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Identification of Mycobiota and Diagnosis of Soybean Seed Diseases

Abstract. Micromycetes that colonise soybean seeds reduce their sowing quality, causing loss of germination, rotting and death of seedlings, and the appearance of diseases on young plants. Infection-free seed is one of the factors that ensure crop growth. Therefore, the study of mycobiota of seeds is relevant for the implementation of preventive disease control measures. The purpose of this study was to identify phytopathogenic fungi that colonise soybean seeds and to establish their species composition. The seed samples were selected in the conditions of the Ukrainian Scientific Plant Quarantine Research Station (Chernivtsi Oblast) of the Institute of Plant Protection of the National Academy of Agrarian Sciences during 2019-2021. Mycological studies of soybean seeds were performed in the scientific laboratory of the Department of Phytopathology of the National University of Life and Environmental Sciences of Ukraine using the biological method from subsequent morphological and microscopic analysis of pathogens. It was established that the species composition of mycobiota of soybean seeds is diverse and is represented by 17 species: *Reronospora manshurica* (Naum.) Syd. (downy mildew pathogen), *Sclerotinia sclerotiorum* de Bary (white mold pathogen), *Botryotinia fuckeliana* Whetzel. (grey mold pathogen), *Diaporthe sp.* (Phomopsis pathogen); pathogens of fusariosis are *Fusarium okhusporum* (Schl.), Snyd. et Hans., *Fusarium graminearum* Schwabe, *Fusarium gibbosum* App. et Wr., *Fusarium solani* (Mart.) Sacc.; *Alternaria alternata* (Fries: Fries) Keissler (Alternaria pathogen), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (cladosporiosis pathogen), *Colletotrichum sp.* (anthracnose pathogen); pathogens of various moulds – *Aspergillus niger* van Tiegh., *Aspergillus flavus* Link, *Trichothecium roseum* Link ex Friet Fries, *Mucor mucedo* Fres. amend. Bref., *Penicillium expansum* Link and *Rhizopus nigricans* Ehrenb. The dominant species were *C. cladosporioides* and *A. alternata*, their frequency of occurrence was in the range of 28.5-47.8%. The obtained data on the symptoms of mycosis of soybean seeds, the species composition of pathogens and their frequency of occurrence are of practical importance for the diagnosis of diseases, the identification of pathogens and the implementation of preventive therapeutic control measures

Keywords: *Glycine max*, micromycetes, endophytic infection, mould, seed germination, types of pathogens

INTRODUCTION

Healthy seeds are one of the factors that ensure the growth of crop yields. At the same time, seed quality is strongly influenced by microbial communities, which consist of endophytes and epiphytes and take part in germination, growth, and development of plants and seed storage [1]. Seed-associated microbial populations consist of 9,000 different synergistic, commensal, and potential pathogenic microbial species [1; 2]. In general, over 80% of plant diseases are caused by fungi or fungi-like pathogens that threaten food safety [3].

Legume seeds are a favourable substrate for damage by various pathogens [4]. Soybean (*Glycine max* L.) is infected with more than 200 types of phytopathogenic microorganisms, among which fungi are the most dangerous [5]. At the same time, about 30 types of pathogens can cause considerable economic losses [6]. Phytopathogenic fungi that colonise soybean seeds reduce its seeding quality, causing loss of germination, rotting and death of seedlings, disease manifestations on young plants [7; 8]. Infected seeds contribute to the spread of pathogens locally and over long distances [9]. Some fungal pathogens that colonise soybean seeds can produce mycotoxins [10; 11], dangerous for humans and animals.

In general, pathogens affecting soybean seeds, in addition to reducing germination and yield, also cause

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biochemical changes in the seed. Thus, the fungi *Phomopsis longicolla* and *Cercospora kikuchii* affect the quality of soybean seeds, reducing the content of isoflavone, protein, oil, and fatty acids, with malonligenistin showing the greatest reduction [12]. Therefore, the study of the phytopathological state of the seeds, the identification of pathogens is relevant for the implementation of preventive disease control measures. The latter require information about the quality of the seed, its pathological condition since the seed can be a carrier of plant diseases [13; 14].

In various countries of the world, research is being conducted on the species composition of fungi that affect soybean seeds and reduce their quality. Phytopathogenic species of fungi of the *Diaporthe/Phomopsis* complex, which cause seed rot, pod, and stem rot on soybeans, are harmful in European countries and are represented by the species *D. longicolla*, *D. caulivora*, *D. eres*, and *D. novem* [15]. The analysis of the phytopathological state of soybean seeds in the conditions of Serbia confirmed its infection with the fungi *Peronospora manshurica*, *Macrophomina phaseolina*, *Botrytis cinerea*, *Cercospora kikuchii*, as well as species from the genera *Alternaria*, *Diaporthe*, and *Fusarium*. The dominant species of fungi were *Alternaria* and *Peronospora manshurica* [16]. The identification of fungi affecting soybean seeds grown in the Mediterranean region of Turkey confirmed its infection with four species, namely: *Aspergillus spp*., *Penicillium spp*., *Cladosporium spp*., and *Fusarium spp*. [17].

