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Effect of increasing doses of zinc in combination with organic materials on the occurrence of entomopathogenic fungi in the soil

Wpływ wzrastających dawek cynku w połączeniu z materiałami organicznymi na występowanie grzybów entomopatogenicznych w glebie

Summary. The aim of the study was to determine the effect of zinc application in different doses with organic fertilization on the genera composition and the number of CFU (colony-forming units) of entomopathogenic fungi (EPF) in the soil. The experiment was carried out in greenhouse conditions. Soil samples for testing were collected in the third year of the experiment (spring, autumn), where the research objects were I – dose of zinc: control – lack (0) and 200, 400 and 600 mg Zn kg^{-1} of soil; II – no fertilization – 0 (CO), spent mushroom substrate (SMS), chicken (ChM) and cattle manure (CM). Entomopathogenic fungi were isolated using the method of isolation on a selective medium. In the course of the research, EPF of the genus: *Beauveria*, *Metarhizium*, *Cordyceps* and *Lecanicillium* were determined. The conducted research showed that entomopathogenic fungi of the *Metarhizium* genera formed the most CFU in spring. In the autumn there were three times less of them. Statistical analysis showed that the number of CFUs of the identified genus of fungi (on average) in soil samples significantly depended on the dose of zinc applied, organic fertilization and the genus of fungus, but only for *Metarhizium* spp.

Key words: zinc, organic materials, entomopathogenic fungi, CFU

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INTRODUCTION

Soil is most exposed to heavy metal contamination. The average total content of heavy metals (mg kg^{-1}) in Polish soils, depending on the category of soil gravity, is for Cd: 0.29–0.86, Pb: 14.2–25.2, Cr: 8.00–25.6, Zn: 41.7–91.0, Cu: 10.4–20.3, Ni: 5.50–23.4 [Kabata-Pendias and Pendias 1999]. They originate from natural sources and from human activity, both agricultural and non-agricultural, with their excessive amounts posing a threat to living organisms [Micó et al. 2006, Tchounwou et al. 2012, Rutkowska et al. 2015, Briffa et al. 2020, Kuziemska et al. 2020]. Heavy metals in the soil environment combine with mineral parts of the soil, as well as with organic matter, and their forms are more or less available to plants [Singh and Kalamdhad 2011, Jain et al. 2020]. In natural concentrations heavy metals are often needed for a proper function of living organisms, but in excessive amounts they are toxic [Singh and Kalamdhad 2011, Kuziemska et al. 2020]. They have a great impact on biological activity of soil microorganisms, and when taken up by plants, they affect their cellular structures, growth and development [Tscherko et al. 2007, Hassn et al. 2014].

Zinc is a heavy metal whose soil content depends mainly on its amount in soil parent material and on anthropogenic factors; its soil content depends on its amounts absorbed by clay minerals and bound by organic matter [Sadeghzadeh 2013, Recena et al. 2021, Kuziemska et al. 2022]. Zinc is a microelement that is needed by the plant for their proper growth and development, it is present in enzymes, affects the transformation of calcium and phosphorus, as well as the production of chlorophyll. This element regulates the proportions of ingredients at the cell level, affecting the functioning of cell membranes [Bonnet et al. 2000, Nielsen 2012]. Zinc, like other heavy metals, if present in excessive concentrations, is harmful to plants growing in the soil, but also has a toxic effect on microorganisms living in it. However, its mobility and thus toxicity can be reduced by increasing soil organic matter content. Organic fertilizer and organic waste used as fertilizer are the main source of soil organic matter [Kwiatkowska-Malina 2017, Majchrowska-Safaryan et al. 2018, Kuziemska et al. 2022].

Present in the environment and, in particular, in the soil environment, EPF constitute an important part of organic matter and the proper functioning of agroecosystems [Tkaczuk 2008, Tkaczuk et al. 2014, Ghaley et al. 2014, Sharma et al. 2018]. Infecting many arthropod species, including crop pests, they cause disruption of physiological processes in the definite host, leading to its death [Hajek and Leger 1994, Chandler et al. 2000, Quesada-Moraga et al. 2007]. Their severity and pathogenicity depend on their interaction with the host organism, weather conditions and on other biotic and abiotic factors.

