

# First results of the study of entomopathogenic microflora *Cydia pomonella* (Linnaeus, 1758) in apple orchards of Uzbekistan

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In this study, *Cydia pomonella* (Linnaeus, 1758) entomopathogenic microorganisms were isolated from damaged insect carcasses in the territory of Tashkent and Fergana regions of Uzbekistan. Insect specimens were selected using the trapping belt method and 30 strains of micromycetes were isolated from these specimens. In morphological and cultural studies, entomopathogenic micromycetes in dead and infected individuals of *C. pomonella* belong to the genera *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*. The results obtained showed that micromycetes of the species *Penicillium chrysogenum* Thom, *Alternaria alternata* (Fr.) Keissl., *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum* Schldl., *Aspergillus fumigatus* Fresen., *Aspergillus niger* Tiegh., *Aspergillus terreus* Thom were present both in the suspension after washing off the insects and in the homogenized insects.

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## Keywords

Insects, *Cydiapomonella*, entomopathogenic micromycetes, *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, *Mucor*

## Introduction

Increasing the export potential of Uzbekistan to meet the population's needs for environmentally friendly fruit and vegetable products is one of the most important tasks. Management of agrobiocenosis in order to ensure its productivity and the use of scientific methods is a primary problem. Currently, fruit trees are damaged by insects, the damage from which reaches an almost global distribution, causing serious damage to the fruit, damaging the quality of the product and reducing fruit yield by 30-80%. The codling moth (*Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae) is a major apple insect worldwide, ranking among the top 100 invasive species in the world and the most destructive apple insect. Without the use of biological and chemical means of protection, damage from the insect can reach up to 80% in apples and 60% in pears (Jiang et al. 2018, Balaško et al. 2022, Wan et al. 2019). In this regard, biological insect control methods may be a priority approach.

Studying the interaction of microflora with insects contributes to the creation of new biological methods of combating antifungal activity to protect fruit plants. The authors isolated a fungus of the genus *Paecilomyces* Bainier, 1907, which is used for biological control of phytopathogenic fungi and bacteria through various mechanisms of action. The use of this type of fungus on plants had a biostimulating effect on crop yields (Moreno-Gavira et al. 2020). The entomopathogenic properties of the *Cladosporium* Link, 1816 fungus are associated with the production of toxic secondary metabolites. These toxins colonize the structures of insects, ambrosia beetles, leaf weevils and galls (Nicoletti et al. 2024). Researchers have searched for entomotoxic, antifeedant and hormonal substances produced by fungi of the genera *Aspergillus* P. Micheli, 1729 and *Penicillium* Link, 1809 that have insecticidal properties (Berestetskiy and Hu 2021). In a study, a new highly pathogenic strain of the fungus *Aspergillus fijiensis* Varga, Frisvad & Samson was isolated from naturally infected carcasses of adult *Diaphorina citri* Crawford, 1911. Under greenhouse and laboratory conditions, isolated *A. fijiensis* GDIZM-1 is effective against *D. citri* for the development of a new microbial pesticide against *D. citri* (Yan et al. 2022). The authors studied secondary metabolites, polyketides, alkaloids, lysine derivatives and terpenoids, which activate fungal entomotoxicity and the development of an aggressive immune response among insect hosts (Singh et al. 2016). Researchers have isolated rhizosphere bacteria of the genera *Pseudomonas protegens* Ramette et al., 2012 and *Streptomyces globisporus* D. from the soil. These bacteria are used by insects as alternative hosts and potentially act as rhizosphere-competent insect symbionts (Pronk et al. 2022). The works studied the nutritional, ecological and biotic properties of endophytic bacteria associated with their use in agriculture for insect control (Watts et al. 2023). The review article examined the insecticidal properties of secondary metabolites of the genus *Penicillium*, which had high entomopathogenic activity against insects (Nicoletti et al. 2023). In a literature review, the authors studied the microbiota of Lepidoptera and their impact on the insects, as well as potential patterns in the structure and composition of insect microbiota (Mereghetti et al. 2017). The composition and diversity of endophytic fungi isolated from *Taxillus chinensis* (DC.) Danser was studied in specimens of parasites growing on seven different shrubs and fruit plants, *Morus alba* L., *Prunus salicina* Lindl., *Phellodendron chinense* C.K.Schneid., *Bauhinia purpurea* L., *Dalbergia odorifera* T.C.Chen, *Diospyros kaki* L.f. and *Dimocarpus longan* Lour. (Song et al. 2023). The beneficial properties of microorganisms for use in agriculture have been studied. The isolated microorganisms are capable of producing volatile organic compounds (VOCs) with enormous biotechnological potential (Almeida et al. 2022). Scientists isolated a biofumigant from the leaves of *Tithonia diversifolia* (Hemsl.) A.Gray, which were used to treat grain insects with the values LC50 17.86 and 11.49 µg/L (*Sitophilus oryzae* A.Hustache, 1930), 19.80 and 10.29 µg/L (*Rhizopertha dominica* (Fabricius, 1792) and 24.41 and 17.80 µg/L. L air (*Tribolium castaneum* (Herbst, 1797), respectively (Devi et al. 2023). Scientists studied the microflora community, disease resistance and plant growth stimulation in the specimens of *Protaetia brevitarsis* (Lewis, 1879) larva fractions. Microorganisms contributed to plant disease resistance and plant growth (Xuan et al. 2022). In the study, entomopathogenic fungi were isolated from the carcasses of insects, and then they were identified using classical microbiological methods. (Picciotti et al. 2023). Studies have examined

