

IRSTI 61.33.39

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### **Influence of mixed aqueous solutions of polyhexamethylene guanidine hydrochloride and OP-10 on vegetable crop seeds**

**Abstract.** The effect of bactericidal and fungicidal polymer polyhexamethylene guanidine hydrochloride (PHMGH) and its mixture with non-ionic surfactant oxyethylated isooctylphenol (OP-10) on the germination of seeds of tomatoes, cucumbers and sugar beet was studied. PHMGH is known disinfectant. It is odorless, colorless, non-corrosive and non-toxic for humans, PHMGH can be applied to make an innovative product in the disinfection of plants and vegetables and preservation of food products. Vegetable seeds were treated with aqueous solutions of PHMGH, OP-10 and mixed solutions of PHMGH/OP-10. Tests were made in relation to the growth and development of plants treated with bactericides. PHMGH (0.05 %) aqueous solution favors for cucumber seed growth (the germination percentage equals to 94 %), PHMGH/OP-10 complex (at a ratio of 1:1 at initial component concentrations equaled to 0.01%) for tomato seeds (90%). The mixture of PHMG/OP-10 was effective on the growth and development for all of the studied vegetable crops (stem and leaf length). The effect of aqueous solutions of PHMGH, OP-10 and PHMGH/OP-10 on the content of chlorophyll in the leaves of the studied objects was studied. The increasing of chlorophyll amount was observed after treatment with PHMGH complexes that results in resistance of vegetables to action of environmental factors.

**Key words:** polyhexamethylene guanidine hydrochloride, oxyethylated isooctylphenol, bactericidal complexes, fungicidal complexes, germination, chlorophyll, seeds, surfactant.

#### **Introduction**

The consumption of fresh vegetables increases every year in our country. Wide using of chemicals on plant protection leads to the negative environmental, sanitary and other consequences. With the accumulation of negative impact factors, the development of improving methods and means of plant protection, the alternative ways preventing diseases of vegetable crops are growing. For this reason, the importance of the development and production of new bactericidal and fungicidal complexes increases [1].

Vegetable crops as other plants are exposed to diseases, which in turn interfere to obtaining of a stable and high yield. Traditional methods of crop increasing are complex, long-lasting and not always effective [2-3].

Recently, a method of film based on polymers has been successfully used in vegetable growing. This

method allows bactericidal and fungicidal complexes to fix on the surface of the culture, providing them the high germination, as well as further development of a plant. Moreover, the use of such agents eliminates the negative impact of microorganisms, being safe for the environment [4].

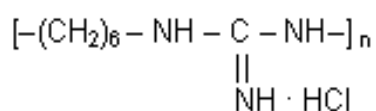
Currently, the environmentally correct, non toxic preparations are necessary for agriculture. In this perspective, complexes with high biological activity based on polyhexamethylene guanidine hydrochloride (PHMGH) and PHMGH/surfactant were used to improve the germination of vegetable seeds. Polymeric guanidines are widely used as disinfectants [5-8]. The many physical and chemical characteristics of this product: odorless, colorless, non-corrosive and non-toxic for humans with a neutral pH make an innovative product in the disinfection of plants and vegetables and preservation of food products. The antimicrobial activity of this product was shown on

many bacteria [9]. Also, PHMGH has a fungicidal activity on various fungal species [10]. Non-ionic surfactant OP-10 was used due to their surface properties and ability to wet the surface of plants (stem, leaves).

The main aim of this study was to show opportunity of using of polyhexamethylene guanidine hydrochloride with respect to the some vegetable crops cultivated in our country such as tomatoes, cucumbers and sugar beet. Therefore, the biological activity of PHGMH and its complexes with non-ionic surfactant was tested for the above vegetable cultures.

## Materials and Methods

*Materials.* The water-soluble polymer PHMGH was used for the preparation the new bactericidal and fungicidal compounds. Polyhexamethylene guanidine hydrochloride (PHMGH) is an antimicrobial biocide of the guanidine group, produced in Russia at the Pokrovsky Plant of Biopreparations,  $M_n = 1.7 \cdot 10^3$ , has the following structural formula:



It is known that PHMGH is polyelectrolyte of cationic type and it keeps the bactericidal and fungicidal properties at interaction with other polyelectrolytes and surfactants [11-13].

