THE BIOTECHNOLOGICAL POTENTIAL OF MICROORGANISMS DETERMINATION FOR CREATING COMPLEX PREPARATIONS WITH INSECT-FUNGICIDAL PROPERTIES

PILYAK Nina*, KHODORCHUK Vasyl**

Engineering and Technological Institute "Biotekhnika" of the National Academy of Agrarian Sciences of Ukraine * https://orcid.org/0000-0002-5074-4011, ** https://orcid.org/0000-0001-6542-0290 e-mail: nceb2017@gmail.com

Summary. The research results of the biotechnological potential of microbial strains with different taxonomic statuses are presented for stable microbial consortia creation in complex preparations with insect-fungicidal properties development. Six strains of microorganisms with fungicidal properties and four with insecticidal properties were selected from the Collection of the Engineering and Technological Institute "Biotekhnika" of the National Academy of Agrarian Sciences of Ukraine. Their biotechnological potential was analyzed toward test objects (phytopathogens and insects). It was found that *Trichoderma* fungi demonstrate the highest fungicidal activity, while *Streptomyces* fungi exhibit the highest insecticidal activity.

Keywords: *microbial strains, biotechnological potential, complex biopreparations, fungicidal properties, insecticidal properties.*

Introduction. It is well known that microbial preparations using is considered appropriate for the significant reduction of chemical pesticide application in agriculture. These preparations are environmentally friendly, promote physiological and biochemical processes intensification in plants, enhance their resistance to diseases, and positively influence soil by increasing the beneficial microflora [1, 2]. Scientists explain such effects, at least in part, by bioactive substances and microbial synthesis [3, 4].

Recent years of research conducted at the Engineering and Technological Institute "Biotekhnika" of the National Academy of Agrarian Sciences of Ukraine (ETI "Biotekhnika" NAAS), have focused on creating new effective complex microbial preparations that combine growth-stimulating, fungicidal, and insecticidal properties [5]. An important scientific and practical task in developing complex preparations is the formation of stable microbial consortia from different taxonomic groups [5, 6]. Microorganisms co-culturing in consortia is possible only through their symbiosis and synergistic action. It is possible to obtain combined action preparations, where the synergistic action exceeds the individual action of each strain, precisely based on such stable consortia.

The research focused on microbial strains with fungicidal and insecticidal properties, which are stored in the ETI "Biotekhnika" NAAS Industrially Valuable Microorganisms Collection.

The aim of the study was promising microorganisms' selection and their biotechnological potential determination for the subsequent formation of stable consortia, serving as the basis for insect-fungicidal complex preparations.

Materials and Methods

There were used commonly accepted techniques and methodological approaches employed in microbiology and biotechnology [2, 7].

The collection cultures screening [7] was performed which permitted ten strains of microbial cultures with fungicidal and insecticidal properties to be selected.

The biotechnological potential of the selected microorganisms was evaluated. The cross streak method [8] was used for fungicidal properties (antagonism) study between selected strains and test objects. A complex of dominant phytopathogens *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium oxysporum*, *Fusarium moniliforme*, and *Fusarium graminearum* were used as test objects. The antagonism index was assessed by considering the zone of test objects' growth inhibition [8].

Insecticidal properties were determined using the English grain aphid (*Sitobion avenae* Fabricius, 1775) was used as a test object.

Results and Discussions. The research on microbial fungicides demonstrates the significant role of antagonistic fungi in suppressing plant pathogens [1, 2]. These microorganisms possess a wide range of antagonistic properties, expressed through substrate competition, antibiotics production, and other substances that inhibit the growth of phytopathogens [2, 9]. *Trichoderma* and *Gliocladium* fungi are commonly used for fungal fungicide creation [1, 2]. The most widely used base for bacterial preparations, which compete with fungal ones in the market are *Pseudomonas* and *Bacillus* [1, 10].

For the development of microbial insecticides, both fungal and bacterial cultures are used. *Bacillus*, *Streptomyces*, *Beauveria*, and *Metarhizium* genus representatives could be pointed among them [1, 2].

The Collection ETI "Biotekhnika" NAAS contains microorganisms with both fungicidal and insecticidal properties. The collection cultures screening resulted in the selection of the most promising strain for microbial consortia creation. There were selected strains with:

– <u>fungicidal properties</u>: *Trichoderma viride* st. T-4; *Trichotecium roseum*; *Gliocladium rozeum*; *Bacillus subtilis* st. 215; *Pseudomonas fluorescens* st. 2; *Pseudomonas aureofaciens* st. 5;

<u>insecticidal properties</u>: Bacillus thuringiensis var. thuringiensis st.
202; Bacillus thuringiensis var. kurstaki st. BTK-4; Streptomyces avervitilis;
Beauveria bassiana.