According to the results of laboratory studies, heavy metals affect the species composition, the course of metabolic processes, and thus also the possibility of insect infection by EPF [Zimmerman and Wolf 2002, Pečiulytė and Dirginčiutė-Volodkienė 2012, Hassn et al. 2014, Łopusiewicz et al. 2019]. The ecotypes of EPF species accumulate heavy metals in different amounts, which means that their pathogenicity varies. There are species of fungi that are characterized by a high degree of resistance to heavy metals or that produce mutants capable of tolerating high concentrations of metals [Zimmerman and Wolf 2002, Tkaczuk 2008, Rajapaksha 2011, Tkaczuk et al. 2019].

In this study, the effect of zinc application in various doses in combination with organic fertilization on the genera composition and the intensity of the occurrence of EPF in the soil was determined.

MATERIAL AND METHODS

Soil samples for testing were taken from a greenhouse where a three-year pot experiment (2014–1016) was conducted in a completely randomized arrangement, in three repetitions, where the number of research objects was 60.

Two research factors were taken into account in the experiment:

– the level of zinc application: control – lack (0) and 200, 400 and 600 mg Zn kg^{-1} of soil which was applied to the soil in the form of $ZnSO_4 \cdot 5H_2O$, only in the first year of the study, before sowing the test plant;

– organic materials, where the research objects were: control – without organic fertilization (CO), spent mushroom substrate (SMS), chicken manure (ChM) and cattle manure (CM). The organic fertilizers used in the experiment were applied separately in a single dose in the first year of the experiment. They were added to the soil in a dose of 2 g C_{org} kg^{-1} and thoroughly mixed with it 14 days before sowing the test plant.

Pots with a capacity of 12 dm^3 were filled with 14 kg of lessive soil taken from arable land (valuation class IVa, agricultural suitability complex 4 – very good rye) with a granulometric composition of 72% sand, 24% silt and 4% clay. The pH of the soil used in the experiment (pH in 1 mol dm^3 HCl) was 6.25, total nitrogen 1.42 g kg^{-1}, organic carbon 15.4 g kg^{-1}, ratio C : N = 10.8 : 1, and total Zn content 54.6 mg kg^{-1}. During the experiment, the soil moisture was regulated by irrigation and was at the level of 60–70% of the full water capacity.

In each year of the experiment, the test plant was cocksfoot of the Amera variety (*Dactylis glomerata* L.). During the growing season, the above-ground parts of the plant were harvested four times, every 30 days [Kuziemska et al. 2022].

Soil samples for research were taken from individual research objects in the last year of the experiment in spring (before the start of vegetation) and autumn (after collecting the last cut). In the laboratory experiment to determine the genus composition of EPF (entomopathogenic fungi) isolated from test objects, the method developed by Strasser et al. [1996] was used using a selective medium. The selective medium was composed of 20 g of glucose, 10 g of peptone and 18 g of agar. The medium components were dissolved in 1 liter of deionized water, and then sterilized using a steam-pressure autoclave at a temperature of 120°C and a pressure of 1 atmosphere. The following selective components were added to the medium after it cooled to 50°C: 0.6 g of streptomycin, 0.05 g of tetracycline 0.05 g of cycloheximide and 0.1 g of dodine.

From each test object, 2 g of soil were taken, to which 18 ml of sterilized deionized water mixed with a preparation reducing surface tension Triton-X was added. From the solution prepared in this way, 0.1 ml of soil solution was taken with an automatic pipette and applied to the surface of the prepared selective substrate. The solution on the surface of the substrate was spread with a glass spatula under sterile conditions in a laminar chamber.

The Petri dishes on which the soil solution was applied were placed in incubators set at 21°C. Colonies of the isolated genus of fungi were counted after 8–10 days of cultivation. The experiment was performed in three repetitions for each research object. The obtained results were presented as the number of colony-forming- units (CFU) for each genus of EPE in 1 g of soil. Colonies grown *in vitro* were defined according to literature recommendations given by Rehner and Buckley [2005], Rehner et al. [2011], Humber [2012], Inglis et al. [2012] based on fungal microstructure morphology. Characterization of fungal isolates was made by the determination of conidial size and shape, conidiogenous cell, and colony morphology [Humber 2012, Inglis et al. 2012]. Since we did not perform molecular studies of the isolated fungi in the experiment, they were classified according to the rank of the genus.

