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POTENTIAL CAPACITIES OF ACTINOBACTERIA IN PEST CONTROL IN ECOLOGICAL AGRICULTURE

Excessive use of synthetic pesticides causes, in addition to the effect on non-target organisms, problems associated with the accumulation of toxic residues and metabolites, environmental pollution and disruption of ecological balance. This necessitates researching and highlighting effective and harmless methods and means of controlling pests, among which a special place belongs to biological means of protection, in particular biological products. The use of biological insecticides developed on the basis of actinobacteria has demonstrated high biological efficiency, significant environmental and economic effectiveness. The article presents a laboratory and field evaluation of the antifungal activity of *Streptomyces* actinomycetes strains by inhibiting the growth and development of Fusarium pathogens. The biological characteristics and technological indicators of *Saccharopolyspora spinosa* biomass production for pest control in protected ground were determined.

actinobacteria; biological preparation; ecological agriculture; plant health

Introduction. Against the background of climate change and the resulting increased impact of pests on agricultural crops, global food security remains at risk in relation to the increasing rates of the global population. Increasing the yield of agricultural activities aimed at maintaining food security and food safety can be achieved by effectively optimizing the phytosanitary status of agroecosystems based on the use of ecologically harmless plant protection products. The strategy and tactics of phytosanitary optimization of agroecosystems using plant protection products with low ecological risk and their application technologies, starting from the worsening of the phytosanitary status of agricultural crops, need to be revised. It has become a pressing opportunity to change the concept of plant protection, which as a scientific discipline is oriented towards to protect against pests organisms

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under the conditions of intensive agricultural development, which requires the optimization of trophic links and the application of biocenotic regulation mechanisms (Voloşciuc, L., 2021).

The solution of phytosanitary problems is indispensably linked to the application of plant protection means, and in order to combine these activities with the optimal preservation of the environment, measures are taken to implement the concept of plant health with the use of biological protection, especially microbiological products. Although the application of biological control agents (viruses, bacteria, microscopic fungi, entomopathogenic nematodes, microsporidia) registers significant results, the rates of expansion of the surfaces protected with biopreparations remain modest, which determines the need to identify new groups of biological control agents. Among the microbiological means of control, the application of preparations based on actinobacteria stands out, which, thanks to their biological peculiarities, can be applied as in protection against pathogenes pathogens and arthropods harmful to a wide range of agricultural crops.

To improve the technological processes of production and application of actinobacterial means, it becomes necessary to implement bio controllable microorganisms, which ensure high indices of biological effectiveness and ecological and economic efficiency with sufficient resistance in the conditions of production of agricultural crops. This determines the need to carry out research on several aspects of the production and application of actinobacteria, including the selection of biological agents with pathogenicity on the target objects, the improvement of culture media and production conditions, the development of optimal formulations in which the biological agent exhibits activity and resistance in the conditions of application, the use of biotechnological achievements aimed at improving the mechanisms and strategies of biological protection of plants (Boincean, B., et al., 2020).

The suppression of the development of harmful organisms by biological methods is based on the antagonism that exists in nature between the individual host plant, living organisms and microorganisms in the composition of the microbiome and the products of their vital activity. Biological preparations based on microorganisms antagonistic to harmful organisms are becoming more and more widespread in agriculture. In addition to the significant results recorded for different crops in various agroclimatic zones, the protective effect shown is determined by the direct biological action depending on the particularities of the abiotic factors, as well as by the relationships between the representatives of the microbiota within the concrete biocenosis.

Actinobacteria represent a diverse variety of natural products, exceedingly more than 22 000 biologically active secondary metabolites. The range of plant protection products based on actinobacteria includes environmentally harmless means based on secondary metabolism products (avermectins,

spinosyns). Biological insecticides and acaricides based on them demonstrate high effectiveness in controlling harmful arthropods. High specificity, lack of adaptation to harmful organisms, low toxicity and sufficient pathogenicity in combating target objects are valuable qualities against the background of maintaining the natural ecological balance. The high biological diversity of actinobacteria and the high ability to adapt to production and application conditions represent valuable qualities for the application of these microorganisms in the natural arsenal of effective means in integrated protection systems for conventional and organic agriculture. Protection against harmful insicts in protected land in conventional and organic farming systems requires the development and application of biological preparations, the most promising of which have become the means obtained from the biomass of actinobacteria (Kaur T., Kumari M., 2013; Karnam V.H., 2016).

Material and methods. Research was carried out during the period in laboratory and field conditions at the Institute of Genetics, Physiology and Plant Protection of the USM. The material for identifying the frequency of occurrence of harmful arthropods was analysed after highlighting vegetable crops in greenhouse conditions (Burtseva, S.A., et al., 2015; Voloşciuc, L., 2021).

