Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2021, 11(3), 86-89, doi: 10.15421/2021_148

ORIGINAL ARTICLE

Influence of Filazonit biopreparation on soybean seed quality

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Received: 11.01.2021. Accepted 20.04.2021

Filazonit determined an essential inhibition of the CFU (number of micromycetes) in soybean seeds in the cultivars *Suzirya* and *Kent*, compared to the control. The number of CFU in the soybean seeds of different varieties ranged from 0.2 x 10^4 CFU/g of seeds to 1.5* CFU/g of seeds, almost 2.5 times less than the number in the control specimen (0.8 * 10^4 CFU/g to 2.7 * 104 CFU/g seeds). We found that the soybean seeds of the cultivars *Suzirya* and *Kent* are dominated by phytopathogenic micromycetes, which belong to the genera *Alternaria, Fusarium,* and *Penicillium*. They are the primary factors of biological pollution of agrophytocenoses and the reduction of product biosafety. The changes in the quality of soybean seeds grown in organic production conditions using biological technologies were determined. We registered that the protein and oil content in the seeds of cultivars *Kent* and *Suzirya* were higher than the standard indicators stated in DSTU 4964: 2008. TU. SOY standards. Moreover, the mass fraction of the seed moisture did not exceed the acceptable norms. We discovered that the cultivar, genotypes, and moisture supply of soybean plants, and cultivation technology influenced the biochemical composition of the soybean seeds. Depending on these factors, the protein content in the seed cultivars ranges from 37.5 to 41.11%, fat content ranges from 19.02 to 21.7%, and the mass fraction moisture ranges from 8.8 to 11.4%.

Keywords: soybeans, micromycetes, biopreparations, agrophytocenoses, biosafety, toxicity, hydrothermal indicators.

Introduction

Soybeans have a functional chemical composition and high nutritional and forage quality. Proteins are the main biochemical component of soybean seeds. The more proteins can be found in soybeans, the higher their nutritional and technological value is. According to various research findings, soybean seeds contain about 40% protein on average (these indicators can range from 30 to 50%) (Ryzhkov, 2015; Batashova, 2014; Kim et al., 2012; Moraes et al., 2006). A specific deficiency of animal proteins in the nutrition of modern people resulted in a more significant consumption of soybean seeds in the modern food industry as an additional substitute for animal proteins. This has brought to increased requirements as to the quality of soybean seeds. Therefore, the main objective of the current soybean selection is to improve the quality of the seeds, optimize their composition and technological properties, and receive higher protein content. Some authors (Yusova et al., 2017; Hardoim et al., 2015; Bellaloui et al., 2015; Yermolina et al., 2011) have been studied the protein content, its polymorphism depending on the species, diversity, and conditions of legume seed cultivation, which show that protein content in the legume seeds also significantly depends on the variety, meaning that it is under genetic control (Vozhehova, 2016).

According to the degree of protein content variability, legumes are divided into two groups: the ones sensitive to cultivation conditions, like beans, soybeans, and the less sensitive ones, like peas, chickpeas, lentils (Petybskaya, 2012). While several researchers consider that when the temperatures during the cultivation period are relatively low, proteins in legume seeds are accumulated in fewer amounts compared to the years with higher temperatures (Song et al., 2016). Watering plants have a significant influence on the seed quality as well. However, some scientists consider that abundant irrigation causes decreases in protein content in soybeans as well as increased fat content (Vozhehova, 2016; Bulyhin, 2014), but some others consider that abundant watering of soybeans causes lower content of both protein and fat (Novikova et al., 2018; Kazanok, 2011). Since soybeans are widely used for nutrition both in Ukraine and worldwide, understanding the influence of environmental factors on the yield and grain quality in different climatic conditions is of great importance. The use of intensive agro-technological approaches has resulted in the deterioration of ecological conditions for the cultivation of many crops, including soybeans, and it has also significantly changed the phytosanitary situation in agrophytocenoses (Borysovskyy, 2014). The anthropogenic disturbances of natural ecosystems bring on the important role of fungi in the cycle of major biophilic elements on a global scale.