The obtained results were statistically processed using the Statistica 13.3 TIBCO Software Inc. program. One-way analysis of variance (ANOVA) and post-hoc Tukey test were performed. The calculated means were combined into homogeneous groups at the significance level $\alpha < 0.05$.

RESULTS AND DISCUSSION

Heavy metals can pose threat to the proper functioning of living organisms. When they occur in high concentrations in the soil environment, they affect the deterioration of the chemical properties of the soil, and also reduce the number and activity of microorganisms living in it [Tscherko et al. 2007, Lenart and Wolny-Koładka 2013, Briffa et al. 2020, Kuziemska et al. 2020]. According to the research conducted by Kwiatkowska-Malina [2017] and Kuziemska et al. [2022], increasing organic matter content in soil is one of the factors decreasing the mobility of heavy metals, and thus decreasing their bioavailability.

In soil samples collected from individual research units (in the spring and autumn), four genera of EPF were found: *Beauveria*, *Metarhizium*, *Cordyceps* and *Lecanicillium* (Tab. 1, 2). Given that only morphological methods were applied during the identification of fungi, they were described to the rank of genus, because, as demonstrated by the latest phylogenetic studies based on DNA sequencing [Bischoff et al. 2006, 2009, Rehner et al. 2011, Kepler et al. 2017], there are numerous fungus species within the genus of *Beauveria*, *Cordyceps*, *Metarhizium* and *Lecanicillium* that are almost impossible to distinguish from each other without the application of molecular methods.

General colony characteristics and the micromorphological features of isolated entomopathogenic fungi, that allowed them to be classified into particular genera are described below.

Colonies of *Beauveria* spp. on SDA reached a size of 52–58 mm on the 20th day of growth at 21°C, cottony at center, radially sulcate to filamentous toward the filiform margin, white. Mycelium superficial and immersed. Hyphae septate, branched, hyaline, smooth, 1–2 μm wide. Conidia solitary, acropleurogenous, globose, subglobose, unicellular, smooth-walled, hyaline, dry with 3.2 μm of diameter. The obtained isolates were morphologically the most similar to the *B. bassiana* species.

Colonies of *Metarhizium* spp. on SDA reached a size of 67–75 mm on the 20th day of growth at 21°C, cottony to floccose at center. At the beginning of growth curled toward the slightly filiform margin that is colored white, with several sporodochial conidiomata, green or olivaceous. At day 20 of growth, the colonies were green and olive in color. Mycelium was superficial and immersed. Hyphae were septate, branched, hyaline, smooth, 1–2 μm wide. Conidia were basocatenulate, cylindrical, truncated at the ends, unicellular, pale olivaceous 6–8 × 1.5–2 μm, accumulating in a columnar, dark olivaceous masses.

Colonies of *Cordyceps* spp. on SDA reached a size of 66–72 mm on the 20th day of growth at 21°C. At the beginning of growth curled is colored white in 20th day of growth light pink. Mycelium superficial and immersed. Pink synnemata up to 10–20 mm long were often produced. The phialides are flask-shaped with a globose to ellipsoidal basal portion, and the conidia were cylindrical to fusiform, hyaline to slightly pink and 3–4 × 1–2 µm. In terms of morphological features, the colonies most closely resembled *C. fumosorosea* species.

Colonies of *Lecanicillium* spp. on SDA reached a size of 21–25 mm on the 20th day of growth at 21°C, they presented an off-white coloration, with a cottony appearance, in addition the colonies presented greater vertical growth in the upper part due to the abundant mycelium, in the lower part of the plate a wrinkling or ridges were observed in the medium during their growth. Conidia size ranged from 2.5 to 7.5 µm in length and 1.5 to 2 µm in width, with conidia present at the end of the phialides w clusters or solitary, glassy in color and cylindrical or ellipsoidal shape.