The fungus *Diaporthe spp.* causes seed decay and deterioration of its quality. In wet production areas of the United States of America, up to 10 species have been extracted from soybean seed. Based on analysis of their morphology, associated species included *D. aspalathi*, *D. caulivora*, *D. kongii*, *D. longicolla*, *D. sojae*, *D. ueckerae*, *D. unshiuensis,* and three new fungi *D. bacilloides*, *D. flavescens,* and *D. insulistroma* [18]. Seven species and six genera were identified from 55 fungal isolates obtained from soybean seeds (USA – southern Virginia, eastern Virginia, and north-eastern North Carolina). Fungi of the genera *Alternaria*, *Diaphorte*, and *Fusarium* were the most common [19]. Soybean anthracnose (pathogen – fungus *Colletotrichum truncatum*) is a dangerous disease in Brazil [20-22]. Potential yield losses from the disease can be up to 50% [22].

Fungi belonging to 13 genera were extracted from soybean seeds grown in Southwest China, among which *Fusarium* (*F. fujikuroi*, *F. proliferatum*, *F. verticillioides*, *F. asiaticum*, and *F. incarnatum*) accounted for 55.0%, *Colletotrichum spp*. – 23.0% [23]. In the conditions of Indonesia, the fungi *Aspergillus flavus* Link, *Aspergillus niger* van Tieghem, and *Cladosporium oxysporum* Berk & M.A. Curtis, *Colletotrichum dematium* (Pers. et Fr.) Grove f. sp. truncata (Schw.) Arx, *Curvularia pallescens* Boedijn, *Fusarium solani* (Mart.) Sacc., *Melanospora zamiae* Corda and *Nigrospora sp.* were extracted from the seeds of various soybean varieties [24].

Scientific literature has reports and experimental confirmations about the possibility of spreading the fungus *Corynespora cassiicola* (Berk. & Curt.) with soybean seeds [25]. It was also established that with an increase in the duration of soybean seed storage (from 0 to 8 months), a change in fungal populations occurs, namely an increase in the incidence of *Aspergillus spp*. from 1.3% to 7.5% and *Penicillium spp*. from 0% to 17.5%, while the infestation of *Phomopsis spp*. decreases from 7.5% to 0-0.3% [26].

In the conditions of Ukraine, studies were previously conducted that confirm the infection of soybean seeds by various microorganisms of fungal and bacterial aetiology [27; 28]. At the same time, this information is insufficient for a comprehensive understanding of the entire spectrum of micromycetes that can be found in infected seeds.

The purpose of this study was to detect and identify micromycetes that infect soybean seeds, to establish the frequency of their occurrence and to investigate the symptoms of pathologies.

MATERIALS AND METHODS

Field research was performed in the conditions of the Ukrainian Research Station of Plant Quarantine, the Institute of Plant Protection of the National Academy of Agrarian Sciences during 2019-2021. For analysis, soybean seeds of the Xenia variety were selected, and an average sample was formed. For further research, the seed material was stored in a paper bag in laboratory conditions at 18-20°C.

Phytopathological analysis of soybean seeds was performed in the scientific laboratory of the Department of Phytopathology of the National University of Life and Natural Sciences of Ukraine according to generally accepted methods [29]. At the bottom of a Petri dish with a diameter of 90 m, three layers of filter paper were placed, which were moistened with distilled water (excess water was drained). Petri dishes, filter paper, and water were sterile. Ten seeds were spread evenly (at 2 cm) on the surface of the filter paper. The total number of seeds under examination was 400 pieces. Next, the prepared seeds were incubated at 25±2°C for 7 days with a 12-hour alternation of light and darkness. After the end of the incubation period, the seeds were examined using a stereomicroscope (SIGETA MS-217 LED 20x-40x Bino Stereo) to identify the morphological structures of the fungi. Further work lied in making temporary microscopic preparations and using a monochrome microscope (Sigeta MB-103 40x-1600x LED Mono) to analyse mycelial morphology and sporulation. Soybean seeds affected by downy mildew were detected by macroscopic examination.

To detect internal infection, surface sterilisation of soybean seeds [30] and its incubation under the conditions given above were performed. To investigate the symptoms of diseases, in the pre-harvest period, soybeans with signs of pathologies were selected and diagnosed.

