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An Approach for the Control of *Caenorhabditis elegans* N2 via the Regulation of Growth Conditions and *Pleurotus ostreatus* Po4

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Abstract: Food resources are essential for the survival and growth of the population. Soil phytopathogenic nematodes cause great damage to agricultural crops, endangering food supplies and resources in general. Different methods have been used to control them. However, this issue still requires a more effective solution. *Caenorhabditis elegans* (CGC strain wild-type N2) was applied as a model with an *Escherichia coli* OP50 feeding substrate for nematodes. Our approach was based on the thermodynamically substantiated creation of growth conditions that are unfavorable for nematodes to suppress them irreversibly. The thermodynamic calculations showed that obligate anaerobic conditions, namely the absence of oxygen and a low redox potential (−100 mV and below), were potentially unacceptable for nematodes. Anaerobic conditions were created using both abiogenic (physicochemical) and biological methods. Abiogenic anaerobic conditions were achieved by preventing oxygen access and adding low-potential sodium sulfide (Eh = −250...−200 mV) to the cultivation medium. By applying biological methods, *Pleurotus ostreatus* Po4 and *E. coli* O_2 was completely removed and the redox potential was decreased from +100. . .+200 mV to −100...−200 mV (in particular, due to the synthesis of H_2S). Even the short-term exposure (1–2 days) of nematodes under anaerobic conditions led to their suppression and death. Thus, the short-term creation of anaerobic conditions in the soil may be an effective method to control, e.g., phytopathogenic aerobic nematodes. This research contributes to the development of foundations to preserve agricultural plants and increase crop yield as well as the development of an approach for the environmentally friendly control of phytopathogens.

Keywords: phytopathogenic nematodes; food resources; thermodynamic prediction; growth control; crop preservation

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1. Introduction

Agriculture is one of the most important areas of the economy and human life since it supplies a steadily growing population with the food resources that are essential to survive [1]. Nematodes are the most spread multi-cellular organisms on the globe [2]. They are considered to be the most abundant among Metazoas in soil. The amount of nematodes in bulk soil reaches 40 individuals/g. Rhizosphere soil can contain up to 200 individuals/g [3]. Depending on the conditions, their density can reach up to 10 million individuals/m^3 of soil [2]. Nematodes reside in water films surrounding soil particles as well as around or even in the roots of plants. They are characterized by a wide range of substrates to consume bacteria, fungi, algae, and protozoa. Special attention is paid to the group of parasitic species against plants and animals [3].

Nematodes were estimated to be the main pathogens for global agriculture and cause greater crop loss than bacteria and viruses. Their activity threatens the stability of food resource maintenance [2]. More than 4100 species of plant-parasitic nematodes have been identified. The species of genera *Heterodera*, *Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, and *Xiphinema* are considered to be the most harmful for agriculture [4]. They threaten valuable agricultural crops such as sugar beet, potato, soybean, maize, wheat, etc. [3–5]. The crop losses caused by nematodes are estimated to be up to USD 100–118 billion annually [4,6]. The specific features of nematodes such as high survival ability, wide variety of plant hosts, and the types of harm cause their wide distribution and encourage the search for pathways of their neutralization and preservation of the yield of valuable agricultural crops [2].

The methods used to control nematodes are mainly concentrated on chemical nematicides, cultural practices, and the development of resistant plant cultivars [6]. For the past decades, chemicals have been widely used and considered the best solution to the problem due to their high efficiency, ease of use, and low cost [2]. However, environmental concerns (for example, toxic compounds such as dibromochloropropane and ethylene dibromide [6]), potential hazards to human health, and resistance development forced the search for an alternative environmentally friendly approach. Biological control methods instead of chemical nematicides are considered to be promising alternatives [2]. The application of natural enemies of nematodes can be useful to prevent their spread in soil [6]. One such biological approach is based on the use of the antagonistic properties of some bacteria and fungi against nematodes. Microorganisms have evolved a variety of mechanisms to control nematodes [2]. For example, a lot of nematophagous bacteria producing hydrolytic enzymes and toxins were studied: *Actinomycetes*, *Agrobacterium*, *Arthrobacter*, *Alcaligenes*, *Aureobacterium*, *Azotobacter*, *Bacillus, Beijerinckia*, *Chromobacterium*, *Clavibacter*, *Clostridium*, *Comamonas*, *Corynebacterium*, *Curtobacterium*, *Desulforibtio*, *Enterobacter*, *Flavobacterium*, *Gluconobacter*, *Hydrogenophaga*, *Klebsiella*, *Methylobacterium*, *Pasteuria*, *Pseudomonas*, *Phyllobacterium*, *Phingobacterium*, *Rhizobium*, *Stenotrotrophomonas*, and *Variovorax* [2,7]. Also, a wide group of nematophagous fungi was explored: Ascomycetes, Basidiomycetes (e.g., *Pleurotaceae*), Chytridiomycetes, and Zygomycetes, as well as Chromistan Oomycetes. They are performed by endoparasitic (obligate parasites of nematodes), nematode-trapping (capture nematodes by trapping devices produced from the vegetative mycelia), opportunistic (colonize nematode reproductive structures and affect their reproductive capabilities), and toxic fungi (produce low-molecular toxic metabolites) [2].

