

## INFLUENCE OF PHILAZONIT BIOPREPARATION ON THE MYCOBIOME OF SOYBEAN PLANTS RHIZOSPHERE

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### Abstract

The article presents the results of analysis of biodiversity and spatial-functional structure of the microbial complex in the soil rhizosphere, and development of ways to regulate the number of phytopathogenic micromycetes in the rhizosphere of soybean plants in organic production. Varieties of plants of soy were grown using the biopreparation «Philazonit», which was developed in the company «Philazonit of Ukraine». The study determined the effect of phylazonite biopreparation on the mycobiome of the rhizosphere of soybean plants. The interaction of plants of soy varieties Kent and Suzir'ja with phytopathogenic micromycetes in conditions of the organic production in the Central Forest-Steppe of Ukraine (Research farm «Skvyrske» of Institute of Agroecology and Nature Management of the National Academy of Agrarian Sciences of Ukraine (IANM of the NAAS) was investigated. The number of phytopathogenic micromycetes in the rhizosphere of plants of different varieties of soy is determined depending on the variety and technology of its cultivation. It was established that the biopreparation Philazonit inhibits the formation of the number of phytopathogenic micromycetes in the rhizosphere of plants of the soy variety Suzir'ja and Kent during the growing season. It is proved that the representatives of genera: *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus* are dominated in the rhizosphere of plants of soy of both varieties. In the rhizospheres of plants of the soy Suzir'ja variety the representatives of genus *Penicillium* (32.8 %) most often occurred. The smallest number of micromycetes were members of the genus *Aspergillus* (9.5 %). In the rhizosphere of plants of the Kent variety dominated by representatives of the genera *Alternaria* and *Fusarium* (35.6 % and 34.1 % respectively). Representatives of the genus *Penicillium* were 15 % and *Aspergillus* – 15.3 % It is proved noted that during the studies there was a

correlation between the development of micromycetes and the hydrothermal coefficient – in the flowering phase ( $r=0.8$ ) and in the ripening phase ( $r=0.9$ ) except for the germination phase.

**Keywords:** soybeans, phytopathogenic micromycetes, rhizosphere, hydrothermal coefficient, ecology, agrocenosis.

DOI: 10.21303/2504-5695.2021.001874

## 1. Introduction

Fertile land and favorable climate determined the rapid spread of production of soy in Ukraine. At the same time, there is a growing of the mass accumulation of phytopathogenic microorganisms in the agrocenoses of soy, among which genera: *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus* are dominated [1–3]. They lead to the development of harm-causing diseases of soybean plants during the growing season and can intensively inhabit the seed material. This causes a deterioration in the quality of the crop by reducing the indicators of protein and fat contents [4]. These micromycetes are toxin-forming. They can damage of the various organs of plants, animals, fungi and are toxic to humans.

A wide range of phytotoxic action have deuteromycetes toxins, which belong to the genera: *Penicillium*, *Aspergillus* and *Fusarium*. In the functioning of the system (soil – microbial community – plants), toxin-forming micromycetes are characterized by greater competitiveness compared to other microorganisms [5–8].

Therefore, more and more attention is paid to the organic production of soy in the world, which is aimed at regulating the number of phytopathogenic microorganisms in agrocenoses. Biological preparations are one of the most powerful factors in regulating of the biotic relationships in agrocenoses, which allows to solve the issue of ensuring a balanced nutrition of plants and resistance to phytopathogenic micromycetes. Retrospective analysis of literature showed that the problems of the effectiveness of the influence of biological preparations on the functioning of agroecosystems were studied by various scientists [9–15]. Their research revealed the main problems such as: optimization of the nutritional regime under the influence of biopreparations, increasing of the crop yields, reproducing of the soil fertility, regulating their microbiological activity. High efficiency of the various biopreparations using was found, among which Philazonit biopreparation has proven itself.

Philazonit is a complex biopreparation based on beneficial soil bacteria.