Micromycetes were also extracted from pure cultures and cultivated on potato-glucose agar [30; 31] in Petri dishes for 7 days at 22±2°C, which allowed evaluating the cultural and morphological characteristics of mushrooms. To identify pathogens, special literature was used [32; 33], where the species composition of soybean diseases in different regions of the world is covered, their symptoms on the vegetative and generative organs of plants are described, and attention is paid to the biological and morphological features of pathogens. Frequency of occurrence [30; 31] of micromycetes species were calculated according to formula (1):

$$Foc = \frac{n \times 100}{N} \quad (1)$$

where *Foc* is the frequency of occurrence, %; *n* is the number of seeds in which this species was found, pcs. *N* is the total number of examined seeds, pcs.;

RESULTS AND DISCUSSION

Three-year studies of the structure of micromycetes that colonise soybean seeds proved that their species composition is diverse and is represented by 17 species (Fig. 1): *Peronospora manshurica* (Naum.) Syd. (downy mildew pathogen), *Sclerotinia sclerotiorum* de Bary (white mold pathogen), *Botryotinia fuckeliana* Whetzel. (grey mold pathogen), *Diaporthe sp.* (Phomopsis pathogen); pathogens of fusariosis are *Fusarium okhusporum* (Schl.), Snyd. et Hans., *Fusarium graminearum* Schwabe, *Fusarium gibbosum* App. et Wr., *Fusarium solani* (Mart.) Sacc.; *Alternaria alternata* (Fries: Fries) Keissler (Alternaria pathogen), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (cladosporiosis pathogen), *Colletotrichum sp.* (anthracnose pathogen); pathogens of various molds – *Aspergillus niger* van Tiegh., *Aspergillus flavus* Link, *Trichothecium roseum* Link ex Friet Fries, *Mucor mucedo* Fres. amend. Bref., *Penicillium expansum* Link and *Rhizopus nigricans* Ehrenb.

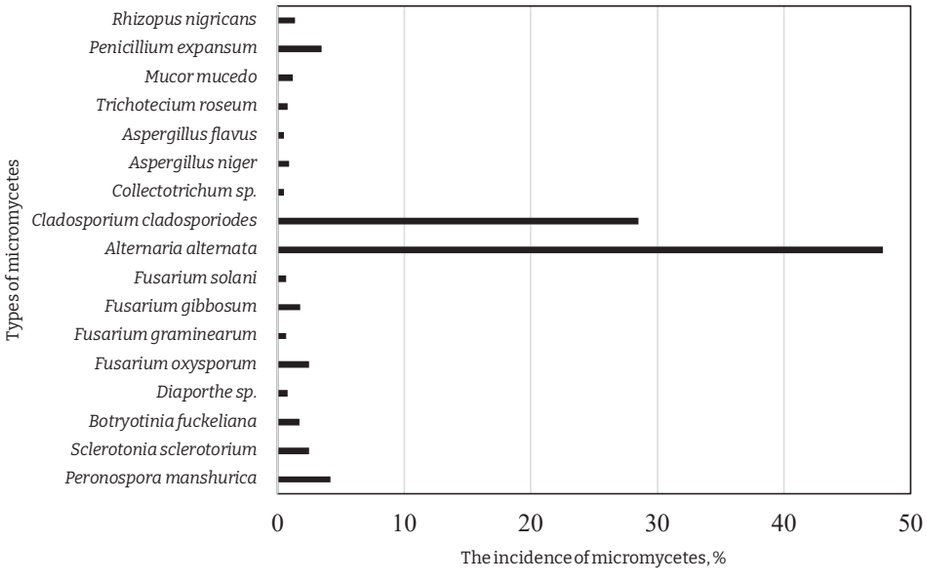

Figure 1. Frequency of occurrence of micromycetes affecting soybean seeds (epiphytic and endophytic mycoflora)

The frequency of the identified representatives of the mycobiota of soybean seeds was as follows: *P. manshurica* (4.2%), *S. sclerotiorum* (2.5%), *B. fuckeliana* (1.7%), *Diaporthe sp.* (0.8%), *F. oxysporum* (2.5%), *F. graminearum* (0.7%), *F. gibbosum* (1.8%), *F. solani* (0.7%), *A. alternata* (47.8%), *C. cladosporioides* (28.5%), *Colletotrichum sp.* (0.5%), *A. niger* (0.9%), *A. flavus* (0.5%), *T. roseum* (0.8%), *M. mucedo* (1.2%), *P. expansum* (3.5%), *R. nigricans* (1.4%).

