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ARTYKUŁ ORYGINALNY

Porównanie patogeniczności i wpływu temperatury na szczepy bakterii *Photorhabdus* i *Xenorhabdus*

Comparative of the pathogenicity and temperature effects on *Photorhabdus* and *Xenorhabdus* bacterial strains

Katarzyna Barszczewska*, Anna Filipiak

Streszczenie

Xenorhabdus (Poinar and Thomas, 1979) i *Photorhabdus* spp. (Boemare, 1993) to entomopatogeniczne bakterie o szerokim zakresie żywicieli, symbiotycznie związane z nicieniami z rodzin Steinernematidae (Filipjev, 1934) i Heterorhabditidae (Poinar, 1976). Entomopatogeniczne nicienie są wektorami, umożliwiającymi bakteriom wniknięcie do ciała owada, a następnie zabicie larw owadów i przekształcenie zwłok w źródło pożywienia odpowiednie dla wzrostu i rozwoju nicieni. W tym badaniu oceniano patogeniczność zawiesiny bakteryjnej *Photorhabdus* i *Xenorhabdus* przeciwko larwom *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). Różne stężenia bakterii (tj. 75, 100 i 125 CFU/ml) zostały wykorzystane do określenia procentowej śmiertelności larw. Śmiertelność przy najwyższym stężeniu osiągnęła 82,5–87,5% po 7 dniach obserwacji. We wszystkich dawkach najwyższą śmiertelność uzyskano po zastosowaniu *Xenorhabdus* sp. wyizolowanego z *Steinernema kraussei*. Aby wybrać odpowiednią temperaturę do dalszych eksperymentów, bakterie poddano działaniu różnych temperatur (15, 20, 25, 30 i 35°C). Wyniki wykazały, że patogeniczność bakterii wzrosła w temperaturze 20°C i spadła w temperaturze 35°C. Przedstawione wyniki sugerują, że bakterie *Photorhabdus* i *Xenorhabdus* mogą być obiecującymi kandydatami jako czynniki biokontroli, ale należy przeprowadzić więcej badań terenowych w celu przetestowania odporności bakterii na różne warunki środowiskowe.

Słowa kluczowe: *Photorhabdus*, *Xenorhabdus*, nicienie entomopatogenne, *Galleria mellonella*, patogenność, toksyczność

Abstract

Xenorhabdus (Poinar and Thomas, 1979) and *Photorhabdus* spp. (Boemare, 1993) are entomopathogenic bacteria with a wide insect host range, symbiotically associated with nematodes of the families Steinernematidae (Filipjev, 1934) and Heterorhabditidae (Poinar, 1976), respectively. Entomopathogenic nematodes are vectors, allowing bacteria to enter the insect's body, then kill the insect larvae and convert the cadaver into a food source suitable for the growth and development of nematodes. In this study, the pathogenicity of the bacterial suspension of *Photorhabdus* and *Xenorhabdus* against *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae was evaluated. Different bacterial concentrations (i.e., 75, 100 and 125 CFU/ml) were used to determine the percent mortality of larvae. The mortality rate at the highest concentration reached 82.5–87.5% at 7-day follow-up. At all doses, the highest mortality was obtained after the use of *Xenorhabdus* sp. isolated from *Steinernema kraussei*. To select an appropriate temperature for further experiment, bacteria were exposed to different temperatures (15, 20, 25, 30 and 35°C). The results showed that bacterial pathogenicity increased at 20°C and decreased at 35°C. The results presented here suggest that *Photorhabdus* and *Xenorhabdus* bacteria may be a promising candidate in biocontrol agents, but more field studies should be conducted to test the resistance and robustness of the bacteria to various environmental conditions.