Mold fungi are the source of many diseases in animals and humans. About 30,000 species of mold fungi were discovered in forage and nutrition products, of which more than 250 species can produce dangerous toxins, which can cause poisoning between animals and humans (Reshetylo, 2020). When the mold fungus contaminates soybean seeds, a significant amount of yield is lost each year. Improper time of harvesting and wrong drying time during storage causes reproduction of phytopathogenic fungi and toxic substances called mycotoxins. This causes spoilage of the forage organoleptic qualities, reduced nutritional value, and seeds unsuitable for technological processing. In animal husbandry, the use of forages affected by fungi can cause chronic toxicity, which can cause the death of livestock and birds (Antonissen, 2014; Boonen et al., 2012).

Phytopathogenic micromycetes can infect most forage crops of plant and animal origin. Lentils, beans, soybeans, and other legumes most often contain phytopathogenic micromycetes. Several pathogens often parasitize soybean plants simultaneously, which reduces the yield of its seeds by 15–20% or more, lowering the protein content by 4–18% and fat content by 1.6–5.6%. The feed can often be contaminated by *Aspergillus, Penicillium, Mucor, Fusarium, Rhizopus,* and *Cladosporium.* However, some species like *Aspergillus fumigatus, Aspergillus niger, Aspergillus amstelodami, Penicillium brevicaulis, Penicillium bicolor, Mucor racemosus, Rhizopus equinus, Rhizopus cohnii,* and *Trichoderma lignorum,* can get into the body with food (to the digestive tract) or with air (to the respiratory tract) and germinate in the internal organs of the animals (Akladious et al., 2019; Verweij et al., 2015; Warrilow et al., 2015). Such diseases as fusariosis, alternariosis, peronosporosis, aspergillus mildew, and aspergillosis are of great significance because they are toxin-forming (Kalagatur et al., 2018). They significantly reduce the ecological safety of the plant products. Among the toxins that produce the listed micromycetes, the following are especially dangerous: *Fusarium oxysporum* Schleht*, F. culmorum* (W.G.Sm.) Sacc.*, F. Sambucinum* Fuckvar. Minus Wr.*, Alternaria alternata, Ascochyta sojaecola* Abr.*, Cercospora sojina* Hara, *Septoria glycines* T. Hemmi., *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* (Lib.) de Bary., and *Peronospora manshurica* Sydow (Alabouvette et al., 2009).

Moreover, according to the academic sources (Levitin, 2012), climate change and higher temperatures, especially in the winter months, cause expansion of pathogens in areas where they did not occur before (Shvartau et al., 2017). Therefore, many scientific studies are aimed at researching the connections between the soil types and climatic conditions during the cultivation season, which are the best factors as to the improvement of the productivity of oilseeds and their resistance to some significant pathogenic diseases (Novikova et al., 2018; Vozhehova et al., 2018).

Therefore, the matter of high-quality soybean in terms of biological cultivation conditions has been studied insufficiently, and it was necessary to research the influence of different technological measures as to the soil types and climatic conditions of its cultivation on the formation of colony-forming units in seed varieties as well as to analyze the biochemical composition of soybean seeds.