The biotechnological potential of the selected collection microbial cultures was studied based on their fungicidal and insecticidal activity against test objects (phytopathogens and insects).

The results showed that *Trichoderma viride* st. T-4 demonstrated the highest fungicidal activity against the phytopathogens complex, with a growth inhibition rate of 93 % (Fig.1). Due to bioactive substances fungal strains *Gliocladium rozeum* and *Trichotecium roseum* exhibited zones of growth inhibition for phytopathogens within the range of 89-91 %. Among the bacterial strains, *Pseudomonas aureofaciens* st. 5 and *Pseudomonas fluorescens* st. 2 were the most active, suppressing the growth of phytopathogens by 89-95% through the action of active metabolites.

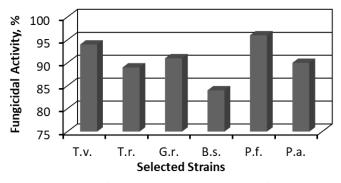


Figure 1. Fungicidal activity of microbial strains selected from the ETI "Biotekhnika" NAAS Collection against the phytopathogens complex.

Notes: T. v. - Trichoderma viride; T. r. – Trichothecium roseum; G. r. – Gliocladium rozeum; B. s. – Bacillus subtilis; P. f. – Pseudomonas fluorescens; P. a. – Pseudomonas aureofaciens The biotechnological potential of the selected microorganisms with insecticidal properties was determined using the English grain aphid (Fig. 2). *Streptomyces avervitilis* culture demonstrated the highest activity at 81 %.

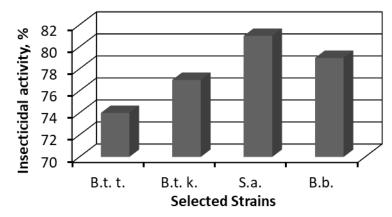


 Figure 2. Insecticidal activity of microbial strains selected from the ETI "Biotekhnika" NAAS Collection against Sitobion avenae.
Notes: B. t. t. – Bacillus thuringiensis var. thuringiensis; B. t. k. – Bacillus thuringiensis var. kurstaki; S. a. – Streptomyces avervitilis; B. b. – Beauveria bassiana

The obtained data analysis indicates that the majority of cultures, selected from the Institute's Collection, exhibit high biotechnological potential, which will allow their use for further microbial consortia formation.

Conclusions: The conducted study has shown that almost all selected microorganisms have a high biotechnological potential. Therefore, screening of fungal and bacterial cultures from the Collection of Industrially Valuable Microorganisms of ETI "Biotekhnika" NAAS, which exhibit high fungicidal and insecticidal activity in monocultural preparations, made it possible to almost accurately identify cultures suitable for the microbial consortia formation – the basis of complex preparations with insect-fungicidal properties creation.

References

- 1. Biopesticides and bioagents. Novel tools for pest management A. Anwer ed. Waretown: Apple academic press, 2018. 402 p.
- Montesinos, E., Bonaterra A. Microbial Pesticides. In *Encyclopedia of Microbiology*, 3rd ed.; Schaechter M., ed. Amsterdam: Elsevier, 2009. P. 110–120.

- 3. Vessey J. K. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil.* 2003. Vol. 225. P. 571–586.
- Olanrewaju O. S., Glick B. R., Babalola O. O. Mechanisms of action of plant growth promoting bacteria. World Journal of Microbiology and Biotechnology. 2017. Vol. 33, No. 11. P. 197–204.
- Krutyakova V.I., Pilyak N.V., Nikipelova O.M. Scientific basis of natural growth regulators with fungicide abilities creation. *In The* systems of agriculture biologization agents manufacturing and using: Monograph. Kyiv: Agrarna nauka, 2022. P. 250–265. (In Ukrainian)
- 6. Krutyakova V.I., Pilyak N.V., Nikipelova O.M. Complex microbial preparations creation methods based on microbial groups with destructive and antagonistic functions. *In The systems of agriculture biologization agents manufacturing and using*: Monograph. Kyiv: Agrarna nauka, 2022. P. 266–275. (In Ukrainian)
- Lertcanawanichaku M., Sawangnop S. A comparison of two methods used for measuring the antagonistic activity of bacillus species. *Walailak J Sci Technol.* 2008. Vol. 5. P. 161–171.
- 8. Ghisalberti E.L., Sovasithamparam K. Antifungal antibiotics produced by *Trichoderma spp. Soil Biol. Biochem.* 1991. № 23. P. 1011-1020.
- Suzuki S., Hel Y., Oyaizul H. Indole-3-acetic acid production in *Pseudomonas fluorescens* HP72 and its Association with suppression of creeping bentgrass brown patch. current Microbiol. 2003 Vol. 4, No. 2, pp. 138–143.