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Original article

Effect of sulfur-containing agrochemicals on growth, yield, and protein content of soybeans (Glycine max (L.) Merr)

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ABSTRACT

In this study, effect of different forms of sulfur-containing agrochemicals on growth, yield, and protein content of soybean grains have been evaluated. Three forms were used, such as powdery, solute, and pasty, in which elemental sulfur is contained in a nanostructured state. Plants treated with powdered and solute sulfur-containing agrochemicals had the highest growth and grain yield values, and the effect of applying pasty sulfur-containing agrochemicals did not differ from the control, in which there was low yield on all variants. The use of powdered and solute sulfur-containing agrochemicals increased all protein fractions in soybeans. The results show that the use of powdered and solute sulfur-containing agrochemicals is necessary to boost the yield of soy and increase the supply of proteins in the grains. A key factor in the availability of sulfur for soybean plants is the conversion of sulfur to a nanodisperse state. This study provides relevant information about sulfur-containing agrochemicals, which can promote higher seed yields and increase the content of protein in soybeans.

1. Introduction

Soybean is the leading crop among grain legumes in terms of protein content in seeds and indispensable in terms of meeting a high demand of the population for protein. The high content of protein and valuable food components allows using it as an inexpensive and useful substitute for meat and dairy products. Soybean (Glycine max (L.) Merr.) seeds contain about 40% protein and a complete set of amino acids including those essential for human nutrition (Dong et al., 2014; Vorobyev et al., 2019). Besides, soybean is also an important oil culture. At present, its area of cultivation is expanding. An increase in soybean yield can be reached through a sufficient supply of nutrients to the plants. Like other legumes, it requires sulphur for proper growth for increased yields in comparison to many other crops. Sulfur is known to constitute the three most important amino acids, namely, cystine, cysteine, and methionine. Therefore, for the accumulation of protein in soybean seeds, sulfur is absorbed relatively more than in other crops, in which protein is not a priority spare substance.

The increase of soybean yields due to sulfur application against the background of N, P, K, Ca, and NPK addition has been established. Sulphur promotes the accumulation of dry matter by soybean plants and increases the 1000 seed weight and yields. The role of sulphur as a food element as described in the review (Gilbert, 1951) and the role of sulphur as a nutrient and fertilizer is greatly underestimated (Palmer et al., 2001).

It was believed that the sulphur present in soil minerals is sufficient to maintain the necessary level of sulphur. Recently, the shortage of Sulphur in the soils of many regions of the world has become clear, and insufficient sulphur has become a significant constraint on the production of basic human food (The Sulphur Institute, TSI, Washington, USA). In this regard, the role of sulphur is increased as the fourth element in addition to nitrogen, phosphorus, and potassium (Ming Xian and Messick, 2007). The shortage of sulphur is global over the world. The greatest problem is observed in America and Asia, especially in China, where 30% of lands are deficient in Sulphur (Messick, 2007, Ming Xian & Messick, 2007). With the intensive cultivation of soybeans, the lack of sulfur becomes a limiting factor in the yield of this crop over time.

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Lastly, it is possible to use the nano sulphur not only as a fertilizer but also for plant protection and plant growth stimulants. Recently, sulfur-containing micro-fertilizers, which are used for foliar feeding of plants, have become increasingly common. Soybean is one of the main crops and it plays a crucial role in providing the population with food. Therefore, the influence of sulphur on the crop yield and quality has been studied intensively (Zhao et al., 1995).

The sulphur application can lead to higher soybean yield, higher dry matter in seeds, while the lack of sulphur followed by nitrates increases in soybean seeds (Anderson and Fitzgerald 2001; Taalab et al., 2008, Wu and Xiao 1998; Zhao et al., 2006).

The high level of soybean consumption in the world stimulates research to increase soybean productivity. In this regard, the study on mineral nutrition of soybean plants using various types and combinations of sulfur-containing fertilizers and growth stimulants is essential for higher yield and better seed quality of crop today. Of high importance is to define an optimal form of sulfur-containing agrochemicals at which sulfur will be better assimilated by plants. Thus, the present study shows the effect of different forms of sulphur-containing agrochemicals on growth and development, therefore, on soybean productivity and also on the biochemical composition of soybean seeds.

2. Materials and methods

2.1. Plant material

The seeds were collected in soybean varieties, Luna and Fortuna. These varieties are known for their high protein content in which protein contains 40% and fat 20%. Noteworthy is that soybean varieties are resistant to drought, lodging, major diseases of soybean like powdery mildew. Soybean earlier non-treated seeds were used for three experiments: (1) Greenhouse experiments; (2) Field trial tests; (3) Production fields. Greenhouse experiments were conducted in during three years (2017–2019), Field trial tests and Production fields – in 2018–2019.

2.2. Preparation of Sulphur-containing agrochemicals

A solution of calcium polysulfide synthesized using the local raw materials in a test reactor of 150-liter in volume was employed for treatment. Oil sulphur obtained during oil purification at Tengiz deposit (Western Kazakhstan) and calcium oxide with a disperse particles of 0.2–0.3 μm in size with the expressed activity of interaction with sulphur, obtained by low-temperature (900 °C) firing of limestone shell of Beineu deposit (Western Kazakhstan), were used as raw materials. Calcium polysulfide solution with a density of 1.24 g/cm^3 was obtained hydrothermally at a temperature of 100 °C. To obtain sulfur nanoparticles (20–40 nm), a solution of calcium polysulfide in the amount necessary to obtain a solution with a concentration of 2% was placed in the measuring cylinder. Water was added to the mixture of CaO and sulfur in the volume necessary to achieve the specified CaO:S: H2O ratios. After two hours of mixing, a two-phase system was formed from an aqueous solution (the upper part) and the sediment (paste) in the lower layer. In the air, a solution of calcium polysulfide decomposes with the release of highly dispersed sulfur-a nanosized sulfur with a particle size of 20–120 nm, easily assimilated by crops. The slow addition of a solution of calcium polysulfide to water and uniform mixing leads to the decomposition of the solution of calcium polysulfide with the release of sulfur nanoparticles. the pH of the water solution increases by 1 unit, the color of the water solution becomes yellow, opaque, and there are no visible particles. The Tyndall effect is detected under the microscope. An aqueous 2% solution of calcium polysulfide was processed immediately after preparation. Sulfur powder was not released, nano sulfur was introduced as a part of an aqueous solution of calcium polysulfide in the form of a nanodispersed suspension of elemental sulfur particles. Dry powder of calcium polysulfide prepared by drying at 600 C of calcium polysulfide solution. The content of calcium polysulfide in dry preparation is 68%. Decomposition of the dry preparation occurs when dissolved with water or when watering with the release of nanodisperse sulfur. The paste-like preparation is obtained as a result of hydrothermal synthesis of a solution of calcium polysulfide in the form of a paste-like precipitate, the content of nanodisperse sulfur in which the composition of calcium polysulfide is 10–12%. The selection of nanosized sulfur occurs when the precipitate is diluted with water in a ratio of 1:10.