Research conducted by Jaworska et al. [1996], Ropek and Para [2003], Rajapaksha et al. [2004], Tkaczuk [2003, 2005], Gorczyca [2005], Quesada-Moraga et al. [2007], Macdonald et al. [2011], Rajapaksha [2011], Pečiulytė and Dirginčiutė-Volodkienė [2012], Hassn et al. [2014], Tkaczuk et al. [2019] prove that EPF isolated from the soil show a different resistance to heavy metals.

In the spring time, the dominant genus of EPF isolated from the soils of the research objects was *Metarhizium* (Tab. 1). The number of CFU ranged from 2.5 CFU × 10^3 g^{-1} in control soil (CO), i.e. without zinc or organic fertilizer treatment, to 14.1 CFU × 10^3 g^{-1} in soil treated with chicken manure (ChM) and with a zinc dose of 200 mg kg^{-1}. On objects where zinc was applied at a dose of 200 mg kg^{-1} of soil in combination with organic materials, fungi *Metrhizium* spp. formed significantly more CFU units than at 0 level of zinc application. The smallest number of CFU units, the fungi of the genus *Metarhizium*, created 600 mg kg^{-1} of soil at the level of zinc application. In case of *Cordyceps* spp. the highest CFU was produced at zero zinc application level from 0.4 CFU×10^3 g^{-1} – CO, to 0.7 CFU ×10^3 g^{-1} – SMS and CM. On the other hand, the fewest or no CFU were found in the objects where Zn was applied at a dose of 600 mg kg^{-1} of soil, and these differences were statistically significant. Research conducted by El-Sharouny et al. [1988], concerning the presence of heavy metals in the soils of Egypt and their toxicity to EPF, proved that the fungi of the genus *Isaria* (= *Cordyceps*) and *Metarhizium* spp. are the most resistant to elevated zinc content in the soil. Żurek et al. [1998], examining the effect of the combined use of heavy metals with pesticides in vitro, found that zinc inhibited the growth of *Paecilomyces fumosoroseus* fungus by 46–43%, while changing the color of the colony. Popowska-Nowak et al. [2004] reports that EPF of *Metarhizium* spp. isolated from soil containing increased amounts of heavy metals show adaptability to adverse environmental conditions.

In the spring time, fungi of the genera *Beauveria* spp. and *Lecanicillium* spp. were also isolated from the soils of the research objects. Bajan [2000] and Pečiulytė and Dirginčiutė-Volodkienė [2012] reported that anthropogenic pollution, including high concentrations of heavy metals, reduces species diversity and the pathogenicity of entomopathogenic fungi. The most of number of CFU was created by fungi of the genus *Beauveria* at the level of zinc application of 200 mg kg^{-1} of soil, and in the objects where SMS and CM were applied, this difference was statistically significant. Barabasz et al. [1997] and Bajan [2000] showed that entomopathogenic fungi of the genus *Beauveria* spp. show the bioaccumulation capacity of zinc ions, and its level depends on the fungus strain. Entomopathogenic fungi of *Lecanicillium* spp. were also isolated from soil samples, with the most number of CFU in the soil with no zinc application but treated with cattle manure (0.2 CFU × 10^3 g^{-1} of soil). Research conducted by Kumar et al. [2018] showed that the fungus *Lecanicillium psalliotae* is relatively resistant to low concentrations of ZnO. By accumulating zinc ions, it contributes to reducing its bioavailability for plants. On research objects where zinc was applied in a dose of 600 mg kg^{-1} of soil, *Beauveria* spp. and *Lecanicillium* spp. were not isolated.

Table 1. The number of colony-forming units of entomopathogenic fungi (CFU × 10^3 g^{-1}) in soils of the research objects (spring term)

