supplied with the mobile potassium (soluble K in AL) 199 – 364 ppm and an alkaline *p*H of 8.0 – 8.2.

The extraradicular fertilization was achieved with the solutions of concentration 2%, in the doses of 5 litres of fertilizer / ha (the first treatment which is applied with equipment for applying of fertilizers) and respectively 10%, in doses of 5 litres of fertilizer / ha (at the second treatment applied by helicopter).

Results and discussions

The use of the collagen hydrolysate in the NPK matrix which contains the chelated microelements leads to the solutions of stable physico-chemically fertilizers in the diverse conditions of deposition and utilization which are specific to these types of liquid fertilizers.

Use of hydrolysate protein has not only the chelate role but also a protective colloid role fact which helps to maintain the stability of the fertilizer and through the components with the high molecular mass (14000 Da) it forms the membranes on the surface of the vegetable tissue. These adherent membranes have an action of gradual cession of the chelated microelements of collagen polypeptides, acting as hydrophilic protector under the factors of environment and also as biostimulator.

The agricultural chemical tests in the Vegetation House were achieved in the vases of Mitscherlich type, in which 20 kg of soil were introduced. The agricultural chemical experiments were performed on two cycles of vegetation, and the tested experimental variants were: 111 AH 1, 111 AH2 and 111 AH4, respectively with 1, 2 and 4% protein hydrolysate in NPK structure of 1:1:1 type with microelements. The used soil in the agricultural chemical tests was a cambic chernozem with the average fertility: humus 3.5%, total nitrogen (N) 0.170 %, 50 ppm mobile phosphor (P), 300 ppm mobile potassium (K) and a *p*H with the values of 6.2 – 6.4.

As the extraradicular fertilizer with the protein substances which are used in the agricultural chemical tests there was selected the variant 111 AH1 (with a content of 1% protein hydrolysate). The tests were achieved towards a foliar unfertilized witness and 3 witnesses at which there were applied the treatments with the classic extraradicular fertilizers with a report $N:P_2O_5:K_2O$ de 3:1:1, 1:3:1 and respectively 1:1:1 (table 1).

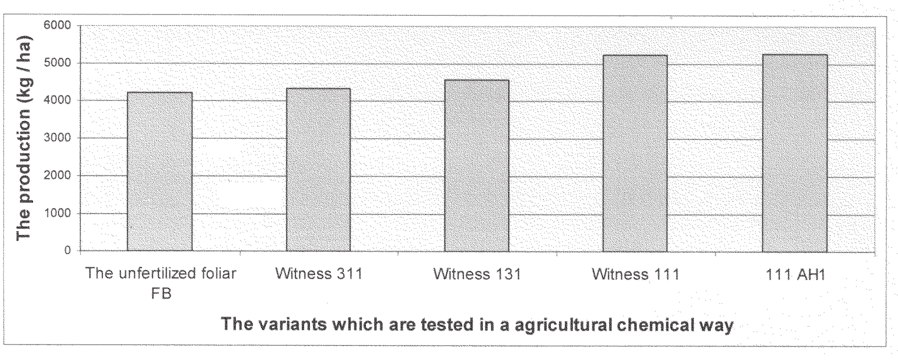

Fig. 3. Evolution of the production (Kg/ha) in terms of the variants which are tested in a agricultural chemical way (the wheat of ROMULUS type)

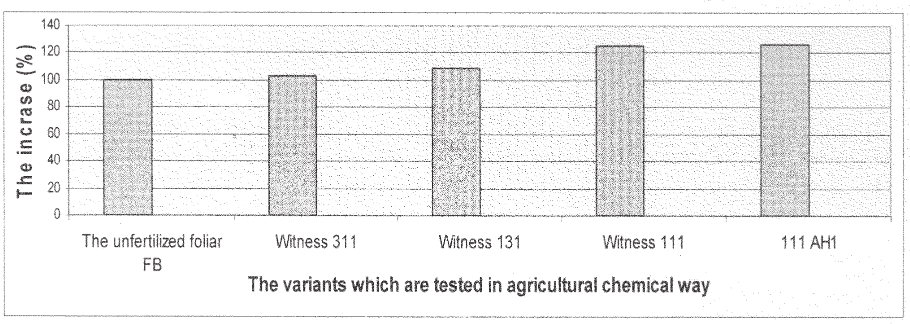

Fig. 4. Evolution of the production increase (%) in terms of the variants which are tested in an agricultural chemical way (wheat of ROMULUS type)