As a surfactant, the oxyethylated isooctylphenol (OP-10) was used. OP-10 is a nonionic surface active agent, product of joining of ethylene oxide to the alkylphenol with formula  $\text{C}_8\text{H}_{17}\text{C}_6\text{H}_4\text{O}(\text{CH}_2\text{CH}_2\text{O})_{10}\text{H}$  ( $\text{C}_7\text{H}_{17}-\text{C}_6\text{H}_4-\text{O}-(\text{CH}_2-\text{CH}_2\text{O})_n$ ). Also the complexes of PHMGH/ OP-10 at a ratio of 1:1 were tested for the investigation.

*Methods.* The germination of seeds was studied in accordance with GOST 12038-84 "Seeds of agricultural crops. Methods for determination of germination". Four samples per 100 seeds of vegetable cultures were taken. Seeds were placed on moistened filter papers in Petri dishes. Petri dishes were previously sterilized in a desiccator SNOL 58/350 "Abutenos Elektrotechnika". The limit of reproducible temperatures ranges from 20 to 300 °C, the error of temperature stabilization is  $\pm 2$  °C for 1 hour at a temperature of 130 °C. Petri dishes wrapped in a tracing paper were placed in a thermostat for three days at a temperature of 27 °C. Germination of seeds was determined as a percentage. For the result of the

analysis, the arithmetic mean results of determining the germination capacity of all analyzed samples were taken.

The main regularities of plant growth and development were studied within thirty days by the standard method [14]. The length of the aerial part of the plants was measured during the experiment and a change of the external characteristics of the plant was observed. The thickness of the stalks of germinated plants was measured with the help of a caliper SHZ I – 125 mm, the division price 0.1 mm, class 2.

The chlorophyll content was determined on a SPEKOL 1500 spectrophotometer. The measuring error was  $\pm 0.3\%$  according to biochemical methods for plant physiology study [15].

The effect of mixtures of PHMGH, OP-10 and PHMGH/OP-10 on seed germination was investigated by two methods:

### 1) Direct influence method on seeds.

Seeds were treated with mixtures of water solutions of PHMGH, OP-10 and PHMGH/OP-10 of 0.01%; 0.05 %; 0.1 % and 0.2 % concentrations. To determine the germination, the treated seeds were placed in a climatic chamber at a temperature of + 28 °C for 3-7 days;

### 2) Method of sprout watering.

After germination of seeds in the soil, the irrigation was carried out with mixtures of PHMGH, OP-10 and PHMGH/OP-10 in water with 0.01 %; 0.05 %; 0.1 %; 0.2 % concentrations (50 ml).

## Results and Discussion

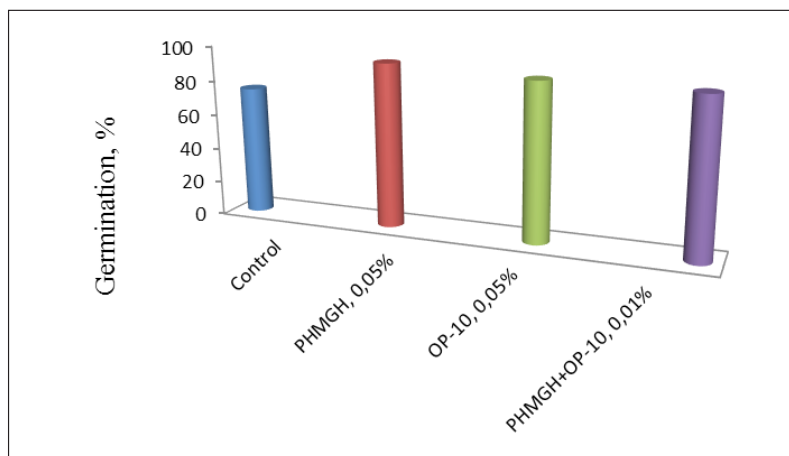
One of the methods for assessment of the sowing quality of vegetable crops is to determine their productivity. In conditions of poor productivity, the possibility of obtaining a high yield reduces. Therefore, before sowing the crop, it becomes necessary to treat the seeds with bactericidal and fungicidal preparations.

