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Seasonal dominance of *Metarhizium* spp. in soils of cereal monocultures in Eastern Poland

Abstract. The aim of this study was to determine the generic composition and the intensity of entomopathogenic fungi (EPF) occurrence in soils cultivated in cereal monocultures in eastern Poland. Soil samples were collected from an individual farm located in the northern part of the Lublin Voivodeship, in the Janów Podlaski commune, in spring and autumn 2024 and spring 2025. A composite sample weighing about 1 kg of soil was created from soil samples taken from fifteen locations at each study site, including arable land where cereals were farmed in monoculture, forest soil, and former agricultural wasteland for comparison.

The entomopathogenic fungi from individual soil samples were isolated using the selective medium method developed by Strasser et al. At each study date, pHKCl in the collected soil samples was determined potentiometrically. The presence of EPFs from the genera *Beauveria*, *Metarhizium*, and *Cordyceps* was demonstrated in the studied soils. Their occurrence intensity depended on the cultivated plant species and the date of soil sampling. Evaluating the mean CFU density of the studied EPF genera, only for soils cultivated in cereal monoculture, demonstrated that *Metarhizium* spp. exhibited the greatest resistance to monoculture cultivation. This indicates that EPF from this genus are highly resistant to agronomic treatments, including mineral fertilization and pesticide protection.

Keywords: cereal monocultures, eastern Poland, entomopathogenic fungi in the soils

INTRODUCTION

The intensive development of agriculture in Europe and monoculture cultivation has resulted in all European Union (EU) member states observing and experiencing negative consequences such as depletion of natural resources, increased erosion and loss of natural soil fertility, increased importance of new diseases and pests, water shortages, limited biomass production and a decline in the biodiversity of organisms [Barros-Rodríguez et al. 2021, Kuźniar et al. 2021, Majchrowska-Safaryan et al. 2023, Dunn et al. 2025]. Frequent successive cultivation of cereal crops disrupts the ecological balance of the environment, resulting in a significant increase in weed infestation and species compensation. The

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increased share of cereals causes, for example, a greater occurrence of cereal broom, cleavers, and chamomile, as well as increased pressure from diseases and pests and also affects the soil microbiology.

Maintaining high microbiological activity in the soil rhizosphere is an important element of crop production. This is also significantly influenced by the composition of exudates from potential hosts. These exudates largely modify the physicochemical properties of cultivated soils and thus influence the soil microbiota [Chaparro et al. 2014, Zhalnina et al. 2018, Egidi et al. 2025]. Although the role of the rhizosphere in plant health, fitness, immunity, and productivity has been known for decades, in recent years it has been increasingly recognized that microorganisms with endophytic capabilities that also colonize above-ground plant organs also have certain implications for host immunity and defense against pathogens [Chen et al. 2020, Li et al. 2022]. Entomopathogenic fungi also play a significant role in these processes. These fungi are parasites of herbivorous insects, very effectively limiting their populations [Tkaczuk 2008]. They also increase nutrient absorption and water use efficiency under stressful conditions [Dara et al. 2017]. Therefore, research should take into account the complex relationships between the soil environment, plant, pest, and entomopathogenic fungus. This is a very dynamic system, changing under the influence of both abiotic and biotic environmental conditions [Majchrowska-Safaryan et al. 2023]. Mc Coy et al. [1992], Uzman et al. [2019], Sharma et al. [2023], report that moisture, temperature, organic matter content, and soil pH are important factors in monoculture crops. Cereal monoculture provides a food base only for a narrow group of pests. The lack of insect species diversity limits the host range, leading to a decline in EPF diversity. The widespread use of plant protection products (especially chemical seed dressings and fungicides) in monocultures is highly toxic to beneficial soil microorganisms [Mc Coy et al. 1992].

Despite numerous studies on the occurrence of EPFs, little is known about their seasonal dynamics within long-term cereal monocultures in Eastern Europe. Therefore, we hypothesized that cereal monoculture reduces the diversity and number of CFUs entomopathogenic fungi in cultivated soils.