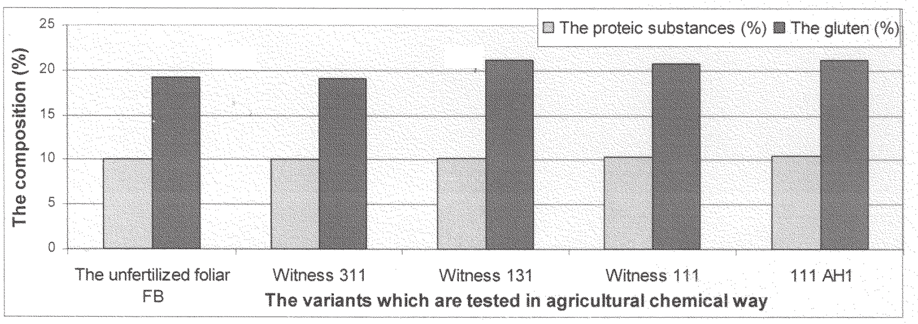

Fig.5. Composition of the wheat grain in the protein substances (%) and in the gluten (%) in terms of the variants which are tested in an agricultural chemical way (wheat of ROMULUS type)

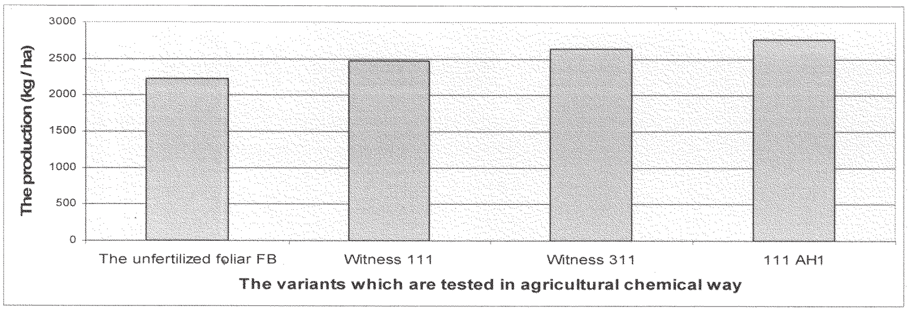

Fig.6. Evolution of the production (Kg/ ha) in terms of the variants which are tested in an agricultural chemical way (soya, the AG0801RR type)

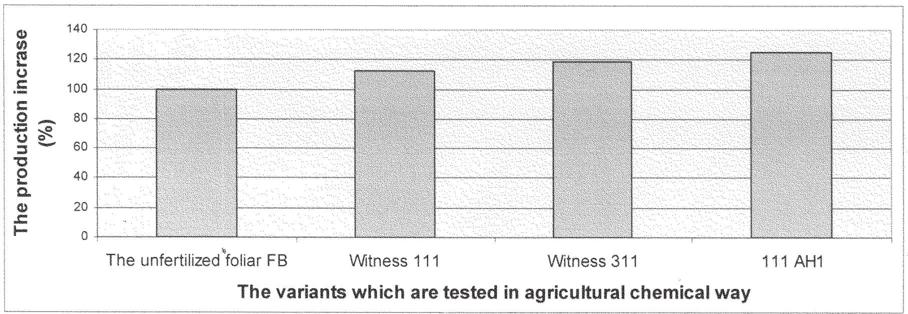

Fig.7.Evolution of the production increase (%) in terms of the variants which are tested in an agricultural chemical way (soya, the AG0801RR type)

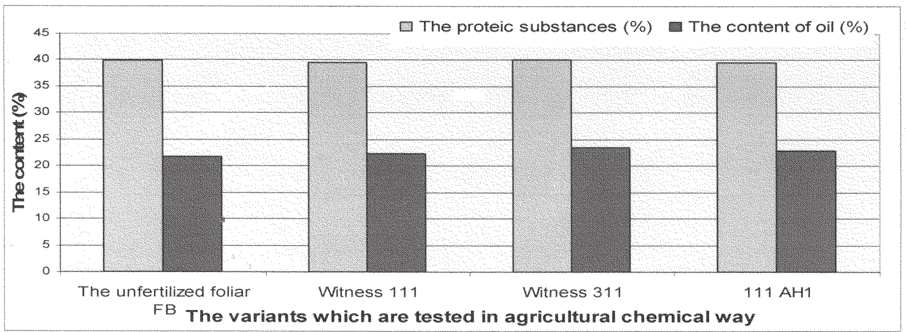

Fig. 8. Composition of the soya grain in the protein substances (%) and in the oil (%) in terms of the variants which are tested in an agricultural chemical way (soya, the AG0801RR type)