Philazonit contains several groups of bacteria: nitrogen-fixing bacteria, phosphate-mobilizing bacteria, cellulose-destroying bacteria. The composition of Philazonit also includes natural B vitamins, which reduce the sensitivity of plants to diseases, as well as hormones that accelerate seed germination and plant growth, in particular gibberellins and auxins. Antipathogenic bacteria prevent and protect plants from fungal diseases, especially *Fusarium oxysporum* and promote the production of immunity in plants. Soy plants respond well to the introduction of biopreparations that contribute to: the productivity of soy; humic acids enhance the properties of plant immunoregulation, biosynthesis of protective substances; contribute to the growth of auxins and cytokinins; regulate of the physiological processes in cells [16, 17].

Consequently, soil microbial cenoses have complex genetic and spatial organization and high biodiversity, the structure of which depends on farming systems, agrotechnical measures and physiological biochemical properties of cultivated plants. Therefore, the study of the effect of the biological product Filazonit on biodiversity and the spatial-functional structure of the microbial complex in the soil rhizosphere is important for regulating the number of phytopathogenic micromycetes in the rhizosphere of plants of soy varieties in conditions of organic production.

So, the goal of the investigation was to develop of the ways to regulate the number of phytopathogenic micromycetes in the rhizosphere of plants of soy varieties in conditions of organic production.

## 2. Materials and Methods

### 2.1. Characteristics of place, soil of researches

Experimental research was carried out during 2018-2020 in the Central Forest-Steppe of Ukraine (Research farm “Skvyrsk” of organic production of the IANM of the NAAS) and in

the Department of Agrobioreources and Environmentally Safe Technologies of the IANM of the NAAS. The best soils for soy are black humus earth, dark gray and chestnut. The most suitable for this crop are soils with a neutral reaction (pH 6.5–7.0). The soil of the research field of the “Skvyrsk” research station of organic production is represented by low-humus black humus earth, and in the mechanical composition – large dusty-medium loamy. The humus content in the upper layer of soil (0–20 cm) is 3.6 %, lightly hydrolyzed nitrogen – 6.6 mg, easily digestible phosphorus – 14.0 mg and metabolic potassium – 15.2 mg per 100 g of soil. The reaction of the soil solution is slightly acidic (pH=6.0).

## 2. 2. Hydrothermal coefficient (HTC)

Hydrothermal coefficient (HTC) was calculated according to the methodology [18].

$$HTC = \Sigma R / 0,1 \cdot \Sigma t_{act > 10^{\circ}}$$

where:

HTC < 0.4 – very strong drought,  
HTC from 0.4 to 0.5 – severe drought,  
HTC from 0.6 to 0.7 – average drought,  
HTC from 0.8 to 0.9 – weak drought,  
HTC from 1.0 to 1.5 – sufficiently moisture,  
HTC > 1.5 – excessively moisture.

## 2. 3. The object of the study

Samples of plants of different varieties of soy were selected during the growing season in the phase: shoots, flowering, maturation. The studies were carried out on plants of soybean varieties: Suzir’ja, selection of the National Scientific Center of the Institute of Agriculture of the National University of Ukraine and Kent variety, selection company “SAATBAULINZ” in Austria. The technologies with using of the biopreparations of various actions is the alternative at the organic production, because the use of mineral fertilizers and plant chemical protection products is prohibited. Therefore, the varieties of plants of soy were grown using the biopreparation “Philazonit”, which was developed in the company “Philazonit of Ukraine”.

We used a biological product Filazonit before sowing soybeans (tillage). Consumption rate – 1.5 l/ha.

To compare the results, we used the control option (soybean varieties without processing of the biological product).

The sampling of the biological samples and counting the number of colony-forming units (CFU) were carried out according to methods, generally recognized in mycology [19].

## 2. 4. Analysis of soil mycobiome

Analysis of soil mycobiome was carried out by soil dilution method [20]. The rhizosphere soil was taken from the roots of plants. Samples were transferred in laboratory conditions to sterile flasks of 90 ml of sterile distilled water and for 5 minutes were shaken on a horizontal orbital shaker (Wise Shake SHO, SHR 2D) to a homogeneous suspension. 1 ml of suspension was transferred from the flask to a sterile test tube of 9 ml of sterile water. From the test tube of the 3rd dilution, 1 ml of the suspension was seeded into sterile Petri cups. After that, 10 ml of Chapek medium was poured, mixed and incubated at a temperature of 25 °C for 3 days, after which colonies were counted on the automatic counter SCAN 4000 (Intercience, France) and transferred them into test tubes for further identification.