Thus, the aqueous solution of the preparation contains elemental sulfur and calcium hydroxide. The content of elemental sulfur in calcium polysulfide with a density of 1.24 g/cm3 was 160–180 g/l, and the sulfur content in a 2% solution was 3.2–3.6 g/l. The ratio of sulfur to calcium hydroxide in solution is almost constant and amounts to 1.47–1.49.

At the first stage of calcium polysulfide decomposition by water dilution, primary nanoparticles with an average size of 20 nm are formed, which are stable for a certain period (10–15 min). Afterward, nanoparticles start to assemble into larger particles (units) with sizes ranging from 80 to 400 nm. Thus, plants were treated immediately after dilution of a concentrated solution of calcium polysulfide with water, which ensured the preservation of the nano-disperse state of the particles of sulfur and calcium hydroxide, which are convenient for plants to absorb. The nano-dispersed particles of calcium hydroxide present in the 2% solution on the surface of plants are carbonized and, together with nano-sulfur, significantly enhance the plant growth. During treatment, two types of nanoparticles are precipitated, namely, sulfur with a size of 20–40 nm and calcium carbonate particles with a size of 25–50 nm. This fact allowed referring to this agrochemical as a growth stimulant.

2.3. Initial seeds treatment with nano-sulphur

Soybean seeds were initially treated using three different methods with nanosulfur application: (A) solution; (2) dry; and (C) pasty.

For the experiment in a greenhouse, the pre-treatment of seeds was carried out in hermetical glass vials, where 100 dry grains of the same size were placed. Then the grains were immersed in 1 L of a 2% solution of calcium polysulfide with the concentration of sulfur in the solution amounting to 3.2–3.6 g/liter. The time of keeping the seeds was 15 min. Afterward, the grains were planted.

For the field experiments and production fields, the seeds were treated with the same solution at the same holding time but in containers of a bigger size.

In all experiments, the control seeds were soaked in water.

2.4. Greenhouse experiments

In greenhouse conditions, the impact of the efficiency of sulphur-containing growth stimulant on soybean crops was studied. The seeds were collected in soybean varieties Luna. Seeds treated with nano-dispersed sulfur and control (non-treated seeds) were sown in pots. Greenhouse location of Al-Farabi Kazakh National University (latitude 43° 13′20 S, longitude 76° 55′06 E with an altitude of 873 m.a.s.l.). In the greenhouse soybean was planted on coconut coir. The size of the university's greenhouse is 300 sq/m^2. In the experiments, each coconut shavings 4 holes, and three times respectively 48 seeds were planted. Plants were grown in the greenhouse for 90 days at 24/12 °C (day/night) with

17/7 h light/dark. The watering regime on the soybean was repeated every two days.

Seed germination was recorded. The ascended plants underwent triple secondary treatment with 100 ml. of the same 2% solution of calcium polysulfide. The treatment was carried out in 10, 20, and 30 days after the seeds were planted using root nutrition. Control plants were treated with water at the same time. When estimating the results, the growth of plants, the number of pods of one plant, the number of seeds of one plant, the weight of seeds from one plant, and the weight of 1000 seeds were taken into account.

Studies were carried out with three-fold repeatability, and all the measurements were carried out also in three-fold biological repeatability.

2.5. Field trial tests

Field experiments were conducted at Almalybak, Karasay region (latitude 43○ 15′16 S, longitude 76○41′06 E and altitude of 770 m.a.s.l.) on the experimental areas of the Kazakh Research Institute of Agriculture and Plant Industry.

2.6. Production fields

Production field location in Factory of the Kazakh Academy of Nutrition "Amiran" (latitude 43○ 40′23 S, longitude 77○ 18′50 E with an altitude of 515 m.a.s.l.) and Almalybak field and Amiran field are located outside the city, in the Almaty region. The soybeans were sown on sandy loam grounds The pre-sowing treatment of seeds was carried out in industrial containers as described above. After planting soybean, daily observations were carried out. The experiment was carried out three times. In all replicates of the experiment, the seeds were watered three times every 10th day since sowing (Day 10, 20, and 30 days after sowing) with 500 ml freshly prepared 2% solution of calcium polysulfide. Control plants were watered at the same time. Plants were selected and samples were taken three times per 1 ha of the field. The length of the plant, pod number, and fruit bean number of each soybean variant were measured.

Seed germination was recorded and plants were grown in the same conditions in greenhouse and field trials until maturing and harvesting. Seed yield was measured from individual plants at harvesting both in the greenhouse and in fields with three types of initial treatment of seeds (1, 2, and 3) as described above.

2.7. Biochemical analysis of glycinins

Seeds were collected during harvest from each variant, and storage proteins - glycinins were extracted with a phosphate buffer pH 6.9. The purified proteins were separated in the polyacrylamide gene in vertical electrophoresis separation system following the method described by Lammley. The concentration of acrylamide in the separating gel was 10 and 12%, the ratio between acrylamide and methylene bisacrylamide was equal to 48. Fixation and staining of proteins on the gel were carried out with 12.5% trichloroacetic acid until the Prism Ultra protein ladder, Catalog No: ab116027, was used as a molecular weight marker ranking from 10 to 180 kDa. The quantitative estimation of content in spectrum components was carried out by densitometry using the Quantum ST4 gel recording system, the relative percentage of individual subunits and groups of proteins was calculated to the content of all components.

2.8. Statistical data analysis

The descriptive statistics of all traits was measured during the experiment. Variability of traits was assessed using Microsoft Excel. The correlation analysis was calculated using the Rstudio software (Rstudio Team, 2015). Statistics of biochemical analysis were performed using the program Statistica 10 (StatSoft STATISTICA 10, 2011).