Pleurotus ostreatus is a predatory mushroom known to produce nematocidal toxins causing immobilization and death [8–10]. There are several compounds produced by this fungus: trans-2-decenedioic nematocidal toxin that paralyzes and kills nematodes and linoleic acid, which reduces nematodes' head sizes. The mechanisms of nematodes' control are still unclear [11–13]. Another substance (3-octanone) produced by *P. ostreatus* was recently shown with the potential to kill *Caenorhabditis elegans* by causing the disruption of cell membrane integrity, calcium influx, etc. [14]. Thus, the use of *P. ostreatus* is considered to be one of the most promising methods to control nematodes. *C. elegans* is a free-living nematode that lives in temperate soil environments. It serves as a model organism in various types of research [15].

Since the issue of plant protection against nematodes is still active, the existing methods do not provide an effective problem solution. Though chemical nematicides are effective enough, they threaten the environment and human health. Biological methods are attractive due to their environmental friendliness. However, the application of some specific bacterial or fungal species can restrict the area of their use to specific species of nematodes.

Our approach is based on the application of the set of factors that affect the growth of nematodes in different ways, providing effective control with no harm to the environment. Therefore, the goal of this work was to investigate the patterns of the effects of pH, redox potential, and O_2 presence as well as *P. ostreatus* Po4 and bacteria (*Escherichia coli* OP50

and soil microbial community) on nematodes to develop foundations for their effective environmentally friendly control, contributing to the preservation of valuable agricultural crops and food resources.

2. Materials and Methods

2.1. Organisms Used in the Experiments

In the experiments, a model organism representing nematodes, *Caenorhabditis elegans* wild-type N2, was used. It was obtained from the Caenorhabditis Genetics Center (CGC), University of Minnesota (USA). It was isolated from the mushroom compost near Bristol (Great Britain) and maintained in the CGC collection. Bacteria were represented by the model bacterium *Escherichia coli* OP50, which served as a food source for *C. elegans* N2 and was obtained from CGC together with *C. elegans* N2. Native bacteria community obtained from the compost was isolated and also served in the experiments as models of naturally occurring soil bacterial community. *P. ostreatus* Po4 used in the experiments was a wild-type mycelium grown from a mushroom fruiting body, which was collected in nature close to Kluczbork city (southwest Poland). The mycelium was stored in the University of Opole collection created and maintained by E. Moliszewska, Ph.D.

2.2. Theoretical Estimation of Conditions for C. elegans N2 Suppression

To evaluate the conditions required to inhibit a model nematode *C. elegans* N2, Pourbaix diagrams of the stability of elements in water solutions were applied [16,17]. We considered two main factors of the environment (pH and Eh) provided in the diagrams to determine the conditions enhancing or suppressing the growth of *C. elegans* N2.

2.3. Measurement of Cultivation Parameters

The values of pH and Eh of the medium and other solutions were detected potentiometrically using pH/conductivity meter CPC-411, electrode pH EPS-1, and electrode redox ERS-2 (Elmetron, Zabrze, Poland), respectively.

2.4. Preparation of pH Buffer and Study of the Range of pH Values for C. elegans N2 Activity

Phosphate buffers with pH values within 3.0–11.0 with step 1.0 were used. The buffers were prepared using stock solutions of NaH_2PO_4 (pH = 4.4) and Na_3PO_4 (pH = 12.1), each at a concentration of 20 g/L. To obtain a buffer with pH = 3.0, the NaH_2PO_4 solution (pH = 4.4) was titrated with 0.1 N HCl. The same titration was used to obtain a buffer with pH = 4.0. To obtain buffers with pH 5.0–11.0, stock solutions were mixed in the appropriate ratios. Prepared buffer solutions were added to the nutrient medium where it was necessary and sterilized via autoclaving. After sterilization, pH changes were insignificant.

To investigate the effect of pH on the growth of nematodes, they were pre-cultivated in Petri plates on PA in the presence of the pure culture of *Escherichia coli* OP50 used as a substrate [18]. After 2 days of cultivation, the effect of pH values in the range from 3.0 to 11.00 with step 1.0 was studied as follows. Holes with a diameter of 5 mm were made in PA with pre-cultivated nematodes. There were 4 holes per plate. The pH buffer (50 µL) creating the specific pH value was added to each hole. As a control, 0.9% solution of NaCl (pH = 7.0) was used. The observations of the influence of pH on the activity and survival of nematodes took place after 1; 12; 24; 48; 96; and 288 h. It was evaluated via the activity of movement of nematodes. Due to the small volume of buffer and long cultivation period, 50 µL pH buffer was added to each hole every 24 h to avoid its exsiccation.

2.5. Preparation of Eh Buffer and Study of the Effect of Eh on the Survival of C. elegans N2

The low-potential buffer that creates Eh = −500. . .−400 mV (pH = 8.0) was prepared as follows: The initial buffer solution with 2.5 g/L concentration of S^{2-} was prepared via the dissolution of 26 g of $Na_2S{\cdot}9H_2O$ salt in 74 mL of distilled water purged with argon (flow rate of 0.5 L/min) for 15 min to remove oxygen. It was used to prepare buffer solutions

used in the experiment with the concentration of 100–500 mg/L of S^{2-} with the step of 100 mg/L.

