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NEW DATA ON INSECTICIDAL ACTIVITY OF SOME NATIVE BACTERIAL AND FUNGAL STRAINS

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Abstract

A wide range of organisms, including insects, which can cause considerable losses, attacks tomato crop. Chemical insecticides continue to be used as the primary pest protection measure. The research aims to identify native strains of Bacillus thuringiensis and Beauveria bassiana to be used in the sustainable management of pests of tomato crops in the Republic of Moldova.
Based on conducted activities regarding isolating and characterizing bacterial strains, seven newly isolated strains were used in bioassay tests. Three other bacterial and ten fungal strains from the collection of the Institute of Zoology were tested against selected pests. Four experimental treatments were set up, two against adults and two against larvae of Leptinotarsa decemlineata. The bioassay tests against adults of Colorado potato beetle revealed two fungal and one bacterial strain with promising results for biological control. Low insecticidal activity was recorded against the larvae of potato beetle with the tested strains.

Key words*: Leptinotarsa decemlineata, Bacillus thuringiensis, Beauveria bassiana, native strains, insecticidal activity.*

INTRODUCTION

In the last centuries, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), known as the Colorado potato beetle, became a leading global pest, widely distributed through the Northern Hemisphere (Cingel et al., 2016). Feeding initially with a few wild hosts, the Colorado potato beetle is now causing severe attacks on a range of plants from the Solanaceae family. Although its' preferred host is potato (*Solanum tuberosum* L.), the tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.) are also essential hosts (Weber, 2003; Vargas-Ortiz et al., 2018). Defoliation by this voracious pest can cause 40-80% of yield losses (Hare, 1980; Alyokhin et al., 2013; Cingel et al., 2016).
Despite all the efforts to implement different agricultural practices to control the Colorado potato beetle, chemical insecticides continue to be used as the primary pest protection measure. However, using chemical insecticides to control the pests is not the best plant protection strategy, especially when dealing with *L. decemlineata*. First of all, due to its diverse life cycle, phenotypic plasticity, high adaptability, and capability to detoxify or tolerate toxins, the Colorado potato beetle has successfully overcome chemical pesticides (Cingel et al., 2016). Moreover, using pesticides creates many problems related to human safety. Also, it leads to the emergence of secondary pest outbreaks, environmental pollution, biodiversity reduction, and pesticide-resistant insects (Damalas & Eleftherohorinos, 2011).
The need to identify an alternative to chemical insecticides stimulated the interest in developing inoffensive methods for humans and the environment, bringing traditional and biological pest control methods back to researchers' attention. Biological control represents a complex of techniques employing some organisms to reduce other harmful organisms below the economic threshold based on natural mechanisms such as predation, parasitism, competition, etc. These involve biological control agents such as parasites, predators, viruses, fungi, and bacteria. Natural enemies of *L. decemlineata* can become a valuable part in the pest management programs. Entomopathogenic organisms are harmless to the farmers, consumers, as well to the environment, and their specific action minimizes the impact on beneficial organisms and other non-targeted organisms (Lacey &

Siegel, 2000; Hokkanen & Hajek, 2003; Mudgal et al., 2013; Kumar et al., 2021). Moreover, entomopathogens favor biodiversity and the natural control of arthropods by parasitoids and predators (Lacey et al., 2015).

Numerous species of bacteria and fungi have been isolated from pests (Jurat-Fuentes & Jackson, 2012; Kumar *et al*., 2021), but few have been used for biopesticide development. Among microbial control agents, the bacterium *Bacillus thuringiensis* Berliner (*Bt*) and fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. (*Bb*) (Kumar et al., 2021) are the most extensively used to control lepidopteran and coleopteran pests. Despite that, entomopathogens represent an underdeveloped and underutilized resource in arthropod pest management. Using more selective biopesticides supported by adequate habitat design can increase the enemies' impact on the Colorado potato beetle population (Cingel et al., 2016).