microbial pesticides active against various microbial pathogens of damaging insects. The conditions for cultivating microorganisms were optimized to obtain active metabolites to increase plant productivity in the field (Lee et al. 2022). Studies have been conducted to isolate bacterial isolates with beneficial properties on Arabidopsis seedlings. Bacteria identified as *Pseudomonas* Migula, 1894, *Serratia* Ballantyne, 2019 and *Stenotrophomonas* Palleroni & Bradbury 1993 had a stimulating effect on plant growth (Tzec-Interián et al. 2020). Scientists studied the antagonistic ability of the fungus *Paecilomyces variotii* Bainier against seven phytopathogens using in vitro and in vivo methods. The results showed a strong effect, reducing disease by 78% and 76%, respectively. *P. variotii* is a potential biological control agent against a number of airborne and soil-borne diseases and the insects (Moreno-Gavira et al. 2020). The study discovered a strain of *B. gladioli* KRS027 with antifungal activity against gray mold caused by *Botrytis cinerea* Pers. It was shown that *B. gladioli* KRS027 can be used for biocontrol of plants in agriculture (Wang et al. 2023). Scientists have studied an extract of the endophytic fungus *Trichoderma* sp., EFI 671, isolated from parts of the stem of the medicinal plant *Laurus* sp., which showed biological activity against phytopathogens (*Fusarium graminearum* Schwabe, *Rhizoctonia solani* J.G.Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary and *B. cinerea*) and insects (*Spodoptera littoralis* (Boisduval, 1833), *Myzus persicae* (Sulzer, 1776), *Rhopalosiphum padi* (Linnaeus, 1758)). Chemicals isolated from a mixture of triglycerides (m1), eburicol (2),  $\beta$ -sitostenone (3), ergosterol (4) and ergosterol peroxide (5) inhibited insect growth (Kaushik et al. 2023). The antifungal properties of entomopathogenic fungi of the genus *Metarhizium brunneum* Petch were studied. In the experiments, insect larvae were treated with a fungal suspension, and corn and wheat seeds were soaked in the culture liquid. *M. brunneum* strains 1154 and 1868 significantly increased wireworm mortality (Razinger et al. 2020). Scientists isolated rhizobacteria of the *Lauraceae* species and studied antifungal activity against *Fusarium solani* (Mart.) Sacc. and *F. oxysporum*. At the same time, 33 isolates significantly reduced the growth of *F. solani*, and 21 isolates inhibited the growth of *F. oxysporum*. Nine bacterial isolates inhibiting fungal growth by more than 20% were identified by analyzing the sequence of the 16S Rdna gene, they belonged to the genera *Streptomyces* Waksman & Henrici, 1943, *Arthrobacter* Conn & Dimmick, 1947, *Pseudomonas* Migula, 1894 and *Staphylococcus* Rosenbach, 1884 (Reverchon et al. 2019). The authors studied the effect of the pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary on the actinobacterial community of soybean root. 70 endophytic actinobacteria belonging to the genera *Rhodococcus* Zopf, 1891, *Kribbella* Park et al., 1999, *Glycomyces* Labeda et al., 1985, *Saccharothrix* Labeda et al., 1984, *Streptosporangium* Couch, 1955 and *Cellulosimicrobium* Schumann et al., 2001 were studied. Four strains of *Streptomyces* produced agroactive compounds and showed antagonistic activity of 54.1-87.6%. (Liu et al. 2019). Scientists isolated a fungus from the bodies of insects of the species *Nasonovia ribisnigri* (Mosley, 1841) and identified it as *Paecilomyces niveus* Stolk & Samson, 1971. When *Nasonovia ribisnigri* (Mosley, 1841) was treated with the pathogenic fungus *P. niveus* for 120 hours, the average insect mortality was 74%. This allowed the use of *P. niveus* culture instead of chemical insecticides (Zawadneak et al. 2019). Twelve strains of entomopathogenic fungi from the genera *Lecanicillium* W. Gams & Zare, 2001, *Paecilomyces* Bainier, 1907, *Beauveria* Vuill., 1912, *Metarhizium* Sorokin, *Cordyceps* (Fr.) Link and *Nomuraea* Maubl. were tested for aphid control. Among the tested entomopathogenic fungi, *L. lecanii* 41185 showed the greatest virulent pathogenicity for both aphids *Myzus persicae* (Sulzer, 1776) and *Aphis gossypii* Glover, 1877. Treatment with fungi for 5 days for the *Aphis gossypii* Glover, 1877 species was 100%. At the same time, the lethal concentration (LC50) of the conidial suspension of the fungus was  $6.5 \times 10^5$  conidia/ml (Vu et al. 2007). Scientists in studies to combat the codling moth used entomopathogenic nematodes of the species *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982 and *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding, 1982, which had a high potential for combating overwintering *Cydia pomonella* larvae at temperatures above 10 - 15°C, respectively (Lacey and Unruh 2005). From diseased and damaged specimens of sunflower plants, fungi were isolated and identified as *Alternaria alternata* (Fr.) Keissl., *Alternaria tenuis* Nees and *F. oxysporum*, *F. solani*. When termites were treated with the biomass of *A. alternata* and *A. tenuis* crops, their mortality rate was up to 80% (Akhmedova et al. 2021). The authors identified bacterial strains of the species *Pantoea agglomerans* (Beijerinck, 1888) Gavini et al. 1989, *Priestia megaterium* (de Bary, 1884) Gupta, Patel, Saini & Chen, 2020

