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Cultural and morphological features of the entomopathogenic fungus *Beauveria bassiana* isolated from the Colorado potato beetle

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Annotation. The article examines the cultural and morphological features of the entomopathogenic fungus Beauveria bassiana, isolated from the Colorado potato beetle, the ways of penetration into the body of the insect and, as a consequence, the physiological processes occurring in it.
Keywords: fungus, enzyme, toxin, Beauveria bassiana, Colorado potato beetle, apressor, gifa, spore, chitinase, mycelium.

Introduction. Currently, scientific work on entomopathogenic fungi is being recorded at a high rate in the world. Most of them are devoted to the biology of highly specialized representatives of the anamorphic *Metarhizium* and *Beauveria* families [1].

Entomopathogenic fungi are common in terrestrial ecosystems and cause epizootics in insect populations. There are more than 2,000 species of entomopathogenic fungi in the world, among which *Ascomycota* is the leader in the number of representatives of the family. many species of entomopathogenic fungi are actively studied and applied to regulate the number of pests in all countries of the world. Their advantage over other biological control agents is that these pathogens have a contact effect, which allows them to be used against agricultural pests. Entomopathogenic fungal-based preparations can be used indoors and outdoors at a time that is conducive to fungal growth. Entomopathogenic fungi produce toxins. Toxins of the fungi *Beauveria, Metarhizium, Aspergillus, Penecillium* are well studied and described. Cyclopeptide toxins are the most common. In addition, entomopathogenic fungi produce enzymes. *B. bassiana* fungus produces proteases that are a necessary component of the infectious process [2; 3].

B. bassiana fungus produces other toxins, particularly bassianin, bassiacridine, bassianolid, boverolides, tenellin, and oosporeins. In addition to their effect on insects, some fungal toxins have antifungal and antibiotic effects, such as oosporein toxin [2].

B. bassiana (Bals.) Vuill. The entomopathogenic fungus was first identified in 1835 by Bassi de Lodi in a silkworm.

B.bassiana (Bals.) Vuill. entomopathogenic fungi occur in a variety of ecosystems, ranging from temperate regions to tropical and equatorial zones [7].

The optimum temperature for the development of *B. bassiana* fungus is 20-26 °C, and the temperature range for growth is 5 and 36 °C. These pathogens cause disease in various insects. These are Lepidoptera, Hymenoptera, Coleoptera, Diptera, Orthoptera and others. Infected insects are covered with white or cotton-like mycelium. Insects are prone to infection at almost all stages of development [5].

Numerous studies have shown that *B. bassiana* fungus is highly effective in protecting potatoes from the Colorado potato beetle [4].

Wraight S.P., Ramos M.E. [9] studies have found that chemicals are more effective than biological drugs against Colorado potato beetle larvae. The mechanism of action of biological drugs is slow, and their use is characterized by weather conditions. Although the effectiveness of the *B. bassiana* fungus against the Colorado potato beetle is lower than that of the chemical drug, it has been observed that it is harmless to plant yields, environment, entomofauna, and the human body.

The pathogenic *B.bassiana* fungus occurs in large quantities in soils under natural conditions. This fungus naturally controls various stages of the Colorado potato beetle, but it has been found that its eggs are not prone to infection by the *B.bassiana* fungus [6].

Research location and work methods. The research was conducted in the laboratory of the Department of Plant Protection of Tashkent State Agrarian University and the Institute of Microbiology under the Russian Academy of Sciences. Methods widely used in microbiology, mycology and entomology were used in the conduct of scientific research.

The distribution of the Colorado potato beetle in the potato crop was carried out on the basis of the methods given by Sh.T.Khojaev [11]. Isolation of pure cultures of entomopathogenic fungi from natural substrates V.I.Bilay and N.Yu. Geshtovt [13,14], their cultivation T.I. Gromovyx [15] and study of the influence of environmental factors on them V.M.Goral [16] methods. Identifiers A.A.Evlakhova, E.Z.Koval, M.A.Litvinov, N.M.Pidoplichko [17,18,20,10] were used to identify species of entomopathogenic fungi. The methods of N.L. Sevnitskaya and A.O. Sagitov [19,12] were used in the selection and propagation of nutrient media for the cultivation of selected strains.

