

Natural Occurrence Entomopathogenic Fungi: *Beauveria Bassiana* and *Metharizium Anizopliae* from the Population of Colorado Potato Beetle, *Leptinotarsa Decemlineata* (Coleoptera: Chrysomelidae) in Georgia

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Abstract

The paper demonstrated the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: hrysomelidae) its natural enemies such as entomopathogenic fungi. Six isolates were obtained from larvae and adults of the population of *L. decemlineata*, at the different geographical and climatic zone of potato production. Morphological studies show that five isolates belong to the genus of *Beauveria* and one isolate of *Metharizium*. Microscopic observation of colony growth and conidia arrangement showed general and typical characteristics of *Beauveria bassiana* in five isolates and one isolates *Metharizium anizopliae*. Distribution of EPF among the insects, horizontal transmission, is a critical issue for the control of *decemlineata*. Experimental results show that adults of the *L. decemlineata* were infected with a fungal disease as a result of movement in the soil and spread naturally among them. The beetles moved on the surface of the soil, as well as deep into the soil. On the 5th day few beetles appeared infected with mycosis; on the 9th day their number increased and infected beetles achieved around 50%; Finally, on the 14th day all the beetles were infected and their mortality reached 100%. The identification and investigation of mycosis infection in the population of Colorado potato beetle, which has developed resistance to many insecticides used, is important to reduce economic losses and save the environment from pollution and is an important pathogen that can be used for the control of this insect.

Keywords: Colorado Potato Beetle, *Leptinotarsa Decemlineata*, Entomopathogenic Fungi, *Beauveria Bassiana*, *Metarhizium Anisopliae*, Biological Control, Potato Pest Management.

Introduction

Potatoes are one of the most important staple foods in the world after wheat and rice and the third largest crop in the world in terms of human consumption [1]. In Georgia, the potato is the most widely grown crop in vegetable production [2]. A wide range of insects can damage potatoes directly by feeding on the tubers and spoiling the crop or indirectly by feeding on leaves or stems. Tuber yield losses due to insects are estimated at 75% without crop protection [3]. The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: hrysomelidae), is a serious pest of potato (*Solanum tuberosum* L.) in the USA and

Europe, as well as in Asia [4-5]. The first considerable damage to the potato was reported in Colorado, USA [6]. By the end of the twentieth century, the pest had spread throughout Europe and Asia and continued to expand its geographic range to new regions of the world (Wang et al. 2017). In Georgia, the pest was first discovered in 1965 in Gagra (Abkhazia), [7-9]. The beetle survives by feeding on cultivated and wild solanaceous plants, like potato, eggplant, and tomato Both larvae and adults of CPB damage the leaves of potato plants. However, the main damage is caused by larval feeding [10]. At present, for the control CPB various chemical insecticides were used. The rapid development

of resistance to various chemical insecticides requires the development of approaches to the control of *L. decemlineata* populations using natural enemies. The most widely used biological agents in biological control are entomopathogens [11]. Among them, entomopathogenic fungi (EPF) especially infects the pest through integument, their ease of production and high adaptability make them potentially more useful than the others. EPF has three mechanisms of action including causing mechanical damage to tissues and producing toxic metabolites. In this way, they can cause the direct death of insects or weaken them, and limit their vital activities [12]. The widely distributed entomopathogenic hyphomycete *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) has been studied for microbial control of CPB [13-16]. *B. bassiana* shows efficient control against adult and all larval stages of CPB and provides an extremely high level of control during the potato growing season because it can continue to reproduce after application. However, its pathogenicity is affected by abiotic factors such as moisture, sunlight, and ultraviolet rays [17-18]. The objectives of this research were to find and identify an entomopathogenic fungus, spread in the population of *L. decemlineata*, at different geographical and different zones of potato production.

Material and Methods

Collection of insects

Adults and larvae of *L. decemlineata* (I-V instars) were collected from four different geographical areas at different altitudes (600-1200 m a.s.l), potato fields: Kumisi, Tetrackaro, Bulachauri, and Tianety, 2023-2024.

Isolation of Entomopathogenic Fungi

Single insects with the symptoms characteristic of entomopatho-

genic fungus have been transferred in the laboratory for the detection of fungi strains. Adults and larvae of CPB were placed in moisture chambers to develop fungi mycelium. Fungi isolated from the insect cadaver were cultivated on artificial media and incubated at $23 \pm 2^\circ \text{C}$ for 12-15 days.