with antifungal activity against phytopathogens of *Aspergillus* fungi, 23 species of *Penicillium*, 25 species of *Fusarium*, 30 species of *Alternaria* and five species of *Curvularia* sp. (Turaeva et al. 2020, 2021).

Due to the relevance of this problem, the purpose of the research was to study the entomopathogenic microflora of groups of insects of the species *C. pomonella* common in apple orchards in the Tashkent and Fergana regions.

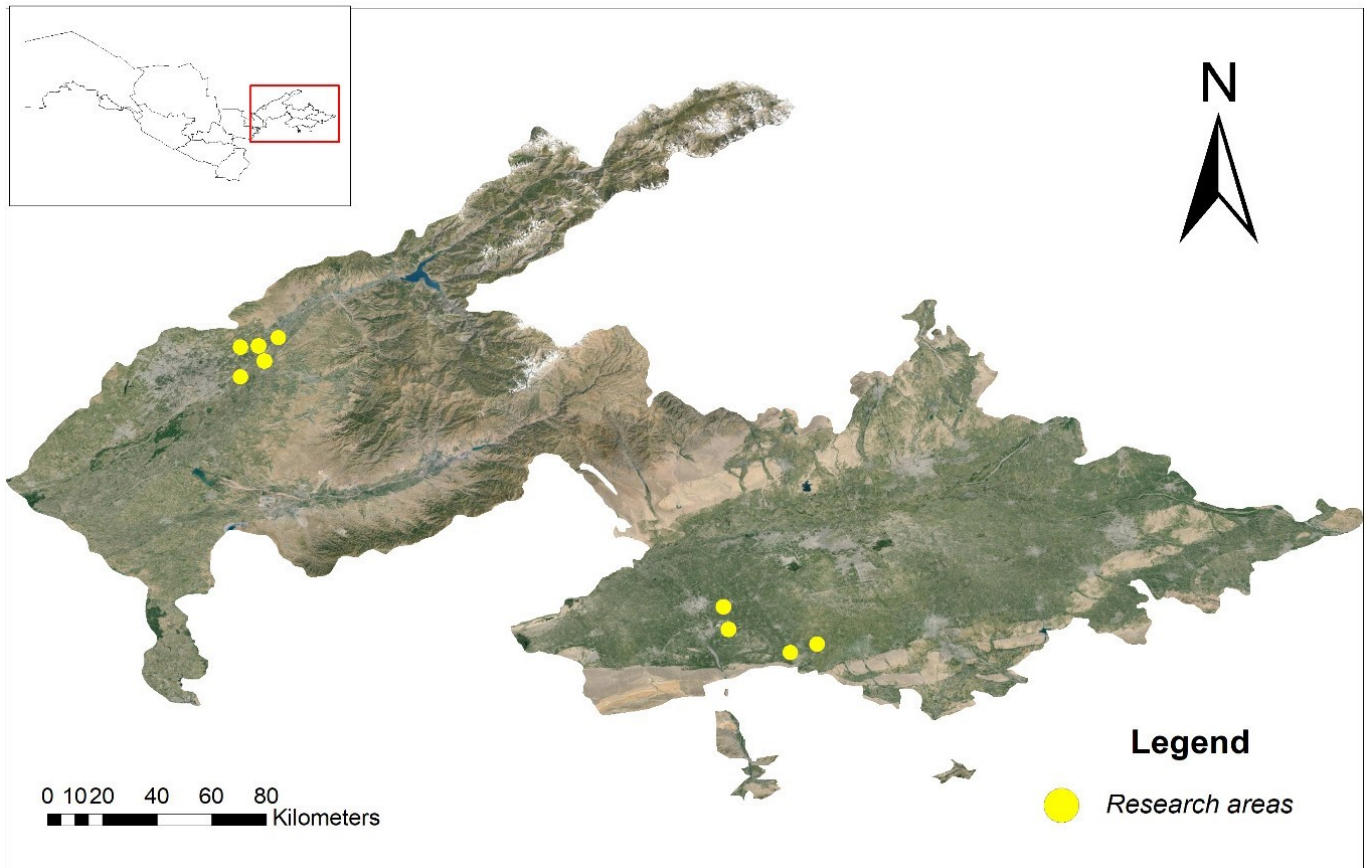
These studies were carried out in the laboratory “Ecology of entomophages and the theoretical foundations of biometod” at the Institute of Zoology of the Academy of Sciences of the Republic of Uzbekistan. The research objects were studied within the framework of the state research program on the topic “Biological basis for controlling the number of insects”.

## Materials and methods

The collection of insects was carried out during the period June–July 2022–2023 in apple orchards growing in the Rishtan and Uchkuprik districts of the Fergana region and in the Kibray district of the Tashkent region of Uzbekistan. (Fig. 1). The insect species *Cydia pomonella*, which belongs to the genus *Cydia* Hübner, 1825, family Tortricidae, was selected for the study.