3. Results

3.1. Greenhouse experiments

Sulfur-containing growth stimulants affected the germination of soybean seeds Luna in greenhouses. Positive interactions were observed for powdered and solute forms. With use of paste, germination was low in all experiments in the greenhouse (Fig. 1.). In greenhouse conditions, when seeds were treated with a sulfur-containing growth stimulant in powder and solution forms, the agrochemical was quickly absorbed into the seeds, which accelerated their germination. In the case of a pasty form, the absorption of the agrochemical into the seeds was much slower.

The beneficial effect of sulfur-containing powdered and solute agrochemicals on morphological parameters, plant height, and development of the vegetation period was revealed, compared to the pasty form and control plants (Table 1).

When using powdered sulfur-containing agrochemicals in greenhouse conditions, the productivity of one plant of the soy variety "Luna" reached an average of 12.89 ± 0.32 g. With a sulfur-containing agrochemical solution, the yield was 11.4 ± 1.16 g. the Productivity when using a pastry sulfur-containing agrochemical was 9.3 ± 1.0 g. The control version had the lowest value of 8.2 ± 0.5 g. The highest productivity was shown by dry powdered sulfur (Fig. 2).

In all experiments, comparative acceleration in the growth and development of soy compared to the control variants was noted. Although when using powder and solute forms of sulfur-containing agrochemicals, the effect is more pronounced.

The average weight of 1000 seeds were: 136,002 g after treatment with sulfur paste, 154,869, g in control, 190,873 g after treatment with solution, and 199,245 g after powder treatment.

Noticeable fluctuations in the yield of soybeans were observed when using powder and solute forms of an agrochemical. In greenhouse conditions, the yield of one plant varied from 8.2 to 12.8 g. Structural analysis showed that pasty form of the sulfur-containing growth stimulant leads to a decrease in productivity.

3.2. Field trial tests

The average plant height, number of pods pieces of one plant, number of seeds of one plant and 1000 seed weight of Luna soybeans in field tests are presented in Table 2.

In the field plots of the Kazakh Scientific research Institute of agriculture and crop production, the soy variety "Luna" when using powdered sulfur-containing agrochemicals, the productivity of one plant on average for 2 years was 23.3 ± 0.65 g. The solution of the sulfur-containing agrochemicals showed productivity-22.7 ± 0.2 g (Table 2). Pasty sulfur-containing agrochemicals −17.4 ± 0.15 g. When using the initial superphosphate, the productivity was 17.8 ± 0.6 g. with the initial ammophos, the worst indicator was 16.5 ± 0.95 g. In the control version, the productivity reached 18.3 ± 1.05 g. High yield showed variants of powdered and solution of sulfur-containing agrochemicals (Fig. 3).

The average plant height, number of pods pieces of one plant, number of seeds of one plant and 1000 seed weight of Fortuna soybeans in field tests are presented in Table 3.

The variety of soya "Fortuna" grown in the same field with the used powdered sulfur-containing agrochemicals showed the best indicator on average 21.2 ± 3.7 g. productivity of one plant for

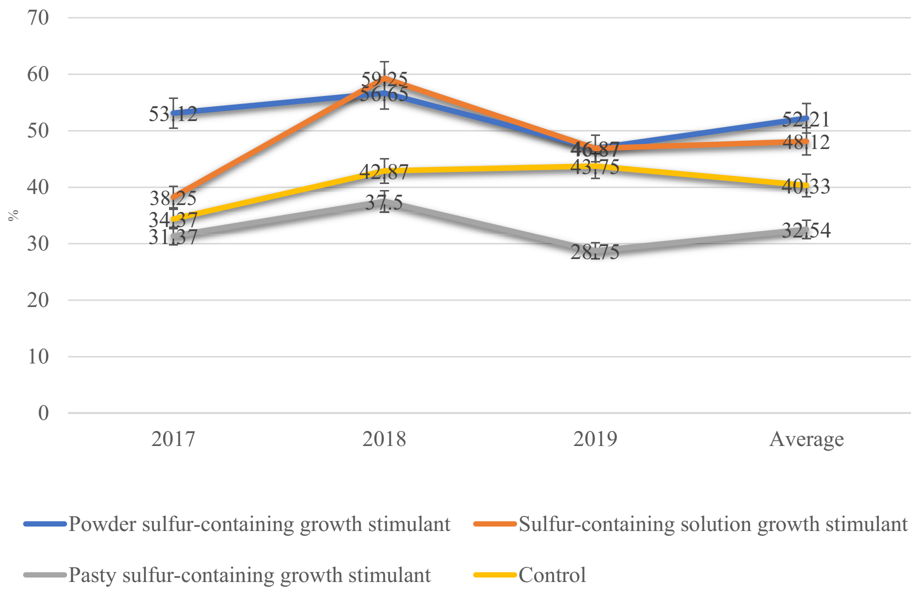

Fig. 1. Seed germination analysis.

Table 1
Mean results of all experiments in greenhouse.

Soybean varieties Luna Variants	Plant height (cm)	Number of pods pieces of one plant	Number of seeds of one plant	1000 seed weight
2017 y.				
Powder sulfur-containing agrochemicals	59.5 ± 2.1	26.7 ± 1.9	60.9 ± 3.4	204.5 ± 4.5*
Sulfur-containing solute agrochemicals	59.8 ± 2.7	26.7 ± 2.4	59.0 ± 3.1	193.3 ± 5.2*
Pasty sulfur-containing agrochemicals	48.9 ± 2.6	26.3 ± 1.9	50.2 ± 2.4	179 ± 3.4
Control	47.7 ± 1.8	20.6 ± 2.4	45.8 ± 2.1	180.5 ± 3.1
2018 y.				
Powder sulfur-containing agrochemicals	52.9 ± 2.9	25.6 ± 1.7	62.7 ± 2.6	210.7 ± 3.4*
Sulfur-containing solute agrochemicals	59.3 ± 2.4	26.9 ± 1.6	56.7 ± 2.5	170.3 ± 3.8*
Pasty sulfur-containing agrochemicals	52.8 ± 2.1	25.3 ± 1.9	51.4 ± 1.7	159.6 ± 2.7
Control	49.7 ± 1.9	19.5 ± 0.8	46.3 ± 1.6	162.6 ± 3.3
2019 y.				
Powder sulfur-containing org. fertilizer	58.9 ± 2.1	23.7 ± 1.3	64.8 ± 2.7	201.7 ± 3.9*
Sulfur-containing agrochemicals	60.7 ± 2.4	29.5 ± 3.2	61.9 ± 2.9	210.6 ± 4.7*
Pasty sulfur-containing agrochemicals	51.8 ± 3.1	24.8 ± 0.8	56.7 ± 2.6	190.5 ± 3.5
Control	48.5 ± 2.4	21.2 ± 0.9	49.3 ± 3.5	182.8 ± 3.7