Materials and Methods

The experimental research was conducted in 2018 - 2020 in the Central Forest-Steppe region of Ukraine (Skvyrska research station of the organic production Institute of Agroecology and Nature Management of NAAS, further – SRS OP IAN NAAS, and in the Department of Agrobioresources and Ecologically Safe Technologies of the Institute of Agroecology and Nature Management of NAAS. The samples of some different varieties of soybean seeds were taken in the ripening phase. The following types of soybean plants were the object of the study: the cultivar *Suzirya*, the selection by the National Research Center of the Institute of Agriculture of NAAS of Ukraine, and the cultivar *Kent*, the selection by company selections SAATBAULINZ in Austria. Since organic production does not allow using mineral fertilizers and pesticides, an alternative to using technologies with biopreparations (biological products) of different actions, therefore, these varieties of soybean plants were cultivated using the biological product "*Filazonit*", which was developed by the company named "*Filazonit Ukraine*". "*Filazonit*" is a biopreparation of the complex action based on helpful soil bacteria. "*Filazonit*" contains some groups of bacteria like nitrogen-fixing bacteria, phosphate-mobilizing bacteria, cellulose-destroying bacteria. The composition of "*Filazonit*" also contains some natural vitamin B, which reduces the plants' susceptibility to diseases, and it includes some hormones that accelerate the germination of seeds and plants, like *gibberellin* and *auxin*. Antipathogenic bacteria prevent and protect plants from fungal diseases, especially *Fusarium oxyporum*, and promote immunity in plants (Havrylyuk et al., 2019). For the research, four samples of 50 seeds of each studied cultivar were taken. The seeds were washed for 30 minutes in sterile water, disinfected for 5 minutes in 0.5% potassium permanganate solution, and then washed with sterile water. The washed seeds were placed on sterile filter paper and dried at 30 ^{o}C in a vacuum oven. They were grinded for 1 minute and sieved through a sieve with a diameter of 1 mm. The samples of the four specimens were mixed, and this way, a mean sample was made. The sample was transferred to sterile flasks of 10 g (3 repetitions), then 90 ml of sterile water was added, and the flasks were shaken for 5 minutes until the homogeneous suspension was received. Then, 1 ml of the received suspension was planted in sterile Petri dishes (3 replicas of each variety). Then 10 ml of the Czapek agar were poured, stirred, and incubated at 25 °C for three days, then the colonies were counted with an automatic counter SCAN 4000 (Interscience, France) and sowed in test tubes for further identification.

The identification of the isolates was performed on 15-day species using a trinocular microscope (DN-200 M) using the base Mycobank and the determinant (Koval, 2016). The average number of fungal colonies per 1 g of dry seeds was determined by the formula (Kurakov, 2001):

$$A = \frac{\text{B} \times \text{V} \times \text{K}}{\text{g}}$$

A – the number of infectious units, micromycetes in 1 g of dry seeds;
b – the average number of colonies in the dish;
v – dilution from which the sowing was made;
K – humidity correction indicator;
g – seed mass (10 g).

The hydrothermal coefficient (HTC) was calculated according to Selyaninov (1928):
HTC = $\Sigma R / 0{,}1 \cdot \Sigma t_{act>10}$,
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 – sufficient moisture,
HTC > 1.5 – excessively moisture.

The indicators of seed quality were determined by the method suggested by DSTU 4964: 2008. TU. SOYA (Burtsev et al., 2010). Statistical processing of the obtained results was performed using the analysis of variance and correlation (p = 0.05) (Markov et al., 2012). We used Statgraphics Plus for Windows to process the data.

Results and Discussion

During the research of 2018–2020, the number of CFU in the soybean seeds of the cultivars *Suzirya* and *Kent* grown using *Filazonite* technology was determined. We detected that with "*Philazonite of Ukraine*," some significant inhibition of the colony-forming units (CFU) formation of micromycetes in soybean seeds of cultivars *Suzirya* and *Kent* could be achieved compared with the control specimen. In particular, in 2018, during the research, the highest efficiency of biopreparation for the soybean cultivar *Suzirya* was detected. In that case, the number of CFU was 1.3 thousand CFU/g of seeds, whereas it was 0.2 thousand CFU/g of seeds, less than the number for the seeds of the cultivar *Kent* (Fig. 1).