MATERIAL AND METHODS

The study material consisted of soil samples collected in 2024 (spring – May, autumn – October) and in 2025 (spring – April) on an individual farm with an area of 11.0 ha, located in the north of the Lublin Voivodeship, in the Janów Podlaski commune, in the villages of Romanów and Jakówki (geographic coordinates: 51°44'26"N 23°23'13"E and 52°11'48"N 23°07'48"E). Soil samples were collected from arable land where cereal crops were grown in monoculture and for comparison forest soil (number 4, 52°10'03"N 23°07'43"E) and agricultural wasteland (number 5, 52°11'12"N 23°09'40"E) from a depth of 20 cm at 15 points spaced approximately 15 m apart in a straight line at each survey location. Soil samples for research were collected in early spring and autumn, when EPFs show the highest biological activity and create the highest density of CFUs, which results from optimal climatic conditions for infecting pests [Majchrowska-Safaryan et al. 2023]. Cereal crops have been cultivated in the given locations for the last five years. The tested soils were classified as, quality class IVa – medium arable soils. This region is dominated by sandy deposits (sands with low clay and loam), composed primarily of sand and silt fractions with low clay content. Organic matter content ranges from 1.5% to 3.0%. The species of cultivated plants, their pesticide protection and mineral fertilization are presented in Table 1.

An average collective sample (about weight approx. 1.0 kg) was prepared from fifteen soil samples. The soil was sieved through a 1 mm sieve to separate larger fractions and contaminants. Before conducting the laboratory experiment, the soil was adjusted to the appropriate moisture content (approximately 15–20% of maximum water capacity), optimal for the growth of entomopathogenic fungi (EPF). From the soil sample, 2 g of soil was weighed and then added 18 ml of distilled water with the addition of a surface tension reducing agent (Triton X-100). Using an automatic pipette, 0.1 ml of soil

solution was collected and transferred to the surface of the selective substrate. Inoculations were performed in four replicates for each soil sample. The dishes with the applied soil solution were transferred to an incubator at 21°C without access to light and after 10 days the number of colony-forming units (CFU) of EPF developing on the culture medium was determined. The number of EPF was presented in CFU g⁻¹ of dry matter of soil. At each date of the research, pH_{KCl} was determined potentiometrically in the collected soil samples (Table 2).

Table 1. Crop area, dose of plant protection products and mineral fertilizers used

Field number (area)	Cultivation	Dose of plant protection products	Dose of mineral fertilizers
spring 2024			
1 (4.0 ha)	<i>Triticum aestivum</i> L. (common wheat)	Mustang 306 SE (0.6 dm ³ ha ⁻¹)	Saletrzak 100 kg ha ⁻¹ NPK 8-20-30 200 kg ha ⁻¹
2 (5.0 ha)	<i>Avena sativa</i> L.(oat)	Mustang 306 SE (0.6 dm ³ ha ⁻¹)	Saletrzak 100 kg ha ⁻¹ NPK 8-20-30 200 kg ha ⁻¹
3 (2.0 ha)	<i>Hordeum vulgare</i> L. (barley)	Mustang 306 SE (0.6 dm ³ ha ⁻¹)	Saletrzak 100 kg ha ⁻¹ NPK 8-20-30 200 kg ha ⁻¹
autumn 2024			
1 (4.0 ha)	<i>Hordeum vulgare</i> L. (barley)	Mustang 306 SE (0.6 dm ³ ha ⁻¹)	Saletrosan 150 kg ha ⁻¹
2 (5.0 ha)	<i>Hordeum vulgare</i> L. (barley)	Mustang 306 SE (0.6 dm ³ ha ⁻¹)	Saletrosan 150 kg ha ⁻¹
3 (2.0 ha)	<i>Triticum aestivum</i> L. (common wheat)	Mustang 306 SE (0.6 dm ³ ha ⁻¹)	Saletrosan 150 kg ha ⁻¹
spring 2025			
1 (4.0 ha)	<i>Hordeum vulgare</i> L. (barley)	Mustang 306 SE (0.6 dm ³ ha ⁻¹)	Saletrzak 100 kg ha ⁻¹ NPK 8-20-30 200 kg ha ⁻¹
2 (5.0 ha)	<i>Hordeum vulgare</i> L. (barley)	Mustang 306 SE (0.6 dm ³ ha ⁻¹)	Saletrzak 100 kg ha ⁻¹ NPK 8-20-30 200 kg ha ⁻¹
3 (2.0 ha)	<i>Triticum aestivum</i> L. (common wheat)	Mustang 306 SE (0.6 dm ³ ha ⁻¹)	Saletrzak 100 kg ha ⁻¹ NPK 8-20-30 200 kg ha ⁻¹

Table 2. pH_{KCl} value of the tested soils

Field number	Spring 2024	Autumn 2024	Spring 2025
1	4.51	5.51	4.50
2	5.05	5.09	5.03
3	4.37	4.73	4.80
4	3.96	4.03	3.99
5	4.07	3.96	4.20

The entomopathogenic fungi from individual soil samples were isolated using the selective medium method developed by Strasser et al. [1996], presented in detail in the scientific article by Majchrowska-Safaryan et al. [2025].