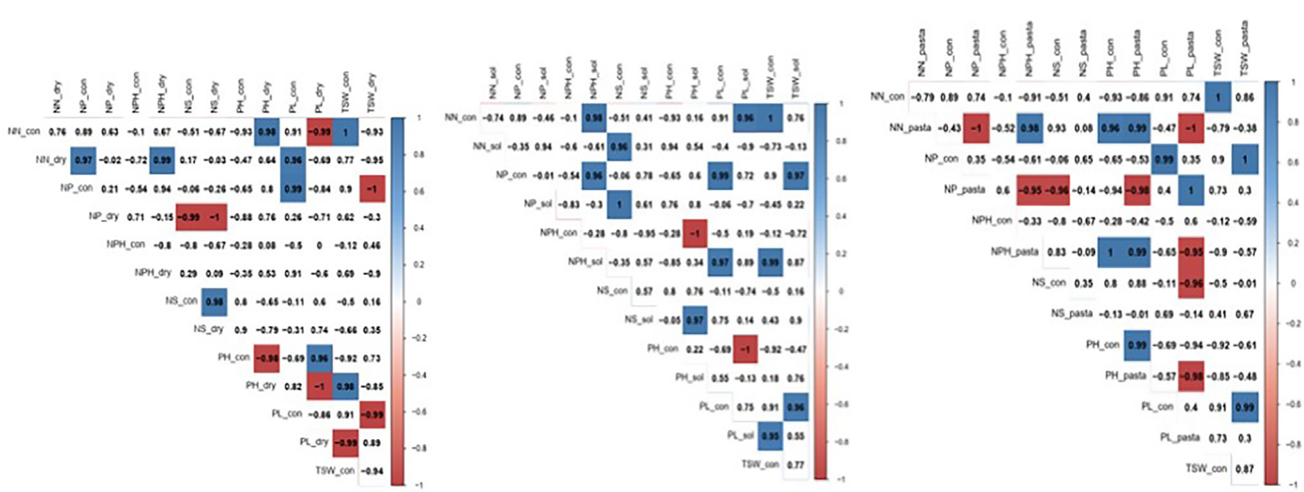

Fig. 2. Correlation analysis of traits experiments in greenhouse, Note: Correlations with $P < 0.05$ are highlighted in color. The color indicates either positive (blue) or negative (red) correlation. (Plant height – PH, Number of nodes – NN, Number of pods – NP, Number of seeds – NS, Pod of length – PL, Number of plants at harvest – NPH, 1000 seed weight – TSW).

Table 2
Mean results of field experimentation soybean varieties Luna in Almalybak, Kazakh Research Institute of Agriculture and Plant growing.

Soybean varieties Luna Variants	Plant height (cm).	Number of pods pieces of one plant	Number of seeds of one plant	1000 seed weight
2018 y.				
Powder sulfur-containing agrochemicals	91.4 ± 4.3	51.0 ± 2.8	129.4 ± 5.1	175.8 ± 4.5
Sulfur-containing solute agrochemicals	100.7 ± 4.9	55.5 ± 3.8	129.9 ± 4.6	176.3 ± 5.3
Pasty sulfur-containing agrochemicals	94.0 ± 3.6	47.3 ± 1.9	109.1 ± 3.4	159.3 ± 4.4
Superphosphate original	87.4 ± 2.9	46.5 ± 2.1	106.0 ± 3.8	162.8 ± 2.9
Ammophos original	97.7 ± 3.1	45.2 ± 2.3	96.9 ± 2.8	161,9 ± 3.2
Control	95.9 ± 2.9	41.4 ± 2.1	107.8 ± 3.3	160.8 ± 5.1
2019 y.				
Powder sulfur-containing agrochemicals	95.8 ± 3.6	54.1 ± 2.1	134.9 ± 4.7	178.2 ± 4.4
Sulfur-containing solute agrochemicals	103.3 ± 3.9	64.0 ± 2.2	131.6 ± 5.1	171.3 ± 4.9
Pasty sulfur-containing agrochemicals	100.1 ± 2.7	50.2 ± 0.9	115.7 ± 2.7	152.6 ± 3.7
Superphosphate original	91.8 ± 2.1	49.2 ± 0.8	111.4 ± 1.6	165.6 ± 3.3
Ammophos original	99.4 ± 3.1	50.1 ± 0.8	115.4 ± 3.3	152.5 ± 4.5
Control	100.2 ± 5.2	47.8 ± 2.4	113.0 ± 5.6	172.2 ± 4.4