Analysis of the distribution of micromycetes shows that the species *C. cladosporioides* and *A. alternata* were dominant. The frequency of their occurrence was within 28.5-47.8%. Pathogens of fusarium wilt were found in 0.7-2.5% of seeds. At the same time, the most common species was *F. oxysporum*. Among other phytopathogenic fungi (*Colletotrichum sp.*, *Diaporthe sp.*, *B. fuckeliana*, *S. sclerotiorum*, *P. manshurica*), which colonise seeds during plant vegetation, their frequency of occurrence was within 0.5-4.2%. Representatives of saprotrophic mycoflora, which cause mould of seeds, had a prevalence ranging from 0.5% to 3.5%. The micromycete *P. expansum* was detected most often.

According to the results of mycological examination, the endophytic mycoflora of *S. sclerotiorum* and *B. fuckeliana* fungi was 95.3% and 92.5%, respectively (Fig. 2). Seeds infected with these fungi lost germination or formed sprouts that rotted (Fig. 3a, b). At the same time, the fungi *A. alternata* and *C. cladosporioides*, which prevailed in the general mycoflora of soybean seeds, were detected only in 4.5 and 2.7% during the analysis of internal infection (Fig. 2). This indicates their localisation in the seed coat. Such seeds created healthy seedlings (Fig. 3c). The internal infection of soybean seeds by the micromycete *P. expansum* was 0.5%.

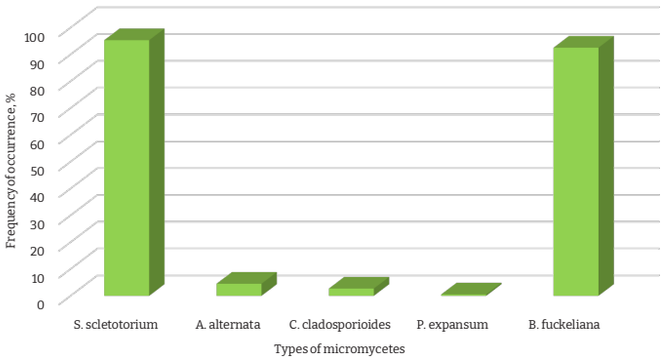

Figure 2. Frequency of occurrence of certain types of pathogens of soybean seeds (endophytic infection)

Figure 3. Soybean seed infected with micromycetes during germination: a – *S. sclerotiorum*, b – *B. fuckeliana* (healthy – left and affected – right), c – *A. Alternata*

As a result of visual diagnosis of affected soybeans with typical disease symptoms, macro- and microscopic analysis of the morphological structures of pathogens, clear pathological changes in the affected seed can be observed. For instance, seeds affected by the fungus *P. manshurica* are covered with a creamy shell of oospores of the fungus, become matte, whitish, and lose their lustre (Fig. 4a). In case of infection of soybeans with *S. sclerotiorum*, the seeds in them become discoloured, lose their lustre, and become thin. In the interseed space, sclerotia can form, which adhere to the seeds (Fig. 4b). Mushrooms *B. fuckeliana* and *Diaporthe sp*. cause the wrinkling of the seeds, the destruction of the skin, putrefaction, and fragility (Fig. 4c, d). Grain affected by *Fusarium spp*. is thin, wrinkled. During germination, it is covered with mycelium (Fig. 4e) and becomes mouldy. The micromycetes *A. alternata* and *C. cladosporioides* cause the darkening of the seed coat, and sometimes fragility. During germination, heavily infected seeds become mouldy (4f, g). Seeds infected with *Colletotrichum sp*. are covered with brown spots, thin and underdeveloped. These symptoms of fungal seed diseases were observed in the case of a strong degree of disease progression on soybeans. Infection of soybean seeds with fungi *A. niger*, *A. flavus*, *T. roseum*, *M. mucedo* Fres., *P. expansum* (Fig. 4h), and *R. nigricans* caused its mould and loss of germination.

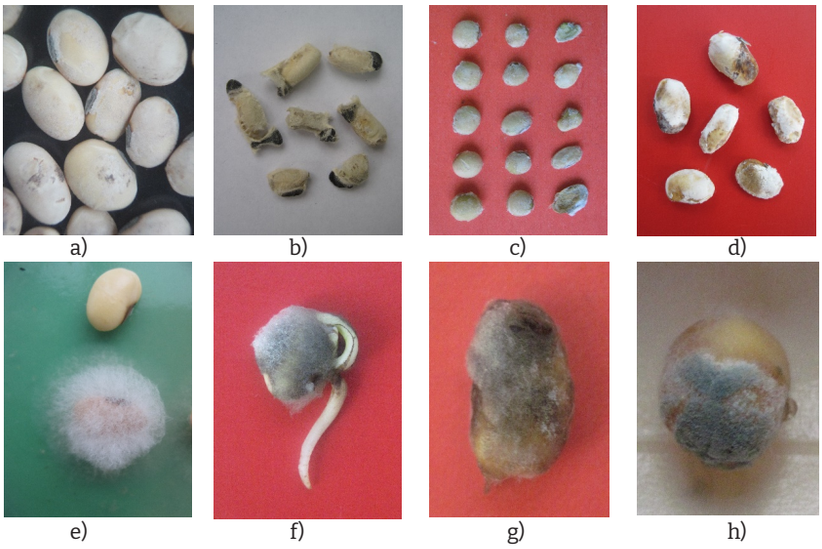

Figure 4. General appearance of soybean seeds severely affected by micromycetes: a – *Peronospora manshurica*, b – *Sclerotinia sclerotiorum*, c – *Botryotinia fuckeliana*, d – *Diaporthe sp*., e – *Fusarium sp*., f – *Alternaria alternata*, g – *Cladosporium cladosporioides*, h – *Penicillium expansum*