The obtained results were statistically processed using the Statistica program v. 13.3 (TIBCO Software Inc, Palo Alto, CA, USA). The first stage of the analysis was to check with the chi-square χ^2 test – whether the distribution of the tested trait (number of CFUs) in the sample followed a normal distribution. Since the data did not have a normal distribution, the transformation $y = \log(x + 0.5)$ was applied. Then, on the transformed data, one-way ANOVA analysis was performed for each factor separately according to the following model:

$$y_{ij} = m + a_i + e_{ij};$$

where: m – the population average; y_{ij} – the value of the examined trait (number of CFUs); a_i – the effect of the i -th level of factor A (sampling date, cultivation); e_{ij} – the random error. When the factor's effect was significant, the Tukey test was used at $p = 0.05$ to compare the means (posthoc analysis). Pearson linear correlation coefficients for pH KCl of the tested soils and the designated EPF genera were also calculated, and the standard deviation was calculated.

RESULTS AND DISCUSSION

Three genera of entomopathogenic fungi (EPF) were detected in soil samples during the spring and autumn study periods: *Beauveria* spp., *Metarhizium* spp., and *Cordyceps* spp. (Tables 3–5). The number of colony-forming units of isolated EPF genera depended on the plant species cultivated in a given field and the date on which the soil was sampled. Studies by Keller and Zimmermann [1989], Landa et al. [2002], Medo and Cagán [2011], Jarmuż-Pietraszczyk et al. [2011], Augustyniuk-Kram and Kram [2012], Steinwender et al. [2015], Dziegielewska and Adamska [2020], Majchrowska-Safaryan and Tkaczuk [2021], Kovač et al. [2021], Majchrowska-Safaryan et al. [2023, 2025], have examined arable soils in Europe and Poland, revealing the highest intensity of EPFs from the aforementioned genera. In the spring of 2024, EPFs of the genus *Metarhizium* spp. were the most abundant, forming $5.37 \text{ CFU } 10^3 \text{ g}^{-1}$ in the soil where spring barley was grown. In the autumn, the number of CFU of this genus was even higher, reaching $8.12 \text{ CFU } 10^3 \text{ g}^{-1}$, and the crop was triticale. Colony-forming units of this genus were not found in soil samples taken from wasteland or forest (Tables 3 and 4). Vänninen [1995] reports that fungi of the genus *Metarhizium*, including *M. anisopliae*, are the species that tolerate agricultural practices such as ploughing, harrowing and the use of chemical plant protection products and monoculture cultivation the best. They also tolerate the periodic absence of potential hosts in the environment better than other species. According to Quesada-Moraga et al. [2007], Tkaczuk [2008], Tkaczuk et al. [2016], this species *M. anisopliae* is more isolated from agricultural soils than from forest environments. In spring and autumn 2024, fungi from genus of *Beauveria* spp. were recorded in soil samples collected from all research sites, with the highest number of CFU units formed on wasteland, which may indicate a high density of their natural pests (no chemical protection used) that are their hosts, as well as a potentially greater abundance of organic matter than in cultivated soils, which is confirmed by research [Zhou et al. 2022]. These soils are characterized by higher humidity, which is favored by the permanent vegetation cover. Natural vegetation limits evaporation, protects the soil from erosion and enriches it with humus.

The analysis of variance showed that there were statistically significant differences in the intensity of occurrence of *Beauveria* and *Metarhizium* fungi depending on how the soils were used.

In both spring and autumn 2024, the lowest number of colony-forming units was found for the genus *Cordyceps* spp., and only in cultivated soils. Cultivated soils are subject to continuous and intense agricultural pressure, which destroys the natural microenvironment of these fungi. The absence of CFUs in forest soils and wasteland suggests that these environments are naturally devoid of these fungi (Tables 3 and 4).