Considering the cost of biopesticides, the local production of biopesticides is significantly more affordable for farmers than importing plant protection products because the prices on the international market exceed their buying capacity (Sarwar, 2015). Also, using local strains as biological control agents can help avoid risks associated with decreased insecticidal activity due to environmental conditions. Thus, the research aimed to identify native strains of *Bt* and *Bb* for local production of biopesticides and promote sustainable management of *L. decemlineata.*

MATERIALS AND METHODS

Bacterial and fungal strains

Ten bacterial and ten fungal strains isolated from various hosts were used for pathogenicity tests against adult insects and larvae of *L. decemlineata* (Table 1). Some of the selected bacterial and fungal strains, *Bt* CNMN-BB-03, CNMN-BB-04, CNMN-BB-05, and *Bb* CNMN-FE-01, were previously described with high insecticidal activity against beetle pests *Neocoenorrhinus pauxillus* (Germ.), *Sitona lineatus* L., *Phyllobius oblongus* (L.), and *Tatianaerhynchites aequatus* L. (Munteanu et al., 2013; 2014a; 2014b). Some degree of activity against beetle species *Sitona lineatus* L. and *Hypera postica* (Gyll.) have shown the following fungal strains: Sl7, Sl9, Cg7, Cg10, Cg11, Cg12 (Moldovan et al., 2022), while others where not tested before (Table 1).

The *Bt* and *Bb* strains with high insecticidal activity are deposited in the Collection of Non-pathogenic Microorganisms of the Institute of Microbiology and Biotechnology, Technical University of Moldova. At the same time, the rest are preserved in the Collection of the Biological Invasions Research Center (BIRC), Institute of Zoology, Moldova State University.

Table 1. Data regarding bacterial and fungal strains used in the bioassay test

No.	Strain	Identity	Host	Reference
1.	CNMN-BB-03	*Bacillus thuringiensis*	*Neocoenorrhinus pauxillus* (Coleoptera)	Munteanu et al., 2013, 2014a, 2014b, Moldovan et al., 2017b
1.	CNMN-BB-04		*Phyllobius oblongus* (Coleoptera)	
2.	CNMN-BB-05		*Tatianaerhynchites aequatus* (Coleoptera)	
3.	S01	*Bacillus* spp.	*Helicoverpa armigera* (Lepidoptera)	Moldovan et al., 2018
4.	S02			
5.	S03			
6.	S04		*Spodoptera exigua* (Lepidoptera)	
7.	S05			
8.	S06			
9.	S07			
10.	CNMN-FE-01	*Beauveria bassiana*	*Sitona lineatus* (Coleoptera)	Moldovan et al. 2017a
11.	Sl7			
12.	Sl9			
13.	Cg7	*Beauveria* spp.		Moldovan et al., 2022
14.	Cg10			
15.	Cg11			
16.	Cg12			
17.	Hp2Cg	*Beauveria* spp.	*Hypera postica* (Coleoptera)	Moldovan et al., 2022
18.	Hp3Cg			
19.	Hp4Cg			

Culture media and growth conditions

For bioassay test bacterial strains were cultivated on solid T3 medium (Travers et al., 1987) at 30±0.2°C for 2-3 days in the darkness. Fungal strains were grown on PDA (Potato Dextrose Agar, Merck) at 25±0.2°C for 7-10 days, in the darkness, until complete sporulation. CFU and conidia were harvested using 10 µl sterile inoculation loop and transferred in tubes with sterile distilled water for inoculum preparation.

Insect rearing

For the bioassay purpose, a rearing colony was established from two wild populations of *L. decemlineata* collected in an infested tomato greenhouse (Orhei district) and a home garden (Causeni district) in the Republic of Moldova. No chemical or biological plant protection treatments were applied to control the wild Colorado beetle population. Sampled insects were placed in sterile cages with fresh tomato leaves and transported to the laboratory of BIRC, Institute of Zoology.

In the laboratory, insects were individually placed in new cages, fed with fresh tomato leaves, and monitored for three days to select only healthy specimens. By five healthy females and males were placed together in sterile insect-rearing cages for 24 hours for further insect rearing. After that, females were separated from males and kept individually for egg laying (Figure 1). Laid eggs were collected and stored in sterile Petri dishes until hatching. Healthy first-instar larvae and adults were taken for a bioassay test.

Bioassay test

Four bioassay experiments have been conducted to identify the most virulent bacterial or fungal strain. In the first and second experiments, eight bacterial strains and only one fungal strain (CNMN-FE-01, with known high insecticidal activity) were tested against both adults and larvae. In the third experiment, only fungal strains were tested; while in the fourth experiment, only bacterial strains were assessed. In the bioassay test, tomato leaves were used as a diet. At least ten insects and 2-5 leaves were used per repetition. Three repetitions were made for each tested strain. Sterile distilled water was used as a control. Insect mortality was recorded during ten days of incubation at 24°C, and a photoperiod of 14 h. Dead insects were removed from Petri dishes or cages every day.

