ENGINEERING AND TECHNOLOGICAL JUSTIFICATION FOR THE BIOTECHNOLOGICAL PRODUCTION OF A MYCOBIOLOGICAL PREPARATION

Viktor Tesliuk[1], Yulia Kolomiyets[1], Viktor Baranovsky[2], Raisa Kovbasenko[3]
[1]National University of Life and Environmental Sciences of Ukraine, Ukraine;
[2]Ternopil Ivan Puluj National Technical University, Ukraine;
[3]Institute of Cell Biology and Genetic Engineering, Ukraine
vtesluk@ukr.net, julyja12345@gmail.com, baranovskyvm@ukr.net, rayasenko@ukr.net

Abstract. Addressing scientific and practical challenges related to increasing crop yields and quality is critical to ensuring food security and meeting the ever-growing needs of the population amid limited land resources. An analysis of global bioengineering research indicates that scientific and practical solutions are focused on the development and use of chemical-based products that are highly effective yet harmful to biota, while bioengineering efforts to create biologically derived products are significantly limited. The development and creation of new, effective and environmentally safe biotechnologies to protect cultivated plants from disease is a priority and a pressing issue. It has been noted that the polysaccharides chitin, chitosan, and glucans possess elicitor properties. Their use in seed and plant treatment enhances plant resistance to biotic stresses, particularly disease-causing pathogens. It has been established that wood-decaying fungi-the true tinder fungus-serve as an accessible raw material for obtaining these polysaccharides. An agro-engineering model technological scheme for the biotechnological production of fungal polysaccharides, which are the active ingredient of the mycobiopreparation, has been developed and proposed. Engineering solutions for the technical infrastructure and technological regulations for the production of the mycobiopreparation Mycosan are presented. It has been established that Mycosan provides protection for potato and tomato plants against late blight under field conditions. Spraying plants with Mycosan resulted in increased yields of tomatoes and potatoes: it was calculated that the protective efficacy of the microbial preparation for treating potato plants against late blight was 76.5%, that of the synthetic preparation Efal was 78.2%, and that of a mixture of half the recommended doses of both was 79.2%; the technical efficacy of the mycobiological preparation on tomatoes was 71.9%, that of Efal was 73.4%, and when half the recommended dose of each preparation was applied, it was 76.2%; potato yield in the treatment with Mycosan increased by 6.5 $t \cdot ha^{-1}$ compared to the control, and tomato yield by 47.8 $t \cdot ha^{-1}$.

Keywords: biotechnology, plant protection, Mycosan, indicators, yield.

Introduction

Addressing scientific and practical challenges related to increasing crop yields and quality is critical to ensuring food security and meeting the ever-growing needs of the population amid limited land resources. Achieving these goals is possible through a comprehensive approach that involves the development and implementation of zone-specific adaptive technologies, the use of high-quality and environmentally safe materials, and the latest engineering principles for technological operations.

One of the measures listed for increasing the gross yield and quality of agricultural products is the reduction of crop losses caused by biotic and abiotic factors, particularly pests, diseases, and weeds. Protecting plants from pests and diseases in the technological process of crop production has been and remains one of the fundamental challenges of modern agricultural production. Pests and diseases can damage crops, causing annual yield losses of 30-50% [1]. An analysis of the problems caused by negative factors and their consequences leads to the development and implementation of measures aimed at mitigating the impact of these factors in order to preserve the genetic potential for yield and quality in cultivated plants. Each of the known measures ensures a positive outcome, but many genetic and natural-climatic factors influence their effectiveness.

Among the known methods for mitigating the harmful effects of negative influences, the use of synthetic pesticides to protect cultivated plants is quite effective in reducing yield losses, but it is accompanied by negative side effects caused by the accumulation of toxic substances in plants and food products, as well as a negative impact on the natural environment [2]. Another area of focus for scientific research is the development and introduction of disease-resistant plant varieties characterized by high biological yield potential. However, pathogenic microorganisms overcome the barriers created against them and develop virulence genes to counter resistance genes much faster than new resistant plant varieties can be developed, Moreover, new strains of pathogenic microorganisms are becoming more aggressive compared to older ones, so work in this area is actively continuing.

Furthermore, the focus of breeding efforts in recent decades on increasing crop yields has led to a significant weakening of their resistance [3].

The situation surrounding this controversial issue necessitates an in-depth scientific analysis, the search for alternative, effective, and environmentally safe methods of plant protection, as well as bioengineering solutions.