### 1. Effect of fungicidal and bactericidal complexes on the germination of vegetable seeds.

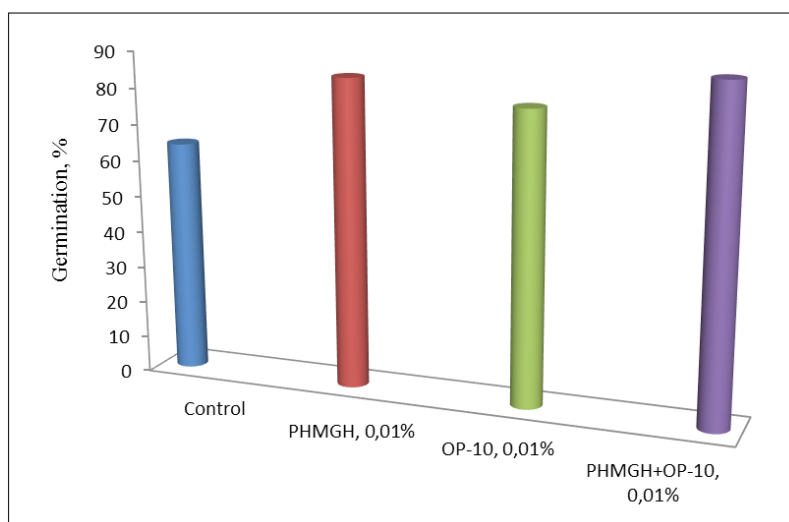
The results of the studies of the effect of PHMGH, OP-10 and mixed solutions of PHMGH/OP-10 on seed germination are shown in Figures 1-3.

According to Figure 1, the best results were achieved by means of processing with a mixture of 0.05 % solution of PHGMH. Germination of seeds of cucumbers reached 90%.

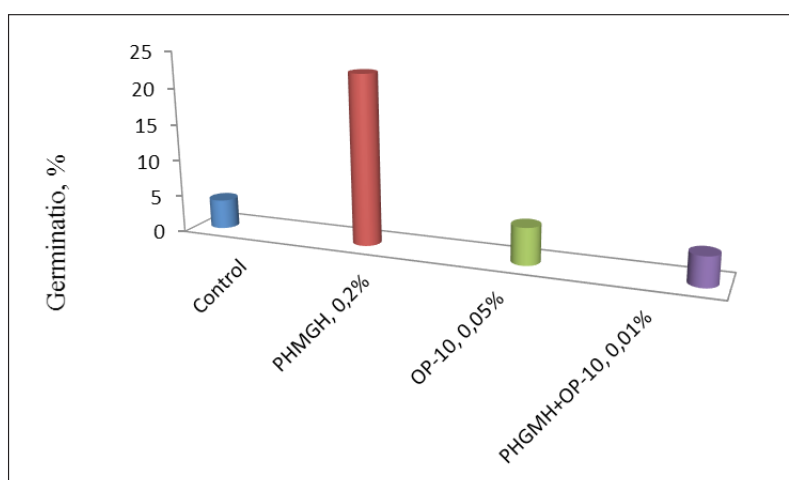
The results of analyzes carried out with respect to tomato seeds are provided in Figure 2.



**Figure 1** – The germination of cucumber seeds treated by solutions of PHGMH, OP-10 and PHMGH/OP-10



**Figure 2** – The germination of tomato seeds treated by solutions of PHGMH, OP-10 and PHMGH/OP-10



**Figure 3** – The germination of sugar beet seeds treated by solutions of PHGMH, OP-10 and PHMGH/OP-10

As seen in Figure 2 the germination reached to 90 % after the treatment by PHGMH/OP-10 mixed solutions of 0.01 % at a ratio of 1:1 (vol.) of 0.01 % solutions. In the next experiment with respect to sugar beet seeds the concentrations of components were changed according to optimal data obtained

previously (Figure 3). As can be seen in Figure 3 the best results was observed after treatment by 0.2 % PHGMH.