## 2. 5. Identification of isolates

Identification of isolates was carried out on 15-day cultures using a trinocular microscope (DN-200 M) using the Mycobank database base [21].

## 2. 6. The count of colonies

The average number of colonies of fungi per 1 g of dry soil was determined by the formula [22]:

$$A = \frac{b \cdot c \cdot K}{g},$$

where:

$A$  – the number of infectious units of micromycetes in 1 g of dry soil;

$b$  – the average number of colonies in a cup;

$c$  – dilution of the solution, from which inoculation is made;

$K$  – humidity amendment;

$g$  – soil weight (10 g).

## 2. 7. Statistical analysis

Statistical analysis of the obtained results was carried out using dispersion and correlation analysis ( $p=0,05$ ) [23]. For the process of the results, standard mathematical methods of data analysis and charting were used using the Microsoft Office Program Package, Statgraphics Plus for Windows, Excel 2000.

## 3. Results

The air temperature exceeded the norm of indicators during the field research and ranged from 14 °C to 29 °C (Fig. 1).

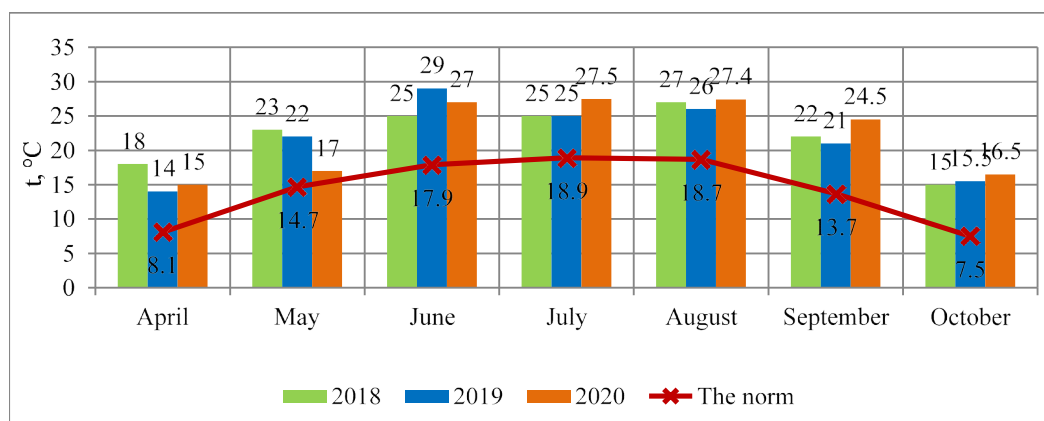


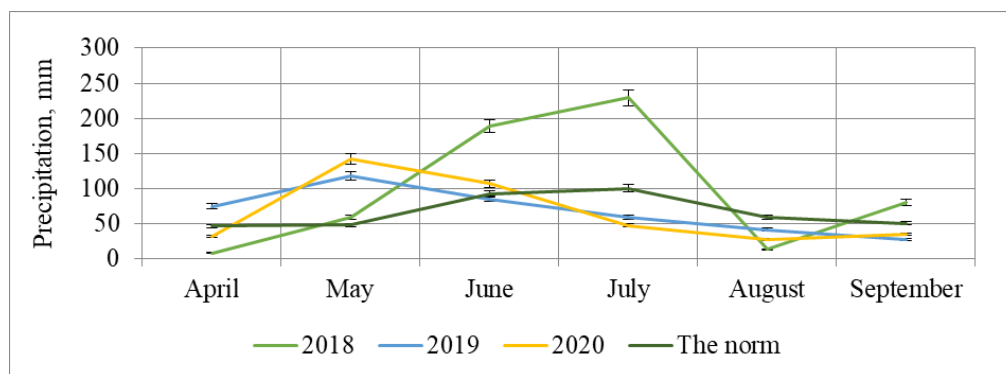
Fig. 1. Air temperature during field research (Research farm “Skvyrske” of IANM of the NA)

It is known that the optimum temperature for germination of soy seeds is 15–20 °C; for flowering – 19–25 °C; for the formation of beans and seeds – 17–23 °C; for maturation – 13–20 °C. The average daily temperature at which the reproductive organs of soy are formed – 18–23 °C [24].