Materials and methods

In the course of our research, we employed biotechnological (to investigate the induced resistance of cultivated plants to diseases, as well as the technological processes for obtaining and utilizing polysaccharides from the cell walls of the true tinder fungus), organoleptic, and biometric (determination of the physical properties of fungi and ground fungal biomass); static and dynamic (determination of the mechanical properties of fungi); visual and physicochemical (determination of the physicochemical parameters of the fungal extract and mycobiopreparation); field (determination of the biological efficacy of the mycobiopreparation).

The study of the technical effectiveness of the use of the biofungicide Mikosan against late blight of potatoes and tomatoes was carried out in 2020–2023 at the experimental field of agricultural plants of the Institute of Mechanics and Automation of Agroindustrial Production of the National Academy of Agrarian Sciences of Ukraine, Kyiv region, Borova village, on potatoes of the Lugovskaya variety and tomatoes of the Lahidny variety.

Research conditions – soils in the experimental fields are black soil, the mechanical composition of the soil is sandy loam, humus content is 1.6%, pH 5.6. The predecessor of vegetables during the research years was winter wheat. Soil cultivation was carried out in the following sequence: stubble peeling (5-7 cm), winter plowing (23-25 cm), moisture closing (3-4 cm), pre-planting cultivation (12-15 cm), application of mineral fertilizers (according to the standard): nitrogen – 90 $kg \cdot ha^{-1}$, phosphorus – 90 $kg \cdot ha^{-1}$, potassium – 90 $kg \cdot ha^{-1}$ in the spring for cultivation, organic fertilizers – 40 $t \cdot ha^{-1}$ were applied for winter plowing.

Crop care measures on both the treated and control plots were carried out according to the accepted technology of growing crops. The area of the experimental plots was 75.6 m^2, the area of the accounting plots was 56 m^2, the repetition was fourfold, the placement of the plots was single-row. The working fluid consumption rate was 300-400 $l \cdot ha^{-1}$. The timing of application of drugs during the growing season was based on the forecast of the appearance of diseases.

Phases of crop development during the period of application of drugs-crop formation. Method of application-continuous treatment. For the application of drugs in small-scale experiments, the sprayer “Orion-6” was used, and for production experiments – MRZ-1200.

The work was carried out by conducting laboratory, field and vegetation studies. Phytopathological records of plant damage were carried out according to the generally accepted methodology. Scheme of the experiment using the biofungicide Mikosan on potatoes and tomatoes (2020-2023): 1. Control – seeds without treatment. 2. Mikosan, with a consumption rate of 10.0 $l \cdot ha^{-1}$. 3. Efal, with a consumption srate of 3.0 $l \cdot ha^{-1}$ (standard). 4. Efal, with a consumption rate of 1.5 $l \cdot ha^{-1}$ + Mikosan, with a consumption rate of 5.0 $l \cdot ha^{-1}$.

Results and Discussion

Scientific research on plant immunity has shown that it is possible to enhance plant resistance to diseases by stimulating and regulating the immune system using biologically active agents. Over the past few decades, many developed countries have begun actively developing such biological plant protection agents (Fig. 1).

Our screening of plant protection methods has revealed that inducing natural resistance is one of the most promising approaches. This method is based on stimulating the phytoimmune potential of plants in a manner analogous to natural processes [4-8].

The essence of induced resistance lies in enhancing the synthesis of enzymes that provide resistance to the penetration of pathogenic infections and in shifting certain metabolic reactions from the biological synthesis of compounds involved in constitutive metabolism to the synthesis of phytoalexins and other antipathogenic substances [9].