To study if the low potential of the culture medium affects the activity or survival of nematodes, the following experiment setup was used: The holes (5 mm in diameter) were prepared in PA with the pre-cultivated nematodes with *E. coli*. Buffer solution with the specific concentration of S^{2-} was added to each hole. Solution of 0.9% NaCl (pH = 7.0) was used as a control. The observation of the effect was carried out after 5; 30; and 60 min. The estimation of the inhibition was conducted via the percentage of the actively moving nematodes.

2.6. Preparation of Bacterial Microbiomes to Study Their Effect on C. elegans N2

To study the effect of conditions created by microorganisms on the survival of *C. elegans* N2, two types of bacterial microbiomes were used: heterotrophic and sulphate reducing bacterial microbiomes. They were obtained from the compost of plant residues. The heterotrophic microbiome was obtained via inoculation of 50 mL of peptone broth (PB) (BioMaxima S.A., Lublin, Poland) with 1 g of compost. The cultivation took place in the hermetically sealed vessels with the volume of 150 mL for 14 days at 20 °C. Sulfate-reducing microbiome was obtained in modified Posgate's B medium, g/L: KH_2PO_4—0.5; NH_4Cl—1.0; Na_2SO_4—2.0; $NaC_3H_5O_3$—3.5; $FeSO_4$—0.5. The inoculation of 50 mL of medium with 1 g of the compost sample was followed by cultivation in 150 mL vessels for 14 days at 20 °C. The maintenance of the metabolic activity of each microbiome was carried out via periodical reseeding of samples with a volume of 5.0 mL into the appropriate fresh nutrient medium.

2.7. Investigation of the Influence of Bacterial Microbiomes on C. elegans N2 with Open-Air Access

The effects of heterotrophic and sulphate-reducing bacterial microbiomes was studied as follows: The PA with the pre-cultivated nematodes with *E. coli* OP50 was perforated with holes (5 mm in diameter). The aliquots (20 µL) of the medium with each microbiome were added to the appropriate holes. As a control, 0.9% solution of NaCl (pH = 7.0) was applied. The exposition took place for 2 days with open-air access at 20 °C. The evaluation of the survival rates of *C. elegans* N2 was carried out by the percentage of the actively moving nematodes as well as following the cultivation of the sampled aliquot (0.5 mL) containing nematodes in the fresh medium.

2.8. Study of the Effect of Microorganisms on C. elegans N2 under Anaerobic Conditions

The specific chambers in the Petri plates were created for this purpose (Figure 1). The PA (BioMaxima S.A., Lublin, Poland) with sodium resazurin solution as the indicator of the redox potential of the medium was used for cultivation. *C. elegans* N2 with *E. coli* OP50 were pre-cultivated on PA for 2 days. Narrow channels were made in the PA, which were filled with a mixture of paraffin and petroleum jelly mixed in a volume ratio of 1:1. That created isolated chambers, providing the opportunity to create different growth conditions (Figure 1). Chamber 1 contained *C. elegans* N2 and *E. coli* OP50 as a substrate with open-air access. Chamber 2 contained *C. elegans* N2, *E. coli* OP50, and heterotrophic bacterial microbiome with open-air access. Chamber 3 was covered with a plastic transparent film to avoid air access and create anaerobic conditions. It contained *C. elegans* N2, *E. coli* OP50, and heterotrophic microbiome. The aliquot containing a heterotrophic bacterial microbiome was 0.5 mL. The cultivation took place for 4 days at 20 °C.

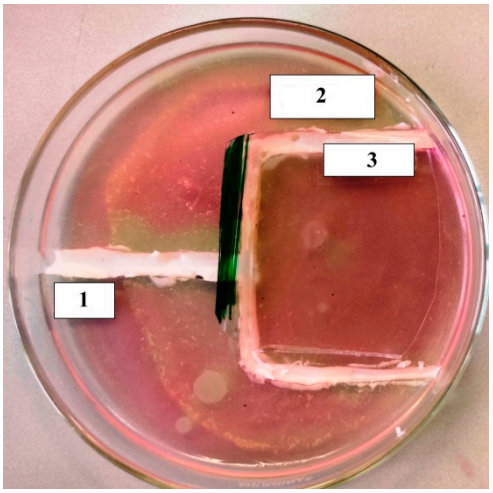

Figure 1. The cultivation system used to evaluate the effect of microorganisms on the activity of *C. elegans* N2: 1—chamber with *C. elegans* N2 and *E. coli* OP50 as a substrate with open-air access; 2—chamber with *C. elegans* N2, *E. coli* OP50, and heterotrophic bacterial microbiome with open-air access; 3—chamber with *C. elegans* N2, *E. coli* OP50, and heterotrophic bacterial microbiome covered with plastic transparent film to avoid air access and create anaerobic conditions.2.9. The Effect of *P. ostreatus* Po4 on the Activity of C. elegans N2.

To evaluate the approach to control *C. elegans* N2 via the application of *P. ostreatus* Po4, the following experiment set up was used: *P. ostreatus* Po4 was pre-cultivated for 7 days in PB at 25 °C to obtain mycelium covering the surface medium in Petri plate. Obtained mycelium was layered on the surface of the PA in the Petri plate with *C. elegans* N2 and *E. coli* OP50 that were pre-cultivated for 2 days. PA contained sodium resazurin to indicate the formation of anaerobic conditions. The effect of *P. ostreatus* Po4 was evaluated via the moving activity of *C. elegans* N2 on the medium. Cultivation was carried out for 7 days at 20 °C.