Organic fertilizer	Zn dose (mg kg^{-1})	Genera of entomopathogenic fungi			
		Beauveria spp.	*Metarhizium* spp.	*Cordyceps* spp.	*Lecanicillium* spp.
Without organic fertilization (CO)	0	0.1 ±0.08^a	2.5 ±0.75^b	0.4 ±0.14^a	0.1 ±0.08^a
	200	0.1 ±0.08^a	6.6 ±1.20^a	0.4 ±0.08^a	0.1 ±0.0^a
	400	0.1 ±0.0^a	7.4 ±1.01^a	0.2 ±0.08^b	0.0 ±0.0^b
	600	0.0 ±0.0^a	2.6 ±0.67^b	0.0 ±0.0^c	0.0 ±0.0^b
Spent mushroom substrate (SMS)	0	0.1 ±0.0^b	6.5 ±2.29^b	0.7 ±0.05^a	0.1 ±0.12^a
	200	0.3 ±0.14^a	9.4 ±0.81^a	0.5 ±0.08^b	0.1 ±0.0^a
	400	0.1 ±0.0^b	7.6 ±0.48^b	0.3 ±0.04^c	0.0 ±0.0^b
	600	0.0 ±0.0^b	4.5 ±0.40^c	0.1 ±0.0^d	0.0 ±0.0^b
Chicken manure (ChM)	0	0.1 ±0.0^{ab}	4.2 ±0.16^c	0.6 ±0.16^a	0.1 ±0.08^a
	200	0.2 ±0.08^a	14.1 ±0.37^a	0.5 ±0.09^a	0.1 ±0.0^a
	400	0.1 ±0.0^{ab}	9.7 ±0.57^b	0.2 ±0.1^b	0.1 ±0.0^a
	600	0.0 ±0.0^b	3.5 ±0.41^c	0.1 ±0.0^b	0.0 ±0.0^b
Cattle manure (CM)	0	0.1 ±0.0^{bc}	6.6 ±0.48^c	0.7 ±0.14^a	0.2 ±0.08^a
	200	0.4 ±0.15^a	12.6 ±0.50^a	0.5 ±0.12^b	0.1 ±0.0^{ab}
	400	0.2 ±0.12^b	9.7 ±0.45^b	0.4 ±0.1^b	0.0 ±0.0^b
	600	0.0 ±0.0^c	5.4 ±0.29^d	0.0 ±0.0^c	0.0 ±0.0^b

$^{a, b, c, d}$ Significance at the level $\alpha < 0.05$, for homogeneous groups of means, the values marked in the columns with the same letters do not differ significantly; ± – standard deviation

In the autumn (after harvesting the test plant), CFU of *Beauveria* spp., *Metarhizium* spp., *Cordyceps* spp. and *Lecanicillium* spp. were found in soil samples collected from research variants (Tab. 2). Pečiulytė and Dirginčiutė-Volodkienė [2012] indicate that EPF such as *Beauveria bassiana, Metarhizium anisopliae, Isaria farinosa* and *Lecanicillium lecani* are relatively highly resistant to increasing zinc doses. This was also confirmed by earlier research done by Arnebrandt et al. [1987] and Nordgren et al. [1985].

In autumn, EPF of the genus *Beauveria* and *Lecanicillium* formed the lowest number of CFU units in the soils of the studied fertilization objects. Statistically significantly more CFU of the genus *Beauveria* were found on the fertilization object where cattle manure was applied, Zn application level 200 mg kg^{-1} of soil (0.3 CFU $\times 10^3$ g^{-1} of soil). Entomopathogenic fungi of *Lecanicillium* spp. formed the most CFU (0.2 CFU $\times$ 10^3 g^{-1}) in control soil, not treated with organic fertilizer.

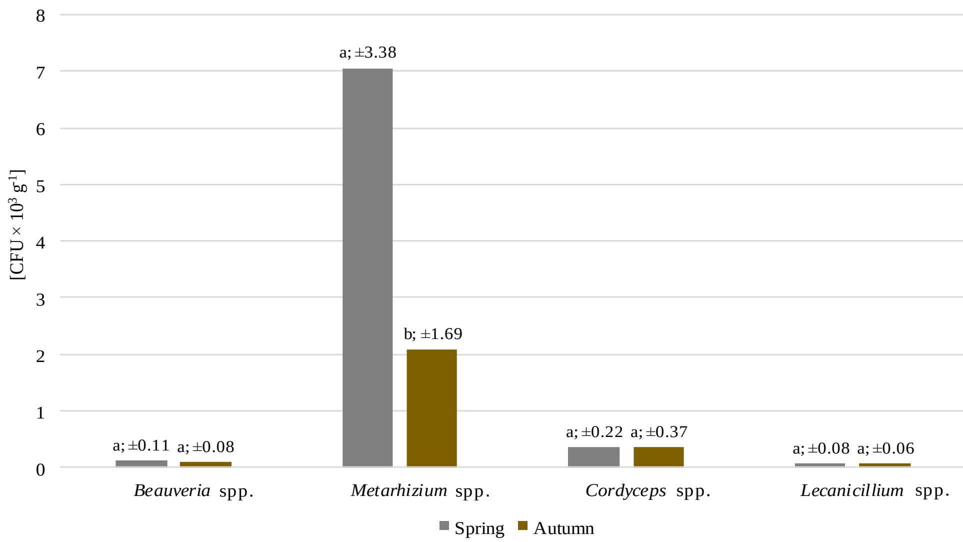
Table 2. The number of colony-forming units of entomopathogenic fungi (CFU $\times$ 10^3 g^{-1}) in soils of the research objects (autumn term)