Key words: *Photorhabdus*, *Xenorhabdus*, entomopathogenic nematodes, *Galleria mellonella*, pathogenicity, toxicity

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Wstęp / Introduction

One of the challenges of agriculture is to improve methods in order to control pests and plant pathogens more effectively and safely. The use of chemical pesticides is a growing problem that affects human and animal health, environmental pollution, and increasing the resistance of agrophages to applied pesticides (Gaines 1969; Xiao and Wu 2019). One of the solutions is the use of biopesticides, which can consist of, for example, microbial organisms (bacteria, fungi), viruses, entomopathogenic nematodes, substances of plant origin. Globally, the demand for organic food is growing, which is driving the market for biopreparations (Copping and Menn 2000). The research that led to the discovery of the entomopathogenic bacterium allowed the development of bacterial insecticides, most of which were based on the bacterium *Bacillus thuringiensis*. The aim was to kill the insect by using insecticidal proteins (Cry proteins) (Federici 2005). In contrast, Toxin complexes (Tcs), which have a high molecular weight and are produced by both Gram-negative and Gram-positive bacteria, have been discovered in *Photorhabdus* and *Xenorhabdus* bacteria. Tcs toxins induce immunosuppression in insects by inhibiting the synthesis of eicosnoid (Waterfield et al. 2001). *Photorhabdus* are symbiotically associated with entomopathogenic nematodes (EPN) of the family Heterorhabditidae, while *Xenorhabdus* are associated with nematodes of the family Steinernematidae (Askary and Abd-Elgawad 2021). EPNs provide an alternative control method to insecticides because it is an environmentally safer option (le Vieux and Malan 2013). Nematodes are vectors that allow bacteria to enter the insect's body (Hinchliffe 2013; Hussein et al. 2022). The bacteria kill the insect larvae and convert the cadaver into a food source suitable for the growth and development of nematodes (Askary et al. 2022). The characteristic of *Photorhabdus* or *Xenorhabdus* is their phenotypic variability, i.e. a primary (phase I) and secondary (phase II) form (Akhurst and Boemare 1988). The phase I cells are able to produce crystalline inclusion bodies and antibiotics, but also stimulate cells to lysate red blood cells, ensuring motility and pigment production. The phase II form is responsible for maintaining the growth rate of nematodes, providing them with protection against antagonistic bacteria (Sicard et al. 2005). One bacterial species is associated with several nematode species, e.g., *Xenorhabdus beddingii* (*Xenorhabdus nematophila* subsp. *beddingii*) (Akhurst, 1982) is associated with the nematodes *Steinernema kraussei*, *Steinernema feltiae*, *Steinernema affine*, or *Photorhabdus luminescens* (Boemare et al. 1993) is associated with the nematodes *Heterorhabditis bacteriophora* and *Heterorhabditis indica*. Many studies have been carried out to assess the pathogenicity of entomopathogenic nematodes and bacteria against *Galleria mellonella* (Won et al. 2017; Santhoshkumar et al. 2021; Guide et al. 2023). The rearing of this insect is economical, because without the use of special equipment and at low cost a large number of larvae can be obtained. An additional advantage is their short life cycle, which makes it possible to conduct large-scale studies (Tsai et al. 2016). Plant protection products containing entomopathogenic nematodes are used only for certain areas of crop production, e.g., *S. feltiae* (Akhurst, 1982) and *H. bacteriophora* (Boemare et al., 1993) perform best in field conditions, where they are exposed to many environmental factors. Entomopathogenic nematodes are applied topically, so they are only effective against soil-feeding larvae. They cannot be applied to the above-ground parts of the plant as a spray, as this leads to desiccation and death (Torrini et al. 2017). Bacteria are also affected by desiccation, UV radiation, etc., but can be applied by spraying leaves, stems, soil or dipping roots into the bacterial solution, allowing for a wider range of practical applications (Purnawati et al. 2014; Preininger et al. 2018).

The aim of the study was to evaluate the pathogenicity of *Xenorhabdus* and *Photorhabdus* bacterial suspensions against *G. mellonella* larvae during a 7-day *in vitro* test and to select an appropriate temperature for high bacterial efficacy.

Materiały i metody / Materials and methods

Izolacja bakterii / Isolation of bacteria

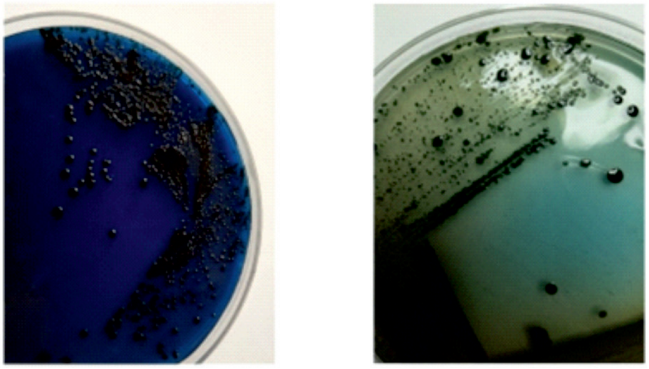
The study evaluated the pathogenicity of three strains of *Xenorhabdus* sp. and two strains of *Photorhabdus* sp. bacteria (tab. 1). All strains of *Xenorhabdus* and *Photorhabdus*