2.9. Data Analysis

The experiments were performed in triplicate. The analysis of obtained data was performed via Microsoft Excel, version 14.0.7116.5000 (Microsoft Corporation, Redmond, WA, USA). Graphs were created via OriginLab software, version 8.5.1 (Northampton, MA, USA). Mean values and standard deviations (SDs) were determined with a 95% confidence level. The values (x) were presented as the mean ± SD. The significance of the influence of redox potential of the medium created by S^{2-} on *C. elegans* N2 survival rate as well as the effect of microbiomes on the activity of *C. elegans* N2 was determined via the one-way ANOVA test with the post-hoc test (Bonferroni correction).

3. Results

3.1. Theoretical Substantiation of Conditions for C. elegans N2 Suppression

To develop an approach for the inhibition of nematodes, the method of thermodynamic prediction via Pourbaix diagrams [16,17] was used (Figure 2). Illustrating the pH-Eh dependence, the diagrams allow for the calculation of the stability of any organic and inorganic compound. This dependence can also be effectively used in relation to all living organisms in order to determine the optimal or unfavorable conditions for their functioning. The obtained calculations are useful to create conditions for stimulation or, conversely, for the inhibition of the growth of organisms.

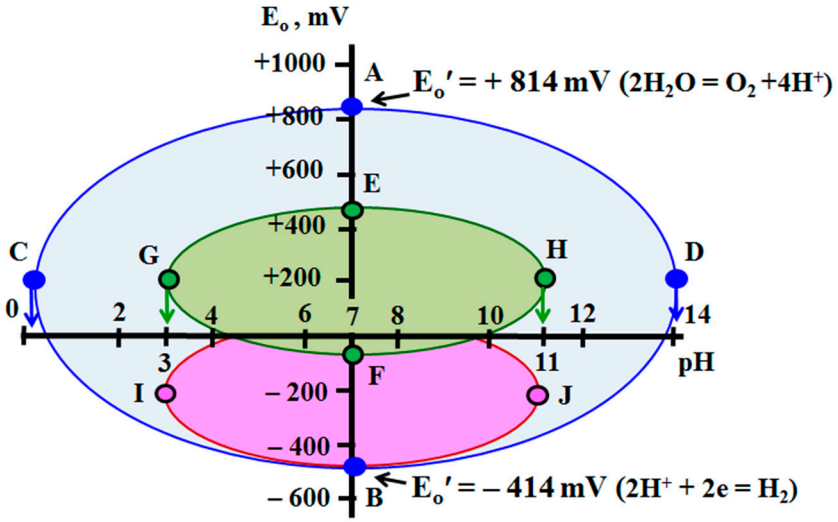

Figure 2. Pourbaix diagrams based on substantiation of the zone of thermodynamic stability of water (blue background); the zone suitable for the existence of aerobic (green background) and anaerobic organisms (pink background).

A model representative of nematodes, *C. elegans* N2, is an aerobic organism with pH = 7.0, which is optimal for growth [19]. Therefore, it can be assumed that pH shifts to acidic or alkaline values will lead to the inhibition or death of nematodes. *C. elegans* N2 is expected to be adversely affected by anaerobic low-potential conditions at Eh < −50 mV.

3.2. The Effect of pH on Survival of C. elegans N2

According to the thermodynamic estimation, there is no restriction of nematode growth in a wide range of pH. The theoretical considerations were experimentally confirmed. The cultivation of nematodes during 12 days in the presence of pH buffers in the range from 3.0 to 11.0 did not reveal any significant inhibition of their activity. During the first 12 h of incubation, the speed of movement of the nematodes was observed to be about 1.5-fold lower at pH = 3.0 and 11.0. However, this effect was not observed during the following cultivation. Moreover, resistance to the increasing level of salinity was found. Since a fresh portion of buffer solution was added every 24 h for 12 days, an 11-fold increase in the concentration of the buffer also did not cause any inhibitory effects.

3.3. The Inhibition of C. elegans N2 via Low Redox Potential

Since it is an aerobic organism [19], its optimum Eh should be within +200. . .+300 mV. Following the thermodynamic calculations, a low redox potential will inhibit the activities of nematodes. This was experimentally confirmed. The exposition of *C. elegans* N2 in the presence of S^{2-} during 60 min. caused the death of 100% of nematodes via the criterion of the complete loss of mobility (Figure 3).

The decrease in the activity (mobility) of nematodes in the concentration range of S^{2-} served as the criterion for the inhibitory effect of a low potential of the medium. Already after 5 min. of exposition, the movements of the nematodes were slower compared to the control. At a 100 mg/L concentration of S^{2-} (Eh = −422 mV), 60% of the nematodes survived after 5 min, and 27% survived after 30 min. The higher concentrations of S^{2-} and correspondingly lower redox potential of the medium (Eh = −476. . .−452 mV) provided survival for only 3 to 7% of nematodes after 5 min of exposition, decreasing these rates by more than twice after 30 min. of incubation. The followed cultivation of the aliquots of the culture medium taken after 60 min of incubation in the presence of S^{2-} in the fresh one showed the absence of the movements of nematodes confirming that a low redox potential is fatal for nematodes.