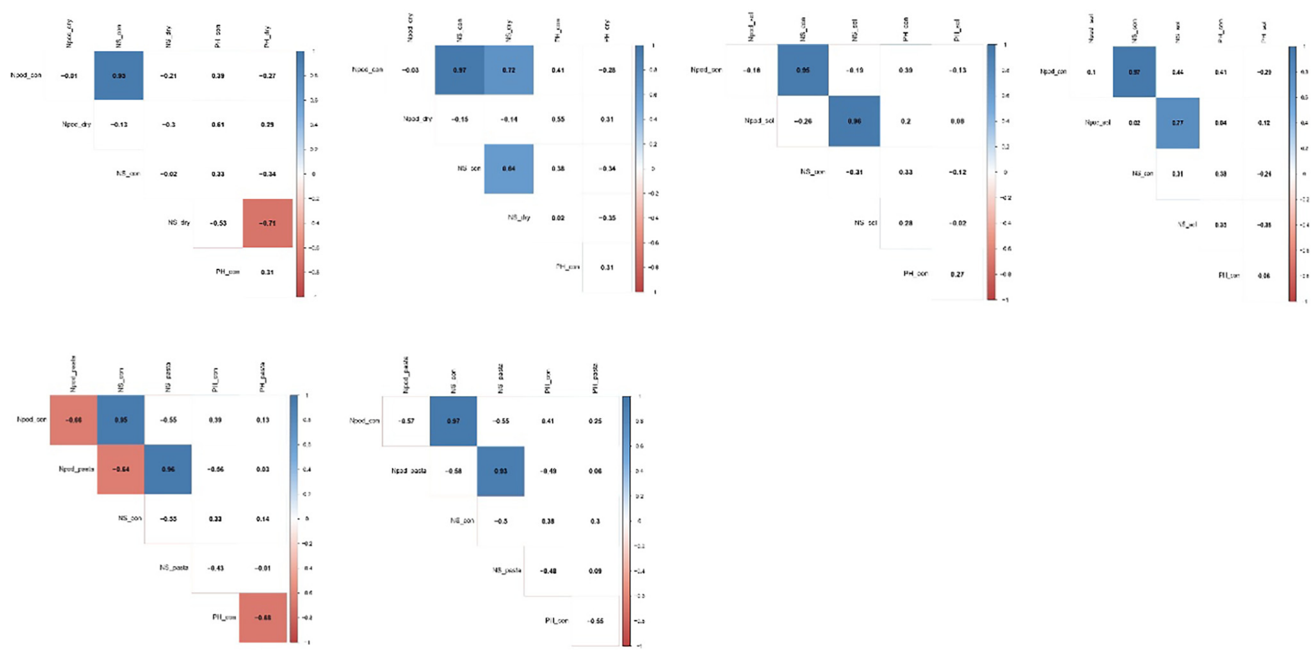

Fig. 3. Correlation analysis of traits experiments soybean varieties "Luna" in Kazakh Research Institute of Agriculture and Plant growing.

Table 3
Mean results of field experimentation soybean varieties Fortuna in Almalybak, Kazakh Research Institute of Agriculture and Plant growing.

Soybean varieties Fortuna Variants	Plant height (cm).	Number of pods pieces of one plant	Number of seeds of one plant	1000 seed weight
2018 y.				
Powder sulfur-containing agrochemicals	59.3 ± 2.5	35.6 ± 0.9	99.8 ± 2.4	175.8 ± 4.5
Sulfur-containing solute agrochemicals	36.8 ± 1.8	41.5 ± 2.4	119.2 ± 6.4	160.9 ± 5.2
Pasty sulfur-containing agrochemicals	54.5 ± 3.6	27.8 ± 1.9	82.3 ± 3.4	159.3 ± 4.4
Superphosphate original	52.9 ± 2.9	37.9 ± 2.1	98.2 ± 3.4	161.8 ± 6.4
Ammophos original	63.7 ± 3.3	49.5 ± 2.2	109.9 ± 3.7	159,9 ± 3.9
Control	47.5 ± 1.6	24.4 ± 2.3	54.8 ± 0.6	160.8 ± 5.1
2019 y.				
Powder sulfur-containing agrochemicals	62.6 ± 2.8	36.9 ± 2.1	122.4 ± 3.4	204.2 ± 4.4
Sulfur-containing solute agrochemicals	40.5 ± 1.4	54.5 ± 2.6	124.5 ± 4.5	171.3 ± 3.9
Pasty sulfur-containing agrochemicals	59.0 ± 2.7	33.2 ± 0.9	85.7 ± 2.7	163.6 ± 3.7
Superphosphate original	55.8 ± 2.1	40.9 ± 0.8	101.3 ± 1.6	164.6 ± 3.3
Ammophos original	66.4 ± 3.1	52.8 ± 0.8	115.3 ± 4.8	162.5 ± 4.8
Control	40.5 ± 2.4	26.5 ± 0.9	59.1 ± 0.5	182.2 ± 4.4

2 years. The solution of the sulfur-containing agrochemicals also reached 21.3 ± 1.05 g. Pasty sulfur-containing agrochemicals was 10.2 ± 3.7 g. Pasty sulfur-containing agrochemicals was 10.2 ± 3.7 g. With using the initial superphosphate, the productivity was-16.2 ± 0.4 g, with the initial ammophos – 18.1 ± 0.6 g. and the control variant showed the worst indicator of 9.7 ± 0.95 g. the

Best data were obtained when using a dry and solution of a sulfur-containing agrochemicals (Fig. 4).

Research showed the effect of sulfur-containing agrochemicals on the growth of soybean plants in field trials compared to controls. Various types of sulfur-containing agrochemicals processing have shown very high efficiency in the growth and development of soybean plants. As a result, the yield of soybean seeds increased significantly in areas with plants treated with a sulfur solution, and the lowest yield was in the control version. Noticeable increases were observed in the field, productivity fluctuated in the Luna variety 16.5–23.3 g., and the Fortuna variety showed from 9.7 to 21.3 g (Tables 2 and 3).

3.3. Production fields

The average plant height, number of pods pieces of one plant, number of seeds of one plant and 1000 seed weight of the Luna variety obtained during the experiment on Production fields are presented in Table 4.

In the production fields of the Kazakh Academy of nutrition "Amiran", the grown variety of soy "Luna" showed high productivity when using powdered 29.35 ± 0.05 g and a solution of sulfur-containing agrochemicals 30.2 ± 1.1 g. Pasty sulfur-containing agrochemicals −17.05 ± 0.55 g. A control variant showed the lowest indicator 16.2 ± 2.6 g. Compared with the control, the increase in yield in the areas, where powder and solute sulfur-containing agrochemicals were used, was 81% and 86%, respectively (Fig. 5).

In manufacturing conditions, productivity was 86% higher, especially in the dry form and in the form of a solution with the addition of sulfur compared to the control. The study presents data on the assessing the effect of a sulfur-containing agrochemicals introduced into the soil on the absorption and growth of soy plants.

3.4. Biochemical analysis of glycinins

Storage proteins from soybean seed were separated in polyacrylamide gel, the spectrum of all globulin groups (β-conglycinins and glycinins) is present in Fig. 6.