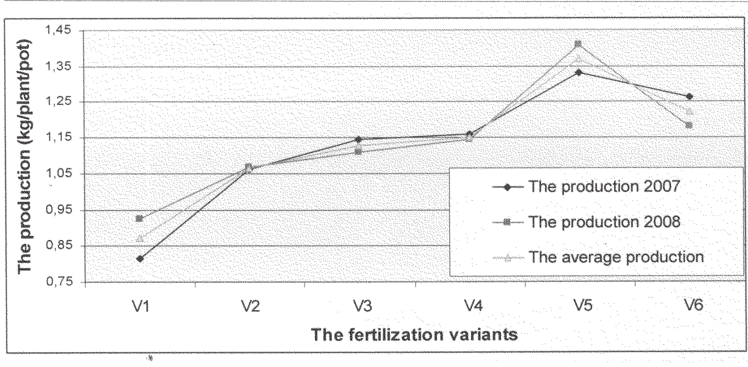

Fig. 9. Evolution of the production at the tomatoes (kg/planta/vase) in terms of the experimental variant, the tests were achieved 2007, 2008 and the multiannual average

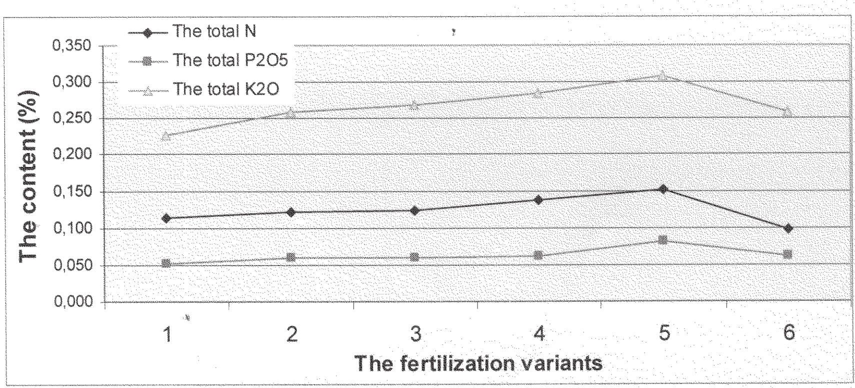

Fig. 10. Evolution of the average of concentration (%) of the nitrogen, the phosphor and of the potassium in the tomatoes, the tests were achieved on two cycles of production in the Vegetation House

The results concerning the increases of production and quality of the crop which are obtained using the experimental fertilizers are showed in the figures 3-8.

In the case of the agricultural chemical tests which are achieved on the tomatoes in Vegetation House (Dacia Pontica type), the experimental variants were the following: basal V1 – unfertilized, basal V2 – fertilized with N 100 mg/kg of soil, P_2O_5 100 mg/kg of soil, K_2O 100 mg/kg of soil (unfertilized foliar), basal V3 – fertilized, fertilized foliar with the witness of 111 type (without the protein hydrolysate), basal V4 – fertilized, fertilized foliar with 111AH1 (cu 1% protein hydrolysate), basal V5 – fertilized, fertilized foliar with 111AH2 (with 2% protein hydrolysate), basal V6 – fertilized, fertilized foliar with 111AH4 (with 4% protein hydrolysate).

The results of the agricultural chemical experiments, the increases and the composition of the finished products (N,P,K and microelements) are shown in the figures 9 and 10.

The three extraradicular fertilizations which are applied on the vegetation cycle were achieved with the solutions of concentration of 1% in the dose of 30 mL of fertilizer which was applied on the vegetation vase.

The subject of these paper has been also studied by other researches [19-21].

Conclusions

Within the experimental activity there were obtained 3 fertilizers with the extraradicular application which was remarked through their complexity due to of the association in the matrix of a classic fertilizer of NPK type, with the microelements, of some organic substances – the protein hydrolysate of collagen – with the chelate and biostimulator role.

The three extraradicular fertilizers with the protein substances of 111AH1, 111AH2 and 111AH4 type and the witness of 311, 131 and 111 type were characterized in an agricultural chemical way and the agricultural chemical tests in the Vegetation House and the experimental field.

The results of the agricultural chemical tests which were achieved in the experimental field, on two cycles of vegetation, were favorable for the wheat and less favorable for the soya because of the insufficient rainfalls from the period May –July.

The cultures of wheat and soya reacted best at the application of the extraradicular fertilizers. The most efficient products being the variants which had an engrafted structure of the protein hydrolysate on the classic matrix of NPK type. Even in the case of the difficult climatic conditions the use of the extraradicular fertilizers with the organical substances (the protein hydrolysate) with the biostimulator role managed at the getting of production increases which were statistically assured, significant.

At the cultures which were tested in the Vegetation House and in the experimental field, the use of the extraradicular fertilizers which showed in the structure of the organic compounds with the biostimulator role managed at the significant production increases which were comprised between 15 and 40%.

In the case of the agricultural chemical experiments which are achieved on tomatoes, the extraradicular fertilizers with a content of 4% of protein substances (111 AH4), registered the production increases which were less than the variants which had 1 and respectively 2%, the fact which determined by the increase of vegetable mass in the detriment of the production.

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