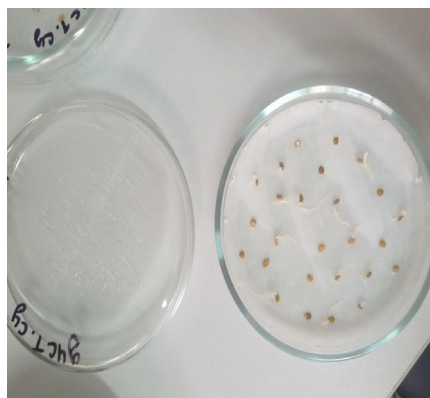
Figure 4 presents the germination of seeds untreated and treated with mixed solutions of PHMGGH/OP-10 at a ratio 1:1 (vol.)



1



2



3



4



5



6

**Figure 4** – Vegetable seeds untreated and treated with mixed solutions of PHMGGH/OP-10 at a ratio 1:1  
 (1 – untreated cucumber seeds, 2 – treated cucumber seeds, 3 – untreated tomato seeds,  
 4 – treated tomato seeds, 5 – untreated sugar beet seeds, 6 – treated sugar beet seeds)

2. *The influence of bactericidal and fungicidal complexes on the growth and development of vegetable crops.*

To observe the growth and development of cultures, we carry out the following experiments: the seeds were planted in 500 g soil in four replicates. After emergence of seeds, the sprouts were watered with solutions of PHGMH, OP-10 and PHGMH/OP-

10 by 50 ml with different concentrations equaled to 0.01 %; 0.05 %; 0.1 %; 0.2 %. The results of the data obtained are presented in Figures 5-7.

As shown in Figure 5 the best results were reached after watering by PHGMH/OP-10 at initial concentrations of components equaled to 0.01 %.

Similar tests were carried out with relation to tomatoes (Figure 6).

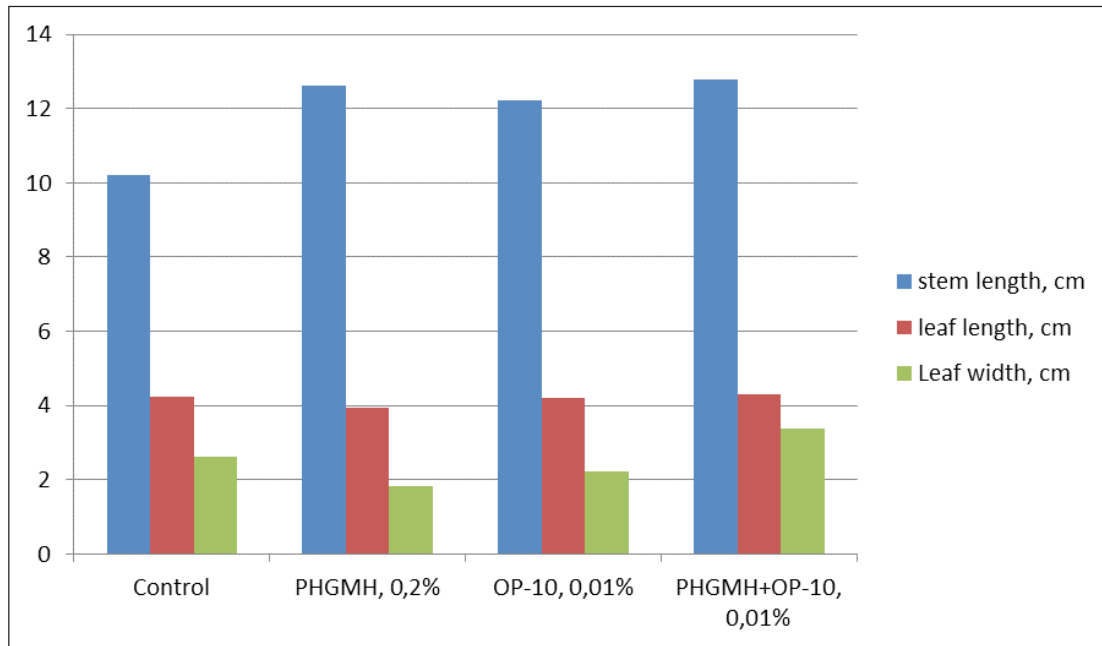


Figure 5 – Influence of solutions of PHGMH, OP-10 and PHGMH/OP-10 on growth and development of cucumber