Tabela 1. Gatunki bakterii *Xenorhabdus* i *Photorhabdus* wykorzystane w badaniach
Table 1. Species of *Xenorhabdus* and *Photorhabdus* bacteria used in the study

Gatunki nicieni, z których wyizolowano bakterie Species of nematodes from which bacteria were isolated	Gatunek bakterii Bacteria species	Referencje References	Nazwa szczepu Strain name
Steinernema feltiae	*Xenorhabdus bovienii*	Akhurst (1982)	ScP
Steinernema arenarium	*Xenorhabdus kozodoii*	Tailliez et al. (2006)	S-03
Steinernema kraussei	*Xenorhabdus bovienii*	Burnell and Stock (2000)	S-06
Heterorhabditis bacteriophora	*Photorhabdus luminescens* subsp. *laumondii*	Boemare et al. (1993)	H-04
Heterorhabditis downesi	*Photorhabdus temperata* subsp. *temperata*	Maher et al. (2017)	Veg

bacteria were isolated from entomopathogenic nematodes from the collection of the Department of Biological Pest Control (Institute of Plant Protection – National Research Institute, Poznań, Poland). For bacterial infection, *G. mellonella* larvae were infected with the invasive nematode (IJ) stage (Bedding and Akhurst 1975). *Photorhabdus* and *Xenorhabdus* were isolated from the haemolymph of dead *G. mellonella* (surface disinfected larvae) and cultivated on nutrient bromothymol blue triphenyltetrazolium chloride agar (NBTA, i.e., nutrient agar with 0.004% triphenyl tetrazolium chloride and 0.025% bromothymol blue) and incubated at room temperature in the dark for 4 days (Thanwisai et al. 2012). On NBTA medium, *Xenorhabdus* in the phase I (pathogenic) form is characterized by a dark blue color (photo 1A), while *Photorhabdus* bacteria in the phase I form are dark green (photo 1B) (Elbrense et al. 2021). A single colony from each isolate was subcultured on the same medium and kept in Luria-Bertani (LB) broth supplemented with 20% glycerol at –80°C for further species identification and bioassay.

A B



Fot. 1. Bakterie w formie pierwotnej wyizolowane na podłoże NBTA (tj. agar odżywczy z 0,004% chlorkiem trifenylotetrazoliowym i 0,025% błękitem bromotymolowym) wyizolowany z hemolimfy zwłok *Galleria mellonella*: A – bakterie *Xenorhabdus* związane z *Steinernema kraussei*, B – bakterie *Photorhabdus* związane z *Heterorhabditis downesi*

Photo 1. Bacteria in phase I cultivated on NBTA medium (i.e., nutrient agar with 0.004% triphenyl tetrazolium chloride and 0.025% bromothymol blue) isolated from the hemolymph of *Galleria mellonella* cadaver: A – *Xenorhabdus* bacteria associated with *Steinernema kraussei*, B – *Photorhabdus* bacteria associated with *Heterorhabditis downesi*

Owady / Insects

Biological tests were carried out on larvae of the greater wax moth, *G. mellonella*. The larvae were reared at room temperature of 22–23°C and fed with wax foundation. For the experiment, larvae in the last developmental stage were used, whose length ranged from 2 to 2.5 cm, the weight ranged from 150 to 700 mg and whose epidermis was cream-colored. After the larvae were selected, 24-hour starvation was carried out for bacterial infection.

Przeżywalność bakterii na węzie pszczelej / Bacterial viability on wax foundation

The wax substrates used in the experiment (stored in the freezer) were crushed, weighed (1 g) and placed in sterile 6-well plates. They were then sprayed with a bacterial suspension containing 75 CFU per ml of sterile water with some mild detergent Tween 20. *Xenorhabdus* and *Photorhabdus* bacteria were used for suspension and cultured on NBTA medium at room temperature of 20–22°C for 4 days to select phase I (pathogenic). Each variant was carried out in 40 replicates and stored at 20–22°C under room conditions. The experiment lasted for 10 days. Each day a sample of wax medium was taken, 1 ml of sterile water was added, gently vortexed, then 200 µl of the suspension was placed on NBTA medium and incubated at room temperature in the dark for 4 days.