However, in all cases, for correct diagnosis of seed pathologies and identification of pathogens, it is necessary to use a biological method and microscopic analysis of pathogens, since pathogens were in the seeds in a latent state. The use of molecular biology methods is also important for the reliable identification of many types of pathogens [15; 34; 35].

Analysis of studies on the structure of populations of micromycetes affecting soybean seeds in different regions of the world show a wide variation in the species composition of pathogens and their frequency of occurrence. Thus, in the Poltava Oblast (Ukraine), damage to seeds belonging to 8 genera was detected: *Alternaria*, *Aspegillus*, *Botrytis*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, and *Peronospora*. The most widespread were *Alternaria sp.* – 26-35% and *Mucor sp.* – 22-47% [28]. In Serbia, the following pathogens of mycoses dominated: *Alternaria sp.* – 28.9%, *P. manshurica* – 44%, and *Fusarium sp.* – 10.3% [16]. In southwestern China, the population of fungi that spread with soybean seeds is represented by *Fusarium sp.*, *Colletotrichum sp.*, *Alternaria* sp., *Corynespora sp.*, *Diaporthe/Phomopsis sp.*, *Stagonosporopsis sp.*, *Chaetomium sp.*, *Podospora sp.*, *Botryosphaeria sp.*, *Thielavia sp.*, *Macrophomina sp.*, *Harzianum sp.*, and *Didymella sp.* Isolates of the genus *Fusarium* dominated, the frequency of which was 55%, and *Colletotrichum sp.* – 22.3% [23]. In Brazilian conditions, among the identified pathogens of soybean seeds, 6 types of fungi were common: *Fusarium sp.*, *Phomopsis sp.*, *Cercospora sp.*, *Colletotrichum sp.*, *Rhizoctonia sp.*, and *Penicillium sp.* [37]. In Ethiopia, damage to soybean seeds by fungi *Fusarium sp.*, *A. niger*, *A. flavus*, *Penicillium sp.*, and *Rhizopus sp.* was detected. The species *A. flavus* (29.18%) and *A. niger* (23.81%) predominated [38]. A study of seed-associated mycoflora in soybean under Indian conditions identified six fungal species, including *A. alternata*, *A. flavus*, *A. niger*, *F. verticillioides*, *Macrophomina phaseolina* and *Rhizopus stolonifer*. The most frequent species were *F. verticillioides* – 19.25%, *M. phaseolina* – 15.00% and *A. alternata* – 13.50% [39].

Research conducted in the conditions of Ukraine (Chernivtsi Oblast) allowed detecting and identifying 17 types of fungi that attack soybean seeds. Therewith, the dominant position was occupied by *C. cladosporioides* and *A. alternata*. The last species also prevailed or was among the main representatives of the mycoflora of seeds in the studies of scientists from different countries [16; 28; 39]. The significant distribution of *A. alternata* is confirmed by its cosmopolitanism [40]. Seed damage by fungi *S. sclerotiorum*, *Colletotrichum sp.*, and *Diaporthe sp.* was also identified, which are distributed in soybean cultivation regions [23; 37], but there is no information about them in Ukraine [28; 36].

CONCLUSIONS

Establishing the species composition of soybean seed pathogens is important for understanding the epidemiology of plant pathologies and developing effective measures to control them. As a result of the research, 17 types of micromycetes that colonise soybean seeds were identified: *Peronospora manshurica* (Naum.) Syd., *Sclerotinia sclerotiorum* de Bary, *Botryotinia fuckeliana* Whetzel., *Diaporthe sp.*, *Fusarium oxysporum* (Schl.), Snyd. et Hans., *Fusarium graminearum* Schwabe, *Fusarium gibbosum* App. et Wr., *Fusarium solani* (Mart.) Sacc., *Alternaria alternata* (Fries: Fries) Keissler, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Colletotrichum sp.*, *Aspergillus* niger van Tiegh., *Aspergillus flavus* Link, *Trichothecium roseum* Link ex Friet Fries, *Mucor mucedo* Fres. amend. Bref., *Penicillium expansum* Link, and *Rhizopus nigricans* Ehrenb. The dominant species were *C. cladosporioides* and *A. alternata*. At the same time, they were localised in the seed coat. Endophytic micromycetes *S. sclerotiorum*, and *B. fuckeliana* caused loss of seed germination or seedling death.