Table 3. Number of colony-forming units (CFU 10^3 g^{-1}) by isolated genera of entomopathogenic fungi in the tested soils (spring 2024)

Specification		Genera of entomopathogenic fungi		
		<i>Beauveria</i> spp.	<i>Metarhizium</i> spp.	<i>Cordyceps</i> spp.
Cultivation	<i>Triticum aestivum</i> L. (common wheat)	1.13 $\pm$ 0.23 ^b	0.87 $\pm$ 0.18 ^{bc}	0.25 $\pm$ 0.18 ^a
	<i>Avena sativa</i> L.	1.0 $\pm$ 0.40 ^b	1.25 $\pm$ 0.27 ^b	0.12 $\pm$ 0.1 ^a
	<i>Hordeum vulgare</i> L. (barley)	1.13 $\pm$ 0.15 ^b	5.37 $\pm$ 0.62 ^a	0.0 $\pm$ 0.0 ^a
Forest soil		1.25 $\pm$ 0.24 ^b	0.0 $\pm$ 0.0 ^c	0.0 $\pm$ 0.0 ^a
Agricultural wasteland		5.0 $\pm$ 0.35 ^a	0.0 $\pm$ 0.0 ^c	0.0 $\pm$ 0.0 ^a
F value		234.68	296.82	2.857
P value		0.0	0.0	0.068

$\pm$ standard deviation; ab – means within columns with the same lowercase letters are not significant at $\alpha < 0.05$

Table 4. Number of colony-forming units (CFU 10^3 g^{-1}) by isolated genera of entomopathogenic fungi in the tested soils (autumn 2024)

Specification		Genera of entomopathogenic fungi		
		<i>Beauveria</i> spp.	<i>Metarhizium</i> spp.	<i>Cordyceps</i> spp.
Cultivation	<i>Hordeum vulgare</i> L. (barley)	1.0 $\pm$ 0.13 ^b	0.75 $\pm$ 0.15 ^b	0.4 $\pm$ 0.14 ^a
	<i>Hordeum vulgare</i> L. (barley)	1.13 $\pm$ 0.20 ^b	0.62 $\pm$ 0.17 ^b	0.25 $\pm$ 0.3 ^{ab}
	<i>Triticum aestivum</i> L. (common wheat)	1.25 $\pm$ 0.10 ^b	8.12 $\pm$ 0.72 ^a	0.0 $\pm$ 0.0 ^b
Forest soil		1.25 $\pm$ 0.10 ^b	0.0 $\pm$ 0.0 ^c	0.0 $\pm$ 0.0 ^b
Agricultural wasteland		6.25 $\pm$ 0.54 ^a	0.0 $\pm$ 0.0 ^c	0.0 $\pm$ 0.0 ^b
F value		357.87	298.75	10.71
P value		0.0	0.0	0.005

$\pm$ standard deviation; abc – means within columns with the same lowercase letters are not significant at $\alpha < 0.05$

In the spring of 2025 (Table 5), fungi of the genus *Beauveria* spp. formed CFU units in soils collected from all research sites, with the highest CFU found in forest soil and wasteland (1.0 CFU 10^3 g^{-1}). Entomopathogenic fungi of the genus *Metarhizium* spp. produced the highest CFU counts in the barley field (6.5 CFU 10^3 g^{-1}). During this research period, EPF of this genus were also determined in the forest soil (0.12 CFU 10^3 g^{-1}), where its presence had not been detected on the previous two research dates. Entomopathogenic fungi of the genus *Cordyceps* spp. produced the highest number of CFU units in the field where barley was grown (0.5 CFU 10^3 g^{-1}). Analysis of variance revealed statistically significant differences only for EPF of the genus *Metarhizium* spp. Studies investigating the influence of cultivated plant species indicate that EPF occurrence intensity depends more on soil than on plants. However, some fungal populations may be associated with vegetation groups [Steinwender et al. 2015, Nishi and Sato 2019].

According to Karg and Bałazy [2009], the introduction of crop simplification and chemical pest control eliminates arthropod pathogens from fields, as well as parasites and predators, as trophic groups with specific environmental and nutritional requirements, which are a source of food for the EPF. The structure of fungal communities is strongly dependent on soil cultivation methods (conventional, simplified, conservation), the use of monocultures, or intensive pesticides protection [Nelly et al. 2019, Gao et al. 2022, Kierzek et al. 2024].