Colony Growth and Morphology

Colony descriptions and measurements were determined from cultures grown on full-strength potato dextrose agar (PDA) (DifcoTM) on Petri dishes (\varnothing 90 mm). The plates were incubated at 25°C in the dark at $23 \pm 2^\circ \text{C}$ in darkness from 10-14 day from inoculation. Terms and notations are used to describe colony coloration, hyphae, and conidia [19-21]. Microscopic measurements of conidiogenous cells and conidia were taken from PDA cultures at 5–15 days and images were acquired with a light microscope. Terminology for conidial shape follows hyphae and conidia measurements were taken [22].

Horizontal Transmission of *Beauveria Bassiana*

Experiments were conducted to determine the biological activity and distribution infection of the fungus *Beauveria bassiana* to the adult of the Colorado potato beetle. In the glass dishes (volume liter), 1/3 volume with moisture soil, were placed 30 adults of CPB, in each other container, with replications.

Results and Discussion

Identification of Entomopathogenic Fungi

The samples were obtained from 4 different geographical sites at different altitudes (600-1200 meters above sea level (m a.s.l.) and climate zones, each representing a unique agricultural ecosystem in Georgia. In nature, we found infected larvae and adults (Figure 1) which were transferred to the laboratory for the study.



Figure 1: Occurrences infected larvae and Adults of *L. decemlineata* in nature: a - larvae with *Beauveria bassiana*; b – beetles with *Beauveria bassiana*; c – adult with *Metharizium anizopliae*

Five isolates of *Beauveria bassiana* and one isolate of *Metharizium anizopliae* were obtained from larvae and adults of *L. decemlineata*, as described in Table 2.

Table 2: Sites of investigation of *Leptinotarsa decemlineata* in cultivated potato field

Name of isolates	Name of sites	Geographical location (lat. N, long. E)	Altitude	Name of isolates	year
CPB 1	Kumisi	410 61' 36", 440 78' 21"	950	<i>B. bassiana</i>	2024
CPB 2	Tetrackaro	410 55' 22", 440 48' 18"	1000	<i>B. bassiana</i>	2024
CPB 3	Shovi, GCM**	420 35' 36", 430 00' 88"	1800	<i>B. bassiana</i>	2023
CPB 4	Shovi, GCM	420 23' 76", 430 10' 13"	1650	<i>B. bassiana</i>	2023
CPB 5	Shovi, Glola, GCM	420 33' 36", 430 00' 58"	1730	<i>B. bassiana</i>	2023
CPB 6	Shovi, Glola, GCM	420 44' 07", 430 77' 01"	1780	<i>M. anizopliae</i>	2023

*LCM- Lesser Caucasian Mountain; GCM**- Grate Caucasian Range

Morphological study of fungal isolation

Colony growth characteristics and appearance are similar among the majority of *Beauveria* species. All six isolates were cultured on full-strength Potato dextrose agar (Ø 90mm) at $23 \pm 2^\circ$ C for 12-15 days grows average 10–40 mm diam. The five cultures appear as white to light yellow colonies having a cottony, powdery, velutinous, and woolly texture that frequently becomes farinaceous as conidia accumulate on the surface of aging cultures.

One isolation was a different color, beginning with cream-yellow, and later green.

Microscopic observation of colony growth and conidia arrangement showed general and typical characteristics of *Beauveria* in five isolates. Five species of *Beauveria bassiana* and one species of *Metarhizium anizopliae* were identified. Morphological characterization of *Beauveria* species is given in Table 2.

Table 2: Morphological characterization of isolates *Beauveria bassiana* and *Metarhizium anizopliae*

Species	Colony description	Colony size (Ø mm)	Size of conidia (µm)	Form of conidia
<i>Beauveria bassiana</i> (CPB 1, CPB 2, CPB 3, CPB 4, CPB,5)	cottony, powdery, velutinous, white or yellow white appressed to the agar surface	13–30	(1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5 (- 3.0)	globose, sub- globose, broadly ellipsoid forming short chains
<i>Metarhizium anizopliae</i> (CPB 6)	sub velutinous, velutinous to cottony, closely appressed to agar surface dark green	14–34	7.5-10.2 x 4.0–4.5 12.5-15.0 x 2.5-3.0	elongated ellipsoidal to cylindrical

Colony of *M.anizopliae* growth on full-strength PDA 14–34 mm diam. at 12 days at 23oC. Surface mycelium sub velutinous, closely appressed to agar surface, at the s yellowish green to

dark green. Conidia aggregated as, 0.1 mm spherical clusters, white in mass, conidia occasionally. Forming a farinaceous sur-

face layer in older cultures. The colony reverses a dark green color. The conidia, which were oval (n = 30), varied 9.0 (7.5–10.2) µm in length and 4.3 (4.0–4.5) µm in width. The matured spores are commonly elongated ellipsoidal to cylindrical, with dimensions 12.5-15.0 x 2.5-3.0 µm (Figure 2, c,d).