Wybór odpowiedniej temperatury / Selecting correct temperature

The experiment was carried out in 6-well sterile plates; 1 g of food in the form of wax foundation (kept in the freezer) (Strojny 1981) sprayed with a 1 ml dose of bacteria was added to each well. A dose of 125 CFU/ml was used in the experiment prepared by adding pure bacterial colonies to sterile water sterile water with some mild detergent Tween 20. The control was performed with 1 ml deionized sterile water with detergent (Tween 20). The infectious dose was confirmed for each experiment by serial dilutions and colony counts. A surface-sterilised insect was then placed in each hole using an alcohol-soaked swab. The analysis was conducted in one repetition, consisting 40 insects, per bacterial strain (15, 20, 25, 30 and 35°C). The study was followed for 10 days.

Testy biologiczne na owadach / Insect bioassays

Bioassays were conducted in 6-well sterile plates; 1 g of food in the form of wax foundation (kept in the freezer for sterility) (Strojny 1981) sprayed with a 1 ml dose of bacteria was introduced into each well. Three different doses were used for the experiment: 125, 100, 75 CFU/ml, which were made by adding pure bacterial colonies to sterile water with detergent (Tween 20). The control was performed with 1 ml of deionized water with Tween 20. The infectious dose was confirmed for each experiment by serial dilutions and colony counts. Insects, surface sterilized with the use of alcohol-soaked swab, were put into the hole. The analysis was conducted in one repetition, consisting 40 insects, per bacterial strain and kept under

room conditions at 25–30°C. The mortality of the insect was confirmed by isolating bacteria from its haemolymph and growing them on NBTA medium. After infection, *G. mellonella* larvae were observed for seven days for mortality.

Analiza statystyczna / Statistical analysis

Data were analyzed for significance of main effects using analysis of variance (ANOVA), Tukey's test and arithmetic means were compared using Duncan's test ($P \leq 0.05$) (Duncan 1955). In addition, a comparison of percent mortality between strains and doses was performed and showed differences in percent mortality at different temperatures. In addition, a comparison of percent mortality between different temperatures was conducted for all five bacterial strains. Analyses were performed using the Statistica 12 program.

Wyniki i dyskusja / Results and discussion

Przeżywalność bakterii na węzie pszczelej / Bacterial viability on wax foundation

Tests carried out to check the survival of the bacteria on the wax foundation showed that the bacteria remained viable for a minimum of 7 days and maximum of 8 days (tab. 2).

Tabela 2. Przeżywalność bakterii *Photorhabdus* i *Xenorhabdus* na węzie pszczelej
Table 2. Surviving *Photorhabdus* and *Xenorhabdus* bacteria on wax foundation

Szczepy bakterii Bacterial strains	Obecność bakterii według dnia obserwacji Presence of bacteria by day of observation									
	1	2	3	4	5	6	7	8	9	10
H-04	+	+	+	+	+	+	+	–	–	–
Veg	+	+	+	+	+	+	+	+	–	–
S-03	+	+	+	+	+	+	+	+	–	–
ScP	+	+	+	+	+	+	+	–	–	–
S-06	+	+	+	+	+	+	+	+	–	–