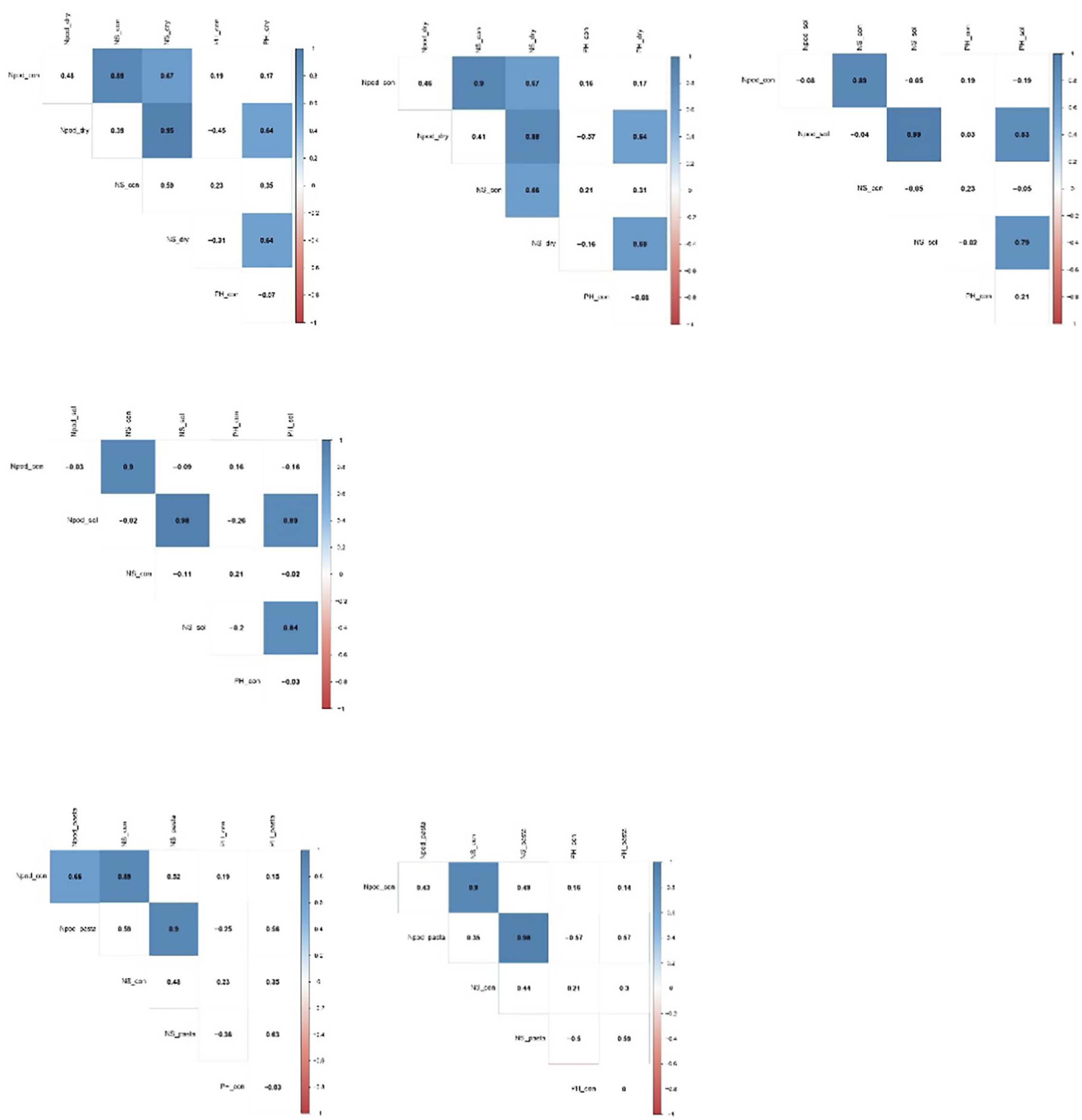

Fig. 4. Correlation analysis of traits experiments soybean varieties "Fortuna" in Kazakh Research Institute of Agriculture and Plant growing.

Table 4
Mean results of experimentation in the Factory of the Kazakh Academy of Nutrition "Amiran".

Soybean varieties Luna Variants	Plant height (cm)	Number of pods pieces of one plant	Number of seeds of one plant	1000 seed weight
2018 y.				
Powder sulfur-containing agrochemicals	115.4 ± 4.8	58.4 ± 2.4	147.7 ± 5.3	199.5 ± 4.4
Sulfur-containing solute agrochemicals	116.8 ± 5.9	50.5 ± 1.9	153.5 ± 6.7	190.0 ± 6.1
Pasty sulfur-containing agrochemicals	101.1 ± 2.7	57.3 ± 0.9	124.6 ± 2.7	134.6 ± 3.7
Control	99.9 ± 2.3	40.5 ± 2.6	118.5 ± 4.9	137.5 ± 4.4
2019 y.				
Powder sulfur-containing agrochemicals	118.4 ± 5.6	56.4 ± 1.7	145.7 ± 3.5	201.5 ± 4.4
Sulfur-containing solute agrochemicals	126.8 ± 6.1	60.5 ± 2.6	163.5 ± 5.1	192.0 ± 6.4
Pasty sulfur-containing agrochemicals	109.1 ± 2.7	53.3 ± 0.9	120.6 ± 2.7	151.6 ± 3.8
Control	108.9 ± 6.4	42.5 ± 2.2	116.5 ± 3.8	141.5 ± 3.4