Table 5. Number of colony-forming units (CFU 10^3 g^{-1}) by isolated genera of entomopathogenic fungi in the tested soils (spring 2025)

Specification		Genera of entomopathogenic fungi		
		<i>Beauveria</i> spp.	<i>Metarhizium</i> spp.	<i>Cordyceps</i> spp.
Cultivation	<i>Hordeum vulgare</i> L. (barley)	0.25 $\pm$ 0.10 ^a	6.5 $\pm$ 0.12 ^a	0.37 $\pm$ 0.12 ^a
	<i>Hordeum vulgare</i> L. (barley)	0.75 $\pm$ 0.30 ^a	1.0 $\pm$ 0.15 ^c	0.5 $\pm$ 0.2 ^a
	<i>Triticum aestivum</i> L. (common wheat)	0.5 $\pm$ 0.15 ^a	3.88 $\pm$ 0.45 ^b	0.13 $\pm$ 0.2 ^a
Forest soil		1.0 $\pm$ 0.25 ^a	0.12 $\pm$ 0.10 ^c	0.0 $\pm$ 0.0 ^a
Agricultural wasteland		1.0 $\pm$ 0.30 ^a	0.0 $\pm$ 0.0 ^c	0.0 $\pm$ 0.0 ^a
F value		34.09	229.24	8.333
P value		0.00033	0.0	0.01

$\pm$ standard deviation; abc – means within columns with the same lowercase letters are not significant at $\alpha < 0.05$

In autumn 2024 date based on the conducted research, a significantly positive correlation was found between the pH value of the tested soils and the intensity of the occurrence of EPF of the genus *Cordyceps* spp. (Table 6) Majchrowska-Safaryan [2022] in her previous studies showed significant correlations between the pH value of meadow soils and the occurrence of EPF of the genus *Lecanicillium* spp.

Table 6. Calculated correlations using Pearson's coefficient between the indicated parameters (autumn 2024)

Correlation	<i>Beauveria</i> spp.	<i>Metarhizium</i> spp.	<i>Cordyceps</i> spp.	pH _{KCl}
<i>Beauveria</i> spp.	1.00	−0.29	−0.43	−0.62
<i>Metarhizium</i> spp.	–	1.00	−0.30	0.15
<i>Cordyceps</i> spp.	–	–	1.00	0.89*
pH _{KCl}	–	–	–	1.00

*marked correlation coefficients are significant for $\alpha < 0.05$

In soil samples collected in spring 2025, the results of correlation coefficient analysis showed statistically significant relationships between the occurrence of the identified EPF genera (at the significance level $\alpha < 0.05$). A strong, significant negative correlation was found between the presence of fungi from the genus *Beauveria* spp. and: *Metarhizium* spp. (−0.982), *Cordyceps* spp. (−0.944) (Table 7). However, a strong, significant positive correlation (0.932) was found between *Metarhizium* spp. and *Cordyceps* spp., which may indicate similar ecological requirements or synergistic co-occurrence of these species in a given environment. With respect to soil pH, no statistically significant relationships were found with any of the isolated EPF.

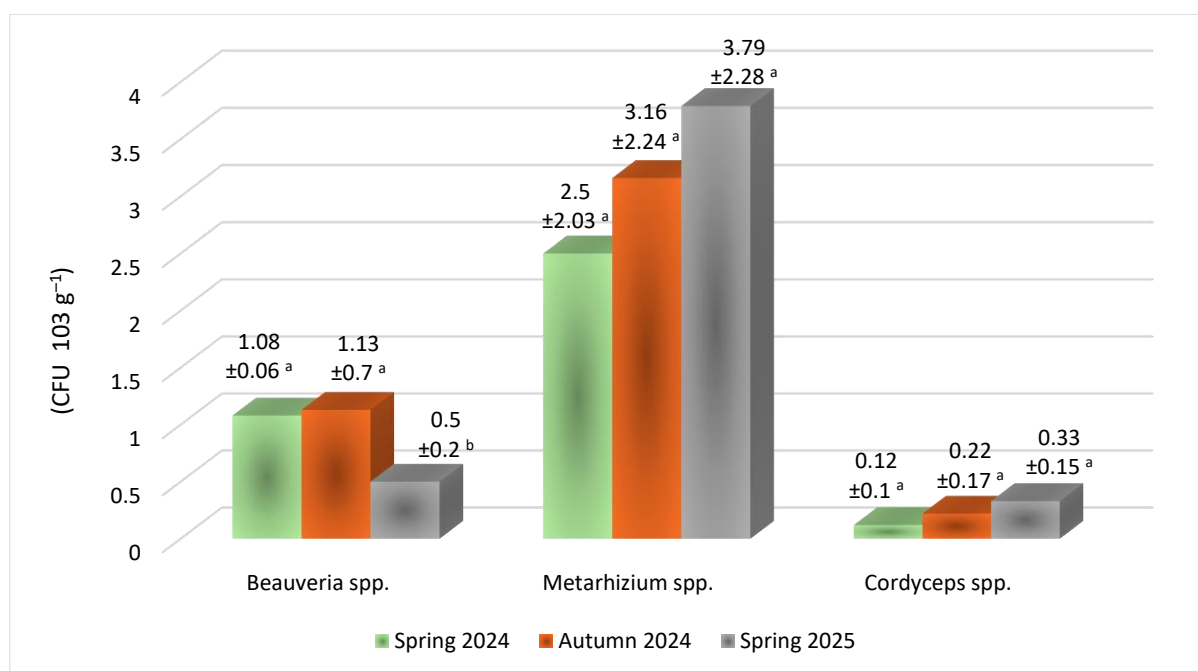
Table 7. Calculated correlations using Pearson's coefficient between the indicated parameters (spring 2025)