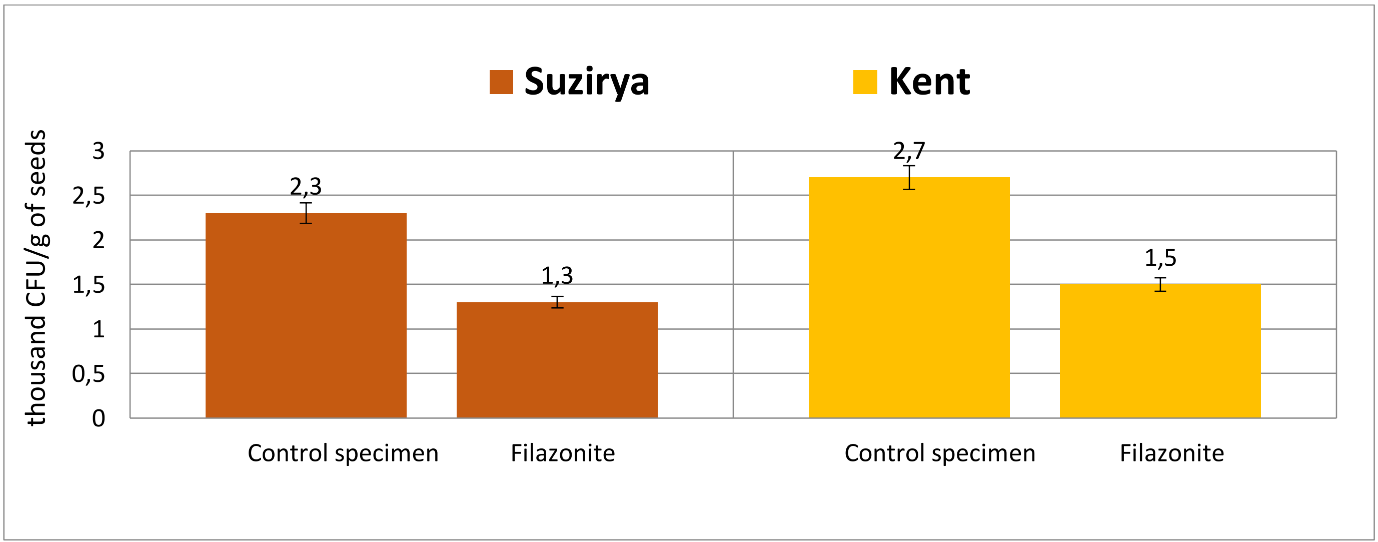


Fig. 1. The number of CFU/g seeds of the micromycete (in thousands) of the soybean cultivars *Suzirya* and *Kent* cultivated by using the technology called "*Filazonit Ukraine*" (2018).

Since the indicators of the CFU numbers in the micromycetes of both varieties turned out to be almost twice lower than the indicator of the control specimen, then the influence of the biopreparation product *Filazonit* on the formation of colony-forming units should be considered inhibitory. In 2019, we revealed that the highest effectiveness of the biopreparation the soybean seeds of the cultivar *Kent* compared with the soybean variety *Suzirya*, where the number of CFU micromycetes was

0.2 thousand CFU/g of seed that was 0.2 thousand CFU/g of seeds less than the number in the cultivar *Kent* (0.4 thousand CFU/g of seeds) (Fig. 2).