The object of research was isolates of entomopathogenic microorganisms isolated from insects, the codling moth, that died in natural conditions. One apple orchard was selected from each district. Specimens of insects that died in natural conditions were collected from five apple trees. The determination of the prevalence of insects and the species of insects was determined by the method (Medvedev 1978, Rakhmonova 2018). To select the insect, the trapping belt method was used. To do this, the burlap was cut into a wide strip about 20–25 cm wide and tightly tied to the trunks of apple trees. Under unfavorable conditions, insects fell into the intended trap. Then the trapped insect carcasses were delivered to the laboratory by separate objects in sterile cups. Insects were examined under a stereomicroscope (40×) brand SMZ-161-T1. Insect carcasses were collected and first subjected to surface sterilization with 70% ethanol for 3–4 minutes, after which they were washed 3 times with sterilized water. Specimens were placed in saline solution 0.7%. Afterwards, suspensions were prepared from the insect larvae using the washing method and sown on Czapek Agar g/l nutrient media: KH<sub>2</sub>PO<sub>4</sub> – 1.0; MgSO<sub>4</sub>–0.5; NaNO<sub>3</sub> – 3.0; KCl – 0.5; FeSO<sub>4</sub> – traces, sucrose – 20g/l; and starch dextrose agar. Insect homogenization was also sown on nutrient media, which was prepared by rubbing the carcasses of larvae. The cups were cultivated at a temperature of 28°C for 3–6 days in a high-precision incubator of the DH210L brand. Next, a quantitative count of the grown colonies of microorganisms was carried out and the strains were screened out onto slanted nutrient media. Identification of the isolated strains was carried out according to morphological and cultural characteristics on a binocular microscope brand BS203 (with a USB camera MICDC MOS 5mp (zoom 400x) according to those specified in the keys (Pidoplichko and Milko 1971; Litvinov 1967; Bilay 1982; Sokirko et al. 2014). To identify microorganisms, a mass spectrometer (MALDITOF) was also used in the sanitary and hygienic laboratory at the Ministry of Health of Uzbekistan.

The work used standard guidelines and generally accepted methods of microbiological analysis (Tepper et al. 2004).



**Figure 1.** Areas where *Cydia pomonella* species have been collected.

## Results and discussion

As a result of research using the trapping belt method, 20 sick and dead insects of the species *C. pomonella* were selected from natural populations of orchards. Microbiological culture was carried out from the selected carcasse specimens. After treatment with 70% alcohol and 3% hydrogen peroxide and washing with distilled water, the specimens were sown under sterile conditions on previously prepared nutrient media and the thermostat was placed at 28°C (Fig. 2). Microbiological inoculation was carried out using the suspension inoculation method, and option 2 was ground in a mortar and larvae were shown on Czapek and CZA nutrient media. On the third and sixth days of growth, the formation and development of a colony of micromycetes was studied. Isolated pure cultures of micromycetes were screened out from the nutrient medium (potato dextrose agar with 1000 ml of water, a decoction was prepared from 200 g of washed and peeled potatoes and 20 g of dextrose, 20 g of agar powder were added, pH value 6,  $0 \pm 0.2$ ).



