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EFFECT OF THE BIOSTIMULANTS OF MICROBIOLOGICAL ORIGIN ON THE ENTOMOPATHOGENIC AND PLANT PARASITIC NEMATODES FROM *MISCANTHUS* × *GIGANTEUS* PLANTATIONS

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ABSTRACT

This study aimed to evaluate the effect of biostimulants derived from *Streptomyces avermitilis* metabolites on entomopathogenic nematodes: *Steinernema carpocapsae*, *Steinernema feltiae*, and *Heterorhabditis bacteriophora*, obtained from the soil of several *Miscanthus* × *giganteus* plantations in 2020–2023. The nematodes were isolated, identified, and cultured using live insects (the greater wax moth *Galleria mellonella*). Three preparations containing biostimulants – Charkor, Stimpo, and Regoplant, were tested for compatibility with entomopathogenic and plant parasitic nematodes. Their effect on nematode survival was evaluated using the Petri dish test. The study showed that the effect of biostimulants on the survival of nematodes depended on the concentration of aversectin contained in the evaluated preparations. Stimpo and Regoplant had an adverse effect on plant parasitic nematodes. The highest *G. mellonella* mortality was observed at the higher dose of Charkor (0.4%), and the lowest at the lower dose of Regoplant (1.22%). The study showed that the virulence of the nematodes decreased after 48 hours of incubation in Regoplant and Stimpo solutions containing aversectin. The degree of interaction between nematodes depended on the nematode species, trophic preferences, and the concentration of active ingredients in the preparations. This conclusion is crucial for the selection of appropriate types of entomopathogenic nematodes and the application rate of plant parasitic nematodes in the assessment of their short- and long-term spread, persistence, and recycling in field conditions.

Key words: abamectin, aversectin, *Galleria mellonella*, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae*, *Streptomyces avermitilis*

INTRODUCTION

A vast area of land across Ukraine has been degraded and deteriorated by organic and inorganic pollution, soil compaction and erosion, including Russia's ongoing military aggression (Pereira et al. 2022; Falko & Zhukov 2023). For the country that once provided the world's food basket and must maintain healthy soil, revitalizing the deteriorated and contaminated areas is an urgent task (Arndt et al. 2023). The phytoremediation of war-damaged land through the cultivation of lignocellulosic energy crops is one of the proposed environmentally friendly solutions in line with the principles of bioeconomy (Alasmary et al. 2020; Fritsche et al. 2021). The approach allows the revitalization of war-damaged land and simultaneously lignocellulose biomass production as an alternative energy source or bioproducts: insulation and construction materials, pulp, and paper (Danielewicz et al. 2018).

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The plants proposed for phytoremediation must grow rapidly and efficiently in the contaminated soil and produce sufficient biomass while improving the soil quality (Yan et al. 2020).

Miscanthus × *giganteus* (*M*×*g*) Greef et Deuter ex Hodkinson et Renvoize has been studied extensively as a phytoagent. It is a rhizomatous, perennial C4 grass species native to Southeast Asia (Lewandowski et al. 2000). The sterile clone *M*×*g* is a high-yielding genotype and a standard cultivar for commercial use. It has an excellent environmental profile with the potential to grow in deteriorated, marginal, and slightly contaminated soil. The recent laboratory and field experiments demonstrated that *M*×*g* could be used for the phytoremediation of soils contaminated with trace elements of different anthropogenic origins (Pidlisnyuk et al. 2020; Nurzhanova et al. 2023). In addition to biomass production, *M*×*g* cultivation significantly contributes to the accumulation of organic matter in the soil (Felten & Emmerling 2012). The plant grew well in poor soils and was recommended for phytoremediation of postwar lands in Ukraine. However, the soil quality in degraded, marginal, or slightly contaminated areas is generally poor, the land is not productive enough, and the quality of *Miscanthus* biomass is relatively low (Kharytonov et al. 2019). To improve soil health (Medkov et al. 2021) and crop production in marginal and slightly contaminated soils, various agronomic practices such as the application of soil fertilizers (Stefanovska et al. 2022) or biostimulants are used (Nebeská et al. 2019; Caradonia et al. 2022)[1].