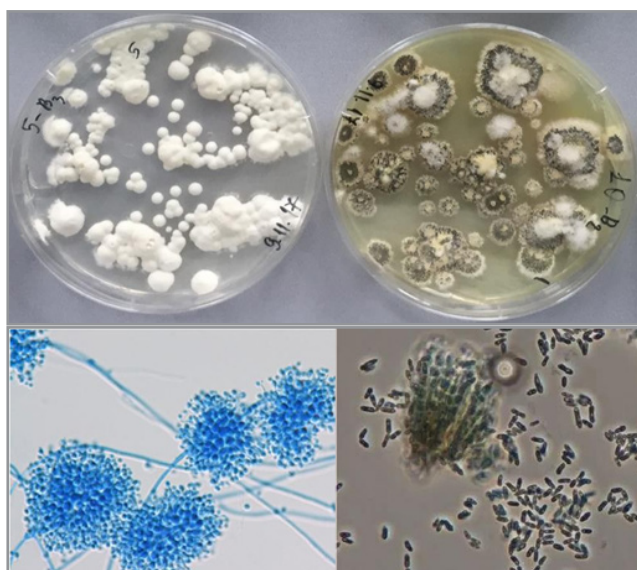


Figure 2: Isolates entomopathogenic fungi from *L.decemlineata*: a - colony of *B.Bassiana*; b – spores of *B.Bassiana*; c-colony of *M.anizopliae* ; d – spores of *M.anizopliae*.

Both fungi are known to produce cuticle-degrading enzymes that facilitate percutaneous infection of the host insect without the need for oral consumption by the target organism [23]. Fungi exhibit qualities that can make them ideal for this infection strategy including a mild-acting nature, the ability to self-replicate, and the ability of fungal conidia to be spread by adults of

L.decemlineata [24-25]. Distribution of EPF among the insects is a critical issue for the control of *L.decemlineata*. Experimental results show that adults of the CPB were infected with a fungal disease as a result of movement in the soil (Figur 3). and spread naturally among them.

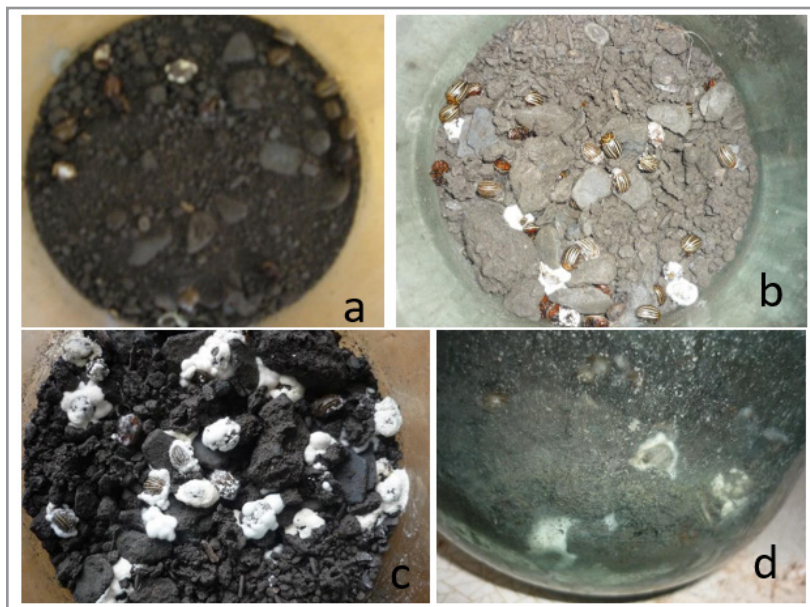


Figure 3: Horizontal transmission of mycosis caused by *B.bassiana* among adults of *L.decemlineata*: 5 days; b – 9 days; c - 14 days; d – infected insects in the soil.

The beetles moved on the surface of the soil, also they were moving deep into the soil. On the 5th day of the beginning experiment, appeared single beetles infected with mycosis, On the 5th day of the experiment, single beetles infected with mycosis appeared, and on the 9th day their number increased and infected beetles achieved around 50%, Finally, on the 14th day all the beetles were infected and their mortality reached 100%.

Conclusion

The identification and investigation of mycosis infection in the population of Colorado potato beetle - *L.decemlineata*, which has developed resistance to many insecticides used, is especially important to reduce economic losses. It has been shown that *Beauveria bassiana* and *Metarhizium anisopliae* isolated from naturally infected insects is an important pathogen that can be used for the control of these insects [26]. Subsequently, mycoses infection and its high efficiency were demonstrated in laboratory and pot experiments. In addition, the optimum condition for the occurrence of maximum pathogenicity of entomopathogenic fungus can be investigated.

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