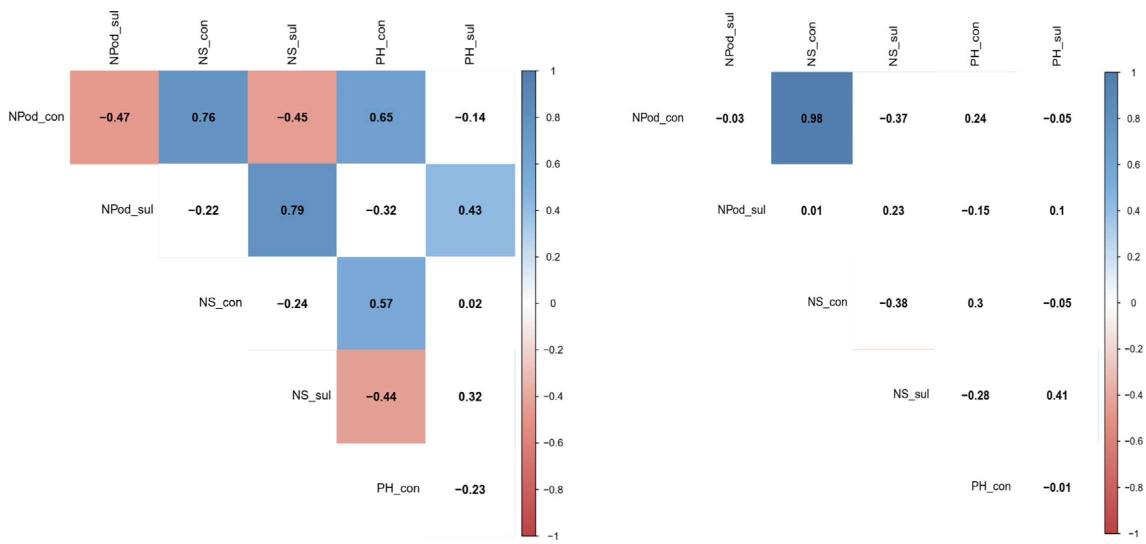

Fig. 5. Correlation analysis of traits experiments soybean varieties "Luna" in Amiran.

The results presented in Fig. 6 showed that treatments of soybean seeds of nano-dispersed sulphur in dry form and solution led to higher glycinin content. Therefore, the increase in sulphur -containing amino acids containing glycinin can improve the nutritional characteristics of protein in general. Treatments of soybean seeds with nano-dispersed sulphur paste did not affect the ratio of protein groups (Table 5).

The storage proteins from soybean seed contain two main groups of proteinase inhibitors: (1) Kunitz trypsin inhibitors (KTi), and (2) Bowman-Birk proteinase inhibitor (Laskowski and Kato, 1980). KTi accounts for about 80% of the total trypsin inhibitory activity of soybean seeds (Kumar et al., 2013). KTi represents the reserve proteins with a molecular weight of 21 kD and is specific for trypsin (Kim et al., 1985; Ryan, 1981). Trypsin inhibitors are sulphur -containing proteins and plant processing may affect their concentration in the protein (Table 6).

The effect of sulphur-containing products on the protein content of soybean seeds was also studied. In the study, the analysis

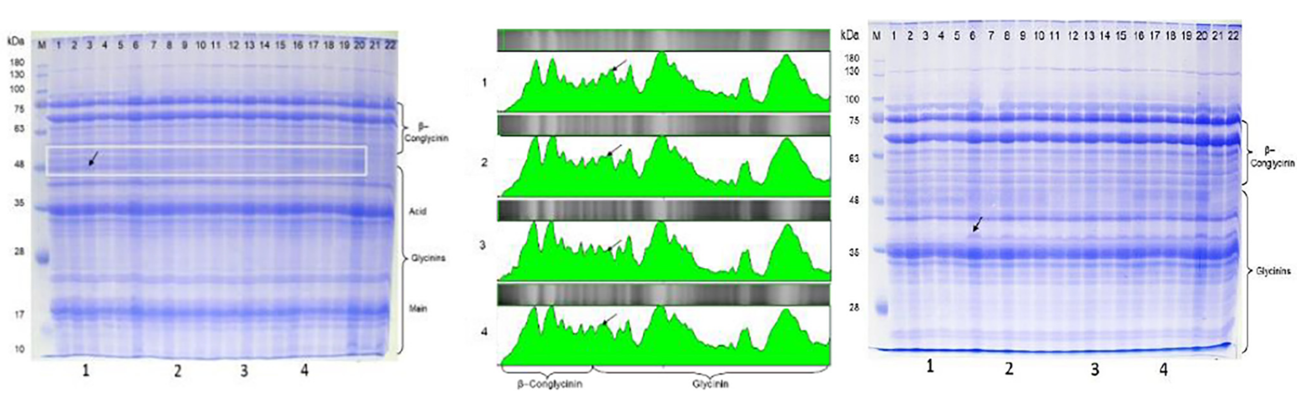

Fig. 6. Electropherogram of storage proteins from soybean seeds. Electrophoresis was carried out in SDS PAG with 12% acrylamide concentration. B. Densitograms of the electrophoretic spectrum of storage proteins from soybean seeds C. Range of soybean seed storage proteins. Electrophoresis in SDS PAG with 10% acrylamide. Note: 1-Powder sulfur-containing agrochemicals; 2-Sulfur-containing solute agrochemicals; 3-Pasty sulfur-containing agrochemicals; 4-Control.

Table 5
The content of β-conglycinin and glycinin, Kunitz trypsin inhibitor, and protein content in soybean seeds after various types of sulphur treatment. Data presented as the average percentage with n = 3.

Protein group	Treatment			
	Powder sulfur-containing agrochemicals	Sulfur-containing solution agrochemicals	Pasty sulfur-containing agrochemicals	Control
β-conglycinin	31.02 ± 0.16	31.44 ± 0.2	32.71 ± 0.8	32.44 ± 0.11
Glycinin	68.98 ± 0.16	68.57 ± 0.2	67.3 ± 0.8	67.56 ± 0.11
Kunitz trypsin inhibitor	2.89 ± 0.12	1.78 ± 0.43	2.65 ± 0.11	2.73 ± 0.13
Protein content	5.0 ± 0.82	36.6 ± 0.77	37.3 ± 1.01	32.9 ± 0.33

showed that the processing of soybean sulphur-containing agrochemicals in different states leads to changes in the concentration of acid glycinin subunits in the zone of proteins with a molecular weight of 48–50 kDa. The content of the Kunitz trypsin inhibitor in soluble seed proteins in samples treated with sulphur slightly increased compared with the control in soybean seeds. The use of sulphur -containing agrochemicals in dry form and in solution leads to an increase in the content of glycinins, and therefore to an increase in sulphur-containing amino acids in the composition of glycinins, which improves the nutritional properties of the protein. The processing of soybean with sulphur in the form of a paste did not affect the ratio of protein groups.

