EVALUATION OF THE BIOSTIMULATORY AND NEMATICIDAL EFFECTS OF *BACILLUS CEREUS* VAR. *FLUORESCENS* ON *SOLANUM TUBEROSUM*

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ABSTRACT

This study evaluated the efficacy of bacterial culture liquid as biocontrol agent in potato (*Solanum tuberosum*) cultivation. In vivo application of the culture liquid derived from *Bacillus cereus* var. *fluorescens* CNMN-BB-07 (6×10^8 cells/mL) resulted in a substantial reduction in *Ditylenchus destructor* infestation, decreasing infection levels to 1–4% compared with 40.42% in the untreated control. The treatment also increased potato yield by 1.7-fold relative to untreated seed tubers (control C1), completely prevented tuber rot during storage, and reduced damage caused by *Gryllotalpa gryllotalpa* to 10.4%. Overall, the tested bacterial strain demonstrated pronounced plant growth–promoting and nematode-suppressive effects, indicating their potential as effective and environmentally sustainable tools for integrated potato protection strategies.

Keywords: *Solanum tuberosum*, biocontrol, *Bacillus cereus* var. *fluorescens* CNMN-BB-07, *Ditylenchus destructor*

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops worldwide, playing a key role in global food security. However, its productivity is significantly affected by a wide range of soil-borne pathogens and pests, among which phytoparasitic nematodes such as *Ditylenchus destructor* are particularly destructive, causing substantial yield losses and deterioration of tuber quality during storage (Jones et al., 2013).

Conventional control strategies rely heavily on chemical pesticides, which may lead to environmental contamination, development of resistance, and negative impacts on human health (Pimentel & Burgess, 2014). In this context, increasing attention has been directed toward sustainable and environmentally friendly alternatives, such as the use of beneficial microorganisms with plant growth-promoting and biocontrol properties (Compant et al., 2005; Lugtenberg & Kamilova, 2009).

Bacterial species *Bacillus cereus* var. *fluorescens* are known to enhance plant growth through mechanisms including phytohormone production, improved nutrient availability, and suppression of plant pathogens (Vessey, 2003; Kloepper et al., 2004). Moreover, these microorganisms can act as biological control agents against nematodes and other soil-borne pests, reducing infestation levels and improving crop productivity (Siddiqui & Mahmood, 1999).

The objective of this study was to evaluate the nematicidal capacity of the culture liquid (CL) produced by the bacteria *Bacillus cereus* var. *fluorescens* CNMN-BB-07, as well as to monitor their impact on ontogenetic development, productivity, and the phytosanitary status of potato crops.

2. MATERIALS AND METHODS

The experiments were conducted at the Institute of Zoology, Moldova State University (MSU), and at the Institute of Microbiology and Biotechnology, Technical University of Moldova (TUM).

The study was performed on seed potato tubers of the Roko cultivar (*Solanum tuberosum*) and the phytoparasitic nematode *Ditylenchus destructor* Thorne, 1945, corresponding to the second stage of ditylenchosis (Melnic et al., 2014; 2016; 2017).

The experimental biological material was obtained through artificial inoculation of tubers with 30–50 individuals of *D. destructor* (females and males), selected from previously infested potato tubers. The inoculated tubers were then incubated for 2–2.5 months, during which nematode penetration and multiplication occurred concurrently with the initiation of tuber germination processes.

The bacterial strain *Bacillus cereus* var. *fluorescens* CNMN-BB-07 (family Bacillaceae, genus *Bacillus*) was isolated in 2005 from fatty carbonate soil (loamy texture, 3.5% humus content) in the Republic of Moldova and deposited in 2014 in the National Collection of Nonpathogenic Microorganisms (CNMN), Technical University of Moldova. The liquid culture (LC) was obtained by cultivating the strain on King B medium at 27 ± 1 °C for 48 h.

Under in vivo conditions, *B. cereus* var. *fluorescens* CNMN-BB-07 was evaluated on seed potato tubers artificially infested with *D. destructor*. The experimental design included four variants: V1—infested tubers treated with LC diluted 1:200; V2—infested tubers treated with LC diluted 1:400; C1—infested, untreated control; and C2—healthy, nematode-free control. Tubers exhibiting 100% infestation (stage II of ditylenchosis) were used for treatment. All treatments were performed by immersion at room temperature (+15 to +20 °C) for 16 h.

During the growing season, systematic phenological observations were conducted to evaluate potential phytotoxic effects and plant growth responses, including the number of shoots (n), plant height (h). At harvest, yield and health-related parameters were assessed, including the number of tubers per nest, average tuber diameter, tuber weight, total yield per nest, the level of infestation by *D. destructor*, incidence of tuber rot and damage caused by mole crickets. Each experimental variant consisted of 10 plants (tubers), and the experiment was performed in three biological replicates (r = 3) under identical controlled conditions. Data were processed using descriptive statistics and are presented as mean values.

3. RESULTS

Previous multi-annual studies performed under vegetative and field conditions demonstrated that the *B. cereus* var. *fluorescens* CNMN-BB-07 (6×10^8 cells/mL) exhibits strong nematicidal activity against *D. destructor*. Undiluted and low-dilution rates (1:50 and 1:100) ensured complete suppression of nematode infestation (0% at harvest), but also induced phytotoxic effects on plant development and tuber formation.