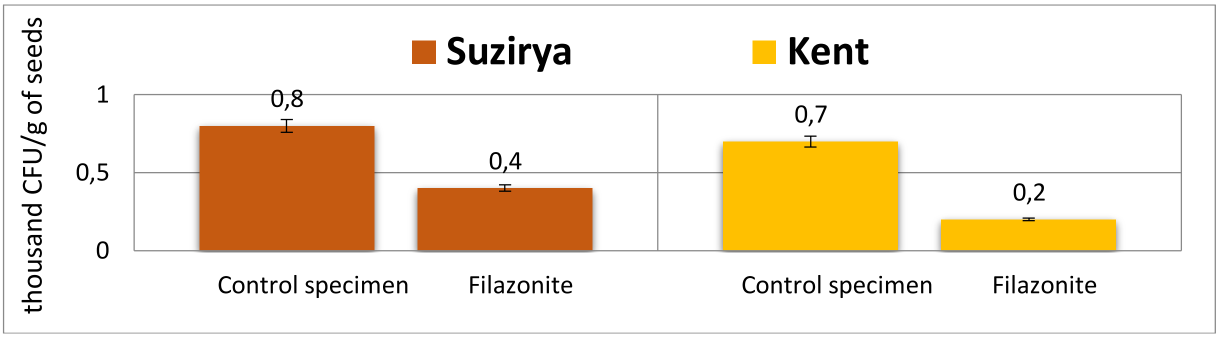


Fig. 2. The number of the CFU/g of seeds of the micromycete (in thousands) in soybean cultivars *Suzirya* and *Kent* cultivated by using the technology called "*Filazonit Ukraine*" (2019).

In 2019, we observed a similar tendency in the CFU formation in two soybean seed cultivars cultivated using the biopreparation "*Filazonit*". We found out that the lowest number of CFU of micromycetes were achieved in the soybean variety *Kent*, which was 0.7 thousand CFU/g of seeds, and that was 3.5 times less compared to the number of control specimens that proved that the antifungal properties of the tested cultivars' soybean plant significantly depend on the genotype of the variety.

According to the results of the research of 2020, similar relationships to the one discovered in 2018–2019 were detected, so the biopreparation "*Filazonit*" proved to be more effective for the cultivar *Kent* (0.6 thousand CFU/g of seed) compared to the soybean cultivar *Suzirya* (0.8 thousand CFU/g of seeds) (Fig. 3).

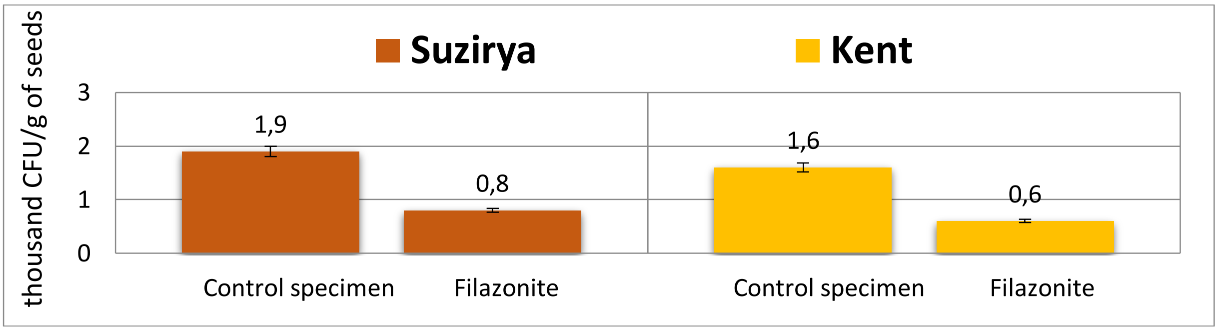


Fig. 3. The number of CFU/g seeds (in thousands) of the soybean cultivars *Suzirya* and *Kent* cultivated by using the technology called "*Filazonit Ukraine*" (2020).

As in the previous year, the micromycetes CFU formation rate in the seeds of both varieties cultivated by "*Filazonit*" technology was almost 2.5 times lower than the rate in the control specimen. Therefore, this shows some significant inhibition on micromycetes CFU and some inhibitory effects of the biopreparation Filazonit. We determined that the CFU number of micromycetes in the soybean cultivar *Suzirya* ranged from 0.4 to 1.3 CFU/g of seeds (in thousands), and in the soybean cultivars *Kent* this number ranged from 0.2 to 1.5 CFU/g of seeds (in thousands) (Table 1).