**Figure 2.** Sampling of insects using the trapping belt method.

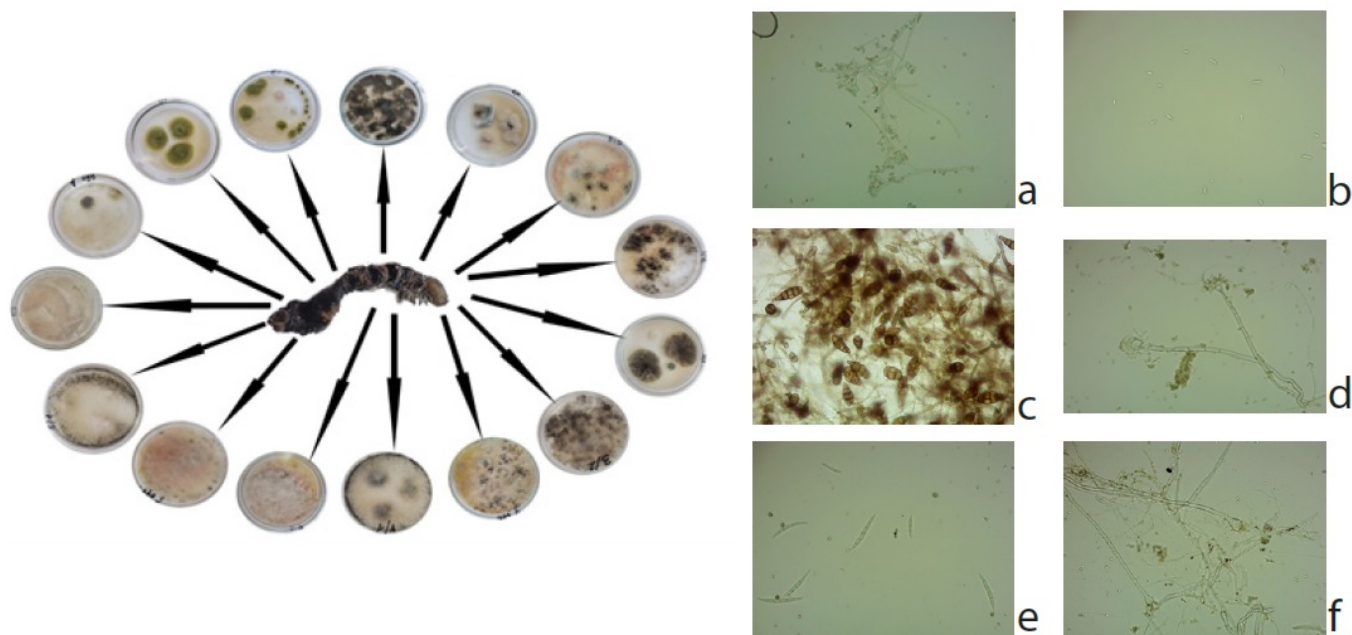
A total of 30 specimens of codling moth insects were selected for the experiment, each of which was collected from 5 apple orchards. Individuals of the 4th age of the codling moth caught on the hunting belt were examined for the presence of entomopathogenic microflora. As a result of research, 40 strains of micromycetes were isolated for the first time from insect specimens from selected gardens in Tashkent and Fergana. In morphological and cultural studies, entomopathogenic micromycetes in dead and infected *C. pomonella* individuals belong to the genera *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*. As a result of microbiological research from the carcasses of insects of the species *Cydia pomonella*, micromycetes belonging to 7 species and 4 genera were identified. Fungi of the species *Alternaria alternata* (Fr.) Keissl., *Fusarium solani* (Mart.) Sacc., *Asperillus niger* Tiegh. were isolated from insect suspension specimens, and when sowing the homogenate, fungi of the species *A. niger*, *A. fumigatus* Fresen., *A. terreus* Thom., *F. oxysporum* Schltdl. and *Penicillium chrysogenum* Thom., *A. alternata* were found (Fig. 3). The number of species in the specimens varied from 1 to 4 species. Fungi of the genus *Aspergillus* dominated the research, with *Fusarium* and *Alternaria* being frequently encountered.

	Entomopathogenic micromycetes	Fergana region		
		Tashkent region Kibray district	Rishtan district	Uchkuprik district
1	<i>Aspergillus</i> sp.	6	5	4
2	<i>Penicillium</i> sp.	4	2	3
3	<i>Fusarium</i> sp.	4	3	2
4	<i>Alternaria</i> sp.	4	2	2