Charkor, Regoplant, and Stimpo preparations containing biostimulants are used in Ukraine to improve the development and biomass production of various agricultural plants, including first-generation energy crops: barley, wheat, sugar beets, fruits, vegetables, and ornamental plants (Tsygankova et al. 2012, 2013; Blyuss et al. 2019). Our previous studies have shown that the use of biostimulants based on secondary metabolites of actinomycetes (*Streptomyces avermitilis* ex Burg et al., Kim and Goodfellow), improves plant growth and biomass production on agricultural land slightly contaminated with trace elements (Nebeská et al. 2019). The ability of actinomycetes to enhance plant growth by cumulative and synergistic effects and to increase ability of plants to protect against pathogens and parasites has been the subject of numerous studies (Wang et al. 2010). Abamectin-based products are commonly used against various pests (Fitzgerald 2004). Abamectin produced by *S. avermitilis* stimulates the gamma-aminobutyric acid (GABA) system in the insect, interfering with nerve and neuromuscular transmission. Abamectin-based products are considered mild chemicals that break down quickly, within 24–48 hours. They are immobile in the soil, so the risk to nontarget organisms and groundwater contamination is considered to be low (Lumaret et al. 2012).

Plant defense is closely related to avermectins, which are a class of polycyclic lactones from *S. avermitilis* and are highly toxic to a broad spectrum of plant parasitic nematodes and soil arthropods (Wang et al. 2010). However, in addition to plant parasitic nematodes, soil fauna also includes beneficial entomopathogenic nematodes, which are lethal insect parasites found in the soil. The two genera, *Steinernema* (over 100 species) and *Heterorhabditis* (29 species), are not closely related phylogenetically, but convergent evolution has led to the appearance of common characters between these organisms. Entomopathogenic bacteria, such as the *Xenorhabdus* spp. in the case of *Steinernema* spp. and *Photorhabdus* spp. in the case of *Heterorhabditis* spp., coexist in symbiosis with species of entomopathogenic nematodes (Stefanovska et al. 2023). This type of symbiosis is mutual. The bacteria eat the host, feed on the nematodes, and form a variety of compounds that prevent other soil organisms from opportunistically colonizing the site of infection. Entomopathogenic nematodes and biostimulants are proposed in integrated pest management programs in horticulture and ornamental crops. However, only a few studies have demonstrate their effect on entomopathogenic nematodes (Meena et al. 2020).

[1] In our previous publications, we used the term plant growth regulators (PGRs). The editor's suggestion changed the terminology and took into account current changes in the regulations regarding microbial products for plant production (Hendriksen 2022).

This aim of the study was to:

– conduct field studies of entomopathogenic nematodes in the soil of *M*×*g* plantations located in eight regions of Ukraine;

– assess the effect of biostimulants based on *Streptomyces avermitilis* metabolites on the following entomopathogenic nematodes: *Steinernema carpocapsae* Weiser, *Steinernema feltiae* Filipjev, and *Heterorhabditis bacteriophora* Poinar, isolated from the soil in *M*×*g* plantations;

– compare the effect of biostimulants on entomopathogenic nematodes and plant parasitic nematodes, i.e., *Heterodera schachtii* (Schmidt) and *Globodera rostochiensis* (Wollenweber), isolated from the vicinity of the *M*×*g* plantations.

MATERIALS AND METHODS

To isolate nematodes, the technique of baiting with live insects of the greater wax moth (*Galleria mellonella* L.) (Lepidoptera: Pyralidae) was developed and used in the laboratory in the following research (Birah et al. 2008). Greater wax moth (GWM) cultures were raised in 1-liter glass containers, kept at 28°C, and fed an artificial diet (Birah et al. 2008). The last instar larvae were heat-treated by soaking in water at 60°C for 10 seconds before use to avoid spinning their silken cocoon and further development to the pupal stage. Heat-treated larvae were stored at 10°C until they were used. The larvae used in the studies weighed on average 0.2–0.3 g.

Nematode isolation, identification and cultivation

Research on entomopathogenic nematodes associated with *M*×*g* was conducted in 2020–2023. Soil samples were collected from 12 *M*×*g* plantations located in eight regions of Ukraine. For four plantations, the survey was carried out twice in different years of plant vegetation. The crop was cultivated there from 1 to 12 years. The total number of soil samples collected in these plantations was 118. Each subsample consisted of approximately 1 kg of soil collected from a depth of at least 15 cm using a straight shovel. Three subsamples for one soil sample were then mixed, homogenized, and placed in a plastic bag to minimize moisture loss. Samples were stored in a refrigerator to prevent temperature rise and exposure to solar radiation.