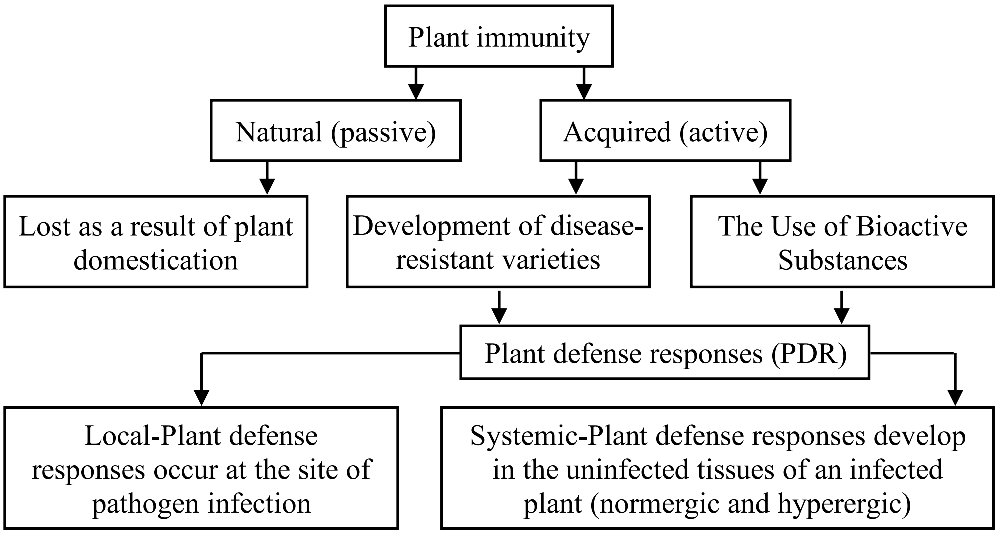

Fig. 1. **Biological bases of stability of plants**

An analysis of the scientific literature indicates that the use of the natural polysaccharides chitin and chitosan helps to increase plant resistance to disease in natural ecosystems. Chitin and its derivatives have become increasingly important in medicine, industry and agriculture in recent years [10]. Consequently, intensive research is being conducted to improve the efficiency of obtaining and applying compositions based on them.

The primary source of chitin is Arthropoda – the shells of prawns, crabs, lobsters, langoustines and crayfish. It is known that crustacean shells are a relatively expensive raw material, the price of which depends on seasonality and is linked to the size and biological species of the crab [11]. The fruiting bodies of higher basidiomycetes have been identified as a promising source of chitin and chitosan polysaccharides, based on knowledge of the structure of the fungal cell wall [12].

In view of the scientific and technical-economic characteristics relevant to our choice of raw materials, the most suitable candidates are the widespread species of wood-decaying phyllophore fungi, specifically the common tinder fungus (Fomes fomentarius (L. Fr.), Gill), which have so far been studied only in a fragmentary manner. As a scientific basis for the biotechnology of the production and application of fungal polysaccharides, we have conducted preliminary studies of the physical and mechanical properties of fungi, developed a biotechnology for the extraction of polysaccharides, and investigated their production and use as an inducer of plant resistance to adverse factors [13]. We have summarised methods for the isolation of chitin from fungal fruiting bodies (Fig. 2) and proposed an engineering and equipment model for the biotechnological production of the mycobiopreparation biofungicide Mycosan (Fig. 3).

The main list of engineering and technical equipment required for the biotechnological extraction of fungal fruiting bodies and the production of fungal polysaccharides, which form the basis of the future formulations of the biofungicide Mycosan, comprises the following items: a dryer for fungal fruiting bodies, a crusher for grinding them, an extraction reactor, a centrifuge for separating the liquid fraction from the insoluble residue, a mixer for combining the components to produce the Mycosan biofungicide, and an automatic machine for packaging and bottling the finished product. As we utilise the insoluble residue in parallel, the biotechnology process is waste-free.

We have determined the biological efficacy of the Mycosan biofungicide, based on fungal polysaccharides, on numerous agricultural crops across various soil and climatic zones of Ukraine. Field studies to assess the efficacy of known and experimental formulations were conducted in accordance with standardised methodologies [14; 15].

The most widespread and damaging disease affecting potato and tomato plants grown in the soil and climatic conditions of Ukraine is late blight. The use of synthetic pesticides at the first signs of the disease helps to protect the plants from infection. However, their main drawback is the accumulation of harmful substances that are transferred to humans through the consumption of produce.