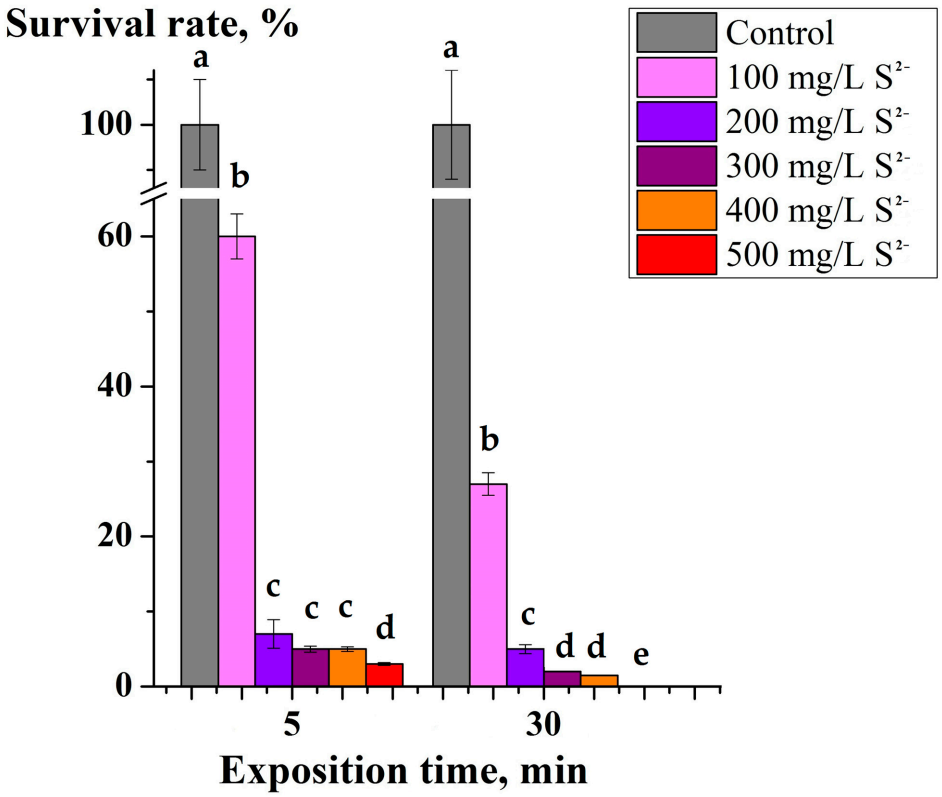

Figure 3. The effect of redox potential of the medium created by S^{2-} at the concentration of 100–500 mg/L on the survival of *C. elegans* N2 (x ± SD, n = 3); $p < 0.05$ with Bonferroni correction, where a, b, c, d, e show the statistical difference between the data set.

Thus, the values of the redox potential significantly influence the survival of *C. elegans* N2. The theoretical considerations were experimentally confirmed, showing the death of nematodes at Eh = −476. . .−422 mV.

3.4. The Influence of the Bacterial Microbiome on C. elegans N2 with Open-Air Access

The metabolic activity of *C. elegans* N2 was affected by two types of bacterial microbiomes obtained from the compost of plant residues: heterotrophic and sulfate-reducing bacterial microbiomes. The heterotrophic microbiome contained free-living rod-shaped and coccoid soil bacteria grown on peptone broth, consuming a wide range of organic substrates. The sulphate-reducing microbiome dominantly contained rod-shaped bacteria, consuming lactate as the basic nutrient, since it was cultivated on the selected sulphate-reducing bacteria medium (Posgate's B). It was shown that the heterotrophic bacterial microbiome did not provide a strong and extended inhibitory effect (Figure 4). The activity rate of the nematodes was almost the same as in the control conditions without the studied microbiomes. After 4–5 h of exposition with the heterotrophic bacterial microbiome, about 80% of the nematodes were actively moving. The movement was completely restored after 24 h of incubation. The sulphate-reducing microbiome caused an inhibitory action after 1 h. About 60% of the nematodes were active. After 5 h, 99% of the nematodes were inactive and remained in this state during the following 19 h. The gradual resumption of the activity of *C. elegans* N2 was observed after 48 h of exposition, which can be explained by the gradual oxidation of sulfides by air oxygen and an increase in the redox potential of the medium with open-air access.