1st experimental setup. Bacterial and fungal suspensions with a concentration of 10^8 CFU/ml were prepared. Fungal spore count has been conducted using a hemocytometer under 400X magnification of Meiji MT5000H microscope. The bacterial CFU count has been prepared by plate method. By 1 ml of each suspension was diluted with 4 ml of distilled sterile water, and the entire volume was sprayed over tomato leaves using a sterile hand-held sprayer. Leaves were left to air dry, and after that, ten adult insects (five males and five females) were transferred onto tomato leaves and left to feed for 24 h. After exposure, adults were transferred to sterile cages with fresh tomato leaf bouquets. Cages were inspected daily, fresh leaves were provided, and mortality data was recorded.

2nd experimental setup. Hatched larvae of *L. decemlineata* from collected eggs were transferred into sterile Petri dishes and fed with tomato leaves treated with bacterial and fungal suspensions as described earlier. After 24 h of exposure, insect larvae were transferred onto fresh leaves. Petri dishes were inspected and swapped daily. Also, fresh tomato leaves were provided. Mortality data was recorded daily.

3rd experimental setup. Following the performed activity of the fungal strain on adults of *L. decemlineata* species, in the 3rd experimental design, more fungal strains were tested. Fungal strains were applied topically by dipping each specimen in test tubes with 1 ml of conidial suspensions (10^7 conidia/ml) for 30 seconds and then transferring them to sterile cages with fresh tomato leaves.

In the *4th experimental setup*, the bacterial strains that performed the best and two additional *Bt* strains were tested against larvae of *L. decemlineata*. In this assay, bacterial suspensions (10^8 CFU/ml) were applied daily, leading to chronic larvae infection.

Statistical analysis

Obtained mortality data were corrected using the Abbott formula. The percentage of insects actually killed by the entomopathogenic bacteria or fungi was estimated by assessing the difference between the percentage of living insects in the control and the percentage of insects living after treatment, divided by the percentage of insects living in the control (Abbott, 1925). Results are presented as mean ± standard error of the mean.

Figure 1. Insect rearing under laboratory conditions.

RESULTS AND DISCUSSIONS

In the first trial, the best results were for one fungal strain *Beauveria bassiana* CNMN-FE-01 causing 50% mortality and two bacterial strains *Bacillus* spp. S03 and *Bt* spp. *kurstaki* CNMN-BB-04, generating the highest mortality respectively, on day seven after treatment (Figure 2). Thus, data on mortality corrected according to the Abbott formula was 50.00 ± 3.21%, 42.59 ± 4.90%, and 28.52 ± 3.29% for best-performing strains, respectively. No significant data regarding insect mortality was recorded for the other tested strains.

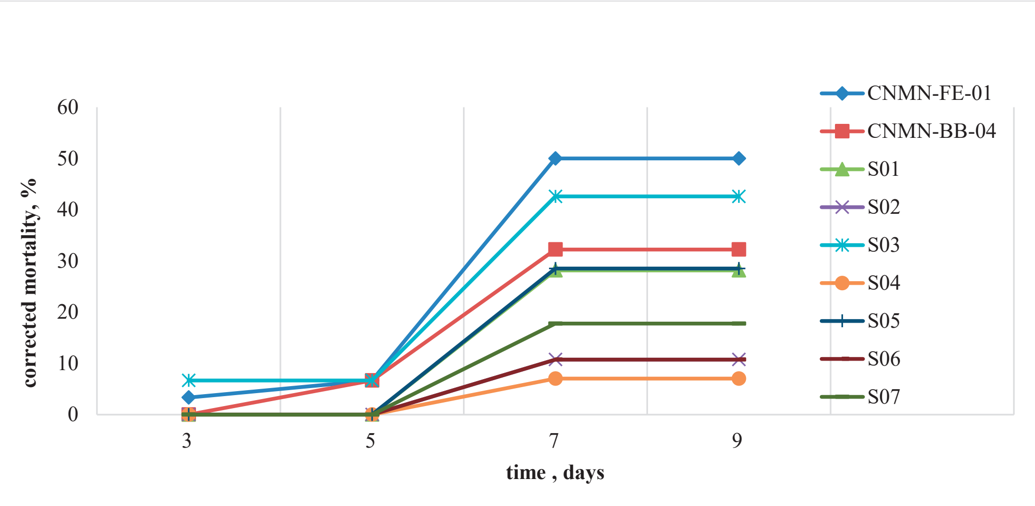

Figure 2. Cumulative mortality of *L. decemlineata* adults after exposure to bacterial and fungal strains. Data corrected according to Abbott formula

In the second experimental setup, larvae obtained from collected eggs were transferred to sterile Petri dishes and fed with tomato leaves treated with bacterial and fungal suspensions. No significant data regarding larvae mortality was recorded, the highest mortality being 33.33 ± 3.33% for *Bt* CNMN-BB-04 and *Bacillus* sp. S03 strains on day 9 after treatment (Figure 3).