The GWM alive insect-baiting technique was used to isolate entomopathogenic nematodes. Upon arrival at the laboratory, each 100 g sample was placed in a plastic container and ten late instars of GWM larvae were introduced into each. The container was then covered with aluminum foil with small holes to minimize moisture and allow gas exchange. For two weeks, the containers were inspected every two days for the presence of dead larvae (cadavers). As a result of the *Heterorhabditis* attack, the cadavers turned red, whereas *Steinernema* turned it yellow or black. The cadavers were placed in White Traps (White 1927), and when infective juveniles (IJs) emerged, they were collected and stored in culture flasks at an average temperature of 10°C. Nematodes species were identified using classical morphological tools (Hunt et al. 2005; Seesao et al. 2017). Several stages of the nematode were randomly selected, i.e., IJs, hermaphroditic females (in the case of *Heterorhabditis* spp.), amphimictic females and males. Adults were extracted from GWM cadavers using Ringer's solution and killed at 60°C. Triethanolamine formalin (TAF) was used for isolated nematode fixation. The isolated nematodes were then processed into anhydrous glycerin (Seinhorst 1966), viewed under a microscope, and identified by species using a key. Isolated and identified species of entomopathogenic nematodes reared according to Kaya and Stock (1997). The newly emerged nematodes were kept at 10°C in tissue culture flasks and used for further laboratory bioassays over a 2–4 week period to see how they were affected by the three biostimulants.

Cyst nematodes belonging to the plant parasitic nematodes, *H. schachtii* (Schmidt) and *G. rostochiensis* (Wollenweber), were used to compare the effect of biostimulants on entomopathogenic and plant parasitic nematodes. The nematodes were initially isolated from sugar beet and potato fields near *M*×*g* plantations. The flotation technique (Kort 1960) was used to extract heteroderid cysts. Of the 1,000 cysts isolated, 100 were selected using a stereomicroscope to observe hatching. The hatching of *Heterodera* spp. was examined in two separate incubation phases. In the first stage, all cysts were exposed to 4°C for 60 days. In the second stage, each population was transferred and used for a biostimulant compatibility experiment. Morphological characteristics were used to identify cyst nematodes (Subbotin et al. 2020).

Assessment of the direct impact of biostimulants on entomopathogenic and plant parasitic nematodes (biotest 1)

Three preparations containing biostimulants were used to test compatibility with entomopathogenic and plant parasitic nematodes: Charkor, Stimpo, and Regoplant. Descriptions of biostimulants are presented in Table 1. Stimpo and Regoplant are qualified for use in organic farming (Certificate No.21-1652-01-01 2023-01-31. 2021).

The effect of biostimulants on nematodes was tested in nine-centimeter Petri dishes using IJ and biostimulants were applied at a dose usually used in field conditions (Pidlisnyuk et al. 2022). The following concentrations of biostimulants were used: Charkor: Ch1 – 0.1%, Ch2 – 0.4%; Regoplant: R1 – 1.22%, R2 – 2.44%; Stimpo: S1 – 0.25%; S2 – 0.50%. Two biostimulants (Stimpo and Regoplant) from the three tested contained aversectin and the concentrations were as follows: R1 – 0.012%; R2 – 1.22%; S1 – 0.00125%, S2 – 0.025%.

Petri dishes were filled with 10 ml of biostimulant solution at each of the tested concentrations and 100 µl of nematode suspension was added at concentrations of 20,000 IJs (entomopathogenic nematodes) and J2 (plant parasitic nematodes) per ml. In the control treatment, only distilled water was used. Petri dishes were kept in the dark at room temperature (22–26°C). Four replications were performed in each experiment. Nematode survival was assessed after 48 hours by taking five 50 µl subsamples from each Petri dish and examining them under a stereomicroscope.

Nematodes that remained immobile even after puncture were considered dead. The nematode solution with the biostimulant was washed three times with sterile water to remove the residues of the compound and to assess the sublethal effect of biostimulant on virulence. The nematodes were incubated for 24 hours in distilled water.