Mature breeds and larvae of the Colorado potato beetle were collected, and those who died from the disease were subjected to microbiological analysis in the laboratory. It was observed that the Colorado potato beetle, which has symptoms of fungal disease, is hard, the upper part of the body is covered with fungal mycelium. The upper body of the Colorado potato beetle was sterilized with 96.0% alcohol. Colorado potato beetle infected with fungal diseases was placed on a Petri dish with moist special paper (wet chamber) and grown at room temperature. To isolate microorganisms from infected and killed Colorado potato beetle, pure specimens were isolated from them grown in Chapek agar nutrient medium. Necessary solutions were prepared from biological materials and fungal species were identified using a binocular microscope brand NLCD-307B. The species composition, cultural and morphological features, biological properties of the grown samples were studied.

Research results. *Beauveria bassiana* Vuill from biological materials collected in the laboratory. the fungus was isolated. *B. bassiana* is an immature fungus belonging to the family *Cordycipitaceae,* the fruit does not form bodies, it is only in the form of mycelium. The bark is white to pale white in color and has a powdery appearance. The hyphae are thin, branched, the branches are opposite, not obstructed, 0.12 μm wide, colorless. Spores are composed of fine sterigmas, oval, 0.15 μm wide, 0.22 μm long, see Fig. 1.

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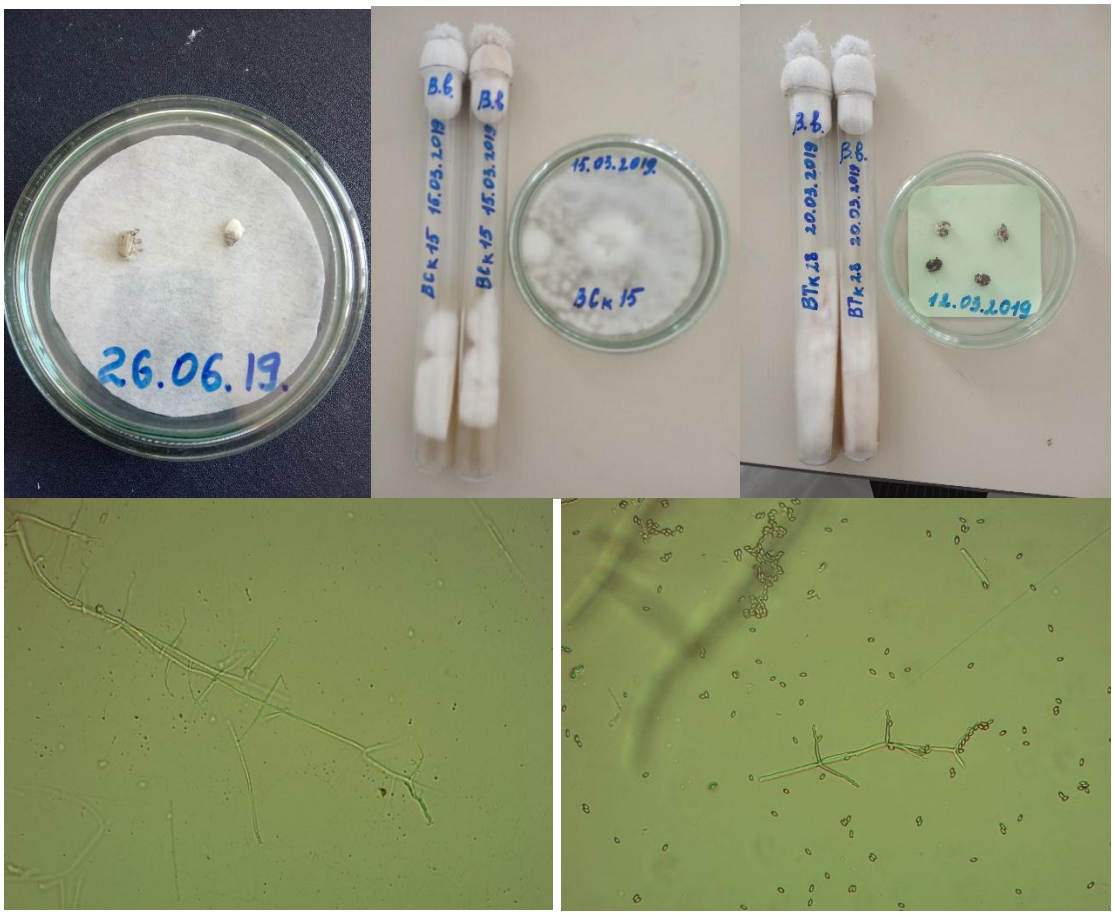