Phytopathogenic fungi *S. sclerotiorum*, *Colletotrichum sp.*, and *Diaporthe sp.* identified among the mycobiota complex of soybean seeds indicate the need for a more complex study of this issue in different soil and ecological conditions of Ukraine because these pathogens during the last decade have not been covered in the studies of Ukrainian scientists. Further research must establish the role of soybean varieties, growing region and meteorological conditions on the structure of the phytopathogenic complex of seed micromycetes.

REFERENCES

[1] Berg, G., & Raaijmakers, J.M. (2018). Saving seed microbiomes. *The ISME Journal*, 12(5), 1167-1170. doi: 1038/s41396-017-0028-2.

[2] Mekonnen, G. (2020). Review on: Impact of seed-borne pathogens on seed quality. *American Journal of Plant Biology*, 5(4), 77-81. doi: 10.11648/j.ajpb.20200504.11.

[3] Agrios, G.N. (2005). *Plant pathology* (5th ed.) London: Elsevier Academic Press.

[4] Kyryk, M.M., Pikovskyi, M.Y., & Azaiki, S. (2013). *Gray mold of plants, biological and ecological properties of its agents (Botrytis cinerea Pers.)*. Kyiv: Phoenix.

[5] Hartman, G.L., Rupe, J.C., Sikora, E.J., Domier, L.L., Davies, J.A., & Steffey, K.L. (2015). Infectious diseases. In *Compendium of soybean diseases and pests* (5th ed.), (pp. 17-136). Saint Paul: The American Phytopathological Society.

[6] Roy, K.W., Baird, R.E., & Abney, T.S. (2000). A review of soybean (Glycine max) seed, pod, and flower mycofloras in North America, with methods and a key for identification of selected fungi. *Mycopathologia*, 150(1), 15-27. doi: 10.1023/a:1010805224993.