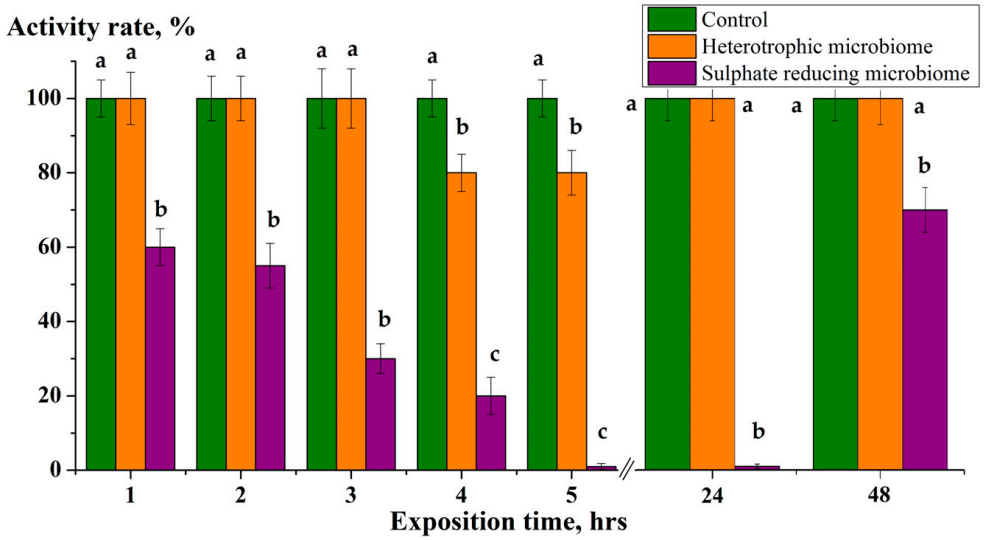

Figure 4. The effect of bacterial microbiomes on the activity of *C. elegans* N2 with open-air access (x ± SD, n = 3); $p < 0.05$ with Bonferroni correction, where a, b, c show the statistical difference between the data set.

Thus, the short-term exposition of *C. elegans* N2 with open-air access either with a heterotrophic or sulphate-reducing microbiome does not lead to the death of nematodes. The inhibitory effect was detected for 1 day with the sulphate-reducing microbiome.

3.5. The Effect of Microorganisms on C. elegans N2 under Anaerobic Conditions

Since the activity of the bacterial microbiome with open-air access did not provide a significant inhibition of the activity of *C. elegans* N2, the application of microorganisms under anaerobic conditions was also studied. The creation of the isolated chambers in the Petri plate provided the opportunity to evaluate the activity of nematodes simultaneously with and without open-air access (Figure 1). A sodium resazurin solution added to the medium as the indicator of anaerobic conditions became colorless after 1 h of cultivation in chamber 3 that had been covered with plastic film to avoid air access. The colorless indicator visualized that the redox potential in chamber 3 was less than −100 mV, confirming anaerobic conditions. At the same time, the sodium resazurin remained pink in chambers 1 and 2, indicating that the Eh >−50 mV, i.e., aerobic conditions.

The results of the experiment confirmed the theoretical considerations. Under aerobic conditions, no activity inhibition was observed. Moreover, in the case of the presence of a heterotrophic microbiome, the nematodes were even more active. Under such conditions, *C. elegans* N2 probably used the heterotrophic microbiome as an additional nutritive substrate. On the contrary, under anaerobic conditions, the movements of nematodes were practically absent after 1 h of cultivation. After 15 h, the degradation of nematodes was observed, resulting in their complete disappearance after 48 h of cultivation. In this case, *C. elegans* N2 served as the nutritive substrate for the microorganisms. The subsequent transfer of nematodes from an anaerobic medium to a medium at aerobic conditions showed a complete lack of activity (mobility) and the absence of nematode growth.

Thus, the anaerobic conditions created by microorganisms cause the death of nematodes. The positive feature of the creation of anaerobic conditions via microorganisms is the opportunity to avoid the application of excessive amounts of chemicals in the environment.

3.6. Enhancement of C. elegans N2 Inhibition via P. ostreatus Po4

The creation of anaerobic conditions via biologically based methods is considered to be more ecologically friendly compared to chemical application. Here, the mycelium of *P. ostreatus* Po4 layered on the surface of the nutrient medium with *C. elegans* N2 that was

pre-cultivated for 2 days with *E. coli* OP50 as a substrate played the role of an insulator of free air access (Figure 5).

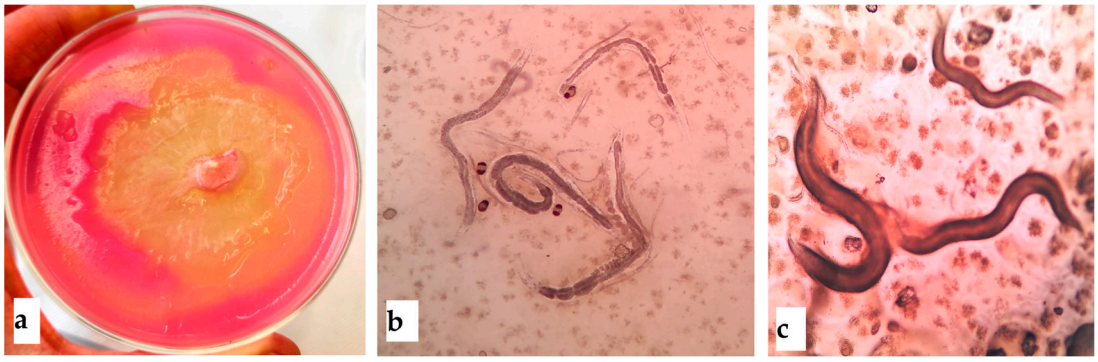

Figure 5. The effect of *P. ostreatus* Po4 as an insulator of free air access to the culture medium containing *C. elegans* N2: (**a**) pink-colored sodium resazurin in medium indicates free air access. Colorless sodium resazurin under *P. ostreatus* Po4 mycelium shows restriction of air access and low redox potential of medium (Eh ≤ -100 mV). (**b**) The appearance of the dead nematodes in anaerobic zone; (**c**) the appearance of active nematodes in aerobic zone.