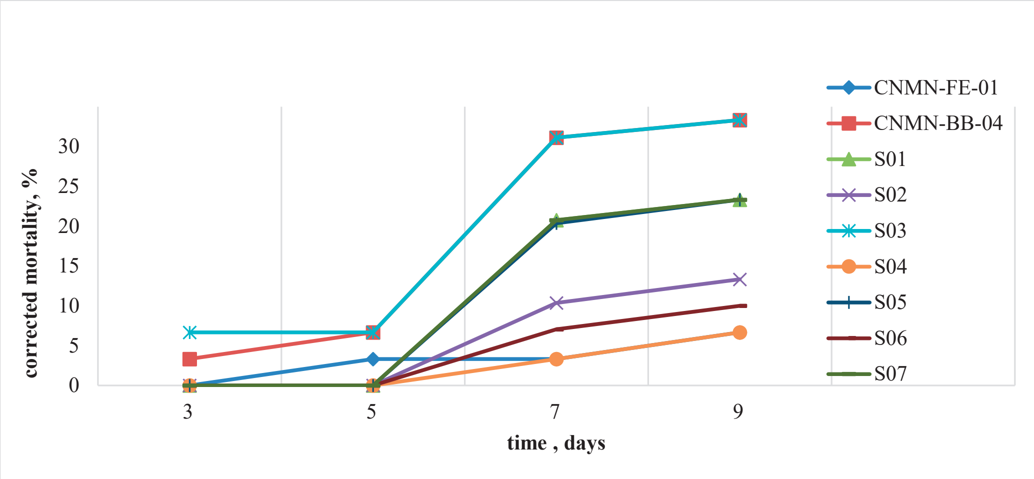

Figure 3. Cumulative mortality of *L. decemlineata* larvae after exposure to bacterial and fungal strains.
Data corrected according to Abbott formula

In the third experimental setup, fungal strain Hp2Cg showed significantly high virulence, causing 53.33 ± 3.33% mortality on day 5 and 100% mortality on day seven after treatment (Figure 4). It was also noticed that insects stopped feeding from day two after treatment compared to the control variant, where insects were feeding actively and laying eggs. Fungal strain CNMN-FE-01 showed entomopathogenic activity with 53.33 ± 3.33% mortality on day seven after treatment. In comparison, the other eight strains have shown very low insect mortality, with values ranging from 33.33 ± 3.33% on day seven after treatment (Figure 4).

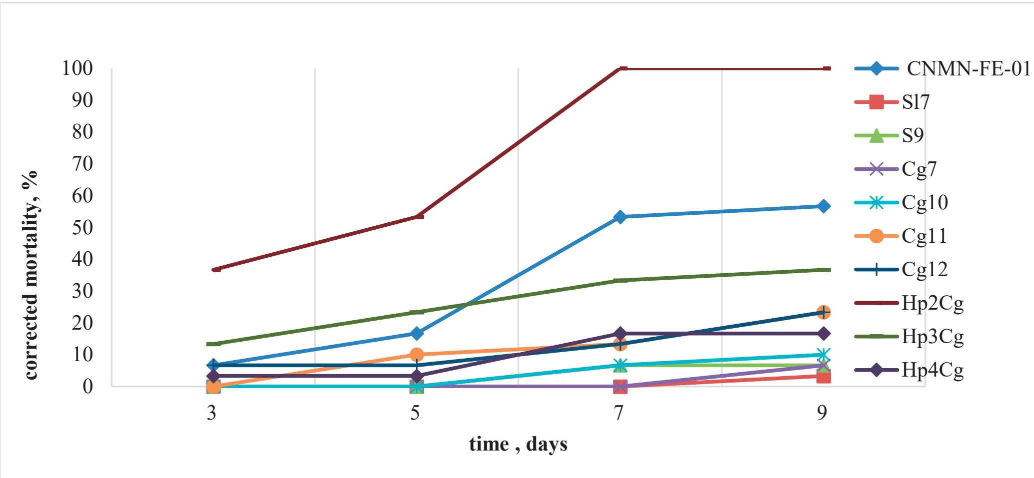

Figure 4. Cumulative mortality of *L. decemlineata* adults after exposure to fungal strains.
Data corrected according to Abbott formula