Table 1. The dynamics of micromycetes number in soybean seeds

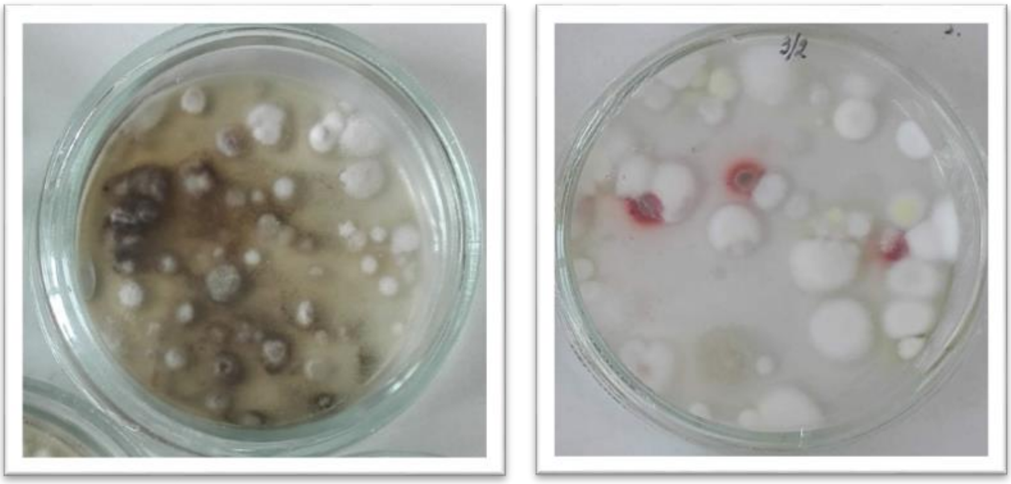
Variety	Biopreparation	The number of CFU micromycetes in ripening phase, thousand			Average
		2018 (HTC=1.35)	2019 (HTC=0.9)	2020 (HTC=1)	
Suzirya	Control specimen	2.3	0.8	1.9	1.6
	“Filazonit”	1.3	0.4	0.8	0.8
HIP_{05}	Year effect				0.32
	Technology effect				0.24
Kent	Control specimen	2.7	0.7	1.6	1.6
	“Filazonit”	1.5	0.2	0.6	0.7
HIP_{05}	Year effect				0.29
	Technology effect				0.24

The difference is significant at $p = 0.05$

The biopreparation "Filazonit" effectiveness for the two soybean varieties was significantly compared to the one for the control specimen. We observed a specific relationship between the development of micromycetes and the biopreparation "*Filazonit*" in the cultivars *Suzirya* and *Kent* (H_{05} = 0.24 and H_{05} = 0.3). Considering the hydrothermal coefficient (HC) observed over the years, the CFU numbers of micromycetes in soybean seeds were also significant in the cultivars *Suzirya* and *Kent*, and they totaled H_{05} = 0.32 and H_{05} = 0.29, respectively. This shows that the value of HC influenced the numbers of micromycetes in soybean seed cultivars Kent and Suzirya. The most significant effect of biopreparation on the soybean cultivars *Suzirya* and *Kent* was detected in 2019, when the number of CFU was 0.2 thousand CFU/g and 0.4 thousand CFU/g of seeds, respectively. That pointed at some significant inhibition of the phytopathogenic micromycetes through the effects of the biopreparation "*Filazonit*". It also can be connected to certain weather conditions. In the second year of the research, the hydrothermal coefficient was 0.9 (weak dryness), which could inhibit the growth of the phytopathogenic micromycetes. The weakest effects of the biopreparation "*Filazonit*" on the soybean cultivars *Suzirya* and *Kent* were observed in 2018, and they came to 1.3 thousand CFU/g and 1.5 thousand CFU/g of seeds, respectively. In that year of the research, the hydrothermal coefficient was 1.35 (quite wet). Excessive moisture is assumed to be unfavorable for developing the phytopathogenic micromycetes (Vozhehova, 2016; Bulyhin, 2014).