Table 1. Characteristics of biostimulants used in the treatment of *M*×*g* (modified from Pidlisnyuk et al. 2022)

Name	Stimpo	Regoplant	Charkor
Origin	natural	natural	synthetic
Principle of operation	It promotes faster cell division, the growth of strong roots, increases leaf area and chlorophyll content, reduces the phytotoxic effects of pesticides and improves the host plant's resistance to pathogenic organisms.	It promotes faster cell division, rhizogenesis, growth of symbiotic microflora in the root system, acceleration of photosynthetic activity and development of leaf surfaces, reduction of phytotoxicity of pesticides and increased tolerance of the host plant to pathogenic organisms.	It accelerates cell division, rhizogenesis, the formation of symbiotic bacteria in the root system, and the development of leaf surfaces. It is a highly effective root formation stimulator, increasing plant resistance to abiotic stress.
Structure	Biostimulant emistim C is a product of the metabolism of *Cylindrocarpon obtusiusculum* (Sacc.) U. Braun contains auxin and cytokinin analogues, saturated and unsaturated fatty acids, amino acids, and carbohydrates.	Biostimulant emistim C is a byproduct of the metabolic activity of *Cylindrocarpon obtusiusculum*, analogues of phytohormones such as auxin and cytokinin, as well as saturated and unsaturated fatty acids, amino acids, and carbohydrates.	Biostimulant emistim C is an analog of phytohormones of auxin, cytokinin, saturated and unsaturated fatty acids, amino acids, and carbohydrates, produced as a product of the metabolism of *Cylindrocarpon obtusiusculum*.
	The natural aversectin C complex is a metabolic product of the actinomycetes *Streptomyces avermitilis*, with the addition of microelements.	The actinomycetes *Streptomyces avermitilis* produces the natural compound aversectin C in the process of metabolism. Part of the biostimulant is the universal microfertilizer "Reakom-Zerno" based on biogenic microelements.	2,6-dimethylpyridine-N-oxide combined with 1-naphthylacetic acid, a synthetic phytohormone analogue is added.
Standard	Manufacturer specification (2020)	Manufacturer specification (2014)	Manufacturer specification (2016)

Virulence of entomopathogenic and plant parasitic nematodes, GWM mortality (biotest 2)

The virulence of nematodes after 48 hours of exposure to biostimulants was tested in the last instar of GWM using 24-well polystyrene plates. One GWM larva was placed in each well. Each well was then half-filled with wet sterile silver sand (10%) on top of the larva. Then, 100 IJ in 50 µl of Ringer's solution was added to each well on the top of the sand. Distilled water was used as a control. The plates were coated with Parafilm and incubated at 25°C. The cadavers were collected after four days to examine nematode infectivity. The virulence experiment was replicated three times.

The Bartlett test with the *bartlett.test* function in R (R Core Team 2020) was used to test for equal variances across experimental groups. Welch ANOVA was used to analyze data with unequal variances across experimental groups. The *oneway.test* function (from the R base) was used to perform Welch's ANOVA in R (with *var.equal* = false option). Post hoc mean values were compared using the Games–Howell test using the *games_howell_test* function (from the *rstatix* library in R).

RESULTS

Field tests for entomopathogenic nematodes

The results showed that of 118 soil samples, 12 contained entomopathogenic nematodes (recovery rate 10%). Of these 12 samples, 8 (55%) contained *Steinernema* isolates and 4 (18.8%) contained *Heterorhabditis* isolates. Three isolates were identified: *H. bacteriophora*, *S. carpocapsae*, *S. feltiae* (Table 2).

The effect of biostimulants on the survival of nematodes

The data presented in Table 3 showed that the effect of biostimulants on nematode survival was statistically significant. However, the effect of Charkor on the survival of *S. carpocapsae* (Fig. 1) was not statistically significant compared to the control (Ch1: $p = 0.21$; Ch2: $p = 0.99$). Stimpo showed a moderate effect. Regoplant had the most significant effect on *S. carpocapsae* survival, reducing nematode survival to 55.8–66.5%. Under experimental conditions, the survival rate of the nematode *S. carpocapsae* was 73.3–77.5%. The effect of Charkor on the survival of *H. bacteriophora* was not statistically significant compared to the control (Ch1: $p = 0.92$; Ch2: $p = 0.92$). The properties determined for Stimpo and Regoplant showed no significant difference related to their impact on the survival of entomopathogenic nematodes, i.e., *S. feltiae*. The tested biostimulants can be classified as follows: Charkor → Stimpo → Regoplant. The effect of Charkor on the survival of *H. schachtii* was not statistically significant compared to the control (Ch1: $p = 0.99$; Ch2: $p = 0.87$), whereas Stimpo and Regoplant significantly reduced the survival of *H. schachtii*, to the level of 42.5–49.3%. The effect of Charkor on the survival of *G. rostochiensis* was not statistically significant compared to the control (Ch1: $p = 0.91$; Ch2: $p = 0.75$). The use of Stimpo resulted in the survival rate of *G. rostochiensis* at the level of 48.0–48.3%, and in the case of Regoplant, the survival rate of this species was at the level of 33.5–36.5%.