Wybór odpowiedniej temperatury / Selecting correct temperature

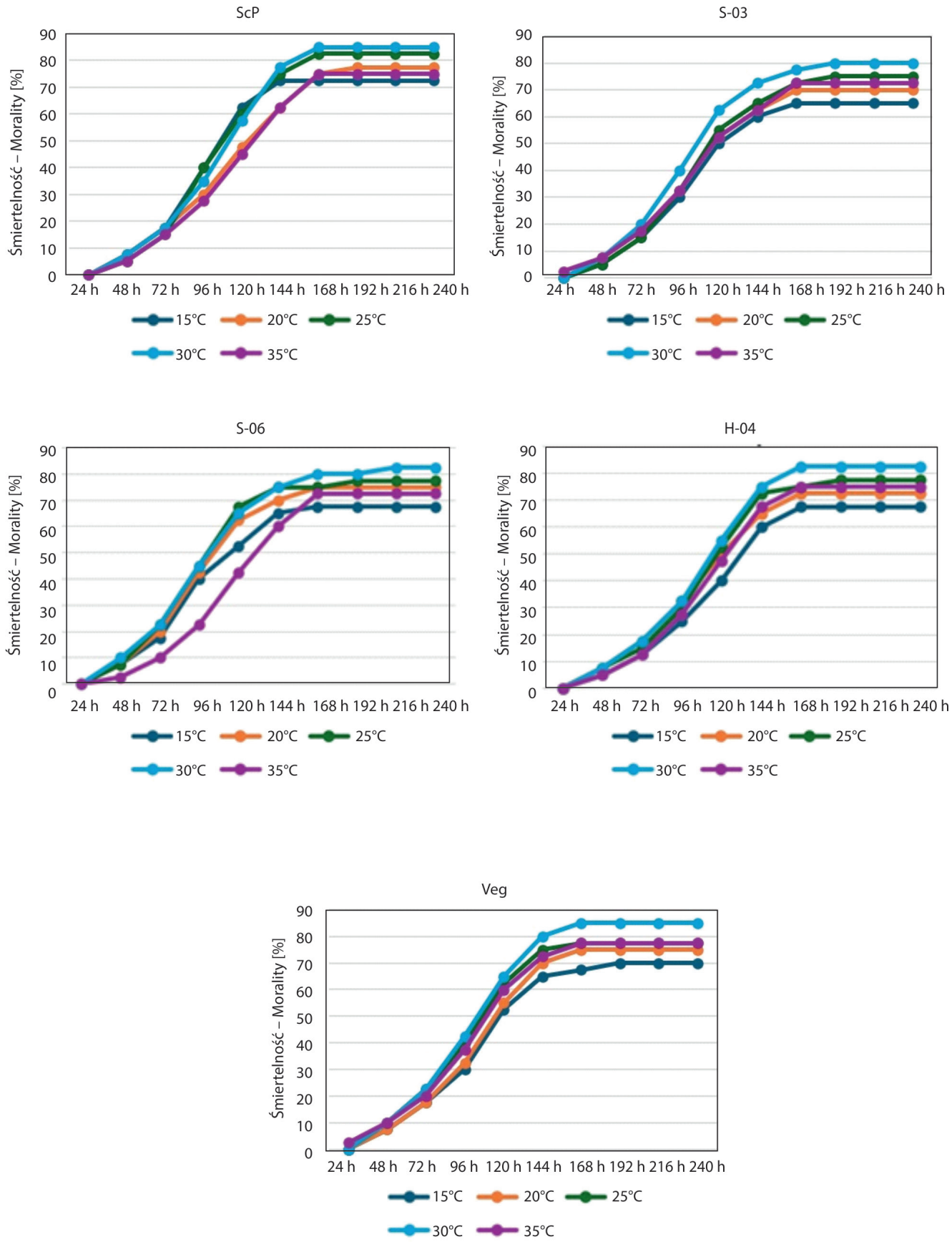
Tests were conducted to expose *Photorhabdus* and *Xenorhabdus* bacteria to different temperatures in order to select the appropriate temperature for the highest mortality at the highest bacterial dose (125 CFU/ml). After 10 days of observation, an increase in insect mortality was observed at 20°C and a decrease at 35°C. All strains had the highest mortality at 25°C and 30°C, indicating that the best temperature for the bacteria is between 25–30°C (fig. 2).

Aktywność owadobójcza szczepów *Photorhabdus* i *Xenorhabdus* / Insecticidal activity of *Photorhabdus* and *Xenorhabdus* strains

The conducted study revealed the pathogenicity of the bacterial suspension (*Xenorhabdus* and *Photorhabdus*) towards *G. mellonella* larvae. The first dead insects were observed on the second day of the experiment. At the higher doses used (i.e., 100 and 125 CFU/ml), each of the strains evaluated caused mortality of *G. mellonella* individuals on that day. In contrast, at the lowest dose used (i.e., 75 CFU/ml), death was observed only for two *Photorhabdus* strains isolated from the nematode *H. bacteriophora* and *H. downesi*. At this dose, the pathogenicity of three *Xenorhabdus* bacterial strains isolated from the nematode *S. feltiae*, *S. arenarium* and *S. kraussei*, was observed only on the third day of the experiment. The highest number of dead larvae was recorded between the 3rd and 5th day, while a decrease in mortality was noted after the 5th day. In the control, after 7 days observation, no dead insects were found (tab. 3). From each dead insect, bacteria were isolated from the hemolymph on NBTA media to confirm the pathogenicity of the bacteria. *Xenorhabdus* bacteria were visible as blue colonies, and *Photorhabdus* bacteria were visible as light green colonies.