Figure 1. *B. bassiana* fungus isolated from the Colorado potato beetle and its microscopic appearance

There are also many species of *B. bassiana* with anamorphic (asexual reproduction) and telemorphic (sexual reproduction) characteristics. It causes white muscarinic disease, which is caused by a fungus in insects. When a fungus enters an insect's body, it grows into the cuticle and kills the insect within a few days.

B. bassiana entomopathogenic fungus enters the body of insects in different ways. Fungi have been found to enter insects through hole holes in the cuticle, sclerites, inter-segmental areas, intestines, as well as spirals or genital openings.

Initially, hydrophobic bonds form between the cuticular lipids and the fungal cell membrane, resulting in the formation of spores. Once fungal spores are formed in insects, they begin to develop. High relative humidity is of great importance at this stage, which is necessary for spore germination and further development of the fungus. The spore becomes a growing tube and apressor formation begins at the site of contact with the cuticle. Apressor is actually a combination of physical strength and lytic enzymes, which significantly facilitates the entry of the pathogen into the interior of the insect. The apressor is then divided. The *B. bassiana* entomopathogenic fungus synthesizes a number of enzymes to penetrate more through the deeper layers of the cuticle. Under the influence of enzymes, cavities are formed in the epicuticle, into which a tube growing from the apressor enters. Once it reaches the procuticle, the penetration tube grows parallel to the lamellae and forms a penetration plate, from which the hyphae begin to spread. The hydrolytic enzymes of the *B. bassiana* fungus formed mainly lipases, proteases, and chitinases. Initially, the breakdown of cuticular proteins occurs under the influence of proteases, resulting in the release of chitin. In parallel, the activity of fungal chitobases and chitinases increases, which hydrolyze chitin to N-acetylglucosamine. In addition,

the activity of fungal chitinases increases, which leads to an increase in “hydrolytic attack” from the pathogen under the influence of pathogenic hyphae and perforation of the exocuticle. In addition, fungal lipases of the already lipid-rich endocuticle are activated. As the fungus grows in insects, melanotic spots appear from the cuticle, which are formed as a result of the onset of prophenol oxidase in the insect’s body. Through the cuticular layers and hypodermis, the fungus enters the hemocoel of the insect. In the hematopoiesis is formed its further development and colonization of internal organs. The fungus first affects the oily body, then the intestines, Malpighian tubes, hypodermis, nervous system, muscle tissue, trachea. After some time, at a sufficiently high humidity (90-100%) fungal hyphae begin to grow on the surface of the insect body in the opposite direction. The hyphae then rupture the cuticular integral and form a conidia. Later, a white form emerges from the dead insect, forming new spores, which grow like white mold. Forms dry, powdery conidia in distinctive white spore balls. Conidia are single-celled haploid and hydrophobic.

Conclusion. *B. bassiana* (Bals.) Vuill entomopathogenic fungus is one of the natural relatives of the Colorado potato beetle. In the local agricultural soils, *B. bassiana* fungus is common, causing natural epizootics in the potato agrobiocenosis where the Colorado potato beetle is widespread. In addition, the fungus *B. bassiana* produces the enzyme chitinase. They hydrolyze chitin to N-acetylglucosamine. In addition, the activity of fungal chitinases increases, which leads to an increase in "hydrolytic attack" under the influence of pathogenic hyphae and perforation of the exocuticle. Therefore, *B. bassiana* fungus has been found to be effective against many pests belonging to the Coleoptera family.

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