Table 2. Frequency of isolated indigenous entomopathogenic nematodes genera found in the researched *M*×*g* plantations

Site (plantation age – year)	Region	Soil samples	*Steinernema* spp.	*Heterorhabditis* spp.
1. Golosyevo (1)	Kyiv	10	1	–
2. Golosyevo (5)	Kyiv	7	1	1
3. Batyeva Gora (9)	Kyiv	5	–	–
4. Batyeva Gora (12)	Kyiv	5	–	–
5. Dolyna (1)	Ivano-Frankivsk	10	–	–
6. Dolyna (5)	Ivano-Frankivsk	10	1	–
7. Tokarivka (2)	Kyiv	5	1	–
8. Tokarivka (5)	Kyiv	5	1	2
9. Kalynivka (10)	Vinnytsia	7	–	–
10. Vremivka (7)	Poltava	7	1	–
11. Shepetivka (7)	Rivne	7	–	–
12. Ksaverivka (10)	Kyiv	10	–	–
13. Zhytomyr (10)	Zhytomyr	5	–	–
14. Barvinkove (6)	Kharkiv	7	1	1
15. Bila Tserkva (5)	Kyiv	10	1	–
16. Borshchiv (8)	Ternopil	8	–	–
Total		118	8	4
Positive percentage of the total number of samples			6.8	3.4
Frequency of occurrence on sites (%)			50.0	18.8

Table 3. Effect of biostimulants on entomopathogenic nematodes and plant parasitic nematodes

Entomopathogenic nematode species	Bartlett's test (*P*-value)	Num Df	Denom Df	*F*-ratio	*p*-level
Heterorhabditis bacteriophora	0.05	6	9.1	41.8	<0.001
Steinernema feltiae	0.55	6	21	134.6	<0.001
Heterodera schachtii	0.26	6	21	161.6	<0.001
Globodera rostochiensis	0.16	6	21	266.6	<0.001
Steinernema carpocapsae	0.005	6	17.1	173.6	<0.001