In the fourth experimental setup, two bacterial strains, that performed the best in previous experiments, *Bt* CNMN-BB-04 and *Bacillus* sp. S03, and two additional *Bt* strains CNMN-BB-03 and CNMN-BB-05 were tested against larvae. Bacterial suspensions were applied daily, leading to chronic infection of larvae. Bacterial strains *Bt* CNMN-BB-05 and *Bacillus* sp. S03 has shown the best results, with 51.48 ± 4.55% and 41.11 ± 4.84% mortality recorded on day nine after treatment (Figure 5).

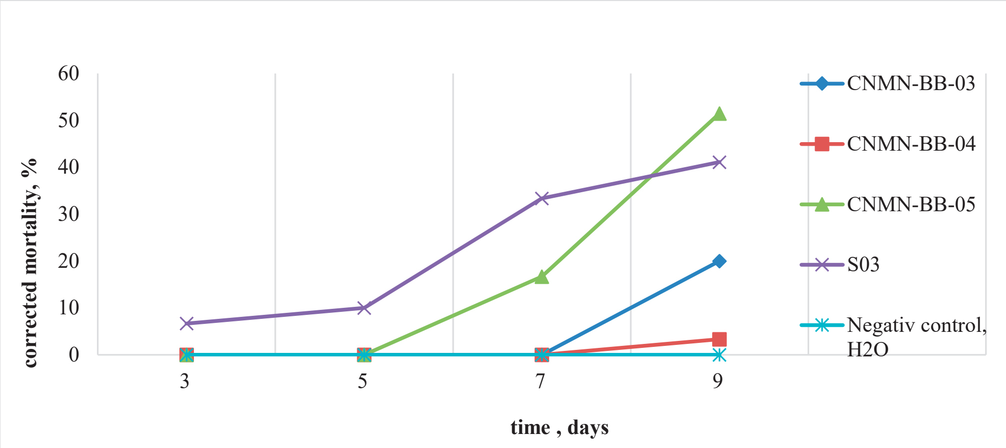

Figure 5. Cumulative mortality of *L. decemlineata* larvae after exposure to bacterial strains. Data corrected according to Abbott formula

In recent years, the scientific community has shown an increased interest in assessing entomopathogenic fungi against Colorado beetles. *Isaria fumosorosea* strain CCM 8367 caused high mortality against Colorado beetle larvae under laboratory conditions (Hussein *et al.*, 2016). Treatment of potato leaves with *Beauveria bassiana* significantly reduced Colorado beetle populations (Wraight & Ramos, 2015). LC50 values were equal to 10^5-10^6 spores/ml in larvae and 10^7-10^8 spores/ml in adults (Duan et al., 2018). New active strains of *B. bassiana* were recently reported from Turkey, causing mortality rates of 96.7 and 100% in larvae at a concentration of 1×10^7 conidia/ml (Baki et al., 2021). Also, highly active strains of *B. bassiana* were reported from the Czech Republic, and mortality of *L. decemlineata* adults caused by new native strains isolated reached up to 100% when beetles were treated with conidial suspensions at a concentration of 1×10^7 spores/ml. Authors recorded a LT50 equal to about seven days (Zemek et al., 2021). The present study reports similar results; the performing strain *Beauveria* sp. Hp2Cg has caused 100% mortality on day seven after treatment, at the same 1×10^7 conidia/ml concentration and the same application method by dipping the insect into conidial suspension. In their work, Zemek et al. (2021) focused on isolating novel native *Bb* strains from cadavers of Colorado beetle. Novel native strains had higher insecticidal activity than the GHA strain, the active ingredient of mycopesticide BotaniGard® WP. The present study was aimed at screening the local collection of *Beauveria* spp. and *Bacillus* spp. strains regarding their insecticidal potential. Strains that have shown some degree of activity were isolated from various hosts. *B. bassiana* strain CNMN-FE-01 has been isolated from *Sitona lineatus*. The strain has high insecticidal activity towards the host from which it was isolated, with LC50 equal to 1.127×10^4 conidia/ml on adults. Screened previously against *Hypera postica* and *Protapion apricans*, CNMN-FE-01 caused mortality rates of 70% and 40% on day seven after treatment (Moldovan et al., 2022). In the present study, this strain also had moderate insecticidal activity against adults and almost no activity against larvae, confirming the idea of host specificity expressed by Zemek et al. (2021). *Beauveria* sp. strain Hp2Cg, isolated from *Hypera postica* cadavers, was not previously assessed against pest beetles. Here, it had high insecticidal potential; thus, evaluating this strain against the host from which it was isolated will be interesting. This paper also reported results regarding assessing bacterial strains against adults and larvae. *Bacillus* sp. strain S03 exhibited some degree of activity against both adults and larvae of the Colorado beetle. Thus, future studies will be oriented toward assessing the synergistic potential of Hp2Cg and S03 strains against adults and larvae (Wraight & Ramos, 2005).