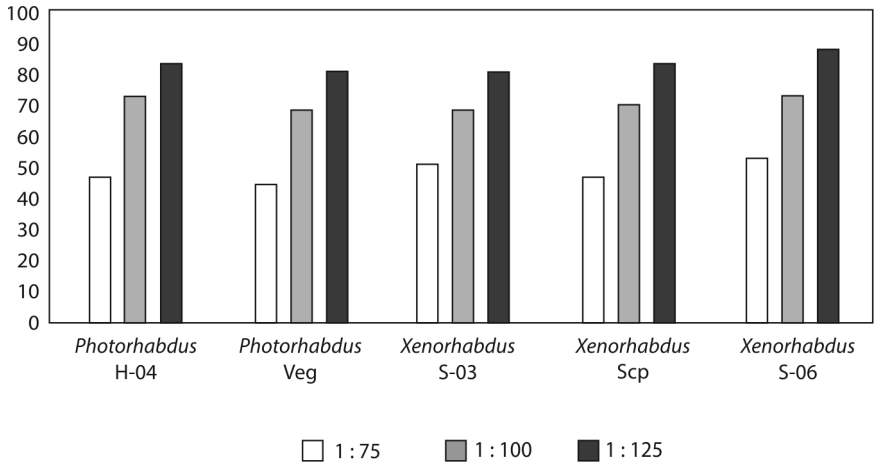
Analiza statystyczna / Statistical analysis

While mortality of all sets of larvae (three bacterial concentrations) did not differ significantly between groups, we detected a significant difference between controls and the *Photorhabdus* treatment, and between controls and *Xenorhabdus* treatment (ANOVA, Tukey's test fed set of larvae with bacteria $F = 81.572$, $p = 0.0013$; ANOVA, Tukey's test fed set of larvae without bacteria $F = 82.945$, $p = 0.0013$). The pathogenicity of the various strains tested was closely related to the concentration used. At the lowest concentration (i.e., 75 CFU/ml), among *Photorhabdus* strains, the highest mortality (47.5%) isolated from the nematode *H. bacteriophora*, while among *Xenorhabdus* strains isolated from *S. kraussei* (52.5%). Similarly, at the highest concentration (i.e., 125 CFU/ml), among *Photorhabdus* strains, the highest mortality was also observed for bacteria isolated from *H. bacteriophora* (82.5%), while among *Xenorhabdus* strains isolated from *S. kraussei* (87.5%). However, at both the highest and lowest concentrations, the *Xenorhabdus* strain isolated from *S. kraussei* was the most pathogenic (fig. 3). There was no mortality in the control group. The viability of *G. mellonella* individuals at the two highest concentrations showed little difference compared to the lowest concentration (tab. 4). For *Photorhabdus* sp. bacteria, the most effective lethal concentration was 82.5% at a concentration of 125 CFU/ml and a confidence interval of mortality of 0.31×10^4–2×10^6. For *Xenorhabdus* sp. bacteria, the most effective lethal concentration was 87.5% (concentration of 125 CFU/ml) and a confidence interval of mortality of 0.79×10^4–9×10^5 (tab. 5).



Rys. 2. Skuteczność *Xenorhabdus* i *Photorhabdus* oceniana jako procentowa śmiertelność larw *Galleria mellonella* przez 10 dni (240 godzin) w czterech wariantach temperatury (15, 20, 25, 30, 35°C). Śmiertelność larw w testach kontrolnych wyniosła 0%

Fig. 2. The efficacy of *Xenorhabdus* and *Photorhabdus* assessed as percentage mortality of *Galleria mellonella* larvae for 10 days (240 h) at four temperature options (15, 20, 25, 30, 35°C). Larvae in control assays showed a 0% mortality



Rys. 3. Porównanie śmiertelności między szczepami bakterii i dawką stężenia bakterii [CFU/ml]
Fig. 3. Comparison of mortality between bacterial strains and bacterial concentration dose [CFU/ml]