The weather conditions for growing soy during the study were within the normal range, despite the fact that the air temperature indicators exceeded the norm. The amount of precipitation during field research was quite contrasting and ranged from 8 mm (lower than the norm by 38 mm) to 229 mm (above the norm by 128.9 mm) (Fig. 2).

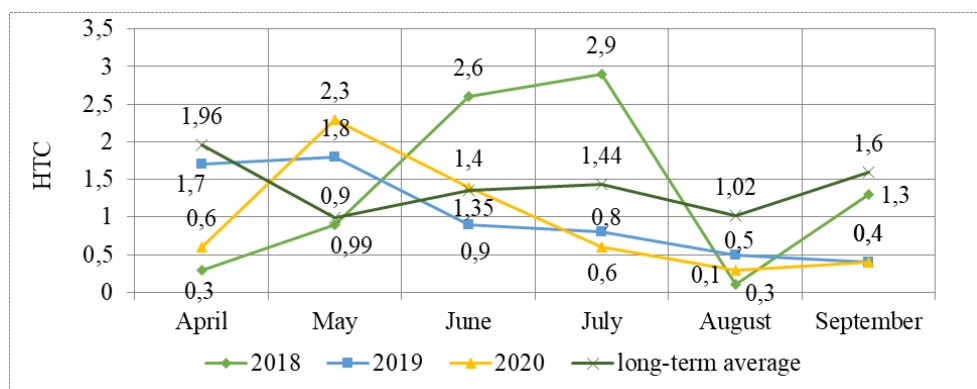
The varieties of soy plants Kent and Suzir’ja are characterized by medium resistance to drought. Given this, 2018 year was the least favorable for growing soy.

Climatic conditions perform both direct and indirect effects on the vital activity of microorganisms. At the same time, temperature and moisture are important components of environmental conditions that regulate the course of soil and biological processes. According to the results of retrospective analysis of literature, hydrothermal coefficient (HTC) determines the vital activity of soil organisms and the activity of soil biochemical processes.



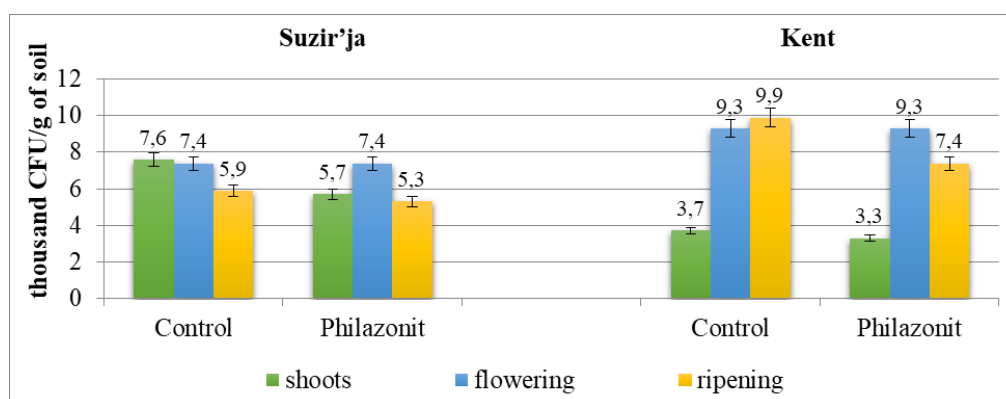
**Fig. 2.** The amount of precipitation during the field research (Research farm “Skvyrskye” of IANM of the NA)

According to the calculated values of the HTC, it was established that the growing season of 2018 was wet enough (HTC 1.35), in 2019 – slightly arid (HTC 0.9) and in 2020 – wet enough (HTC 1) (**Fig. 3**).