Organic fertilizer	Zn dose (mg kg^{-1})	Genera of entomopathogenic fungi			
		Beauveria spp.	*Metarhizium* spp.	*Cordyceps* spp.	*Lecanicillium* spp.
Without organic fertilization (CO)	0	0.1 ±0.08^{a}	1.9 ±0.55^{b}	0.6 ±0.14^{a}	0.1 ±0.08^{ab}
	200	0.1 ±0.08^{a}	2.7 ±0.88^{a}	0.5 ±0.10^{ab}	0.2 ±0.0^{a}
	400	0.1 ±0.0^{a}	1.9 ±0.90^{b}	0.2 ±0.08^{bc}	0.0 ±0.0^{b}
	600	0.0 ±0.0^{b}	0.5 ±0.26^{c}	0.1 ±0.0^{c}	0.0 ±0.0^{b}
Spent mushroom substrate (SMS)	0	0.1 ±0.0^{ab}	1.2 ±0.28^{b}	0.9 ±0.05^{b}	0.0 ±0.0^{b}
	200	0.2 ±0.14^{a}	4.4 ±0.41^{a}	1.4 ±0.12^{a}	0.1 ±0.06^{a}
	400	0.1 ±0.0^{ab}	1.4 ±0.46^{b}	0.1 ±0.08^{c}	0.0 ±0.0^{b}
	600	0.0 ±0.0^{b}	1.1 ±0.40^{b}	0.1 ±0.0^{c}	0.0 ±0.0^{b}
Chicken manure (ChM)	0	0.1 ±0.0^{a}	1.5 ±0.12^{b}	0.2 ±0.16^{b}	0.1 ±0.08^{a}
	200	0.1 ±0.08^{a}	7.2 ±0.80^{a}	0.4 ±0.14^{a}	0.1 ±0.0^{a}
	400	0.0 ±0.0^{b}	1.2 ±0.47^{b}	0.1 ±0.0^{b}	0.0 ±0.0^{b}
	600	0.0 ±0.0^{b}	0.4 ±0.21^{c}	0.1 ±0.0^{b}	0.0 ±0.0^{b}
Cattle manure (CM)	0	0.1 ±0.0^{b}	2.1 ±0.44^{b}	0.2 ±0.14^{b}	0.1 ±0.0^{a}
	200	0.3 ±0.15^{a}	3.6 ±0.30^{a}	0.6 ±0.12^{a}	0.1 ±0.0^{a}
	400	0.1 ±0.08^{b}	1.8 ±0.42^{bc}	0.1 ±0.08^{b}	0.1 ±0.0^{a}
	600	0.0 ±0.0^{c}	1.6 ±0.40^{c}	0.0 ±0.0^{b}	0.1 ±0.0^{a}

a, b, c Significance at the level $\alpha < 0.05$, for homogeneous groups of means, the values marked in the columns with the same letters do not differ significantly; ± – standard deviation

In autumn, *Metarhizium* spp. formed CFU in the soil of all experimental variants. It turned out that the application of zinc at a dose of 200 mg kg^{-1} of soil significantly increased the number of *Metarhizium* spp. CFU in relation to the control (0 dose of Zn). The number of CFU of *Metarhizium* spp. decreased from the highest in the soil treated with a zinc dose of 200 mg kg^{-1} soil to the lowest in response to a zinc dose of 600 mg kg^{-1} soil. Vanninen [1996] reports that fungi of *Metarhizium* spp. show the highest tolerance to the conventional tillage system, to the levels of mineral fertilizers and plant protection products and to increased content of heavy metals in soil.