CONCLUSIONS

Nowadays agriculture faces numerous challenges, including those caused by the overuse of synthetic chemicals in pest control. The development of a sustainable farming system is a priority for the Republic of Moldova, the major goals being to provide food safety, environmental protection, biodiversity conservation, support farmers, and increase of international competitiveness. Microbial biopesticides show great potential in offering sustainable approaches towards efficient pest management.

The present paper reports on new data regarding the insecticidal activity of native bacterial and fungal strains against adults and larvae of Colorado beetle. Among screened strains, *Beauveria* sp. Hp2Cg has shown promising potential to be developed as a Colorado beetle biological control agent.

Future studies will address LC50 and LT50 of the selected strain and synergistic potential of *Beauveria* sp. Hp2Cg and *Bacillus* sp. S03 strains. Physiological characterization of *Beauveria* sp. Hp2Cg strain and field trials must be performed to advance toward local biopesticide production. Also, activities will be oriented towards the isolation of local entomopathogenic bacterial and fungal strains directly from *L. decemlineata*.

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REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, *18*(2), 265–267.

Alyokhin, A., Vincent, C., & Giordanengo, P. (2013). *Insect Pests of Potato: Global Perspectives on Biology and Management*, 1st ed. Oxford, UK: Academic Press.

Baki, D., Tosun, H. S., & Erler, F. (2021). Efficacy of indigenous isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *Egyptian Journal of Biological Pest Control*, *31*, 1–11.

Cingel, A., Savić, J., Lazarević, J., Ćosić, T., Raspor, M., Smigocki, A., & Ninković, S. (2016). Extraordinary adaptive plasticity of Colorado potato beetle: "Ten-Striped Spearman" in the era of biotechnological warfare. *International Journal of Molecular Sciences*, *17*(9), 1538.

Damalas, C. A., & Eleftherohorinos, I. G. (2011). Pesticide exposure, safety issues, and risk assessment indicators. *International Journal of Environmental Research and Public Health*, *8*(5), 1402–1419.

Duan, Y., Wu, H., Ma, Z., Yang, L., Duan, X., & Ma, D. (2018). Identification and virulence of a fungal entomopathogen strain NDBJJ-BFG to Colorado potato beetle *Leptinotarsa decemlineata* (Say). *Journal of Plant Protection*, *45*(4), 751–758.

Hare, D. J. (1980). Impact of defoliation by the Colorado potato beetle on potato yields. *Journal of Economic Entomology*, *73*(3), 369–373.

Hokkanen, H.M.T., & Hajek, A.E. (Eds.). (2003). *Environmental impacts of microbial insecticides: need and methods for risk assessment*. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Hussein, H. M., Skoková Habuštová, O., Půža, V., & Zemek, R. (2016). Laboratory evaluation of *Isaria fumosorosea* CCM 8367 and *Steinernema feltiae* Ustinov against immature stages of the Colorado potato beetle. *PLoS ONE*, *11*, e0152399.

Jurat-Fuentes, J. L., & Jackson, T. A. (2012). Bacterial entomopathogens. In Vega, F.E., Kaya, H.K. (Eds.), *Insect Pathology*, 2nd ed. (pp. 265–349). San Diego, USA: Academic Press.

Kumar, J., Ramlal, A., Mallick, D., & Mishra, V. (2021). An overview of some biopesticides and their importance in plant protection for commercial acceptance. *Plants*, *10*(6), 1185.