Based on these findings, further experiments were conducted using higher dilutions of the bacterial culture liquid: V1 (1:200) and V2 (1:400). Two control variants were included: C1 (inoculated, untreated tubers) and C2 (healthy, untreated tubers). All experimental tubers were planted under open-field conditions in soil free from *D. destructor*. Plant development was monitored throughout the entire growing season, with emphasis on vegetative growth, yield parameters, and pest incidence.

The results (Table 1) demonstrate that the effects of the bacterial treatment are strongly dose-dependent. In variant V1 (1:200), a mild phytotoxic effect was observed, expressed by reduced vegetative growth parameters compared to V2. In contrast, variant V2 (1:400) showed a pronounced stimulatory effect on plant development, with superior biometric indices compared to both control variants.

Table 1. Evaluation of the treatment effectiveness of experimental seed potatoes (*Roko* cultivar), infested with *D. destructor*, in contact with the bacteria *B.cereus* var. *fluorescens* CNMN-BB-07

Batch	Dilution/ exposure time (h)	Phase flowering- ripening		Harvest						
		Shoots n	Plant height (cm)	Tubers / nest n	Avg. tuber diameter d (cm)	Tuber weight m (g)	Yield/ nest m (g)	*D. destructor* (%)	Rots (%)	Mole crickets (%)
V1	1:200/ 16 hours	2.3	47.0	5.0	3.23/4.46	43.4	217.0	0	0	0
V2	1:400/ 16 hours	6.0	67.38	9,6	5.3/4.51	65.3	626.8	1 - 4	0	10.4
C1	inoculated, untreated	3.5	55.6	7,0	3.72/4.63	48.4	377.52	40.42	22.5	40.3
C2	healthy, untreated	4.4	62.36	8,7	5.0 x 3.8	58.3	508.2	0	7.9	12.3

Data are presented as mean values.

Plants from V2 exhibited an average increase in plant height of approximately 12 cm and a two-fold increase in shoot number per plant compared to the infected control (C1). At harvest, the number and mass of tubers per plant were 1.4 times higher in V2 than in C1.

A strong nematicidal effect was recorded in V2, where the infestation level of *D. destructor* decreased from 100% before planting to 1–4% at harvest. In contrast, the infected untreated control (C1) showed a high infestation level of 40.42%.

Additionally, treatment with *B. cereus* CL at 1:400 completely eliminated tuber rot incidence (0%) and significantly reduced damage caused by soil pests (*Gryllotalpa grylotalpa*) to 10.4%, compared to 22.5% in C1 and 40.3% in healthy but untreated control (C2). A proportion of 7.9% of tubers in C2 were affected by bacterial and fungal infections, while 12.3% showed insect damage. At harvest, the number of tubers per nest, as well as the weight of tubers per nest, was on average 1.4 times higher in the experimental batch V2 compared with the control batch C1 (Table 1).

Overall, the results confirm that the biological activity of *B. cereus* var. *fluorescens* CNMN-BB-07 is dose-dependent, with the 1:400 dilution representing the optimal balance between plant growth promotion and nematicidal efficacy (Melnic et al., 2023).

4. DISCUSSION

According to the literature (Avis et al, 2008), rhizosphere-associated bacteria stimulate the synthesis of key secondary metabolites such as phenolic compounds, flavonoids, and phytoalexins. These compounds play an important role in triggering induced systemic resistance (ISR), thereby strengthening plant defense responses against biotic stress. In this context, the presence of such metabolites in the culture liquid may explain not only the direct nematicidal activity against *D. destructor*, but also the improved vegetative performance of potato plants, which may be associated with enhanced physiological status and activation of defense-related pathways.

In vivo experiments demonstrated the biological efficacy of the strain under controlled conditions. The treatment of seed potato tubers (*Roko* cultivar) with culture liquid diluted 1:400 for 16 h resulted in a marked reduction of *D. destructor* infestation, from 100% prior to planting to 1–4% at harvest. In contrast, the untreated infected control (C1) exhibited an infestation level of 40.42%.

Literature data also highlight the antifungal and antimicrobial potential of *Bacillus* spp., including *B. cereus* var. *fluorescens*, suggesting their suitability as biological control agent in sustainable crop protection strategies (Fravel, 2005; Ongena & Jacques, 2008).
Overall, the present data indicate that *B. cereus* var. *fluorescens* CNMN-BB-07 reduces the infestation level of potato tubers by phytoparasitic nematodes while simultaneously promoting plant growth. This multifunctional effect is likely associated with the strain's ability to produce phytohormones and other bioactive metabolites.

5. CONCLUSIONS

The treatment of seed potatoes with culture liquid of *B. cereus* var. *fluorescens* CNMN-BB-07 (1:400, 16 h) significantly reduced *D. destructor* infestation (80–96%) and increased yield by approximately 1.7-fold compared to the untreated control. The strain demonstrated both nematicidal and plant growth–promoting activity, indicating its potential as an environmentally friendly biocontrol agent for sustainable potato protection.

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DECLARATIONS

Conflict of interest: The authors declare that they have no conflict of interest.
Author Contributions: S.R. and D.E. designed the study; O.G. and M.M. performed field experiments; V.S. and V.T. performed microbiological cultivation; O.G. and L.B. analyzed data; O.G. and M.M. writing—original draft preparation; D.E.,S.R.,O.G. writing – review & editing; all authors reviewed and approved the final version.
Data Availability Statement: The datasets generated during the current study are available from the corresponding author upon reasonable request.
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