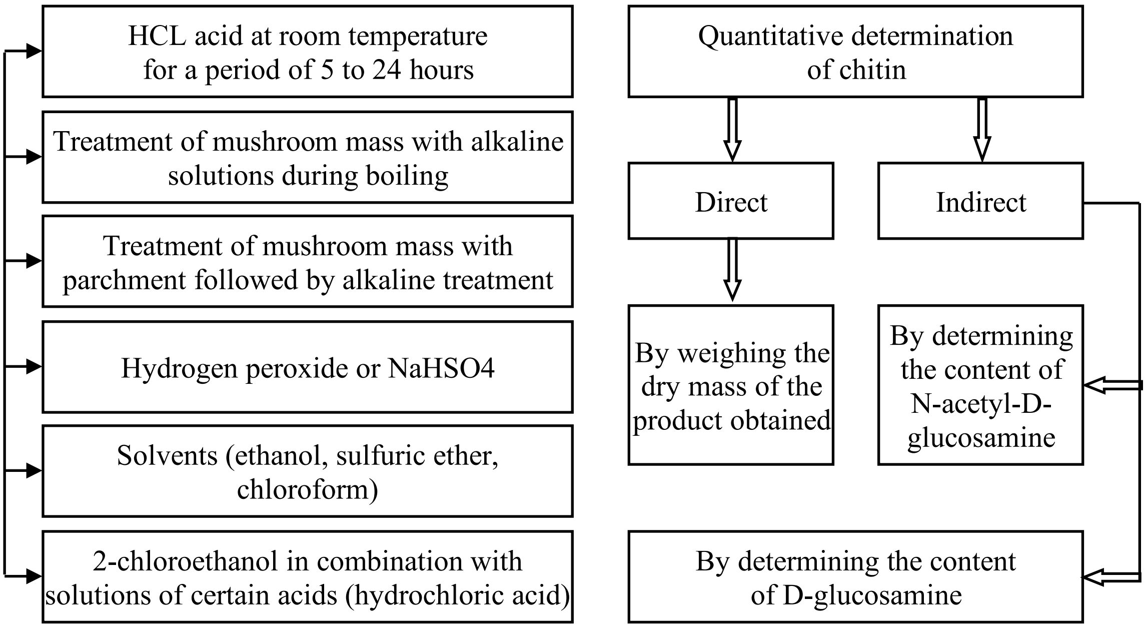

Fig. 2. **Methods for extracting chitin from mushroom fruit bodies**

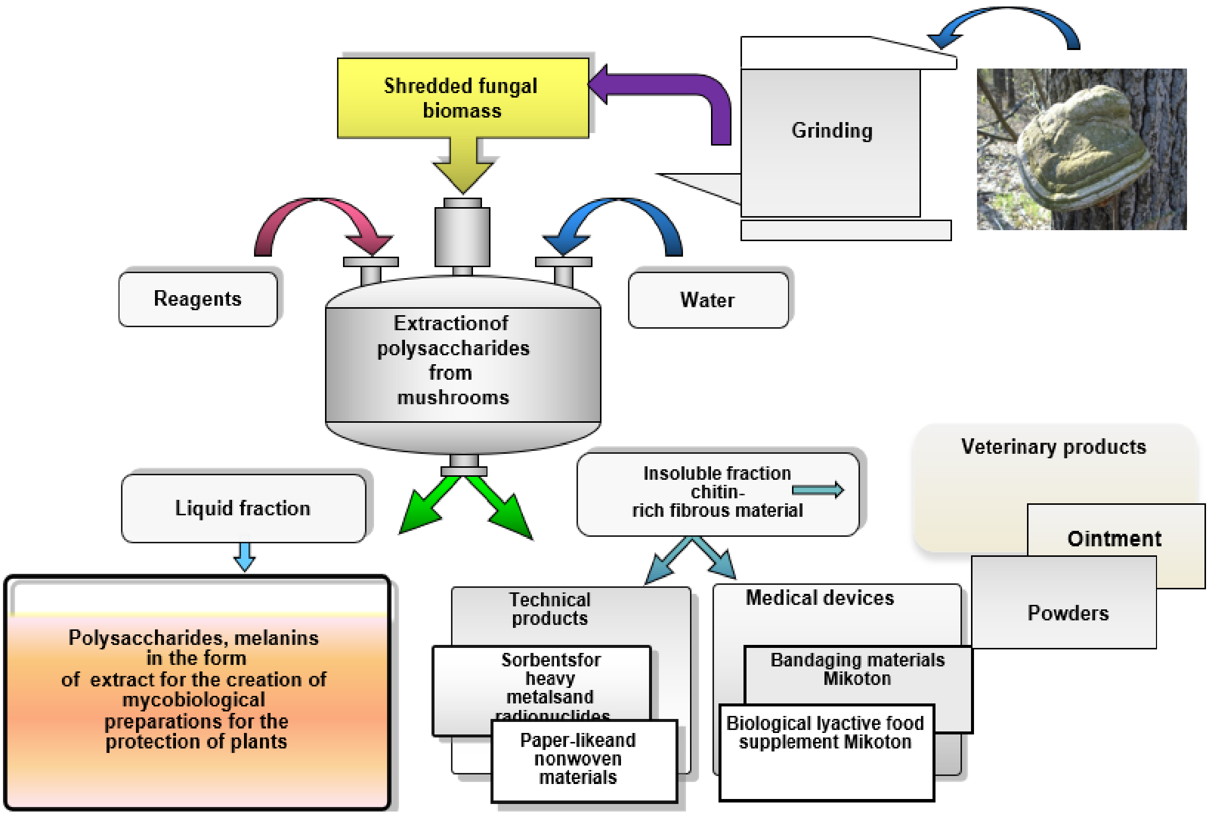

Fig. 3. **Engineering model of the biotechnology behind the biofungicide Mycosan**

Therefore, systematic research into the effectiveness of using a mycobiological preparation to enhance the resistance of potato and tomato plants to late blight was one of the objectives of this scientific study.

The ultimate aim of the work was to develop a technology for the application of a mycobiological preparation to protect potato and tomato plants from late blight and to eliminate the use of synthetic pesticides or reduce their application.

It has been established that Mycosan provides protection for potato and tomato plants against late blight under field conditions. Spraying plants with Mycosan resulted in increased yields of tomatoes and potatoes, Table 1.

It was calculated that the protective efficacy of the microbial preparation for treating potato plants against late blight was 76.5%, that of the synthetic preparation Efal was 78.2%, and that of a mixture of half the recommended doses of both was 79.2%.

The technical efficacy of the mycobiological preparation on tomatoes was 71.9%, that of Efal was 73.4%, and when half the recommended dose of each preparation was applied, it was 76.2%.

Potato yield in the treatment with Mycosan increased by 6.5 $t \cdot ha^{-1}$ compared to the control, and tomato yield by 47.8 $t \cdot ha^{-1}$.

Table 1

Effectiveness of pesticides against late blight on potatoes and tomatoes

Option	**Progression of the disease, %**	**Technical efficiency, %**	**Marketable yield, ton·ha^{-1}**
Potatoes, "Lugovskaya" variety			
Inspection without processing	41.3	0	17.3
Mikosan, 10 $l \cdot ha^{-1}$	9.7	76.5	23.8
Efal, 3.0 $l \cdot ha^{-1}$	9.0	78.2	24.0
Efal, 1.5 $l \cdot ha^{-1}$ + Mikosan, 5.0 $l \cdot ha^{-1}$	8.6	79.2	24.3
NIH $_{05}$	0.6	1.2	0.54
Tomato, "Gentle" variety			
Inspection without processing	37.7	0	1.2