Studies were performed on natural, artificial and mixed culture media. Optimizing the nutrient environment was carried out on several levels — the main source of carbon, nitrogen, pH of the environment, cultivation time. For the cultivation of the mother culture, a composition with several semi-synthetic components is used, which allows a faster start of the culture, in its composition you can find — yeast extract, peptone from casein, starch, glucose.

Cultivation in depth was carried out on liquid media in Erlenmeyer flasks with a volume of 750 ml, the volume of the cultured liquid was 100 ml. Test tubes with agar medium were used for inoculation and were cultivated until a large number of spores were obtained and a compact white-grey lawn was obtained. A volume of 5 ml of sterile water was added to each test tube, with the help of the Drigalski spatula, movements were made on the surface of the medium, thus imposing the detachment of Saccharopolyspora spinosa cells, the obtained suspension was poured into the Erlenmeyer flask, they were placed on the rocker at a temperature of 25—30°C).

To resuspend the culture in 100 ml of culture liquid, NaOH was added to maintain the pH at 7—8, and the sample was centrifuged for 15 min. The sediment was collected, and 5 g of activated carbon was added to the supernatant and centrifuged for 30 min. After this, the sediment is discarded and the supernatant collected. The liquid was placed at a temperature of 40°C to gradually evaporate the solvent, which allowed obtaining small transparent yellowish crystals (Lungu, A., Volosciuc, L., 2023).

The situation in the field of plant and environmental protection was

analysed by applying the methodology of systemic approaches, as a tool for managing complexity and as one of the essential paradigms of the future (Dospekhov B.A., 1989).

Results and discussion. Phytosanitary optimization of agroecosystems becomes a reality when using biological protection based on ecologically harmless means, especially multifunctional biological preparations consisting of microorganisms antagonistic to harmful organisms. Unlike the application of pesticides, biological plant protection agents do not only act on the target objects, but, as producers of biologically active substances, increase the resistance to harmful organisms and the biological activity of agricultural crops. The methodology for the development of multifunctional biological preparations includes the use of technological strains with high biological activity, among which a special role belongs to actinobacteria for combating harmful arthropods, which show resistance to other means of biological control (Herbert A., 2010; Mahajan G., 2012).

Starting from the ability of biologically useful microorganisms to produce antibiosis and antagonism effects on harmful arthropods, measures were applied to highlight and identify microbiological entities by analyzing the cultural properties of growth on different culture media, as well as examining their morphological peculiarities. Most frequently, several cultures of the genus Streptomyces were identified, which, synthesizing several biologically active substances, cause profound changes in the cellular structures of mycotic phytopathogenic agents (Volosciuc, L.F., et al., 2013). After cultivation on the medium with gelatinized oats, some colonies of strains no. 24 and 49 were white in color, and other strains were characterized by different colors (fig. 1).

In the multi-year investigations of microbiological agents useful in combating arthropods, which show resistance to the application of biological means of protection, Saccharopolyspora spinosa was discovered — an aerobic, gram-positive actinobacterium with a mycelium consisting of pale yellow-pink aerial hyphae. The hyphae form long spore chains (about 50

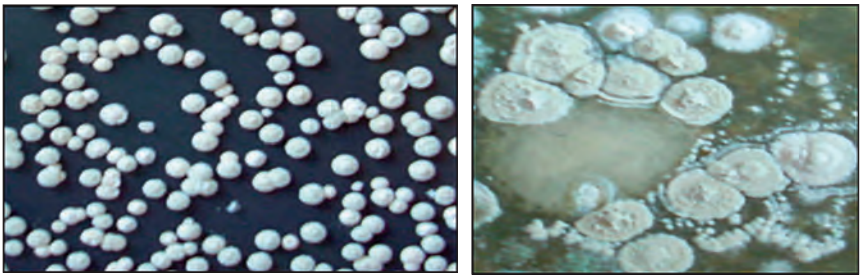

Fig. 1. The particularities of Streptomyces sp, colonies 11, grown on Czapek medium with glucose and oat agar

spores), which are surrounded by spiny spore sheaths. S. spinosa synthesizes spinosad, which is a natural substance produced by actinobacteria in the soil and exhibits toxicity to insects. It is a mixture of two chemicals called spinosyn A and spinosyn D. It is used for the biological control of a wide range of pests: thrips, mining moths, mites, mosquitoes, ants, fruit flies, etc. (Legocki J., Polec I., Zelechowski K., 2010; Yadav N., Yadav A., 2019).

Biotechnological approaches open new perspectives in the creation and improvement of plant protection products and methods. Thus, biotechnological methods have found application in solving the problems of increasing plant resistance to diseases and pests and in creating new plant protection products. In this sense, the cultural particularities of S. spinosa were determined (fig. 2).