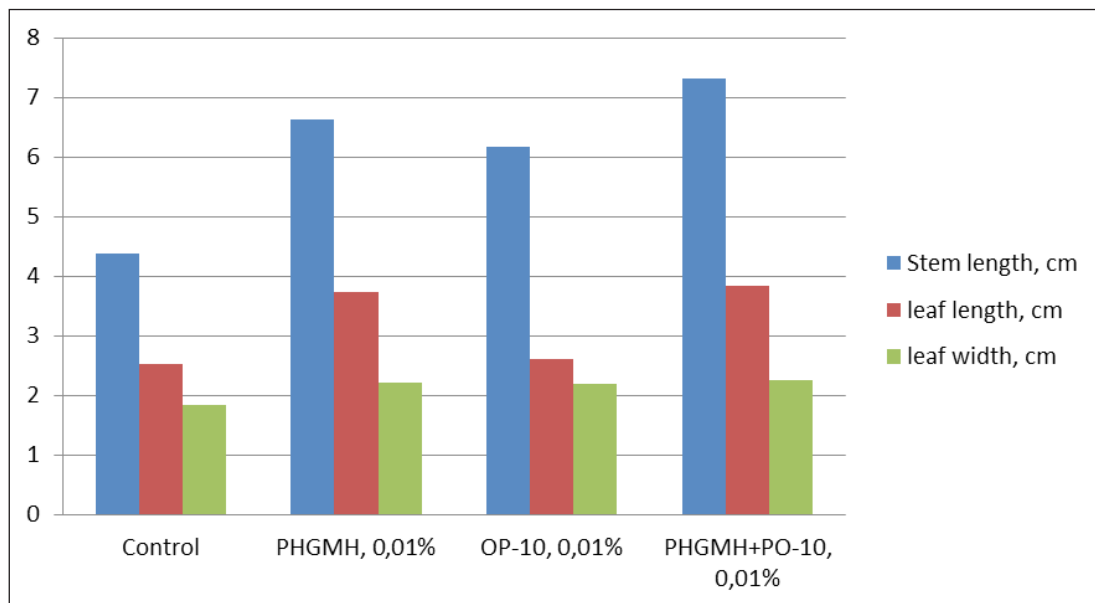


Figure 6 – Influence of solutions of PHGMH, OP-10 and PHGMH/OP-10 on growth and development of tomatoes

According to the results of the analysis of tomato samples, it can be seen from Figure 6 that the watering with complex of PGMG/OP-10 of 0.01% initial aqueous solutions positively influenced on the

growth of the stem length, the length and width of the leaf of the plant.

Similar results were obtained during experiments with sugar beet. The results are shown in Figure 7.