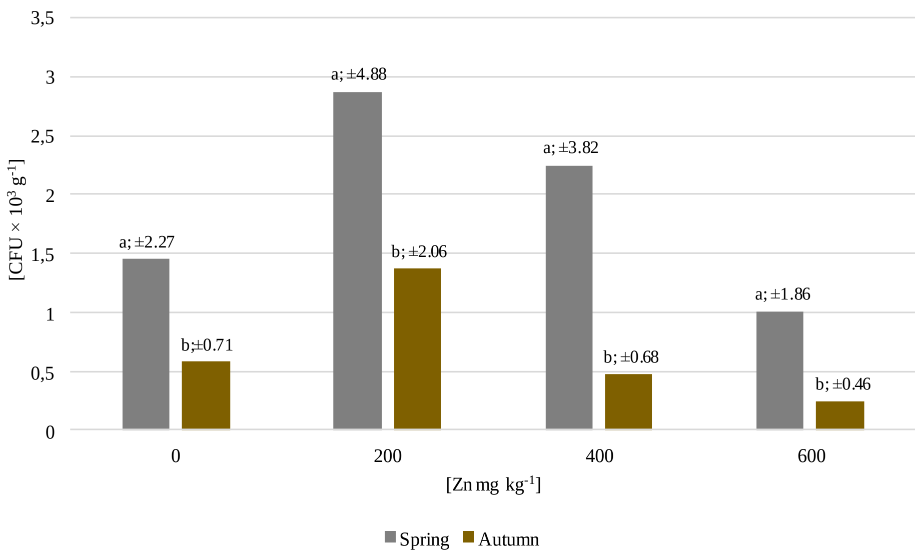
The highest density of CFU of the EPF *Cordyceps* spp. was found in the soil treated with zinc at a dose of 200 mg kg^{-1} of soil (except for the CO object). These differences were statistically significant compared to the control (Zn application level 0). The number of CFU ranged from 0.5 to 1.4 $\times$ 10^3 g^{-1} of soil. At the highest level of zinc application amounting to 600 mg kg^{-1}, the above-mentioned fungi from *Cordyceps* genus did not form colonies on the experimental variant with cattle manure. Research conducted by Hassn et al. [2014] concerning the effect of metal ions on the growth of the fungus *Isaria javanica* showed that the addition of tin ions to the culture medium in a dose corresponding to the natural content in soils did not limit the growth of its colonies *in vitro*. However, Ropek and Para [2003] found that zinc strongly limited the growth of the *Paecilomyces farinosus* (=*Cordyceps*) fungus.



a, b – significance at the level α < 0.05, ± – standard deviation

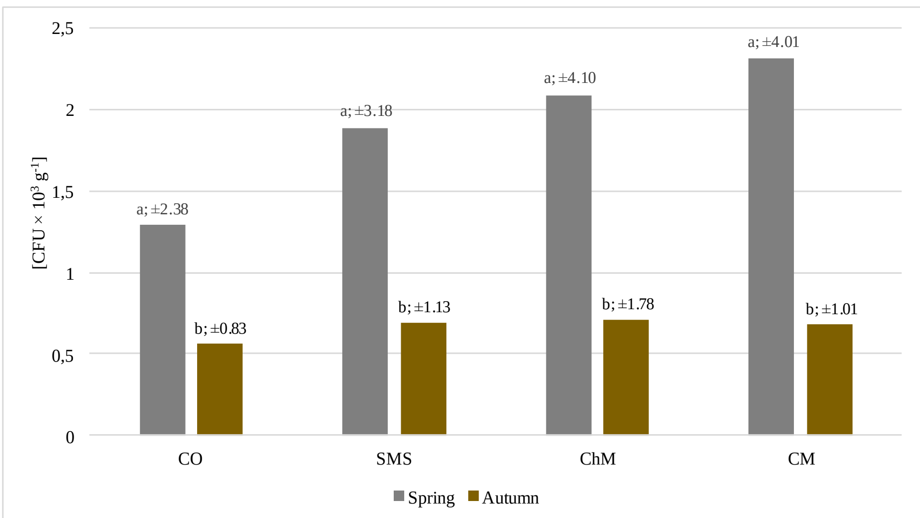
Fig. 1. Average number of colony-forming units [CFU] of entomopathogenic fungi isolated from the tested soils in spring and autumn