**Fig. 3.** Dynamics of indicators of hydrothermal coefficient (HTC) of the vegetation period in the years of research (Research farm “Skvyrskye” of IANM of the NA)

According to the results of studies, it was found that there was a restrained formation of the number of micromycetes in the phase of shoots and maturation in 2018 during the action of the biopreparation Philazonit on plants of soy varieties Kent and Suzir’ja (**Fig. 4**)



**Fig. 4.** The number (CFU/g of soil) of micromycetes in the rhizosphere soil of soybean plants of the varieties Suzir’ja and Kent under the action of the biological product Philazonit (2018)

On plants of soy variety Suzir'ja in the phase of shoots, the number of micromycetes was less by 1.9 thousand CFU/g of soil and in the ripening phase – by 0.6 thousand CFU/g of soil compared to control. At the same time, on plants of soy variety Kent in the phase of shoots the number of micromycetes was less by 0.4 thousand CFU/g of soil, and in the ripening phase – by 2.5 thousand CFU/g of soil compared to control.

It was also found that in the flowering phase both on plants of soy varieties of Suzir'ja and Kent, the indicators of the number of CFU micromycetes were at the level of control and amounted to 7.4 thousand CFU/g of soil and 9.3 thousand CFU/g of soil, respectively. This indicates the low efficiency of the Philazonit biopreparation, which may be due to exceeding temperature standards (by +11.1 °C) and a large amount of precipitation, exceeding the norm by more than 2 times in the period from June to July (flowering phase), which caused the formation and development of phytopathogenic micromycetes and significantly reduced the effectiveness of Philazonit biopreparation on plants of soy varieties Kent and Suzir'ja in the flowering phase.

According to the results of the research, it was established that the formation of mycobiome in the rhizosphere of soy plants was suppressed during ontogenesis during the action of the Philazonit biopreparation on plants of soy varieties Suzir'ja and Kent in 2019 (Fig. 5).

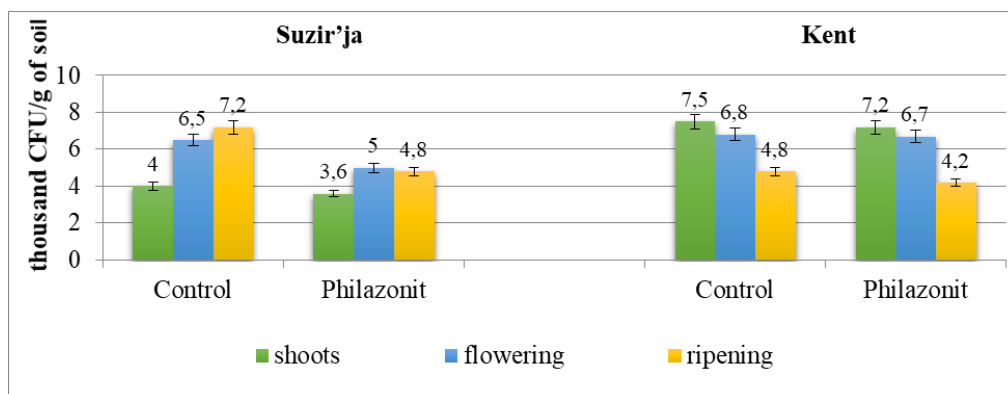


Fig. 5. The number of micromycetes (CFU/g of soil) in the rhizosphere of soybean plants Suzir'ja and Kent under the action of the biological product Philazonit (2019)

The smallest number of phytopathogenic micromycetes was observed in the phase of shoots on plants of soy varieties of Suzir'ja (3.6 thousand CFU/g of soil), and in the ripening phase on plants of soy of Kent variety (4.2 thousand CFU/g of soil). Similar results were obtained in 2020 (Fig. 6).

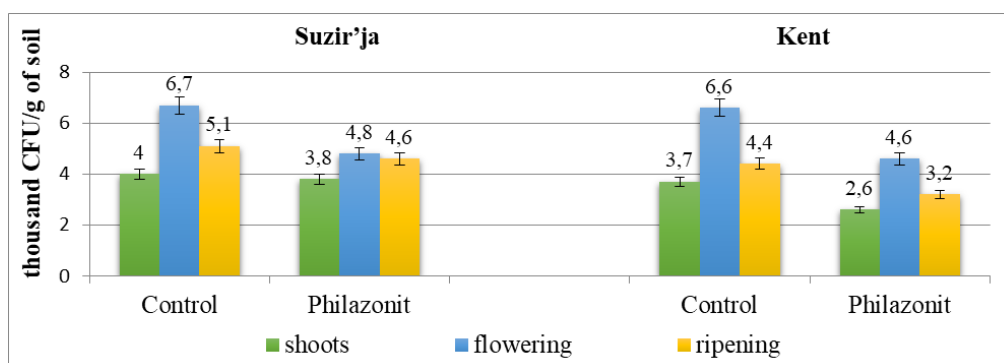


Fig. 6. The number (CFU/g of soil) of micromycetes in the rhizosphere soil of soybean plants of the varieties Suzir'ja and Kent under the action of the biological product Philazonit (2020)