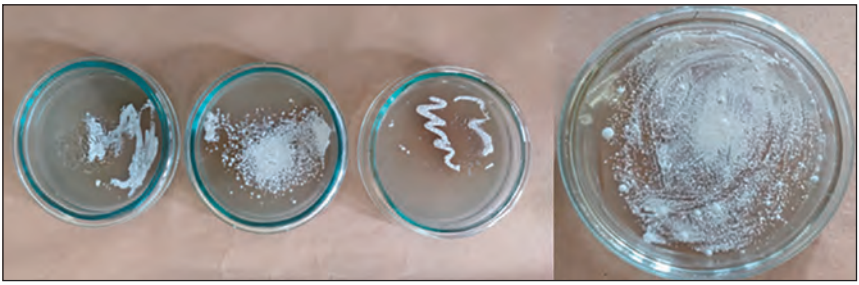

Fig. 2. Cultural peculiarities of *S. spinosa* DSM 44228 according to the effect of temperature on the growth of the actinobacterium

In order to determine the optimal conditions necessary to obtain Saccharopolyspora spinosa biomass, the conditions that ensure the maintenance of the producer's liquid culture and determine the morphological peculiarities of the mycelium were determined (fig. 3).

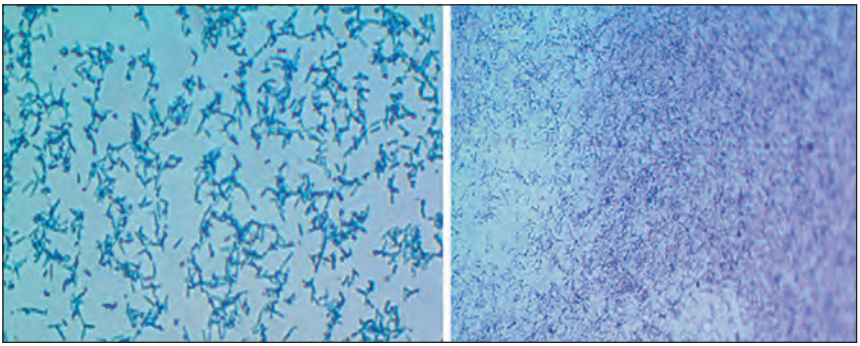

Fig. 3. Samples of *S. spinosa* cultured on liquid medium, Gram-stained preparation, x1000

The development of the biotechnological procedures for the production and application of the actinobacterium Saccharopolyspora spinosa requires the determination of the optimal conditions for the production of biomass and the establishment of the effective preparation form to combat harmful insects, which allowed the establishment of the main indicators for obtaining the biomass of the biological agent (fig. 4).

Fig. 4. Determination of the technological indices of cultivation of *Saccharopolyspora spinosa* Mertz and Yao DSM 44228 on different liquid media

CONCLUSIONS

Actinobacteria constitute a group that occupies an intermediate position between bacteria and fungi, having properties that favor their predominance among other groups of useful microorganisms.

The composition of the nutrient environment plays a significant role in the productivity of the biomass used in the establishment of plant protection means. Strains differ according to morphological and cultural peculiarities, biomass productivity and growth activity.

It was determined that the optimum temperature for cultivation of *Saccharopolyspora spinosa* DSM 44228 is 28—30°C, pH 7—8, and the cultivation time 96 hours at 180 rpm.

The use of methanol records the dissolution of organic substances in the culture liquid. In *Saccharopolyspora spinosa*, after a six-month storage on a nutrient medium inclined at a temperature of 3—4°C, the sporulation capacity is reduced, the capacity is restored after 2—3 recultivations. From the compositions tested, the composition with no. 20. The spinosad separation method with the use of organic solvents and activated carbon as an adsorbent made it possible to obtain the crystalline form of the substance.

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Потенційні можливості актинобактерій у захисті від шкідників в екологічному сільському господарстві

Надмірне використання синтетичних пестицидів спричиняє, окрім дії на нецільові організми, проблеми, пов'язані з накопиченням токсичних залишків і метаболітів, забрудненням довкілля та порушенням екологічної рівноваги. Це зумовлює необхідність проведення дослідження та висвітлення ефективних і нешкідливих способів і засобів захисту зі шкідливими комахами, серед яких особливе місце належить біологічним засобам захисту, зокрема біологічним препаратам. Застосування біологічних інсектицидів, розроблених на основі актинобактерій, продемонструвало високу біологічну ефективність, значну екологічну та економічну результативність. У статті наведено лабораторну та польову оцінку протигрибної активності штамів актиноміцетів *Streptomyces* шляхом пригнічення росту та розвитку збудників фузаріозу. Визначено біологічні особливості та технологічні показники виробництва біомаси *Saccharopolyspora spinosa* для контролю поширення шкідливих комах у захищеному ґрунті.

актинобактерії; біопрепарат; екологічне землеробство; здоров'я рослин

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