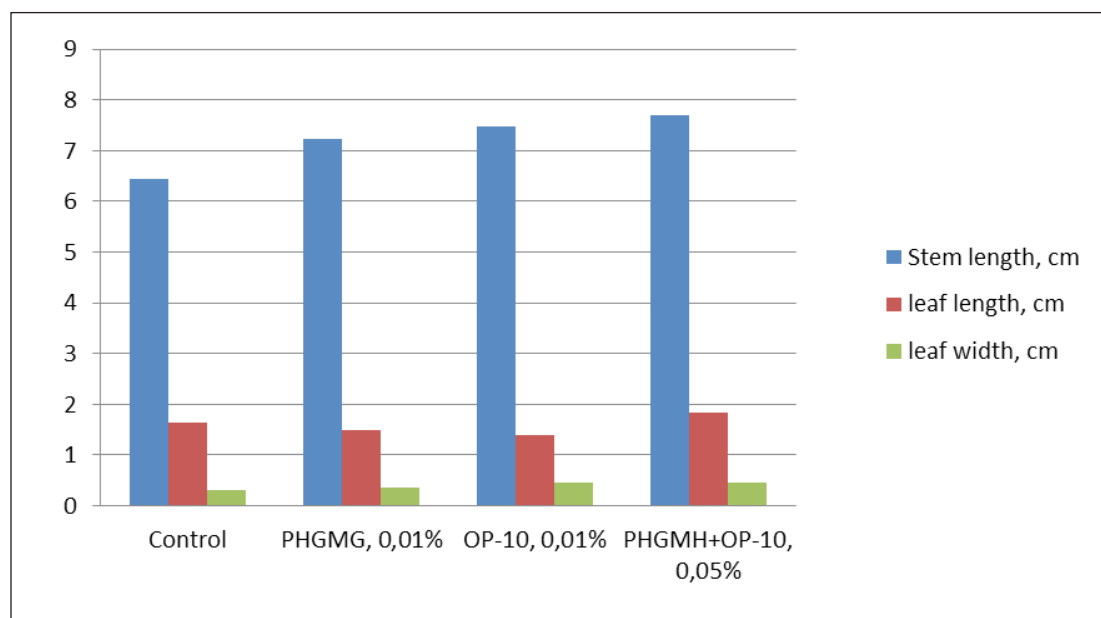


Figure 7 – Influence of solutions of PHGMH, OP-10 and PHGMH/OP-10 on growth and development of sugar beet

Figure 7 shows that good results as in previous tests complexes of PHGMH/OP-10 (0.05 %) exerted with respect to sugar beet growth.

### 3. Determination of chlorophyll on the leaves of cultures

Determination of the amount of chlorophyll in plants gives us the opportunity to assess their resistance to environmental factors. To determine the concentration of chlorophyll it is necessary to separate the pigment from the suspended filtrate of the substance. From the filtrate the chlorophyll concentration was determined by means of analysis conducted on a Spekol spectrophotometer. The results of the studies are presented in Figures 8-10.

As can be seen from Figure 8 that the amount of chlorophyll is most abundant in samples treated with PHGMH complexes (0.05%; 0.1%) and OP-10 (0.01%).

The following experiments were conducted in relation to tomato shoots (Figure 9).

As can be seen in Figure 9, a large amount of chlorophyll was detected in the leaf samples treated with PHGMH (0.01 %) and PHGMH/OP-10 (0.01 %), equaled to 2.6 and 2.97, respectively.

The results on determining the amount of chlorophyll in the leaves of sugar beet are shown in Figure 10.

Figure 10 shows that the most quantity of chlorophyll in the leaves of sugar beet was determined at processing with PHGMH (0.05 %) and OP-10 (0.1 %), equaled to 19.83 and 19.17%, respectively, when in the control sample while the chlorophyll content was 13.74%. The results of experiments confirmed that the crop shoots treated with aqueous solutions of PHGMH, surfactant and their mixture are resistant to environmental factors due to bactericidal and fungicidal activity of guanidine component while non-ionic surfactant provides the surface activity of composition [16-18].