Anaerobic conditions under the mycelium surface were formed after 2 h of cultivation. It was indicated by the change in the sodium resazurin to a colorless appearance (Eh ≤ -100 mV). This took place due to the restriction of air access through the mycelia hyphae film as well as the growth of *E. coli* OP50 on the peptone agar (PA) (BioMaxima S.A., Lublin, Poland) consuming its residual amount.

Under such conditions, after 2 h of cultivation, the movement of nematodes under the mycelium of *P. ostreatus* Po4 was not detected, while *C. elegans* N2 was active in the aerobic zone (pink color of sodium resazurin) where the mycelium was absent. After 48 h of cultivation, active nematodes were detected not only in the aerobic zone on the surface of the PA, but also between and on the hyphae of the aerial mycelium. However, *C. elegans* N2 was completely suppressed in the anaerobic zone formed via *P. ostreatus* Po4 and *E. coli*. The inoculation of the sample from the anaerobic zone and the subsequent cultivation on the fresh medium under aerobic conditions showed the absence of the growth restoration of nematodes.

Thus, the simultaneous application of bacteria and fungi is useful for the control of phytopathogenic nematodes. The effect of the complete suppression of *C. elegans* N2 was confirmed by the creation of unfavorable growth anaerobic conditions via the application of *P. ostreatus* Po4 and *E. coli*. Such an approach is promising for its implementation in agriculture to avoid the pollution of the environment with chemicals.

4. Discussion

With the development of society, the interest in applying biologically based approaches in industry and agriculture is growing. They are considered to be more environmentally friendly compared to physical and chemical ones [6]. Bio-based methods are used for obtaining fuel [20–23], the treatment of soil and water from hazardous xenobiotics [24–27], pathogen control [2,28–30], etc.

Nematodes are estimated to be among the most spread and harmful phytopathogens in soil [31]. There are different approaches applied to control them. However, until recent times, most of them were based on the application of chemicals, threatening the environment [31–33]. Today, attention is being paid to the application of environmentally friendly approaches based on the agents of biological origin. For example, the use of polyphenolic compounds from olive mill waste waters [32] or biosynthesized silver nanoparticles [33] as well as the activities of bacteria (*Lysobacter capsici*, *Bacillus cereus*, *B. thuringiensis*, *Pseudomonas chlororaphis*, *Serratia proteamaculans*, etc. [31]) and fungi [34] (*Arthrobotrys oligospora*,

Drechslerella dactyloides [35], *P. ostreatus* [8,12–14], etc.) are considered to be promising for nematode control.

The proposed research supports the application of a bio-based approach for nematode control. The data in the literature show that the efforts are concentrated on finding the producer of specific lytic enzymes [31,36] or toxins [8] against nematodes. Our study proposes to use microbial and fungal activity to create conditions that are unfavorable for nematodes. This approach is based on the analysis of environmental conditions required for the suppression of nematodes and the creation of such conditions via the metabolic activity of bacteria and fungi. The study showed the response of the model strain of nematodes *C. elegans* N2.

The conditions that are necessary to suppress *C. elegans* N2 were theoretically considered based on the thermodynamic prediction. According to it, water is essential for living organisms [37]. As a chemical compound, water is stable within the zone restricted by the pH range from 0.0 to 14.0 and the range of standard redox potentials (E_o') from −414 to +814 mV (Figure 1). The value of the potential of the upper limit of thermodynamic stability of water (E_o' = +814 mV) is determined by the reversible oxidation of the oxygen of water to molecular oxygen, O_2: $2H_2O = O_2 + 4H^+$. The value of the potential of the lower stability limit is determined by the reversible reaction of the reduction of the proton of water to molecular hydrogen, H_2: $2H^+ + 2e = H_2$. If a compound with a potential higher than +814 mV is introduced into water, the oxygen of water acts as a reducing agent. It is oxidized to O_2, and the potential returns to its original value of +814 mV. If a compound with a potential lower than −414 mV is used, proton plays the role of an oxidizing agent. It is reduced to H_2, and the potential takes its original value (−414 mV). Thus, water is a double redox buffer. All biochemical reactions (and life in general) are possible only in the zone of the thermodynamic stability of water (Figure 1). It should be noted that the potential of the upper limit of water stability equal to +814 mV is created only at a hundred percent concentration of O_2. Since its concentration in air is 21%, in aqueous solutions, the redox potential acquires lower values (+440. . .+450 mV).

It follows that the zone of a theoretically admissible existence for living organisms is within the range of E_o' −414. . .+450 mV and pH 0.0–14.0. However, this does not mean that all organisms can exist in the specified very wide range of pH and E_o' values. There are specific boundaries of pH and E_o' for each organism. Thus, aerobic organisms can exist only in a high-potential (aerobic) zone (E_o' = −50. . .+450 mV) (Figure 1). Anaerobic organisms can exist only in a low-potential zone (E_o' = −414. . .−50 mV). Similar patterns are observed for acidophilic and alkaliphilic organisms. For example, acidophilic bacteria grow only under strongly acidic conditions (pH = 1–3) [38], while alkaliphilic ones grow only under alkaline conditions (pH = 12–13) [39].