We have shown that the content of Kunitz trypsin inhibitor is slightly increased insoluble proteins extracted from seeds treated with dry nano-sulphur compared to Control. However, a significant decrease of KTi was found in seeds treated with nano-sulphur solution, while KTi levels remain unchanged as in Control after treatments with paste nano-sulphur. When studying the protein content of soybean seeds in various types of sulphur treatment, it was shown that the processing of plants with sulphur in the dry form leads to the suppression of the biosynthesis of acid glycine with a molecular weight of 38.4 kDa. Studying the protein content in soybean seeds during various treatments showed that the protein content increases in variants with sulphur-containing agrochemicals compared to the control in Table 5.

Fractionation of proteins in the PAAG system with 10 percent acrylamide showed better resolution in the zone of acidic glycinins. Most of the main glycine subunits run beyond the gel and are not fixed on the electropherogram. Under these conditions, the obtained spectra of the control and the variants with sulfur-containing solution product and pasty sulfur-containing agrochemicals glycinin subunit with a molecular mass of 8.4 kDa, indicated by the arrow in Fig. 6.

However, this subunit was absent in the spectrum of seed storage proteins of soybean treated with ‘powder sulfur-containing org. fertilizer’. Instead, there were two weak components. It is suggested that glycinin subunits have independent genetic control and the expression of some of them may be inhibited or enhanced by sulphur deficiency (Paek et al., 2000). Treatments of plants with dry sulfur-containing agrochemicals leads to the suppression of the biosynthesis of acid glycine with a molecular weight of 38.4 kDa. These results demonstrated a high efficiency, sulphur-containing agrochemicals in the form of a solution and in dry form that increase productivity of soybean when compared with the control and pasty sediment.

Table 6
Protein content of soy seeds in various types of sulfur treatment (%).

Treatment			
Powder sulfur-containing agrochemicals	Sulfur-containing solution agrochemicals	Pasty sulfur-containing agrochemicals	Control
35.0 ± 0.82	36.6 ± 0.77	37.3 ± 1.01	32.9 ± 0.33

4. Discussion

In fields, agricultural systems largely depend on the intensive use of land for crop cultivation with applied inorganic fertilizers (Alvarez et al., 2017; Ashworth et al., 2017; Blanco-Canqui et al., 2015). Modern agricultural systems rely heavily on inorganic fertilization and intensive tillage practices. Various studies have shown that this practice can alter the nutrient balance, biotic interactions, and resource availability, as well as increase production costs and negatively affect soil health and their feedback to regional and global climate (Bogunovic et al., 2018; Buah et al., 2017; Carter, 1988, 2005). The intensification of agricultural production has aggravated the problem of forms of degradation that were not previously typical of soils. The guarantee of ensuring high fertility of soils and increasing the productivity of crops is balanced mineral nutrition for all elements, taking into account the content, distribution, and transformation of them in the soil (Slyusarev, 2007). Sulphur stands alongside such elements as nitrogen, phosphorus, and potassium - the second proteinogenic after nitrogen. Lack of sulfur, like nitrogen, reduces the synthesis of proteins, while the external manifestation of sulfur starvation of plants almost coincides with the signs of a lack in nitrogen nutrition. Its absolute necessity for the processes of respiration, photosynthesis, nitrogen, and carbohydrate metabolism has been established (Bettany et al., 1984). Meanwhile, the removal of sulfur from the soil with a crop yield only slightly inferior to the removal of phosphorus, and in some cases even superior to it. If before the plant nutrition of sulfur was satisfied without additional efforts, now and in the future, the resources of its entry into the soil is reduced, and the need for it in agriculture is growing due to the increased demand for high-quality agricultural products. The main reasons for increased sulphur deficiency are lower atmospheric sulphur gas content, increased use of highly concentrated and ballastless sulphur-free fertilizers, higher crop yields, and increased sulphur removal (Golov, 2012). As a result, the application of sulphur-free agrochemicals became a prerequisite for high yields. The increase in soybean yield from sulfur introduction against the backgrounds of using N, P, K, Ca, and NPK has been established. Sulfur had a positive effect on the formation of tubercular bacteria and the chemical composition of soybean plants (Fageria et al., 2013, 2014, 2016; Fageria and Oliveira, 2014; Espolov et al., 2019).

The results of this research on the effect of sulfur-containing agrochemicals on the yield of soybeans are consistent with the results of other studies (Golov, 2012; Tishkov et al., 2014; Shchegolkov, 2015).

We observed that the S supply significantly increased soybean crop yield, including the weight of 1000 grains, provides greater plant growth and grain yield.

With nano-sulphur in different States leads to changes in the concentration of acid glycinin subunits in proteins with molecular weights of 48–50 kDa The highest intensity of the subunit with a molecular weight of 48 kDa is noted in the treatment variant 'powder sulfur-containing org. fertilizer'. This is confirmed by densitometry of protein spectra in Fig. 6, the subunit is indicated by an arrow. A decrease in the synthesis of β-conglycinins and an increase in the concentration of acidic glycinins was observed in different variants of using sulphur as a fertilizer by many authors (Fujiwara et al., 1992; Gayler and Sykes, 1985; Hirai et al., 1994; Sharma and Sharma, 2018).

Densitometry of a complex of proteins related to β-conglycinins and glycinins shows that the ratio of the two groups of proteins was varied depending on the application of fertilizers (Table 5). The lowest protein content of globulin, prolamine, and glutelin was observed in ESPA. This effect is probably related to the sulfur content in the leaves, since this source of sulfur was below the adequate range of interpretation. In particular, the content of the globulin protein decreased when using ESPA, which can be explained by the lack of S for cysteine synthesis, since the amino acid is the main component of the globulin protein (Chandra and Pandey, 2016). Therefore, it is possible to recommend forms of dry and solution of sulfur-containing agrochemicals to increase the yield of soy on a production scale.

5. Conclusion

The results show that the ideal application of sulfur-containing agrochemicals in the form of dry and solution is necessary to increase the yield of soy. It is due to the nanostructure of sulfur that it penetrated through plants and as a result gave a positive effect. The paste-like form resulted in a lower yield for all tested plants and is not recommended as a fertilizer and growth stimulant. Since not assimilating the thick structure of the paste had a poor translocation, thus did not allow the penetration of seeds, so the germination of soy was low, which led to a decrease in yield. In general, the sulfur-containing agrochemicals in various forms affected the protein content in soy seeds, all fractions of the reserve protein increased compared to the control.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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