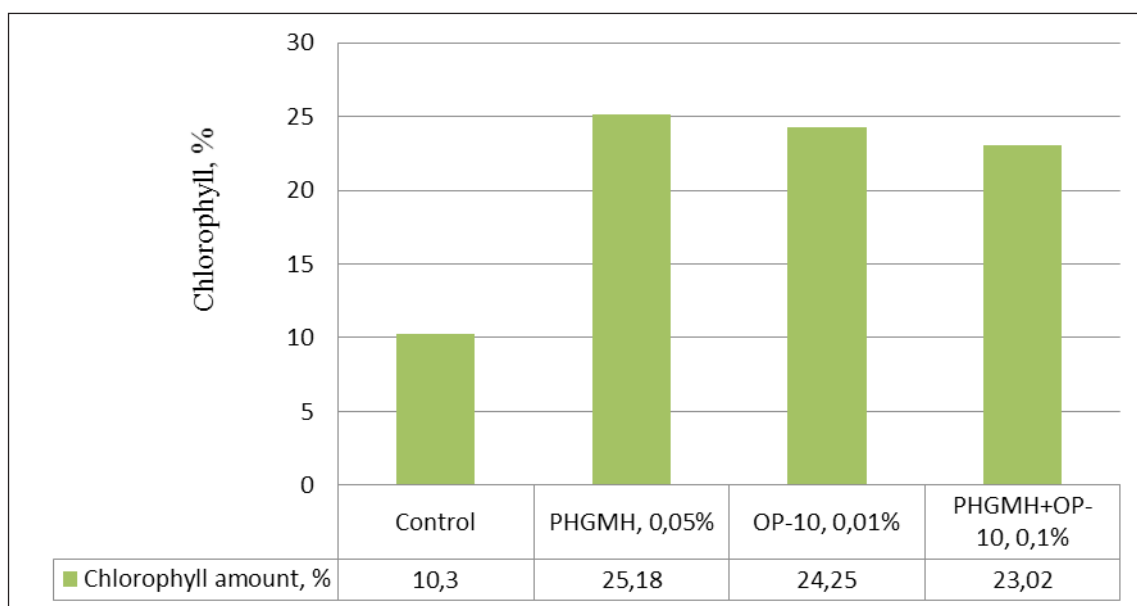


Figure 8 – Chlorophyll amount in cucumber leaf treated with PHGMH, OP-10 and PHGMH/OP-10 solutions