**Table 1.** Entomopathogenic micromycetes isolated from infected apple trees in the Tashkent and Fergana regions

Analysis of the epiphytic microflora of sick and damaged insects of the genus *C. pomonella* showed that the epiphytic microflora of the test samples includes the genus *Aspergillus* 15, *Penicillium* sp., 9 isolates, *Fusarium* sp., 9 micromycetes of the genus *Alternaria* sp. 8 micromycetes. These micromycetes belong to the Aspergillaceae family (Table 1).

During the study period, the largest number of microorganisms from the three studied apple orchards was identified in the Kibray district of the Tashkent region.



**Figure 3.** Microflora of codling moth insects in apple trees: a - *Penicillium chrysogenum*, b - *Fusarium oxysporum*, c - *Alternaria alternata*, d - *Asperillus terreus*, e - *Fusarium solani*, f - *Asperillus niger*.

## Conclusions

At the present stage, fruit growing is one of the priority areas of the agricultural development strategy of Uzbekistan. The climatic conditions of the republic allow growing fruit and berry crops. The main harm to fruit crops, including apple and pear trees, is caused to disease of fruit trees by the codling moth. Moreover these pests leads as well as the lowing quality of the crop production. One of the main methods of pest control is the use of biological preparations based on microorganisms. According to literary data, it is known that metabolites with insecticidal action have been identified in endophytic and phytopathogenic fungi. The results we obtained will serve as the basis for the next stage of research on the study of the insecticidal properties of the isolated micromycetes. The selected strains of micromycetes will be examined by biotesting methods for the study of insecticidal properties. Also, the toxicology and safety of the strains will be determined.

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