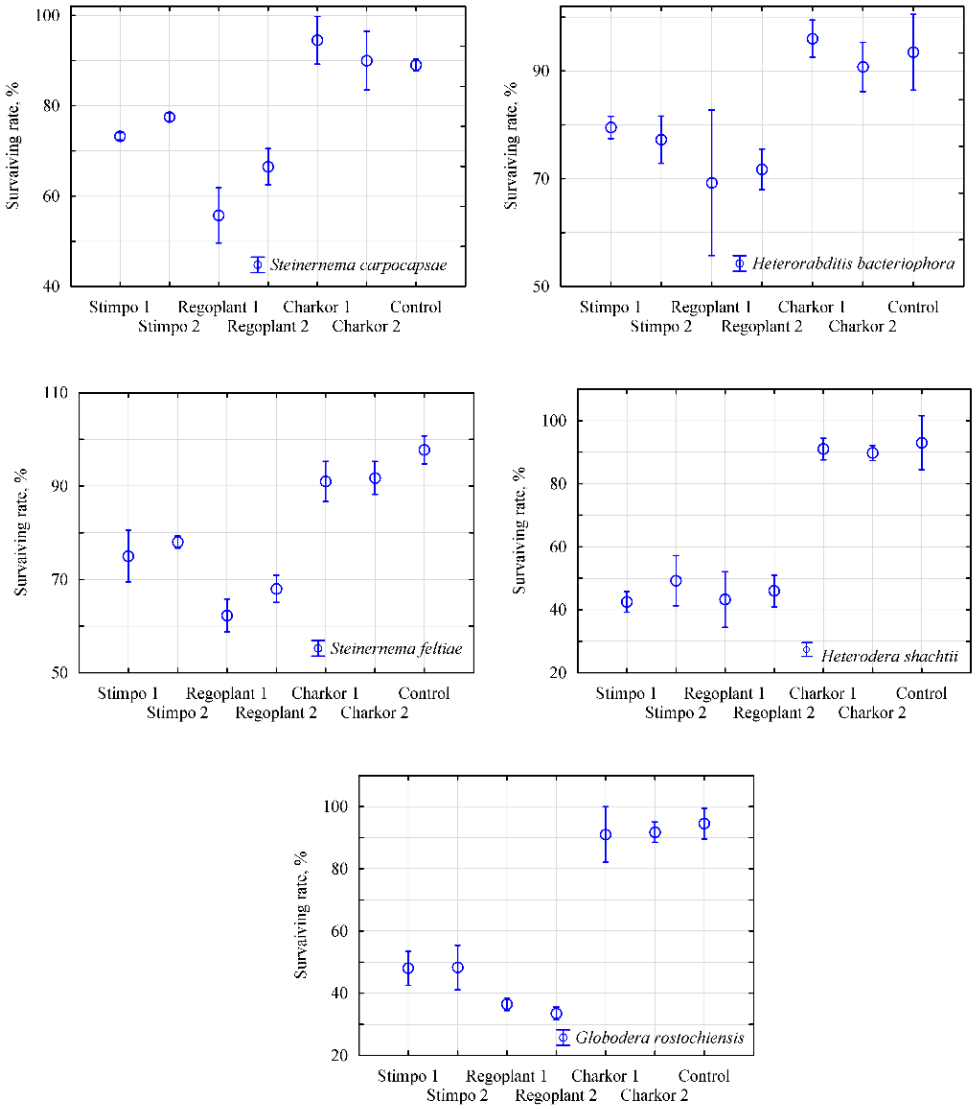


Figure 1. Effect of biostimulants on nematodes surviving (%, mean ± 95% standard deviation)

The mortality of GWM caused by nematodes depended statistically significantly on the use of biostimulants (Fig. 2). *S. carpocapsae* caused the highest GWM mortality when Regoplant was used. Slightly higher mortality was observed for *H. bacteriophora* when Regoplant 2 and Charkor 1 were used. The highest mortality was observed after the use of *S. feltiae*. The highest GWM mortality was observed with the higher dose of Charkor, and the lowest with the lower dose of Regoplant. The action pattern of *H. bacteriophora* and *S. feltiae*, depending on the biostimulant used, was similar. The use of Regoplant had a less negative effect on *S. carpocapsae* than other biostimulants tested.