It should be noted that the most effect of biopreparation was found in the phase of shoots on both varieties, where the number of micromycetes in the mycobiome of rhizosphere plants of the soy Suzir'ja was 3.8 thousand CFU/g of soil, and on plants of soy of Kent variety – 2.6 thousand CFU/g of soil. The efficiency of the Philazonit biopreparation was high despite the fact that 2020 was characterized as wet enough (HTC 1) and the air temperature exceeded the norm. According to statistical analysis, presented in Table 1, the biopreparation Philazonit showed efficacy during the study in all phases of ontogenesis of plants and reduced the number of micromycetes in the mycobiome of the rhizosphere of the plants studied (**Table 1**).

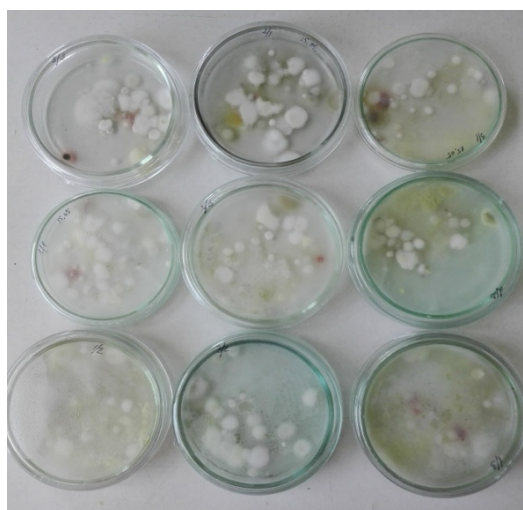
**Table 1**

Dynamics of the number of micromycetes in the rhizosphere of soybean plants during the growing season

Varieties	Biopreparation	The average number of CFU in the rhizosphere of soybean plants, thousand									The average		
		2018			2019			2020			shoots	flowering	ripening
		shoots	flowering	Ripening	shoots	Flow-ering	ripen-ing	Shoots	flower-ing	ripen-ing			
Suzir'ja	Control	65	62	49	35	54	60	35	56	44	45	57,3	51
	Philazonit	47	62	36	26	44	41	25	40	32	32,6	48,7	36,3
	over the years										19,5	9,8	21,4
	for technolo-gies										7,8	8,9	11,7
	Control	37	78	83	62	57	40	31	55	37	43,3	63,3	53,3
	Philazonit	28	78	62	52	45	26	22	42	22	34,0	55,0	36,7
Kent	over the years										11,5	16,0	17,1
	for technolo-gies										8,4	10,7	12,7

Note: The difference is significant at the level of  $p = 0.05$

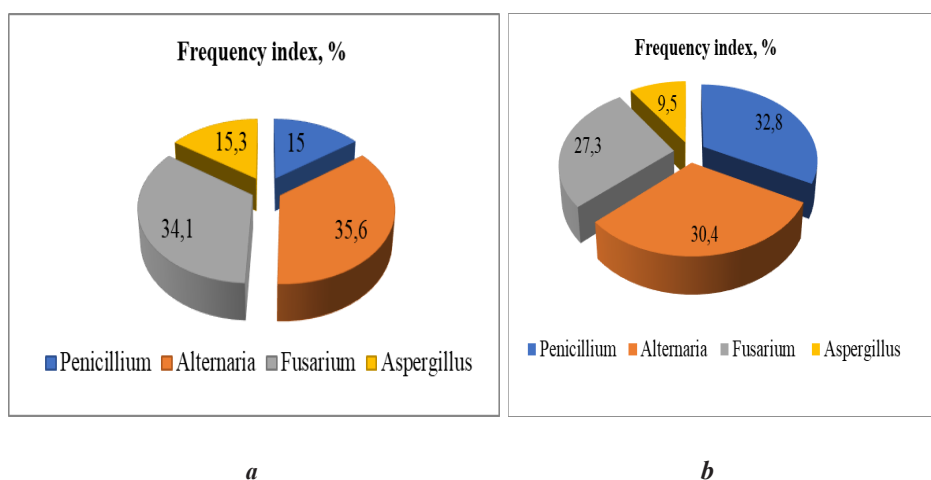
It should be noted that during the studies there was a correlation between the development of micromycetes and the hydrothermal coefficient – in the flowering phase ( $r=0.8$ ) and in the ripening phase ( $r=0.9$ ) except for the germination phase. A significant number of phytopathogenic micromycetes were isolated and identified from the rhizosphere of plants of soy varieties (**Fig. 7**).