At the same time, "*Filazonit*" technology has significantly inhibited the formation and development of micromycetes in both soybean seed cultivars compared to the control specimen indexes. Our research has shown that forming colony-forming units in the soybean varieties *Suzirya* and *Kent* are influenced by the genotype of the variety and cultivation technologies and moisture supply of soybean plants. Depending on these factors, the CFU number of micromycetes in soybean seeds in both varieties ranged from 0.2 to 1.5 thousand CFU/g of seeds. That was significantly less than in the control specimen.

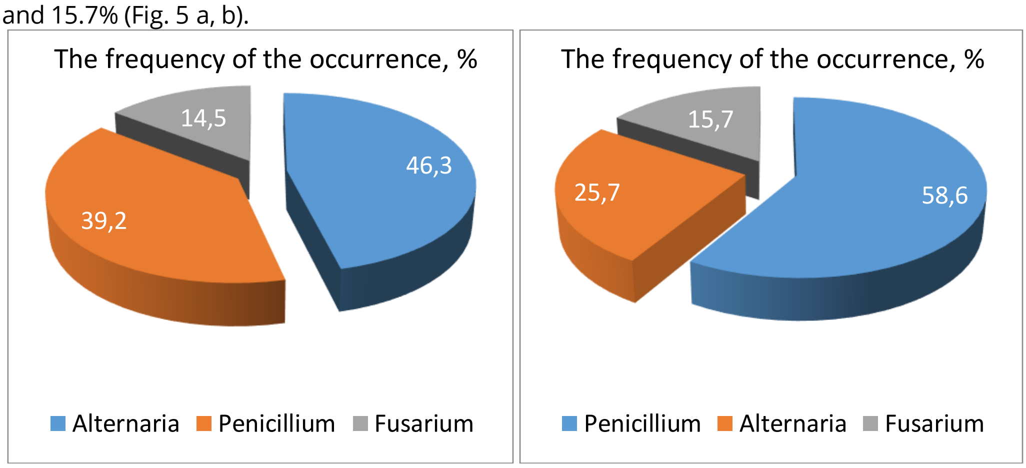
Several phytopathogenic species of micromycetes were isolated and identified in soybean seed cultivars *Suzirya* and *Kent* (Fig. 4).



a b

Fig. 4. The isolates were taken from soybean seed varieties *Suzirya* (a – *Alternaria*) and *Kent* (b – *Penicillium*), five days after emerging.

The analysis showed that the seeds of the two soybean varieties are dominated by species belonging to three genera of micromycetes: *Alternaria*, *Fusarium*, *Penicillium*. In the soybean seed cultivar *Suzirya*, the micromycetes of the genus *Alternaria* are the most common (46.3%). The percentage of micromycetes of the genus *Penicillium* is 39.2%. The genus *Fusarium* can be characterized by the lowest frequency of micromycetes (14.5%). In the Kent cultivar, the micromycetes of the genus *Penicillium* dominated (58.6%), the dominances of the micromycetes of the genera *Alternaria* and *Fusarium* were smaller and came to 25.7% and 15.7% (Fig. 5 a, b).



a b

Fig. 5. The frequency of the occurrence of the isolates taken from soybean seed cultivars *Suzirya* (a) and *Kent* (b) in the laboratory (2018–2020).

Thus, we found that both the soybean seed cultivars *Suzirya* and *Kent* are dominated by phytopathogenic species of micromycetes belonging to the genera *Alternaria, Fusarium, Penicillium*. They are the factors of biological contamination of agrophytocenoses and product biosafety reduction. Therefore, some changes were determined in the quality indicators of the research soybean varieties, which were grown under organic production conditions using biological technologies. According to 2018–2020, we found that the protein and oil content indicators in the soybean seed cultivars *Kent* and *Suzirya* in all variants exceeded the standard indicators (protein norm – 35%; oils – 12%) suggested in DSTU 4964: 2008. TU. SOY. At the same time, the indicators of the mass fraction of seed moisture did not exceed the permissible norms (Table 2).