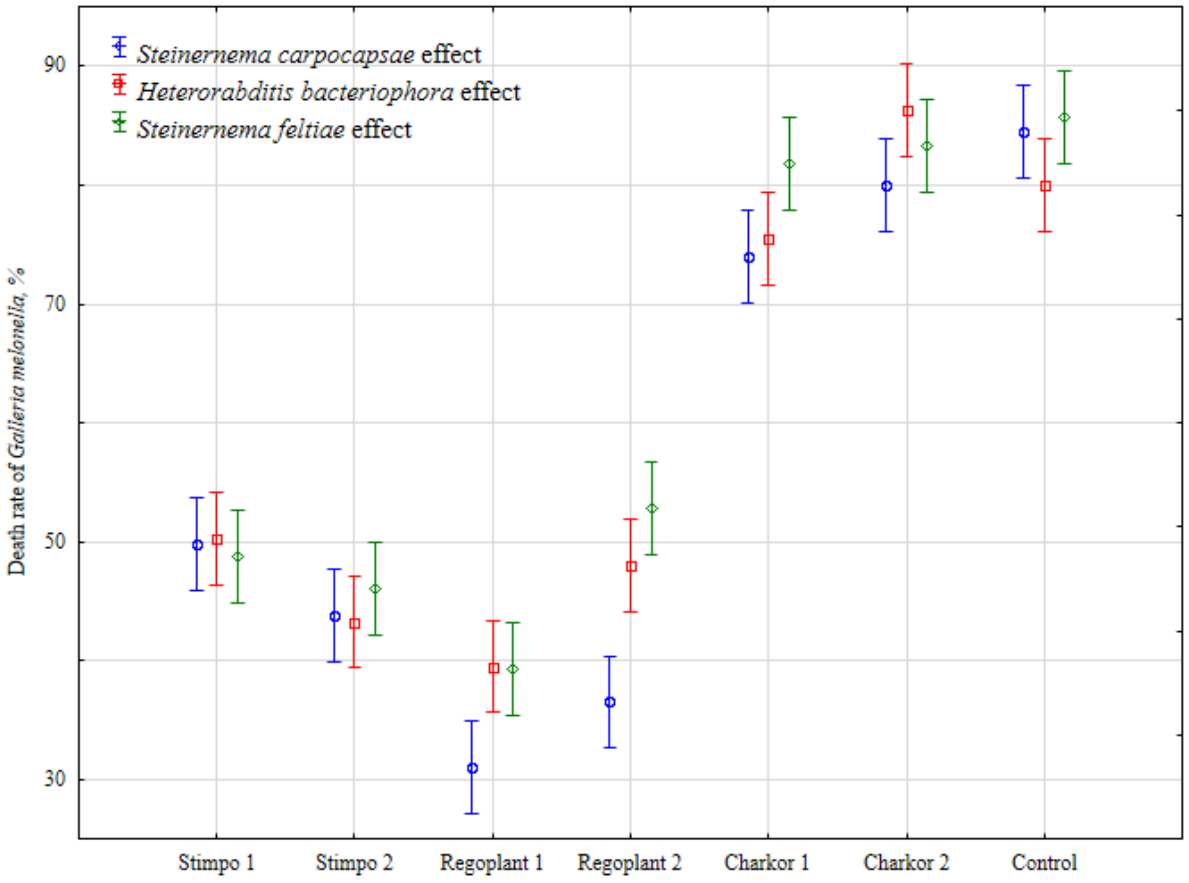


Figure 2. Mortality of wax moth *G. mellonella* from nematodes exposed to biostimulants (%, mean ± 95% standard deviation)

DISCUSSION

Our studies highlighted the specificity of the interactions of biostimulants with entomopathogenic nematodes and plant parasitic nematodes. Two preparations containing biostimulants (Stimpo and Regoplant) provided different concentrations of aversectin C, while the third preparation tested (Charkor) did not contain this substance. The results showed that the effect of biostimulants on the biological activity (virulence) of nematodes depended on the content of aversectin C in the product. This substance certainly influenced the virulence and survival of entomopathogenic and plant parasitic nematodes. The use of Charkor had a moderate effect on the survival of entomopathogenic and plant parasitic nematodes at the lower concentration, which was more pronounced because this particular preparation did not contain aversectin. Charkor treatment only resulted in maximum nematode survival at higher doses. In the case of entomopathogenic nematodes *H. bacteriophora*, *S. feltiae*, and *S. carpocapsae*, survival rates ranged from 90% to 100%. There was no statistically significant difference between the survival of *S. carpocapsae* and *H. bacteriophora* treated with Charkor compared to the control treatment. The concentration of aversectin in Stimpo was 0.00125–0.025%, the average survival rate of entomopathogenic nematodes (*S. carpocapsae*, *S. feltiae*, and *H. bacteriophora*) was 70–80%. The survival rate of the nematode *S. carpocapsae* was 78%.

Regoplant had a higher percentage of aversectin (1.22–2.44%) than Stimpo; consequently, the average survival rate of entomopathogenic nematodes ranged from 55.0% to 78%. The lowest percentage of *S. carpocapsae* (55.8–66.5%) survived after treatment with R2 at a concentration of 2.44%.

The findings of this investigation are consistent with a previous report by Kary et al. (2021) that found a synergistic interaction between lower concentrations of *H. bacteriophora* (LC30) and abamectin (LC10 and LC30 vs. LC50) in a laboratory experiment. Using a higher concentration of nematodes resulted in a decrease in treatment effectiveness. The results for *S. carpocapsae* were comparable to those of Kary et al. (2021).