Tabela 3. Dzienna liczba martwych larw *Galleria mellonella* w trzech stężeniach bakterii
Table 3. Number of dead larvae *Galleria mellonella* per day for three bacterial concentrations

Szczepy bakterii Bacterial strains	Liczba martwych owadów według dnia obserwacji Number of dead insects by day of observation																				
	1			2			3			4			5			6			7		
	dawka bakterii [komórki/ml] bacterial dose [cells/ml]																				
	75	100	125	75	100	125	75	100	125	75	100	125	75	100	125	75	100	125	75	100	125
H-04	0	0	0	1	6	4	4	9	8	8	8	9	3	3	6	3	2	3	0	1	3
Veg	0	0	0	1	4	2	4	8	8	3	4	9	6	4	7	2	5	4	2	2	2
S-03	0	0	0	0	7	5	5	9	9	5	7	9	5	2	5	2	2	2	3	0	2
ScP	0	0	0	0	6	4	8	9	9	6	8	9	4	2	8	3	2	3	0	0	2
S-06	0	0	0	0	7	4	3	9	9	8	7	9	4	3	7	2	2	3	2	0	1
Kontrola Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Tabela 4. Wpływ symbiontów bakteryjnych *Photorhabdus* i *Xenorhabdus* na śmiertelność larw *Galleria mellonella*
Table 4. Effect of the bacterial symbiont *Photorhabdus* and *Xenorhabdus* on the mortality of *Galleria mellonella* larvae

Gatunki bakterii Bacterial species	Śmiertelność [%] Mortality [%]			Długość życia*[dni] Length of life*[days]		
	dawka bakterii [komórki/ml] bacterial dose [cells/ml]					
	75	100	125	75	100	125
H-04	47.5	72.5	82.5	5.4 ± 0.7 ab	3.3 ± 0.7 a	2.9 ± 0.3 ab
Veg	45.0	675	80.0	4.8 ± 1.1 ab	3.7 ± 0.6 ab	3.0 ± 0.6 bc
S-03	50.0	67.5	80.0	4.6 ± 2.4 a	3.7 ± 0.6 ab	3.0 ± 0.6 bc
ScP	47.5	70.0	82.5	5.4 ± 0.7 ab	3.5 ± 1.1 a	2.9 ± 0.3 ab
S-06	52.5	72.5	87.5	4.2 ± 0.4 a	3.3 ± 0.7 a	2.6 ± 0.2 a
Kontrol – Control	0	0	0	9.7 ± 1.5 c		

*średnie nie różnią się istotnie (P < 0,05) – the averages are not significantly different (P < 0.05)

Toxin proteins, which are found in the bacterium *Photorhabdus* sp., have long been identified as an alternative to *Bacillus thuringensis* in the control of insect pests (Sheets and Aktories 2017; Clarke 2020). Tcs toxins were tested on a model insect: *G. mellonela*, because it is considered the best surrogate for toxicological assays. Although it has been shown that *Photorhabdus akhurstii* (Akhurst, 1993) can cause marked cytotoxicity when injected directly into the haemocoel of *G. mellonella*, the oral administration of bacteria has not been studied in detail (Mathur et al. 2019). In the present study, instead of using protein toxin injections into the insect, dietary application was undertaken along with spraying of *Photorhabdus* and *Xenorhabdus* bacteria. A few studies have already reported the efficacy of force feeding for toxicological analysis (Maguire et al. 2016), but in this research we focused on obtaining results of practical application of these bacteria. This research was conducted to determine the toxicity of five bacterial strains belonging to the *Photorhabdus* and *Xenorhabdus* genera. It was observed that with increasing bacterial doses, the mortality of *G. mallonella* larvae increased. The highest mortality rate was 87.5% when using a dose of 125 CFU/ml. Despite such a large dose applied to the food surface, 100% mortality was not achieved as in the case of injection.