Table 2. The quality indicators of the soybean seed cultivars *Kent* and *Suzirya* within the research period

Variant	Year	The quality indicators of soybean seed, %					
		Proteins		Oil		Moisture	
		Suzirya	Kent	Suzirya	Kent	Suzirya	Kent
Control specimen (without processing)	2018	37.11	38.5	19.3	19.2	10.5	11.2
	2019	39.07	40.23	20.5	19.53	8.8	9.7
	2020	38.8	39	19.9	18.7	9.8	11.4
"Filazonite Ukraine"	2018	37.5	38.3	19.4	19.2	10.2	11.4
	2019	39.21	41.11	21.23	21.7	8.8	9.6
	2020	38.8	38.7	19.02	19.1	9.9	10.3
Norm		35		12		12	

Thus, the protein content in the soybean seed cultivar *Suzirya* grown by using the technology "*Filazonite*" ranged from 37.5% to 39.21%, and in the seed soybean variety *Kent* it ranged from 38.3% to 41.11% and were within the norms of the control version. The soybean seed cultivar *Kent* was characterized by a higher protein content of 1.9% than the soybean variety *Suzirya*.

The lowest protein indicators in the soybean seed cultivar *Suzirya* (37.5%) and the soybean seed cultivar *Kent* (38.3%) were observed during the 2018 research. That can be caused by the remarkable connections between the soil types and moisture supply of soybean plants. Similarly, in the research of the same period, the hydrothermal coefficient was 1.35 (relatively high moist). As is known, excessive moisture causes a reduction in the protein content in soybean seeds. The soybean seed cultivar Suzirya cultivated the oil content by using different technologies ranging from 19.02% to 21.23%, and in the soybean seed cultivar Kent, the same index ranged from 19.1% to 21.7%, both found themselves within the control variant. In the soybean seed cultivar *Kent*, the oil indicator is 0.47% higher than the soybean seed cultivar *Suzirya*.

We registered the highest protein and oil content in the soybean seeds of cultivar *Kent* (41.11% and 39.21%, respectively). This was caused by the genotype variety and the influence of "*Filazonit*" biopreparation. The indicators of the moisture mass fraction in soybean seeds did not exceed the permissible norms (12%). The lowest moisture indicator was discovered in the soybean seed cultivar *Suzirya* (8.8%), and the highest indicator was discovered in the soybean seed cultivar *Kent* (11.4%). Our research has shown that the soybean seeds' biochemical composition is influenced by their genotype variety and the cultivation technology and moisture supply of soybean plants. Depending on these factors, the seed protein content was 37.5-41.11%, the fat content – 19.02-21.7%, and the moisture mass fraction ranged from 8.8% to 11.4%.

Conclusions

We found that the soybean seed cultivars named *Suzirya* and *Kent* are dominated by phytopathogenic micromycetes, which belong to the genus types *Alternaria, Fusarium, Penicillium*. They are the factors of biological contamination of agrophytocenoses and the reduction of biosafety of the products.

We determined that the biochemical composition of the researched soybean seeds is influenced both by the genotype of the cultivars and the cultivation technology and moisture supply of soybean plants. The biological product *Filozanit* significantly inhibited the formation and growth of micromycetes in the seeds of both cultivars of soybean compared to the control specimen of the research. Thus, we experimentally proved the physical possibility of regulating the number of phytopathogenic micromycetes in soybean seeds by the biological product Filozanit, which can increase biosafety in soybean agrocenoses and improve their quality.

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Citation:

Parfenuk, A.I., Havryliuk, L.V., Beznosko, I.V., Pasichnik, L.P., Turovnik, Y.A., Ternovyi, Y.V. (2021). Influence of Filazonit biopreparation on soybean seed quality. *Ukrainian Journal of Ecology, 11* (3), 86-89.