Corelation	<i>Beauveria</i> spp.	<i>Metarhizium</i> spp.	<i>Cordyceps</i> spp.	pH _{KCl}
<i>Beauveria</i> spp.	1,00	−0,98*	−0,94*	−0,49
<i>Metarhizium</i> spp.	–	1,00	0,93*	0,33
<i>Cordyceps</i> spp.	–	–	1,00	0,41
pH _{KCl}	–	–	–	1,00

*marked correlation coefficients are significant for $\alpha < 0.05$

Comparing the intensity of the occurrence of EPF isolated only from the soils where cereals were grown in monoculture, it was observed that in each of the research dates the most CFU were formed by fungi of the genus *Metarhizium* spp. (Figure 1), with the highest number in the spring of 2025. Fungi of the genus *Beauveria* spp. showed a tendency to reduce the number of CFU with the increasing duration of cereal monoculture, where in the spring of 2025 the number of CFU was 50% lower than in the two previous research dates, and this was a statistically significant difference. The number of CFU of *Cordyceps* spp. fungi remained at a similar level regardless of the date of soil sampling. In the cultivation soils Konopickà et al. [2022] stated that the effect of sampling location on the number of CFUs of the genus *Beauveria* was not significant ($F = 1.3379$; $df = 7.36$; $P = 0.2614$).

Figure 1. Density of colony-forming units of entomopathogenic fungi (CFU) isolated from soils where cereals were grown in monoculture during the study period (average for cereals in the year)



± standard deviation; ab – means within columns with the same lowercase letters are not significant at $\alpha < 0.05$.

Furthermore, research by Gorczyca et al. [2018] on the occurrence of EPF in agricultural and natural soils of southeastern Poland showed that *B. bassiana* was the dominant species during the autumn study period. In the arable soils of Slovakia maintained under the system of intensive pesticide protection, where cereals (including maize) were grown, it was found that EPF of the genus *Metarhizium* spp. produced significantly more CFU in spring, and the genera *Beauveria* spp. and *Cordyceps* spp. in autumn [Majchrowska-Safaryan et al. 2025]. Studies conducted by Wyrebek et al. [2011], Popowska-Nowak et al. [2016], and Dzięgielewska and Adamska [2020] indicate significant seasonal variability in the intensity of EPF occurrence in soils, closely related to soil temperature and moisture. Bruck and Lewis (2002) found that the frequency and intensity of rainfall, and ultimately the humidity of the environment, influence the possibility of EPF spore dispersal.

CONCLUSION

1. The presence of entomopathogenic fungi from the genera *Beauveria*, *Metarhizium*, and *Cordyceps* was demonstrated in the tested soils. The intensity of their occurrence depended on the cultivated plant species and the date on which the soil samples were collected.

2. The dominant EPF genus in each of the study dates (spring and autumn) was *Metarhizium* spp. Moderate temperatures in spring and autumn, coupled with high soil moisture, favour the development of pathogens.

3. Assessing the average density of colony-forming units of the tested EPF genera in soils cultivated in cereal monoculture established that *Metarhizium* spp. is the least sensitive to this cultivation system.

4. Analysis of variance showed that the generic composition and the intensity of EPF in the studied soils were significantly dependent on the date of soil sampling and the type of crop in some cases.

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