Mikosan, 10.0 $l \cdot ha^{-1}$	10.6	71.9	49.0
Efal, 3.0 $l \cdot ha^{-1}$	9.3	73.4	49.3
Efal, 1.5 $l \cdot ha^{-1}$ + Mikosan, 5 $l \cdot ha^{-1}$	9.0	76.2	49.5
NIH $_{05}$	0.6	1.1	0.58

Technical and regulatory documentation has been developed for the production of the biofungicide Mikosan (Technical Specifications and Technological Regulations, as well as recommendations for use.

This area of research is relevant both in fundamental terms (establishing a theoretical basis for a new approach to plant protection, and developing and refining engineering solutions for biotechnological production of preparations) and in practical terms (introducing a new generation of plant protection products into production.

Conclusions

1. Induced plant resistance is one of the most promising areas of modern crop protection, as it allows activating natural immunity mechanisms without the negative environmental impact typical of synthetic pesticides.
2. The polysaccharides chitin and its derivatives chitosan and glucans, which possess elicitic properties, participate in the protection of cultivated plants against diseases by activating defence genes and the synthesis of antipathogenic phytoantibiotics-phytoalexins. The use of polysaccharides of fungal origin (in particular, chitin and chitosan) as the basis of biological products demonstrates high efficiency in increasing plant resistance to diseases, in particular late blight, while simultaneously increasing yield.
3. An engineering and equipment scheme for the biotechnological production of fungal polysaccharides from the cell wall of the tinder fungus-Fomes fomentarius (L. Fr.), Gill), in particular, the biofungicide Mycosan has been developed, which is recommended for seed treatment and spraying of growing plants.
4. The developed biofungicide of the "Mikosan" type provides a level of protective action comparable to chemical preparations, which confirms the feasibility of its implementation as an environmentally safe alternative or component of integrated plant protection systems.