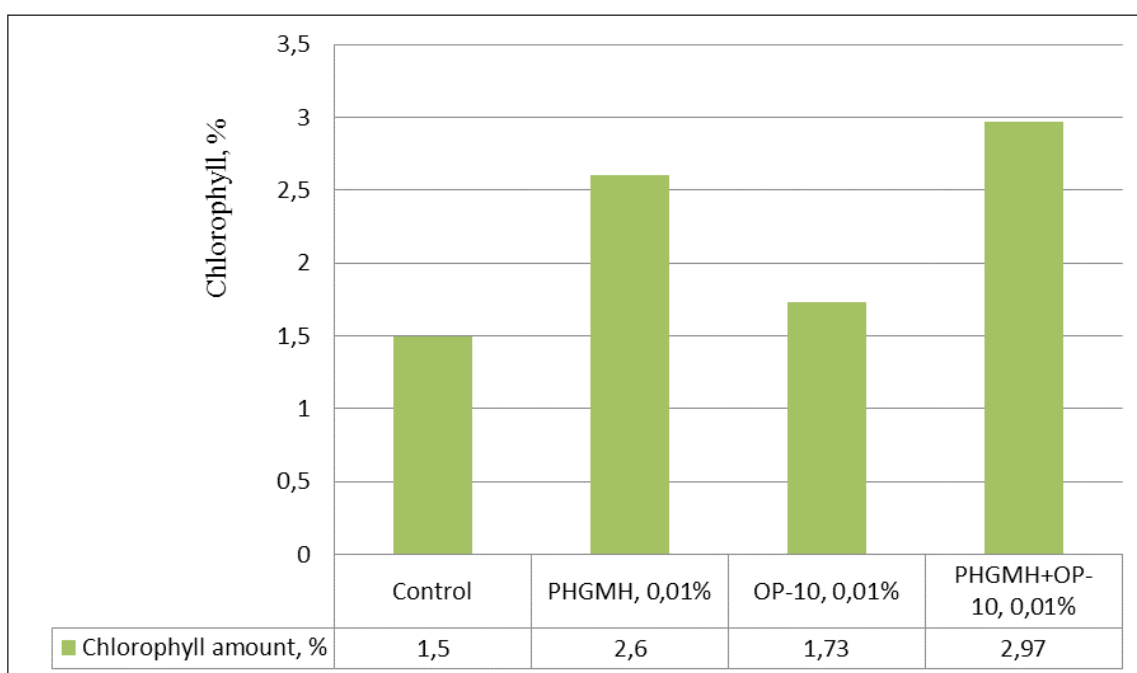
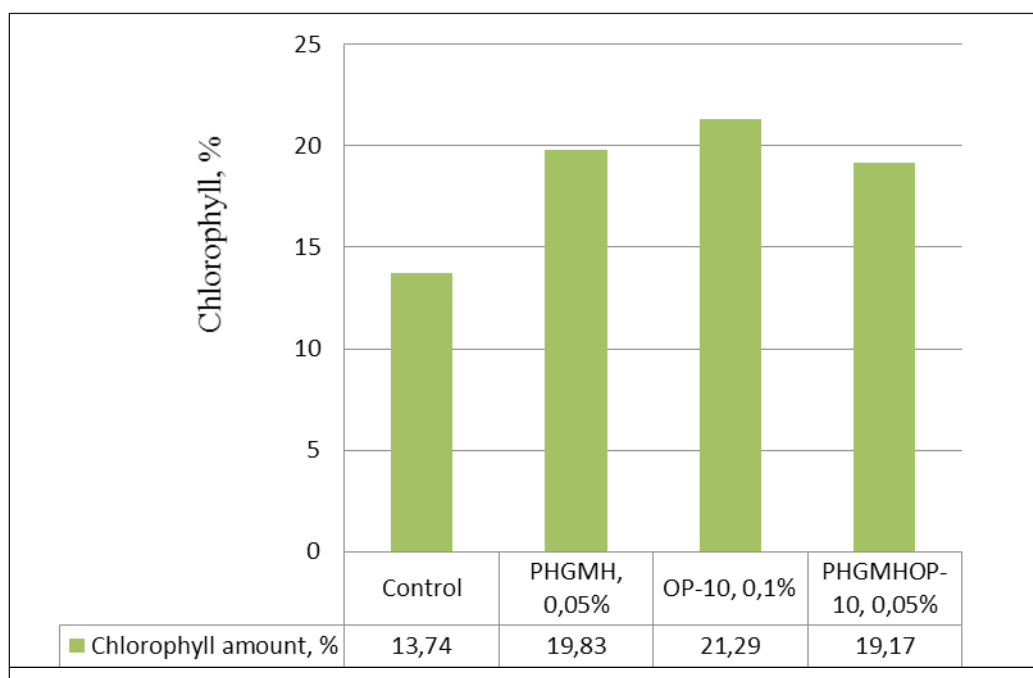


Figure 9 – Chlorophyll amount in tomato leaf treated with PHGMH, OP-10 and PHGMH/OP-10 solutions

## Conclusions

PHGMH, OP-10 and PHGMH/OP-10 complex influence on the germination of vegetable seeds was studied. PHGMH (0.05 %) aqueous solution was more favorable for cucumber seeds (the germination

percentage equals to 94 %), PHGMH/OP-10 complex (at a ratio of 1:1 at initial component concentrations equaled to 0.01%) for tomato seeds (the germination percentage was 90%), and 0.2 % aqueous solution of PHGMH with respect to sugar beet seeds (the germination percentage was 23%).



**Figure 10** – Chlorophyll amount in sugar beet leaf treated with PHGMH, OP-10 and PHGMH/OP-10 solutions

The mixture of PHMG/OP-10 was effective on the growth and development for all of the studied vegetable crops (stem and leaf length). Thus, the sprouts of cucumbers and tomato show good results after treatment with a complex with initial concentration equaled to 0.01 % and sugar beet sprouts – at treating with 0.05% of complex of PHGMH/OP-10.

The amount of chlorophyll in the cultivated shoots of vegetables was determined after treatment with disinfectant and their complexes. The amount of chlorophyll was significantly higher in comparison with the control sample leaves (without treatment) of tomato, cucumber and sugar beet.

The results of the studies showed that the seeds treated with preparations are highly productive and show the increasing of the chlorophyll amount in the leaves providing the resistance of cultures to environmental factors.

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