**Fig. 7.** The spectrum of micromycetes in the rhizosphere soil of soybeans plants of the Kent variety (5 days after seeding)

The number of isolates isolated from the rhizosphere of soybean varieties

Analysis showed that the species, belonging to four genera of micromycetes: *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus* in the rhizosphere of plants of both varieties of soy are dominated. In the rhizospheres of plants of the soy Suzir'ja variety the representatives of genus *Penicillium* (32.8 %) most often occurred. The smallest number of micromycetes were members of the genus *Aspergillus* (9.5 %). In the rhizosphere of plants of the Kent variety dominated by representatives of the genera *Alternaria* and *Fusarium* (35.6 % and 34.1 % respectively). Representatives of the genus *Penicillium* were 15 % and *Aspergillus* – 15.3 % (Fig. 8 *a, b*).



**Fig. 8.** Frequency of isolates isolated from the rhizosphere of soybean plants of varieties:  
*a* – Suzir'ja; *b* – Kent in the laboratory (2018–2020)

Biotic environmental factors significantly affect the number and dynamics of the population of phytopathogenic micromycetes in agroecosystems. One of the important factors is the selective pressure of cultivated plant varieties, which can affect the quantitative and qualitative indicators of the phytopathogenic background in agroecosystems.

Thus, it was established that in the rhizosphere of plants of soy varieties Suzir'ja and Kent the species of phytopathogenic micromycetes, belonging to the genera *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus* are dominated, which are factors of biological contamination of agroecosystems and decrease in product biosafety.

#### 4. Conclusions

The possibility of regulating the number of phytopathogenic micromycetes in the microbiome of soy plants by using biopreparation *Philazonit* has been experimentally proved, which will increase biosafety in soy agroecosystems and improve of the crop quality. According to statistical analysis, the biological product “*Filazonit*” has shown efficiency in research at all stages of plant ontogenesis.

It is proved that the representatives of genera: *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus* are dominated in the rhizosphere of plants of soy of both varieties. In the rhizospheres of plants of the soy Suzir'ja variety the representatives of genus *Penicillium* (32.8 %) most often occurred. In the rhizosphere of plants of the Kent variety dominated by representatives of the genera *Alternaria* and *Fusarium* (35.6 % and 34.1 % respectively). Biotic environmental factors significantly affect the number and dynamics of the population of phytopathogenic micromycetes in agroecosystems. One of the important factors is the selective pressure of cultivated plant varieties, which can affect the quantitative and qualitative indicators of the phytopathogenic background in agroecosystems.



Therefore, the control of microscopic fungal contamination of the main contaminants of biotic origin is relevant in the issue of feed safety. Systematic mycological studies of feed and feed materials for the presence of mold saprophytes will not only determine the taxonomic affiliation and identify toxin-forming species, but also contribute to the creation of a system of measures to prevent human poisoning and feed toxicosis in farm animals.

### Conflicts of interest

The authors declare no conflicts of interest.

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*Received date: 15.04.2021*

*Accepted date: 18.05.2021*

*Published date: 31.05.2021*

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**How to cite:** Parfenuk, A., Havryliuk, L., Beznosko, I., Pasichnik, L., Turovnik, Y., Ternovyi, Y. (2021). Influence of Philazonit bio-preparation on the mycobiome of soybean plants rhizosphere. EUREKA: Life Sciences, 3, 11–20. doi: <https://doi.org/10.21303/2504-5695.2021.001874>