Entomopathogenic nematodes have a stage found in the soil called the infective juveniles (IJs). This is the stage that has the ability to actively seek out a host insect. The third stage, the IJs, can survive in the soil environment for many months due to its considerable resistance to environmental factors. The IJs enter the body cavity of the insect mainly through its natural openings, i.e., mouth, anus, or spiracles. *Steinernema* and *Heterorhabditis* nematodes contain the bacteria *Xenorhabdus* and *Photorhabdus* in their anterior gastrointestinal tract, which are released immediately after the IJs nematode enters the insect's haemolymph (Askary 2010). Our experiment was based on feeding food sprayed with bacteria to *G. mellonela* larvae. Each dead insect was checked for the presence of bacteria in the hemolymph, so we assume that the supplied food, along with the bacteria, moves to the digestive system through the mouth, as in the case of bacteria being transmitted by nematodes. Every infected insect showed the presence of bacteria in the hemolyph, so we assume that the bacteria move into the haemolymph and release their toxins.

Biochemical taxonomic studies on genus *Xenorhabdus* under laboratory conditions showed the pathogenic ability of the bacterial symbiont *Photorhabdus* and *Xenorhabdus* for the greater wax moth (*G. mellonella*) at doses of 100 and 1000 CFU/ml (Boemare and Akhurst 1988). Mortality was observed as early as the third day of observation, while more deaths were recorded at the higher dose. The number of bacterial cells has an impact on the rate at which larvae are killed, so there is a direct correlation between mortality and the spray dose. This is confirmed by the results obtained in this study, which showed that all concentrations used (i.e., 75, 100 and 125 CFU/ml) allowed a reduction in the number of larvae compared to the control, achieving mortality rates in the range of 45–87.5%. In this study, all five strains of entomopathogenic bacteria caused mortality in *G. mellonella*, but there were no significant differences between the strains. The different virulence of these bacteria may be related to the number of bacteria ingested by the tested insect. The highest percentage of mortality was obtained using *Xenorhabdus bovienii* bacteria isolated from the nematode *S. kraussei*. This nematode occurs mostly on slightly acidic soils, often in areas overgrown by coniferous forests (Tumialis et al. 2014). *Steinernema kraussei* has been shown to be effective against the grubs *Melolonta melolontha* L., *Melolonta hippocastani* L. and *Amphimallon solstitiale* L. (Kowalska 2001; Matuska-Łyżwa et al. 2012).

This study analysed the performance of five bacterial strains of the *Xenorhabdus* and *Photorhabdus* genera at different temperatures. Temperature is responsible for modeling biological processes of both hosts and parasites (Mahar et al. 2005; Shapiro-Ilan et al. 2006), so we expected different levels of infectivity of bacteria symbiotically associated with nematodes at five different temperatures (15, 20, 25, 30 and 35°C). This test showed higher susceptibility of *G. mellonella* to all bacterial strains within 240h (i.e., > 80% mortality) at 30°C, probably due to better growth conditions for *Xenorhabdus* and *Photorhabdus* bacteria (Chen et al. 1996). Bacterial infectivity decreased signifi-

Tabela 5. Porównanie patogeniczności symbiontów bakteryjnych *Xenorhabdus* i *Photorhabdus* dla larw *Galleria mellonella* dla dawki 125 CFU/ml

Table 5. Comparison of average pathogenicity of strains belonging to the *Xenorhabdus* and *Photorhabdus* genera for *Galleria mellonella* larvae for a dose of 125 CFU/ml

Rodzaj Genera	Dawka bakterii [komórki/ml] Bacterial dose [cells/ml]	95% C.L.	Slope ± S.E.	Variancja Variance	X^2
Photorhabdus	125	0.31×10^4–2×10^6	0.7 ± 7.1	0.5	3.84
Xenorhabdus	125	0.79×10^4–9×10^5	0.8 ± 8.2	2.3	5.99

cantly at 15 and 35°C, which was expected because bacteria have temperature minimums and maximums at which they lose their ability to feed (Wang et al. 2008). With these tests, we determined the optimal temperature at which the bacteria showed the highest percentage of mortality for *G. mellonella* (25–30°C).

Wnioski / Conclusions

So far, research has not found benefits or agricultural applications with *Photorhabdus* and *Xenorhabdus* bacteria, but this is a promising future in the agricultural industry. The use of these bacteria as a biological control agent would enable effective control of crop pests. This would save time, through easy application in cultivation and in protecting the environment, human and animal life by avoiding harmful chemicals. One important capability of the bacteria is its wide range of insect hosts, making it a viable alternative. Conducted research confirmed that by spraying food with a bacterial suspension, a high percentage of pest mortality can be achieved. However, further research needs to be conducted to test the resistance and robustness of the bacteria to various environmental conditions.

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