Lacey, L. A., & Siegel, J.P. (2000). Safety and ecotoxicology of entomopathogenic bacteria. In Charles, J.-F., Delecluse, A., Nielsen-LeRoux, C. (Eds.), *Entomopathogenic Bacteria: From Laboratory to Field Application* (pp. 253–273). Dordrecht, The Netherlands: Kluwer Academic Publishers.

Lacey, L.A, Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., & Goettel M.S. (2015). Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology*, *132*, 1–41.

Moldovan, A., Toderas, I., Leclerque, A., & Munteanu-Molotievskiy, N. (2017a). Isolation and identification of fungal community of alfalfa pest weevils (Coleoptera: Curculionidae) in the Republic of Moldova. *IOBC WPRS Bulletin*, *129*, 70–73.

Moldovan, A., Toderas, I., & Munteanu-Molotievskiy, N. (2017b). Noi agenți bacterieni de control biologic al insectelor dăunătoare in Republica Moldova. In *Actual* *problems of zoology and parasitology: achievements and prospects* (pp. 303–309).

Moldovan, A., Munteanu-Molotievskiy, N., & Toderas, I. (2018). Studii preliminare privind agenții de control biologic al dăunătorilor tomatelor în Republica Moldova. In *Functional Ecology of Animals* (pp. 287–289).

Moldovan, A., Munteanu-Molotievskiy, N., & Toderas, I. (2022). *Fungii entomopatogeni*. Chisinau, Republic of Moldova: F.E.-P. "Tipografia Centrala".

Mudgal, S., De Toni, A., Tostivint, C., Hokkanen, H., & Chandler, D. (2013). Scientific support, literature review and data collection and analysis for risk assessment on microbial organisms used as active substance in plant protection products–Lot 1 environmental risk characterisation. *EFSA Supporting Publications*, *10*(12), 518E.

Munteanu, N., Toderas, I., Moldovan, A., Malevanciuc, N., Toderaș, L., & Railean, N. (2013). Tulpina *Bacillus thuringiensis* subsp. *kurstaki* – insecticid biologic pentru combaterea coleopterelor curculionide. Patent No MD 4196. *The Official Bulletin of Industrial Property*, *2*, 19.

Munteanu, N., Toderas, I., Moldovan, A., Malevanciuc, N., Toderas, L., Bacal, S., & Railean, N. (2014a). Tulpina de bacterii *Bacillus thuringiensis* subsp. *kurstaki* – bioinsecticid pentru combaterea lepidopterelor din genul *Lymantria*. Patent No MD 4304. *The Official Bulletin of Industrial Property, 9,* 25.

Munteanu, N. V., Danismazoglu, M., Moldovan, A. I., Toderas, I. K., Nalcacioglu, R., & Demirbag, Z. (2014b). The first study on bacterial flora of pest beetles *Sciaphobus squalidus*, *Tatianaerhynchites aequatus* and *Byctiscus betulae* in the Republic of Moldova. *Biologia*, *69* (5), 681–690.

Sarwar, M. (2015). Biopesticides: an effective and environmental friendly insect-pests inhibitor line of action. *International Journal of Engineering and Advanced Research Technology*, *1*(2), 10–15.

Travers, R. S., Martin, P. A., & Reichelderfer, C. F. (1987). Selective process for efficient isolation of soil *Bacillus* spp. *Applied and Environmental Microbiology*, *53*(6), 1263–1266.

Vargas-Ortiz, E., Gonda, I., Smeda, J. R., Mutschler, M. A., Giovannoni, J. J., & Jander, G. (2018). Genetic mapping identifies loci that influence tomato resistance against Colorado potato beetles. *Scientific Reports*, *8*(1), 7429.

Weber, D. (2003). Colorado beetle: pest on the move. *Pesticide Outlook*, *14*, 256–259.

Wraight, S. P., & Ramos, M. E. (2005). Synergistic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. *Journal of Invertebrate Pathology*, *90*(3), 139–150.

Wraight, S. P., & Ramos, M. E. (2015). Delayed efficacy of *Beauveria bassiana* foliar spray applications against Colorado potato beetle: impacts of number and timing of applications on larval and next-generation adult populations. *Biological Control*, *83*, 51–67.

Zemek, R., Konopická, J., Jozová, E., & Skoková Habuštová, O. (2021). Virulence of *Beauveria bassiana* strains isolated from cadavers of Colorado potato beetle, *Leptinotarsa decemlineata*. *Insects*, *12*(12), 1077.