[7] Kyryk, M.M., Pikovskyi, M.Y., & Azaiki S. (2015). *Diseases of seeds of agricultural crops*. Kyiv: Komprynt.
[8] Solomiychuk, M.P., & Pikovskyi, M.Y. (2021). The influence of *Pseudomonas fluorescens* bacteria and substances of stimulating nature on the productivity of the soybean plant and the damage of seeds by pathogens. *Plant and Soil Science*, 12(4), 28-36. doi: 10.31548/agr2021.04.028.
[9] Agarwal, V.K., & Sinclair, J.B. (1996). *Principles of seed pathology*. Boca Raton: CRC Press.
[10] Garcia, D., Barros, G., Chulze, S., Ramos, A.J., Sanchis, V., & Marín, S. (2012). Impact of cycling temperatures on *Fusarium verticillioides* and *Fusarium graminearum* growth and mycotoxins production in soybean. *Journal of the Science of Food and Agriculture*, 92(15), 2952-2959. doi: 10.1002/jsfa.5707.
[11] Egbuta, M., Mwanza, M., Phoku, J., Chilaka, C., & Dutton, M. (2016). Comparative analysis of mycotoxigenic fungi and mycotoxins contaminating soya bean seeds and processed soya bean from nigerian markets. *Advances in Microbiology*, 6(14), 1130-1139. doi: 10.4236/aim.2016.614102.
[12] Lee, J.H., Hwang, S.R., Lee, Y.H., Kim, K., Cho, K.M., & Lee, Y.B. (2015). Changes occurring in compositions and antioxidant properties of healthy soybean seeds [*Glycine max* (L.) Merr.] and soybean seeds diseased by *Phomopsis longicolla* and *Cercospora kikuchii* fungal pathogens. *Food Hemistry*, 185, 205-211. doi: 10.1016/j.foodchem.2015.03.139.
[13] Dutta, B., Gitaitis, R., Smith, S., & Langston, Jr.D. (2014). Interactions of seedborne bacterial pathogens with host and non-host plants in relation to seed infestation and seedling transmission. *PLoS One*, 9(6), article number 99215. doi: 10.1371/journal.pone.0099215.
[14] Sharma, K.K., Singh, U.S., Sharma, P., Kumar, A., & Sharma, L. (2015). Seed treatments for sustainable agriculture – A review. *Journal of Applied and Natural Science*, 7(1), 521-539. doi: 10.31018/jans.v7i1.641.
[15] Hosseini, B., El-Hasan, A., Link, T., & Voegele, R. (2020). Analysis of the species spectrum of the *Diaporthe/Phomopsis* complex in European soybean seeds. *Mycological Progress*, 19, 455-469. doi: 10.1007/s11557-020-01570-y.
[16] Krsmanović, S., Petrović, K., Ceran, M., Djordjevic, V., Randjelovic, P., Jaćimović, S., & Miladinov, Z. (2020). Diversity of phytopathogenic fungi on soybean seed in Serbia. *Field and Vegetable Crops Research*, 57(3), 80-86. doi: 10.5937/ratpov57-27516.
[17] Üstün, R., Çat, A., Çatal, M., & Uzun, B. (2021). Identification of seedborne fungi on soybean (*Glycine max* L.) seeds grown in mediterranean region of Turkey. *Turkish Journal of Agricultural Research*, 8(3), 367-373. doi: 10.19159/tutad.1014598.
[18] Petrović, K., Skaltsas, D., Castlebury, L.A., Kontz, B., Allen, T.W., Chilvers, M.I., Gregory, N., Kelly, H.M., Koehler, A.M., Kleczewski, N.M., Mueller, D.S., Price, P.P., Smith, D.L., & Mathew, F.M. (2021). Diaporthe seed decay of soybean [*Glycine max* (L.) Merr.] is endemic in the United States, but new fungi are involved. *Plant Disease*, 105(6), 1621-1629. doi: 10.1094/PDIS-03-20-0604-RE.
[19] Escamilla, D., Rosso, M., & Zhang, B. (2019). Identification of fungi associated with soybeans and effective seed disinfection treatments. *Food Science & Nutrition*, 7(10), 1-12. doi: 10.1002/fsn3.1166.
[20] Rogério, F., Ciampi-Guillardi, M., Barbieri, M.C., Bragança, C.A., Seixas, C.D., Almeida, A.M., & Massola, N.S. (2017). Phylogeny and variability of *Colletotrichum truncatum* associated with soybean anthracnose in Brazil. *Journal of Applied Microbiology*, 122(2), 402-415. doi: 10.1111/jam.13346.
[21] Rogério, F., Gladieux, P., Massola, N.S., & Ciampi-Guillardi, M. (2019). Multiple introductions without admixture of *Colletotrichum truncatum* associated with soybean anthracnose in Brazil. *Phytopathology*, 109(4), 681-689. doi: 10.1094/PHYTO-08-18-0321-R.
[22] Boufleur, T., Guillardi, M., Tikami, Í., Rogério, F., Thon, M., Sukno, S., Massola, N., & Baroncelli, R. (2021). Soybean anthracnose caused by *Colletotrichum* species: Current status and future prospects. *Molecular Plant Pathology*, 22(4), 393-409. doi: 10.1111/mpp.13036.
[23] Chang, X.L., Li, H., Naeem, M., Wu, X., Yong, T, Song, C., Liu, T.-G., Chen, W., & Yang, W. (2020). Diversity of the seedborne fungi and pathogenicity of *Fusarium* species associated with intercropped soybean. *Pathogens*, 9(7), article number 531. doi: 10.3390/pathogens9070531.
[24] Soesanto, L., Hartono, A., Mugiastuti, E., & Widarta, H. (2020). Seed-borne pathogenic fungi on some soybean varieties. *Biodiversitas Journal of Biological Diversity*, 21(9), 4010-4015. doi: 10.13057/biodiv/d210911.
[25] Goulart, A., & Utiamada, C. (2020). *Corynespora cassiicola* in soybean seeds – Incidence and transmission. *Bioscience Journal*, 36, 259-265. doi: 10.14393/BJ-v36n0a2020-45550.
[26] Carvalho, E.R., Reis, L.V., Rocha, D.K., Penido, A.C., Mavaieie, D.P., Valquíria, F.F., & Oliveira, J.A. (2021). Incidence of fungal species in stored soybean seeds in relation to cooling before packing and to packing material. *Journal of Agricultural Sciences*, 44(2-3), 193-202. doi: 10.19084/rca.24610.
[27] Pikovskyi, M.Y., & Kyryk, M.M. (2012). Symptoms of white mold of soybean. *Quarantine and Plant Protection*, 7, 2-5.
[28] Pospyelova, H.D. (2015). Species composition of phytopathogenic flora of soy bean seeds. *Bulletin of Poltava State Agrarian Academy*, 2, 44-48.
[29] ISTA: International rules for seed testing, international seed testing association. (1996). *Seed Science and Technology*, 24.