The study showed that the number of CFUs of EPF of the genus *Metarhizium* in spring was by more than 3 times higher than in autumn, and this difference was statistically significant (Fig. 1). Organic fertilization (2.09 CFU × 10^3 g^{-1} – CM) and the level of zinc application (2.87 CFU × 10^3 g^{-1} – 200 mg kg^{-1} of soil) were factors that significantly affected the number of CFUs of the isolated genus of fungi. In both cases (on average) the number of colony-forming units of the marked fungal genus was significantly higher in the spring time of the experiment (Fig. 2, 3). Research conducted by Quesada-Moraga et al. [2007] showed that soils rich in organic matter provided by organic fertilization have high cation exchange capacity, which in turn can adsorb and retain a large amount of EPF spores. Such soils also contain a greater amount of soil humus, which has the ability to bind and retain heavy metals in its structures, thus limiting their bioavailability. Bueno-Pallero et al. [2020] and Afandhi et al. [2022] indicate that a higher content of organic matter supplied to arable soils with cattle manure and composts increases the intensity of EPF. Organic fertilizers increase the number of soil arthropods, which are potential hosts of EPF, and are also vectors in the spread and transmission of EPF spores [Ali-Shtayeh et al. 2002, Anslan et al. 2018, Lin et al. 2019]. Clifton et al. [2015] and Ramos et al. [2017] found that the soils of organic farms enriched with organic matter through organic fertilizers show a greater species diversity and frequency of EPF occurrence than soils from a conventional cultivation system.



a, b – significance at the level α < 0.05, ± – standard deviation

Fig. 2. Average number of colony-forming units [CFU] of entomopathogenic fungi depending on the level of zinc application in spring and autumn



CO – without organic fertilization, SMS – spent mushroom substrate, ChM – chicken manure, CM – cattle manure; a, b – significance at the level α < 0.05, ± – standard deviation

Fig. 3. Average number of colony-forming units [CFU] of entomopathogenic fungi depending on the organic material used in spring and autumn

Among soil microorganisms, there are EPF, which include species with varying degrees of tolerance to environmental stress [Rajapaksha 2011, Tkaczuk et al. 2019]. Studies indicate that elevated concentrations of heavy metals in the soil is a factor that exerts selective pressure on soil microorganisms [Zafar et al. 2007]. According to Pečiulytė and Dirginčiutė-Volodkienė [2012], one of the elements of defense developed by EPF, which allows them to show resistance to elevated concentrations of heavy metals, is the fact that they do not accumulate them in their biomass, but bind them on the cell surface. Zimmermann and Wolf [2002] report that EPF are relatively resistant to heavy metals or produce mutations capable of tolerating high concentrations of them.

CONCLUSIONS

In the soil samples collected from experimental units, the colonies of EPF of the *Beauveria*, *Metarhizium*, *Cordyceps* and *Lecanicillium* genus were found. Determined by using a selective culture medium, fungi of *Metarhizium* spp. were dominant in soil samples in both spring and autumn. Assessing the average density of CFU of the species of EPF across the levels of zinc application, it was found that fungi of *Metarhizium* spp. were more numerous in the spring by over 70% than in the autumn, and this difference was statistically significant. The research results indicated that the highest level of zinc application across the organic materials used limited the number of colony-forming units of the *Beauveria* spp., *Metarhizium* spp., *Cordyceps* spp. and *Lecanicillium* spp. in the spring and autumn. Statistical analysis showed that the number of CFUs of the identified genus of fungi (on average) in soil samples significantly depended on the dose of zinc applied, organic fertilization and the genus of fungus, but only for *Metarhizium* spp.

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