Different species of entomopathogenic nematodes are known to interacted with abamectin in different ways (Kary et al. 2021), and our results suggested a similar pattern. Regoplant and Stimpo had a more negative effect on the survival of *S. feltiae*, while *H. bacteriophora* remained unaffected. This is consistent with the findings of Laznik and Trdan (2014), who found that the compatibility of avermectin with entomopathogenic nematodes varied among species. Kary et al. (2021) investigated the effect of abamectin on *H. bacteriophora* and *S. feltiae*. However, they demonstrated an adverse effect of abamectin on both entomopathogenic nematodes, and to a lesser extent on *H. bacteriophora.*

Current study results have shown that after 48 hours of incubation in Regoplant and Stimpo solutions, the virulence of entomopathogenic nematodes decreased. Thus, laboratory analysis of GWM mortality under the influence of entomopathogenic nematodes showed that the higher dose of Charkor caused the death of most individuals, and the lower dose of Regoplant killed the least. Entomopathogenic nematodes are effective against many below- and aboveground insect pests, including larvae (Koppenhöfer et al. 2020). On the other hand, avermectins are mainly used against ticks and some insect species. By using these two agents together, a wider range of *Miscanthus* pests can be controlled, increasing overall pest control effectiveness. Reducing the number of pests can be achieved by selecting optimal concentrations of avermectin-based products and optimizing the technology of application of entomopathogenic nematodes. According to laboratory and field studies (Fetoh et al. 2009), the combined use of nematodes and semisynthetic avermectin-based products increased the effectiveness against the black cutworm *Agrotis ipsilon* (Hufn.).

At half of the recommended application rate of abamectin, the additional effect was detected when mixed with entomopathogenic nematodes and used to control two subterranean termites – *Psammotermes hypostoma* (Desn.) and *Anacanthotermes ochraceus* (Burm.) (El-Sayed et al. 2022). In the current study, a different trend was detected related to the effect of the researched biostimulants on plant parasitic nematodes. Stimpo and Regoplant reduced the survival of *H. schachtii* and *G. rostochiensis* to a greater extent than entomopathogenic nematodes. The most effective agent was Regoplant with a higher concentration of avermectin. It can be assumed that the effect of the spread of plant parasitic nematodes can be determined based on the toxicity induced by avermectin. Both biostimulants used in the treatment, Regoplant and Stimpo, reduced the survival of plant parasitic nematodes – *H. schachtii* and *G. rostochiensis.* The survival time of plant parasitic nematodes was correlated with the aversectin content in the preparation.

The degree of interaction of biostimulants with entomopathogenic and plant parasitic nematodes depended on the nematode species, trophic preferences, and the concentration of active substances in the biostimulant. It is crucial to select the appropriate types of entomopathogenic nematodes and the application rates of biostimulants in order to further assess their short- and long-term spread, persistence, and recirculation in field conditions. The interaction between biostimulants and entomopathogenic nematodes affects potential effectiveness and should be taken into account when evaluating the effectiveness of recommended doses of biostimulants.

Plant parasitic nematodes can negatively affect the growth and development of *M*×*g* (Arundale et al. 2014; Emery et al. 2017). Commercial production of *M*×*g* requires additional investigation of the adverse effects of plant parasitic nematodes, which can cause serious yield losses during long-term crop cultivation, taking into account the different environmental conditions and geographical location of the plantations. In this regard, improving the *M*×*g* production technology with careful monitoring of plant parasitic nematodes and the use of biostimulants will prevent the risk of reduction in biomass yield from plant parasitic nematodes (Stefanovska et al. 2021).

CONCLUSIONS

This study has indicated the degree of interaction of preparations containing biostimulants – Charkor, Stimpo, and Regoplant, with entomopathogenic nematodes – *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora*, plant parasitic nematodes – *H. schachtii* and *G. rostochiensis*, i.e., nematode species, trophic preference, and concentration of active ingredients in these preparations. The obtained results are important for the selection of appropriate types of entomopathogenic nematodes and the application rates of biostimulants in order to evaluate their short- and long-term spread, persistence, and recycling in the field. The interaction between biostimulants and entomopathogenic nematodes affected potential efficacy. Recommended field-scale application rates of these compounds should be taken into account during development. The combined use of biostimulants and entomopathogenic nematodes will reduce the use of chemical pesticides. From an economic perspective, abamectin-containing biostimulants are needed to suppress plant parasitic nematodes in crop production, which makes their combined use with entomopathogenic nematodes in integrated pest management programs an extremely promising and environmentally friendly option.

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Data availability statement

The datasets used and analyzed in this study are available in the public domain.

Conflicts of interest

The authors declare no competing interests.

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