[30] Dhingra, O.B., & Sinclair, J.B. (1995). *Basic plant pathology methods* (2nd ed.). Boca Raton: CRC Press.
[31] Bilay, V.I. (1982). *Methods of experimental mycology*. Kyiv: Scientific Thought.
[32] Peresypkin, V.F., Kyryk, N.N., Lesovoi, M.P., Kovalenko, S.N., Koroleva, Y.B., Koshevskyi, Y.Y., Shelekhova, L.N., & Mynko, N.D. (1989). *Diseases of cereals and legumes* (Vol. 1). Kyiv: Harvest.
[33] Watanabe, T. (2002). *Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species* (2nd ed.). Boca Raton: CRC Press.
[34] Sousa, R., Arguelles, O.P., Osório, A., Nosé, N., Leda, A.G., Ferreira, T., Haesbaert, F., & Santos, G. (2022). Detection and transmission of *Fusarium verticillioides* in corn seeds according to the plant stage. *Acta Scientiarum. Agronomy*, 44, 3-11. doi: 10.4025/actasciagron.v44i1.53213.
[35] Šišić, A., Oberhänsli, T., Baćanović-Šišić, J., Hohmann, P., & Finckh, M. (2022). A novel real time pcr method for the detection and quantification of *Didymella pinodella* in symptomatic and asymptomatic plant hosts. *Journal of Fungi*, 8(1), 1-17. doi: 10.3390/jof8010041.
[36] Mikolayevskiy, V., Sergiyenko, V., & Marchenko, A. (2013). Microflora of seed of soy. *Agrobiology*, 10, 38-42.
[37] Cortina, J.V., Theodoro, G.F., & Walker, D.R. (2013). Identification of fungi on diseased soybean seeds harvested during a high rainfall period in Mato Grosso do Sul, Brazil. *Bioscience Journal*, 29(2), 386-391.
[38] Alemu, K. (2014). Seed borne fungal pathogen associated with soybean (*Glycine max* L.) and their management in Jimma, Southwestern Ethiopia. *Journal of Biology, Agriculture and Healthcare*, 4(25), 14-19.
[39] Dhawan, S.S., Magar, S.J., Rothe, A.S., Mulekar, V.G., & Jaiswal, K.L. (2019). Detection of seed mycoflora of soybean by seed health testing methods. *International Journal of Universal Science and Technology*, 5(1), 26-31.
[40] Thomma, B.P. (2003). *Alternaria* spp.: From general saprophyte to specific parasite. *Molecular Plant Pathology*, 4(4), 225-236. doi: 10.1046/j.1364-3703.2003.00173.x.

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Ідентифікація мікобіоти та діагностика хвороб насіння сої

Анотація. Мікроміцети, які колонізують насіння сої, знижують його посівні якості, викликаючи втрату схожості, загнивання і загибель проростків, проявлення хвороб на молодих рослинах. Вільне від інфекцій насіння є одним із чинників, які забезпечують зростання врожаю. Тому вивчення мікобіоти насіння є актуальним для проведення превентивних заходів контролю хвороб. Мета роботи – виявити фітопатогенні гриби, які колонізують насіння сої і встановити їхній видовий склад. Відбір зразків насіння проводили в умовах Української науково-дослідної станції карантину рослин (Чернівецька область) Інституту захисту рослин Національної академії аграрних наук протягом 2019–2021 рр. Мікологічні дослідження насіння сої здійснювали у науковій лабораторії кафедри фітопатології Національного університету біоресурсів і природокористування України використовуючи біологічний метод із наступним морфологічним і мікроскопічним аналізом патогенів. Встановлено, що видовий склад мікобіоти насіння сої є різноманітним і представлений 17 видами: *Peronospora manshurica* (Naum.) Syd. (збудник пероноспорозу), *Sclerotinia sclerotiorum* de Bary (зб. білої гнилі), *Botryotinia fuckeliana* Whetzel. (зб. сірої гнилі), *Diaporthe sp.* (зб. фомопсису); збудниками фузаріозу – *Fusarium* oxy*sporum* (Schl.), Snyd. et Hans., *Fusarium graminearum* Schwabe, *Fusarium gibbosum* App. et Wr., *Fusarium solani* (Mart.) Sacc.; *Alternaria alternata* (Fries: Fries) Keissler (зб. альтернаріозу), *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (зб. кладоспоріозу), *Colletotrichum sp.* (зб. антракнозу); збудниками різних пліснявінь – *Aspergillus niger* van Tiegh., *Aspergillus flavus* Link, *Trichothecium roseum* Link ex Friet Fries, *Mucor mucedo* Fres. emend. Bref., *Penicillium expansum* Link і *Rhizopus nigricans* Ehrenb. Домінуюче становище мали види *C. cladosporioides* та *A. alternata., частота їх трапляння була в діапазоні* 28,5–47,8 %. Одержані дані про симптоматику мікозів насіння сої, видовий склад патогенів і частоту їх трапляння, мають практичне значення для діагностики хвороб, ідентифікації збудників і проведення профілактичних терапевничних заходів контролю

Ключові слова: *Glycine max*, мікроміцети, ендофітна інфекція, пліснявіння, проростання насіння, види патогенів