In this regard, acidic or alkaline conditions as well as anaerobic low-potential ones were considered to be useful to suppress *C. elegans* N2. For this purpose, physical, chemical, and biological methods as well as their combination were used. Physical methods involved the isolation of nematodes from the contact with air via a plastic film or a layer of paraffin. The literature shows some efforts to control phytopathogenic nematodes via physical methods. For example, soil solarization is based on the increase in its temperature via the sun. Heat is trapped via the covering of soil by plastic film [40]. Such an approach showed its efficacy for *Meloidogyne* spp. [41], *Rotylenchus reniform*, and *Pratylenchus* spp. [42]. However, such an approach can be useful for greenhouses. Its application in the open soil is restricted. The chemical method provided the creation of low-potential conditions (−300...−150 mV) via sodium sulfide ($Na_2S{\cdot}9H_2O$). Different strategies to apply chemicals were studied to control nematodes. For example, in addition to nematicides [43], plant extracts such as garlic extract containing nematicidal polysulfide compounds [44,45], nematicidal essential oils [46,47], or soil amendments are used to improve its quality as well as the resistance of plants [48]. Such approaches showed promising results; however, some of them such as plant derivatives and oils require frequently repeatable treatments, increasing costs and work. The biological method was used to create low-potential conditions via metabolically

active microorganisms. Such methods were also applied in practice, for example, anaerobic soil disinfestation [49,50].

As an example of combined methods, the restriction of air access to nematodes via the mycelium of *P. ostreatus* Po4 and the simultaneous reduction in the redox potential via microorganisms was applied. As a result, the enhancement of *C. elegans* N2 suppression via the application of the mycelium of *P. ostreatus* Po4 together with microorganisms to create low-potential anaerobic conditions was observed. Moreover, the following increase in the efficiency of nematode control can be reached via the stimulation of the synthesis of nematicidic toxins by *P. ostreatus* Po4. The important result of the study indicates that anaerobic conditions (O_2 absence and Eh < −100 mV) are necessary and enough for the death of nematodes. In agriculture, it can be used in several ways. For example, if the soil contains organic compounds in a high concentration (chernozem), it is quite enough to fill the soil with a high layer of water for a short time. In this case, anaerobic low-potential conditions are created due to the low rate of oxygen dissolution and its rapid consumption by soil microorganisms followed by the anaerobic fermentation of organic compounds with a strong decrease in redox potential. Another way is to introduce concentrated non-toxic organics (for example, easily fermented vegetable residues—such as sugar beet, etc.) into soil. In this case, the concentration of electron donors (organic compounds—such as sugars, etc.) will be much higher than the concentration of electron acceptors (atmospheric oxygen in soil), and an inevitable shift in the metabolism of the soil microbiome to the anaerobic low-potential zone will occur. Finally, to create low-potential conditions, sparingly soluble sulfate (gypsum, $CaSO_4 \cdot nH_2O$) can be introduced into soil. The low solubility of gypsum (40–50 mg/L) will provide a slow reduction of SO_4^{2-} anion to S^{2-} in non-toxic trace concentrations via sulfate reducing bacteria. Therefore, low-potential sulfide will lead to the death of nematodes, but will not be hazardous to the soil microbiome, plants, and soil animals.

A somewhat similar approach was used via anaerobic soil disinfestation. It was also based on the creation of anaerobic conditions in soil via the addition of organics and covering the soil surface with plastic film to avoid air access and create anaerobic conditions. A field study in the Netherlands was shown to be effective against phytopathogenic fungi and nematodes. However, high costs and the hazard of plastic spreading in the environment was its drawback [49]. Anaerobic soil disinfestation was also shown to be effective against phytopathogenic fungi *Rhizoctonia solani* AG-5 and *Pratylenchus penetrans* [50]. Thus, the data in the literature indicate that the approach proposed in the current study is promising for application. Our approach is based on the creation of anaerobic conditions that are unfavorable for nematodes via the application of only natural components including natural organic compounds (for example, sugar beet residues, potato residues, etc.) and soil microbial communities to reduce the redox potential and *P. ostreatus* as the possible contributor to the air access insulation as well as the predator for the active killing of nematodes. Since the approach includes several mechanisms of nematode control, it is promising to be further developed for the following implementation in agriculture promoting bio-based environmental technologies.

5. Conclusions

The application of *C. elegans* N2 as a model to study the patterns of nematode control showed its efficiency. The creation of a low potential of the environment via microorganisms provided the effective control of *C. elegans* N2. Heterotrophic and especially sulphate-reducing bacterial microbiomes producing S^{2-} were useful to create a low redox potential of the medium. However, in free-air access conditions, it is not enough to control *C. elegans* N2. The simultaneous application of microorganisms to reduce the redox potential as well as *P. ostreatus* Po4 as an insulator of free air access provided the effective inhibition and death of *C. elegans* N2. The application of the bio-based approach is of great interest to develop environmentally friendly approaches contributing to the reduction in the amounts of chemicals required for soil treatment. A microbial-based approach for

nematode suppression is promising for crop preservation, contributing to food resource protection.

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