5. The obtained research results can be used to further improve the processes of development and introduction into production of effective preparations for protecting agricultural crops from pests and diseases.

Author contributions

Tesliuk V.V. – conceptualisation of the research direction; engineering and biotechnological solutions for equipment parameters and operating modes; justification of raw material properties; evaluation of the resulting compositions. Kolomiets Y.V. – formal analysis; review and editing of the material. Baranovsky V.M. – experimental design, data supervision, processing of experimental data. Kovbasenko R.V. – analysis, review, field studies (design, recording, initial data processing). All authors have read and agreed to the published version of the manuscript.

References

[1] Rai A.K., Sahoo L.P., Sahoo M., Sethand T. Chakradhar Patra. Induced Resistance Mechanism in Plant and Its Importance in Agriculture. International Journal of Plant & Soil Science, vol. 36 (5), 2024, pp.1-22. DOI: 10.9734/IJPSS/2024/v36i54497
[2] Popp J., Pető K., Nagy J. Pesticide productivity and food security: A review. Agronomy for Sustainable Development, vol. 41, 2021, pp.1-15. DOI: 10.1007/s13593-020-00663-y
[3] Pingali P.L., Aiyar A., Abraham M., Rahman A. Transforming Food Systems for a Rising India. Cham: Springer, 2021. DOI: 10.1007/978-3-030-14409-8
[4] Walters D.R., Fountaine J.M. Practical application of induced resistance to plant diseases. Plant Pathology, vol. 71(1), 2022, pp. 15-30. DOI: 10.1111/ppa.13465
[5] Mauch-Mani B., Baccelli I., Luna E., Flors V. Defense priming: An adaptive part of induced resistance. Annual Review of Plant Biology, vol. 72, 2021, pp.475-502. DOI: 10.1146/annurev-arplant-080620-070811
[6] Conrath U., Beckers G.J.M., Langenbach C.J.G., Jaskiewicz M.R. Priming for enhanced defense. Annual Review of Phytopathology, vol. 60, 2022, pp. 97-119. DOI: 10.1146/annurev-phyto-020620-100356
[7] Pandey G., Kumar A., Sharma P., et al. Induced resistance in crops: Mechanisms and applications. Agronomy, vol. 12(5), 2022, pp. 1134. DOI: 10.3390/agronomy12051134
[8] Walters D., Newton A., Lyon G. Induced resistance for plant disease control: Maximizing the efficacy. Frontiers in Plant Science, vol. 12, 2021, pp. 678. DOI: 10.3389/fpls.2021.678589
[9] Hammerschmidt R. Phytoalexins: What have we learned after 60 years? Annual Review of Phytopathology, vol. 59, 2021, pp. 1-20. DOI: 10.1146/annurev-phyto-020620-121621
[10] Sharma S., Kumar A., Kumar R., et al. Chitin and chitosan: Functional biopolymers in agriculture. International Journal of Biological Macromolecules, vol. 183, 2021, pp. 2044-2055. DOI: 10.1016/j.ijbiomac.2021.05.123
[11] Younes I., Rinaudo M. Chitin and chitosan preparation from marine sources. Marine Drugs, vol. 20(3), 2022, pp. 201. DOI: 10.3390/md20030201
[12] Kumar M., Brar A., Vivekanand V., Pareek N., Jaswal R. Fungal polysaccharides: Production and applications. Journal of Fungi, vol. 6(4), 2020, pp. 321. DOI: 10.3390/jof6040321
[13] El Hadrami A., Adam L.R., El Hadrami I., Daayf F. Chitosan in plant protection. Marine Drugs, vol. 21(2), 2023, pp. 112. DOI: 10.3390/md21020112
[14] European and Mediterranean Plant Protection Organization. EPPO. PP 1/152 (4): Design and analysis of efficacy evaluation trials. Paris: EPPO, 2022.
[15] Fry W.E., Birch P.R.J., Judelson H.S., Grünwald N.J., Danies G., Everts K.L., Gevens A.J., Gugino B.K., Johnson D.A., Johnson S.B., McGrath M.T., Myers K.L., Ristaino J.B., Roberts P.D., Secor G.A., Smart C.D. Five reasons to consider Late blight a reemerging disease. Phytopathology, vol. 112(1), 2022, pp. 27-37. DOI: 10.1094/PHYTO-07-21-0301-RVW